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Dottorato di Ricerca in Scienze Chimiche e Farmaceutiche

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Coordinatore: Prof. Stefano Mangani

INVESTIGATION OF NOVEL THERAPEUTIC TOOLS AGAINST INFECTIOUS DISEASES

Part 1. Medicinal Chemistry Investigation of MMV019918 Derivatives as Dual Schizonticide And Gametocytocidal Agents Against *Plasmodium falciparum*

Part 2. Investigation of 5-Aryl-Heterocycles As Potential Inhibitors of Metallo β-Lactamase Enzymes

Tutor:

Prof. Giuseppe Campiani

Dottoranda:

Alessandra Vallone

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Part 1

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CHAPTER 1

INTRODUCTION

1.1 Background: global malaria action plan

Malaria can be defined a critical public health challenge, historically being responsible for the deaths of millions, particularly young children and expectant women.

The **World Malaria Day** provides a common platform for countries to show their successes in malaria control and unify diverse initiatives in the changing global context. According with **World Malaria Report** of 2015, the number of malaria cases globally have declined by 18%[1]. Achieving this goal was made possible by improved usage of high-quality combination medicines, insecticide-treated (ITNs), and indoor residual spraying. However, many serious problems, such as the crescent resistance of the pathogen towards insecticides and drugs, linked to social and economic state of the endemic areas, make the risk of contagion still very high.

Actually, malaria is still endemic in many countries of the world and about 3,2 billion of people in 97 countries and territories, almost half of the world population, are at risk of malaria. Malaria is endemic in a broad band around the equator: Africa, some areas of South America, and many parts of Asia (**Fig. 1**)[2]. The highest number of cases occur in Sub-Saharan Africa, where children under 5 years old and pregnant women are the most affected. In many temperate areas, such as western Europe[3] and the United States, economic development and public health measures have succeeded in eliminating Malaria.

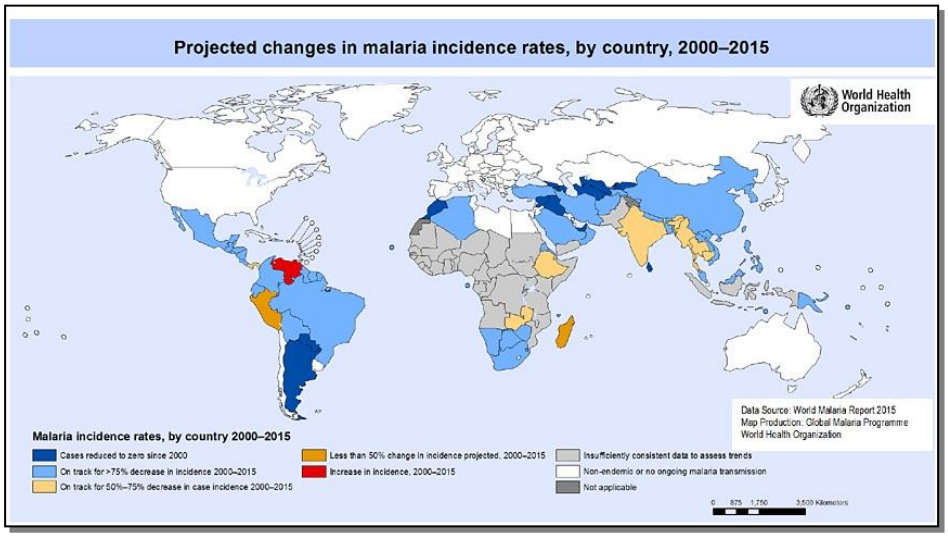


Fig.1 *Endemic areas of malaria*[4].

In the last decades, both public and private sectors increase the attention toward malaria disease. An example of this renewed interest is the establishment in 2007 of the Malaria Eradication

Research Agenda (**malERA**)[5]. Aimed at supporting the discovery, development and delivery of new antimalarial drugs, and ultimately at the eradication of the disease towards the vision of a malaria free world. Through this and ensuing initiatives, it is expected that over the next decade, with sustained support from governments and funding bodies, the number of malaria cases will continue to decrease, with several countries embarking on ambitious plans for national elimination. Several organizations have decided to invest on malaria: the **Roll Back Malaria**[6], the principal financial partnership of WHO; the **Bill and Melinda Gates Foundation**[7] which recently decided to increase their foundation's malaria budget; and of particular importance is the **Medicine For Malaria Venture**[8,9] (MMV) a leading product development partnership (PDP) in the field of antimalarial drug research and development. Because current tools are not sufficient to achieve global eradication, they are investing in a range of new interventions: control malaria to reduce the current burden and sustain control as long as necessary, eliminate malaria over time country by country and investigate new tools and approaches to support global control and elimination efforts. This is the key message of the **global malaria action plan**.

1.2 Malaria: transmission and symptoms

Malaria is a parasitosis caused by a protozoan of the genus ***Plasmodium*** of phylum ***Apicomplexa***. There are more than 100 species of *Plasmodium*, which can infect many animal species such as reptiles, birds, and various mammals. Four species of *Plasmodium* have been recognized to infect humans in nature: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Recently it has been discovered a fifth species of protozoan that caused Malaria in humans or in animals, in particular in monkeys, *P. knowlesi*. The species responsible for the most deaths is the *P. falciparum*, host of the main carrier African malaria[10].

Morphologically, *Plasmodium* shares many features of *Apicomplexa* parasites. Of these, most characteristic are the apical complex and apicoplast. The first is formed by cytoplasmic vescicles secreting proteins involved in the cell invasion. While the second is formed by four membranes and is involved in the lipidic metabolism and in particular in the formation of the parasitophorous vacuole. This is essential for *Plasmodium* survival and undergoes several morphological changes during infection in the host cells.

The disease is transmitted to humans by the female *Anopheles* mosquito, a kind of twilight mosquito that lives near ponds of stagnant water. Infact, malaria is prevalent in tropical and subtropical areas of the world where *Anopheles* mosquitoes can survive and multiply, and at the same time, *Plasmodium* can complete his growth cycle in the vector. There are 430 species of *Anopheles* mosquitoes, of which only 40 transmit malaria to humans, and of these only the female individuals[11].

It is clear that the outcome of the disease is influenced by several factors such as species of infecting parasites, the way of parasite transmission, the immune status of the host and the chemoprophylactic use of antimalarial drugs. This can cause a clinical picture that goes from asymptomatic infection to uncomplicated malaria that can lead to complicated or severe malaria and death. Of the four species of malarial parasites that commonly cause human infection, *P. falciparum* is responsible for virtually all severe cases and deaths. The other species, *P. vivax, P. ovale* and *P. malariae*, cause mainly a febrile illness and only rarely lead to severe disease[12].

The incubation period is defined as the interval between infection and the onset of symptoms, ranges from 9 to 14 days in *P. falciparum* infection and it may be prolonged by the preventive use of antimalarial drugs that can impact on the parasite replication. The characteristic symptom of malaria is fever. The fever is usually irregular at first and the temperature rises with shivering and mild chills. After some days, fever tends to become periodic and cyclic. This classical paroxysm presents three stages: a cold stage typically sudden,

followed by an hot stage with a rapid rise in temperature (39- 41°C), and a terminal sweating stage. Serious complications can develop at any stage, particularly in non-immune people, in which the infection may progress very rapidly to severe malaria unless appropriate treatment is started[13]. Most of severe malaria complications involve central nervous system (cerebral malaria), pulmonary system (respiratory failure), renal system (acute renal failure) and hematopoietic system (severe anemia). This progression can be rapid and potentially fatal. In areas with intense transmission, repeated plasmodial infections may generate a long-term stimulation of the immune system and determine a syndrome named hyper reactive malaria splenomegaly (HMS) with a huge spleen, abdominal distension, and abdominal pain[13].

The clinical manifestation of malaria depends also on the immune state of the host. In endemic areas, children up to 5 years of age commonly develop the symptoms of severe malaria, while symptoms in older children and adult are milder because the acquisition of partial immunity. Non immune pregnant women in their second and third trimester are at increased risk of developing severe malaria, particularly susceptible to develop pulmonary edema and hypoglycemia. Fetal death and premature labor are also common.

1.3 *Plasmodium* life-cycle

The Malaria parasite has a complex multistage life cycle consisting of two distinct phases: an **asexual stage** (schizogony), which occurs in the vertebrate host, and a **sexual stage** (sporogony), which occurs in the vector mosquito (**Fig. 2**). The asexual stage can be also divided into exoerythrocytic and erythrocytic schizogony.

When an infected mosquito bites an uninfected vertebrate host, it injects with saliva into subcutaneous capillaries **sporozoites**, the infective forms of *Plasmodium*. Within approximately 1 hour these disappear from the blood and enter in liver parenchimal cells. Inside the hepatocytes, each sporozoites begins an asexual reproduction resulting in the formation of **schizonts**, which contain **merozoites**. Their ensuing rupture, leads to the liberation of merozoites in the bloodstream[14]. From the clinical point of view, the hepatic schizogony represents the incubation period of parasite and it is infact, asymptomatic.

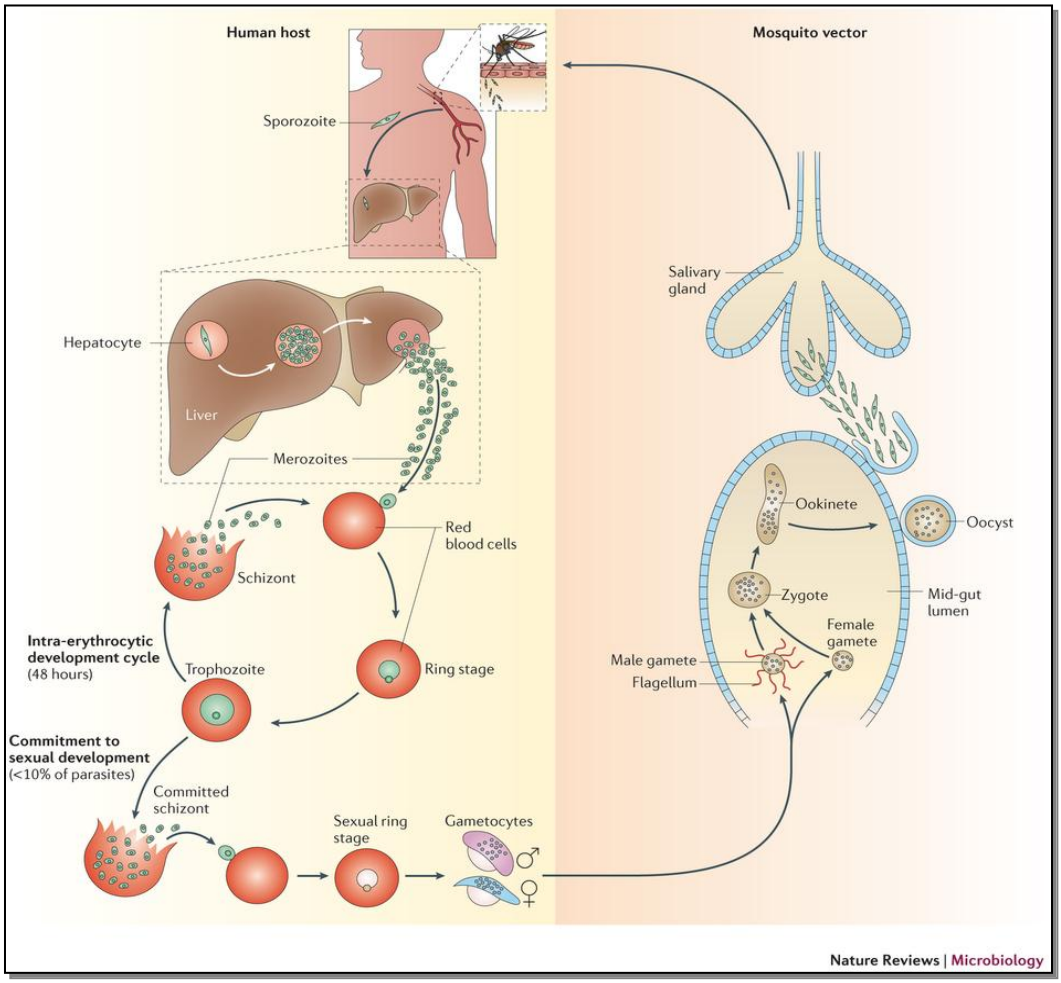


Fig.2 *Plasmodium life-cycle*[15].

Once in the bloodstream, merozoites reaches and invades red blood cells (RBCs) rapidly. The invasion is facilitated by molecular interactions between distinct ligands on the merozoites and host receptors of erythrocyte membrane. Within the RBCs, merozoites start a period of asexual multiplication in which they mature from a ring stage to **trophozoites**, and finally **erythrocytic schizonts**. This schizogony takes about 48 hours and comprises the formation of young rings first (after 12 h of erythrocytic infection); than mature rings (18 h); early trophozoites (24 h); mature trophozoites (30 h); early schizonts (36 h) and finally mature schizonts (42-48 h)[16].

Of particular interest is the morphology and biology of mature trophozoites; there is a parallel maturation of a large central **food vacuole** (FV), an acidic membrane-bound compartment involved in the erythrocytic heme detoxification (**Fig. 3**).

Infact, *Plasmodium* is not able to synthesize *de novo* amino acids, so it consumes up to 80% of the host cell haemoglobin. Haemoglobin is taken up by the parasites into the acid FV and it is released as ferri/ferroprotoporphyrin IX. This chemical species, in its free form is toxic for *Plasmodium*, generating oxidative stress. Therefore, parasite has developed a detoxification process of hematin, converting it, through a process called biomineralization, into insoluble and chemically inert beta-hematin crystals called **hemozoin**[17].

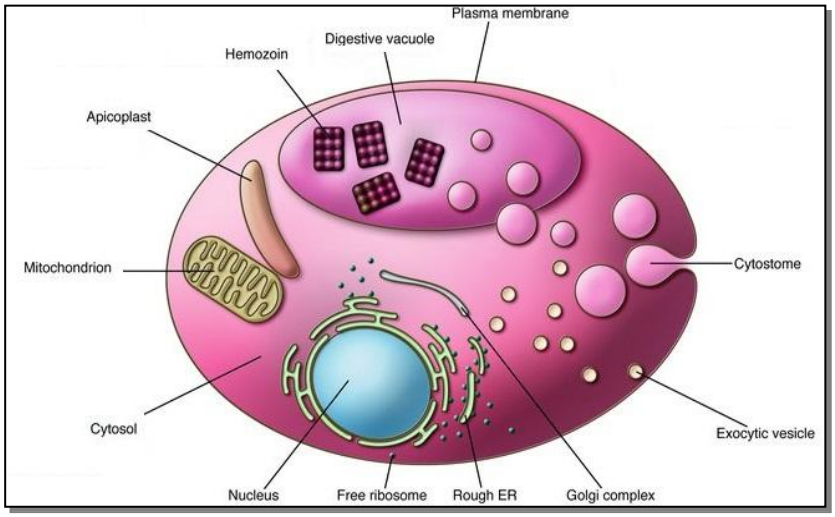


Fig. 3 *Representation of Plasmodium digestive vacuole*[18].

When schizonts became mature, a rupture of the RBCs membrane occurs with liberation of a new generation of merozoites. These invade again new erythrocytes and start a new schizogony cycle.

Erythrocytic schizogony occurs every 2-3 days, and each cycle is responsible of typical malaria symptoms.

During each cycle, a small proportion of asexual parasites divert from asexual replication and produce sexual progeny that differentiate into male and female sexual forms, known as ***gametocytes***. The gametocytes remain in the erythrocytes and are taken up by *Anopheles* mosquitoes after a blood meal. In the mosquito they rapidly mature into ***gametes***. The male gametocyte divides into eight flagellated microgametes, while the female gametocyte develops into a single macrogamete. Fertilization of a macrogamete by a microgamete results in the formation of a ***zygote***, which undergoes meiosis and develops into an invasive ***ookinete*** that penetrates the mosquito gut wall. The ookinete forms an ***oocyst***, within which parasite asexually replicates, forming several thousand ***sporozoites*** (sporogony). Upon oocyst rupture, these sporozoites migrate to the salivary glands, where they can be transmitted back to a new human host during a blood meal and start again a new life cycle[19].

1.4 Sexual reproduction: gametocytogenesis

Despite the real sexual reproduction takes place in the mosquito vector, the actual switch to sexual development occurs in the vertebrate host, with the gametocytogenesis. It represents the merozoites maturation into male and female gametocytes, the gamete precursor, and it is a prerequisite for transmission of malaria.

In *P. falciparum*, gametocytogenesis lasts 10-12 days and it is generally divided into five stages (I-V), according to a widely used classification system. During these stages, both the parasite and the host cells undergo morphological and metabolic changes that will lead to mature gametocytes liberation in the peripheral circulation until a new mosquito blood meal (**Fig.4**)[20].

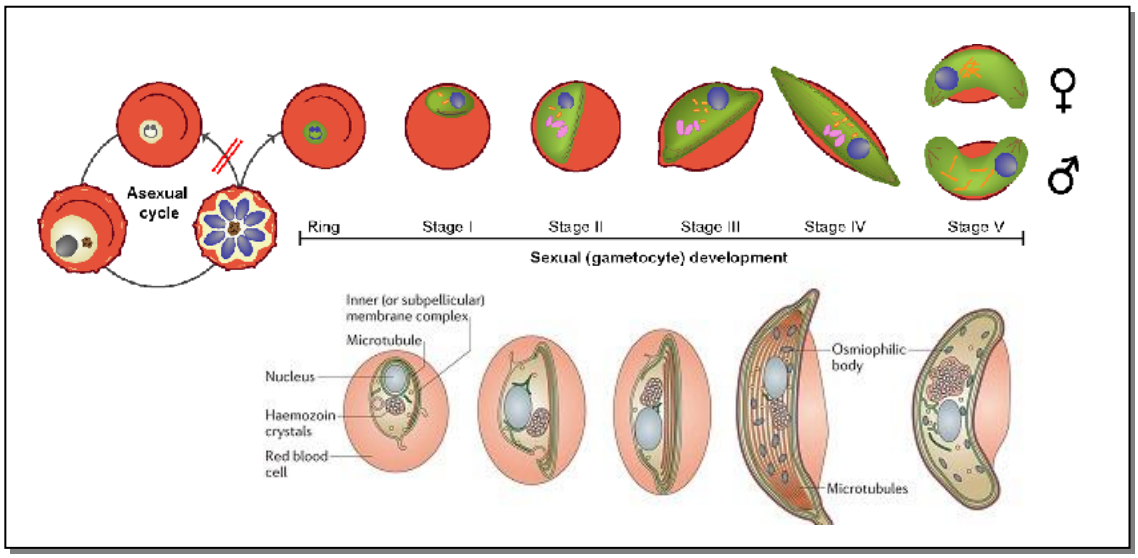


Fig.4 *Gametocytogenesis*[21,15].

- **Stage I**

After 30 h post-invasion, gametocytes are indistinguishable from young trophozoites, they are roundish with a small pointed end, and a pigment pattern. However, prior to any sign of morphological change, gametocytes produce two specific proteins, *Pfg27*, an abundant phosphoprotein and *Pfs16*, a parasitophorous vacuolar membrane protein. The role of both proteins is currently unknown, but seem to be necessary for cell transformation in gametocytes maturation[22].

- **Stage II**:

Between days 3-4, specific morphologic features become apparent. It begins to form a tri-laminar membrane structure known as subpellicular membrane complex, (SMC), responsible of the typical crescent shape of gametocytes. It consists of an outer plasma membrane, a parasitophorous vacuole membrane and a closely subpellicular membrane, all subtended by a layer of structural microtubules. Furthermore, now gametocytes start to synthesize ribosomes and the endoplasmic reticulum[23].

- **Stage III**

This stage starts approximately at day 4. The further development of SMC generates a dramatic cellular rearrangement, with one straight side and one curved side (D-shape). Importantly, male and female gametocytes begin to differentiate, there is infact alpha-tubulin expression in male gametocytes, while in female starts to be produced Pf377, a specific marker for osmiophilic bodies[24].

- **Stage IV**

Between days 6-8, SMC surrounds gametocytes completely making them extremely stiff with a symmetrical shape and round end points. In this stage, the sexual dimorphism is pronounced, infact female gametocytes show an increase in ribosomes, endoplasmic reticulum, Golgi and mitochondria, while for male parasites there is a parallel decrease. This stage also shows expression of some proteins (Pfs25 and Pfs28) with down-regulation of genes involved in glycolysis, protein synthesis and hemoglobin catabolism[24].

- **Stage V**

Although gametocytes remain in this quiescent state for several days, they are still highly sensitive to environmental stimuli. At stage V, between days 10-12, in both male and female gametocytes there is a breakdown of the subpellicular complex resulting in a smoothing of the gametocyte edges and a curved appearance, a character more pronounced in female gametocytes[24].

1.4.1 Gametocytes sequestration

The developing gametocytes (I-IV stages) are generally absent from the peripheral circulation, and only once they are mature (V stage), they are released in the circulation and are accessible to mosquito during a blood meal. Infact, recent studies suggest that mechanical properties associated with the appearance of adhesive properties play an important role in the **sequestration** phenomenon of gametocytes[25].

At first, mature asexual trophozoites are sequestered away from the peripheral circulation, and thus they are able to avoid the splenic clearance. This sequestration is possible due to the characteristic cytoadherence of parasite to the endothelium of the host erythrocytes. This adhesion is mediated by a parasite protein, the *P. falciparum erythrocytes membrane protein-1* (PfEMP1). This protein is exported in the erythrocyte cytoplasm and is anchored to protrusions termed "knobs" on the erythrocytes surface. These knobs structures, are of parasite origins and appear after 16 h from invasion, with the parasite *knob-associated histidine-rich* proteins (KHARP) as the principal components. It has been shown that KHARP play a major role in the sequestration of asexual parasite in various organs[26].

Early-stages gametocytes are also sequestered away from the peripheral circulation, but in sites with reduced vascular flow such as spleen and bone marrow. They appear to lack knobs and to adhere with lower efficiency. It is therefore clear that the mechanism of sexual stage sequestration is different from the asexual one. Infact, the gametocytes sequestration is now mediated by mechanical properties: the crescent ring shape and the characteristic rigidity of gametocytes in these stages allows them to avoid the splenic clearance.

In this context, other surface proteins are involved such as *subtelomeric variable open reading frame* proteins (STEVOR), and repetitive interspersed family (RIFIN). Their real activity is not completely known and clear but it has been shown that they play a significant role both in the adherence and in the maintaining rigidity[27].

Late-stages gametocytes are released into the circulation. This is made possible by several characteristic events. Regarding the adhesive properties, there is a proteolytic degradation of cytoplasmic domain of PfEMP1, furthermore there is a reorganization of RIFIN and STEVOR proteins that are exported in the cytoplasm. On the other hand about the mechanical properties, important is the breakdown of the subpellicular membrane complex which causes loss of rigidity and acquisition of deformability. Under these conditions gametocytes are able to cross the fenestration of sinusoids of spleen and bone marrow and return to the peripheral circulation[28,29].

1.4.2 Gametocytes activation

Mature gametocytes released in the peripheral blood system become infectious to mosquito after a further two or three days of circulation. During a blood meal, mosquito takes up gametocytes into the midgut and here, they undergo to environmental signals that activate the gamete development and the egress from erythrocytes (**Fig.5**).

The activation signals for both male and female gametocytes include a drop of temperature to approximately 5 °C, an increase of pH from 7 to about 8, a rise in intracellular Ca^{2+} concentration and the formation of a mosquito-derived metabolic intermediate xanthurenic acid (XA)[30].

For male gametocytes, activation consists in a rapid three rounds of genome replication followed by the assembly of flagella on the gametocyte surface. This process called ***exflagellation***, leads to the formation of eight flagellated and motile microgametes. As for male gametocytes, also the female ones are activated by the mosquito environmental factors. But DNA replication does not occur and the macrogamete, once released from erythrocyte, undergoes the adhesion and fusion with microgamete resulting in the zygote formation. Nuclear fusion is followed by the genome replication and meiosis in the zygote, which develops in a motile ookinete and penetrate the mosquito gut wall[31].

Important in this context, is the ***gametocytes egress*** from erythrocytes after activation to micro and macro gametes. This phenomenon is linked to the presence of osmiophilic bodies, secretory organelles identified in the stage III of maturation, most abundant in female parasites. These bodies migrate towards plasma membrane and interact with specific markers. The subsequent rupture of parasitophorous vacuole membrane (PVM) before, and erythrocytic membrane (EM) after, makes it possible the egress[31].

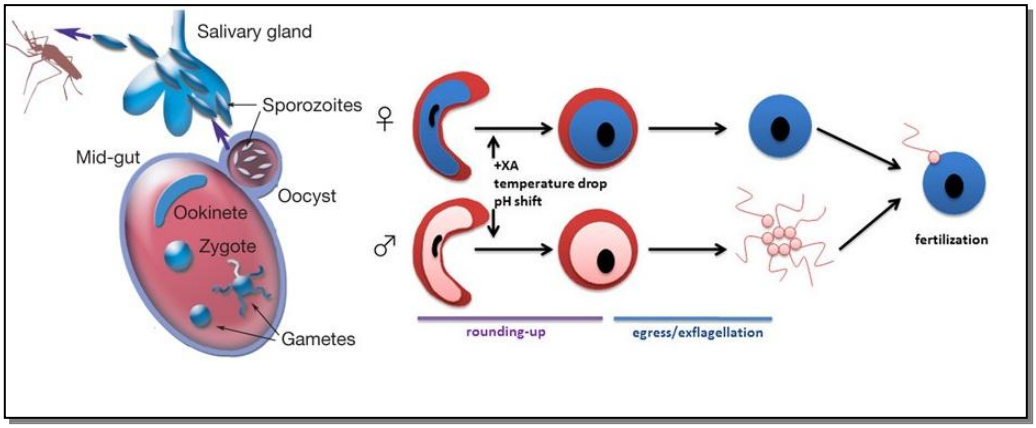


Fig.5 *Gametocytes activation*[32].

1.4.3 Trigger factors of gametocytogenesis

During the parasite life cycle, there are some transitions to different stages which involve complex correlations between the parasite's changing environment and its gene expression pattern[33]. The factors that trigger some of these transitions are well known such as the gametocytes transport from the bloodstream into the mosquito midgut, or the sporozoites release from the salivary gland of mosquito into human bloodstream. However, environmental and genomic changes related with the switch from asexual to sexual replication, the "genesis" of gametocytogenesis, are still an obscure point of plasmodium biology. Different studies have shown that the manipulation of the culture conditions or the addition of pharmacological agents can determine the beginning of gametocytogenesis and affect the number of gametocytes. But none of these experimental studies has been conclusively. It is difficult to predict when or if gametocytogenesis will start. First of all, early stages are sequestered and only the mature forms are released in the bloodstream; and then the beginning of sexual replication is different between the various species of *Plasmodium*. For example, in *P. vivax*, gametocytes appear at the same time of asexual parasitaemia, while for *P. falciparum* they appear later and the development is much longer[34].

What certainly emerges from several works, is that the decision to become a gametocytes appears during asexual stage, in particular, from a single schizont, all merozoites may be pre-committed to asexual or sexual development, and from the second case, either male and female gametocytes can be generated.

1.5 Historical development of antimalarial Drugs

For century, there was no specific treatment for malaria. In XVIIth century, Spanish colonizer brought back from Peru the bark of *Cinchona* tree, from which **quinine** was later extracted. It remained the only possible cure for decades, until XXth century when synthetic alternatives to quinine were developed. Of these, **chloroquine** was the most successful. However, its widely and indiscriminate use has caused the emergence of resistant parasite strains. This drug resistance phenomenon has rapidly spread in endemic areas making it more and more difficult to treat the disease[35]. During the same decade, Chinese scientists extracted from *Artemisia annua* plant the drug **artemisinin**, which has proved to be very effective against chloroquine-resistant malarial parasites[36]. But artemisinin-resistant strains of *P. falciparum* have recently appeared in Southeast Asia with a strong indications of rapid spread[37]. The use of two antimalarials in combination, particularly when they have different mechanism of action, has the potential for delaying the development of resistance to either of the components. Infact, today ***Artemisinin-Based Combination Therapy*** (ACT) is the approved treatment of uncomplicated falciparum malaria, while chloroquine and primaquine combination remains the first-line regimen for radical cure of vivax malaria[38].

For a very long time malaria was considered a neglected disease, but the lasts increasing numbers of mortality cases in young children and pregnant women, has attracted attention. From the beginning of the XXI th century, the attention toward malaria disease has been increased, and the official malaria eradication agenda (***malERA***) was established[5]. This institution brought together biologists, drug developers, clinical investigators, control officials, and financial foundations focusing the attention on distinct themes relevant to malaria eradication. These themes include: new drugs, vaccines, vector control, monitoring and surveillance, integration strategies, and health operations.

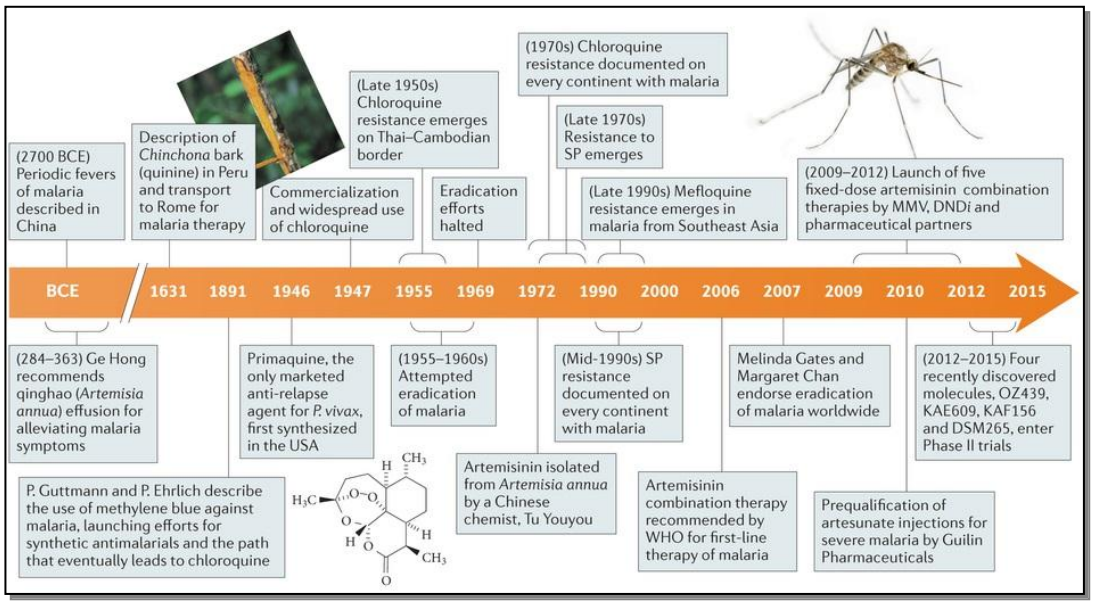


Fig.6 *Landmarks in malaria research*[39].

1.5.1 Antimalarial drug resistance

For decades the gold standard for the treatment of malaria was chloroquine (CQ), characterized by efficacy, low toxicity and affordability. However, its massive and widely use led to the appearance of CQ-resistant strains. This resistance has spread to the vast majority of malaria-endemic areas, rendering this drug increasingly ineffective. Today, antimalarial drug resistance represents one of the most important problem in malaria treatment and control.

The genetic events that confer antimalarial drug resistance are spontaneous, rare and independent of the drug used. They are usually mutations in the copy number of genes, and can be a single mutation, or multiple unlinked events[40].

In the case of CQ, the mechanism of resistance has been demonstrated by the identification of a mutated transporter protein on the surface of the food vacuole of 3D7 strains, named "*Plasmodium Falciparum Chloroquine-resistance transporter*" (PfCRT). This mutation causes a passive transport of CQ which is expelled from the FV[41].

The use of two antimalarials in combination, particularly when they have different mechanism of action, has the potential for inhibiting the development of resistance to either of the components and it is the rational of the introduction of ACT.

A further problem is represented by the phenomenon of *multidrug-resistance*, when the parasite is resistant to more than two antimalarial compounds of different chemical classes and modes of action. Infact, the emergence of a potential spread of artemisinin-resistant parasites requires new urgent strategy of therapy and control.

1.5.2 Antimalarial drugs classification

One of the way of classifying antimalarial drugs is according to their action on particular stages of *Plasmodium* life-cycle and to their different purposes, both in prevention and cure of individual infection or bigger in malaria eradication program[42].

- **Tissue schizonticides** drugs act on the exo-erythrocytic forms of the parasite, when they are inside the liver. This class of drugs is used for casual prophylaxis or in the radical cure of infections caused by *P. vivax* or *P. ovale*, because of their ability to generate hypnozoites, the dormant liver forms. Belonging to this class are the *8-aminoquinolines* primaquine, tafenoquine, pamaquine and some *antimetabolites* such as proguanil and pyrimetamine[42].

- **Hematic schizonticides** act on the asexual erythrocytic forms of all species of malaria parasite destroying schizonts and preventing erythrocytic schizogony. They are usually used during the fever cycles, and in some cases they can eradicate the infection. Belonging to this class are the *4-aminoquinolines* chloroquine, amodiaquine, and *endoperoxides* such as with artemisinin and its derivatives[42].

- **Gametocytocidal** drugs act destroying sexual forms of the parasite in the blood and thereby prevent transmission of the infection to mosquito. Chloroquine has gametocytocidal activity against *P. vivax* and *P. malariae* but not against *P. falciparum*. Primaquine and artemisinin have activity against gametocytes of all plasmodia, *including falciparum*[42].

- **Sporontocides** prevent the development of oocysts in the mosquito thus hampering the transmission from mosquitoes to humans. This class is used as prevention and include some *antimetabolites* such as proguanil, chlorproguanil and pyrimetamine[42].

1.6 Transmission blocking strategies

Historically, antimalarial research and development was focused on molecules targeting the asexual intra-erythrocytic stage of *Plasmodium* life cycle. This stage is responsible for the clinical symptoms of malaria and happens to be the point in which the parasites are most abundant and metabolically active. However, these drugs often do not affect other more inert parasite stages that do not lead to symptoms. These unaffected forms remain effective for transmission from human to mosquito, and from mosquito to human.

The elimination agenda proposed by Bill and Melinda Gates Foundation (BMGF) and WHO in 2007[5,7] will only be realized if the cycle of transmission can be broken. Thus in recent years, with the idea of disease eradication, the research focus has shifted to sexual stages and in particular towards **gametocytes**, sexual forms essential for malaria transmission. Drugs that block transmission could prove powerfully complementary therapy when combined with other interventions and they could provide synergies in an integrated program of antimalarial therapy. So gametocytes became one of the newest pharmacological target of the so-called **Transmission blocking strategies** (TBS)[43].

There are three main TBS that are being pursued:

- **transmission blocking vaccine**: it is an area of intensive research initiated by Malaria Vaccine Initiative (MVI). Current approaches are based upon antigens expressed on the surface of the sexual parasites and mosquito mid-gut. These antigens could be the targets of antibodies induced by vaccination of the host and ingested with the parasites during a mosquito blood meal. The antibodies act by inhibiting the parasite's development within the mosquito itself and they thereby prevent the transmission. Various vaccines have reached the state of clinical trials, but most demonstrated insufficient immunogenicity[43].

- **refractory mosquitoes**: a certain percentage of mosquitoes is refractory to parasite, incapable to transmit the disease. The genes that permit malarial infections in mosquito can be identified and then replaced or altered. Researchers are exploring possible way to emulate this refractoriness mechanism to shift the mosquito population towards a *Plasmodium*-resistant vector phenotype. The candidate system is the use of bacteria, fungi or even viruses as vehicles of refractory genes inside the mosquitoes[43].

- **gametocytocidal drugs**: the rational is to block transmission by clearing most gametocytes in the human host to render him non-infectious to mosquito. This because even if

the patient is clinically cured and asexual forms are eliminated, *Plasmodium* could be transmitted again. In addition, it has been demonstrated that certain antimalarials can increase gametocytemia. Most of the research is focused on the late gametocyte stage, but it is also possible to target the early stage or the mosquito stage[43,44].

1.6.1 Antimalarials with gametocytocidal activity

Gametocytocidal properties of existing antimalarial drugs are known and have been reported in many animal models *in vivo*. But relatively few molecules have reached the state of human clinical trials and further development into commercial therapies due to their toxicity or poor pharmacokinetic features.

The gametocytocidal activity of a drug can be exerted on early gametocytes (stages I-III), which being similar to trophozoites, are particularly vulnerable to drugs that affect hemoglobin metabolism such as artemisinin, or against late stage gametocytes (stages IV-V). At this stage gametocytes are more metabolically quiescent and are more likely to be affected by drugs with cytotoxic properties such as some 8-aminoquinolines. There is also the possibility to inhibit transmission after gametocytogenesis with a drug with sporontocidal activity such as pyrimetamine which directly damages ookinete and with proguanil, inhibiting dihydrofolate reductase[45].

1.6.2 Antimalarial endoperoxides

Artemisinin is a natural component of *Artemisia annua*, a plant used for a very long time in traditional Chinese medicine for the treatment of fever. When the component responsible for its action, qinghaosu or artemisinin has been isolated, its activity against *P. falciparum* was subsequently demonstrated and a series of semi-synthetic derivatives were also prepared and included in antimalarial therapy.

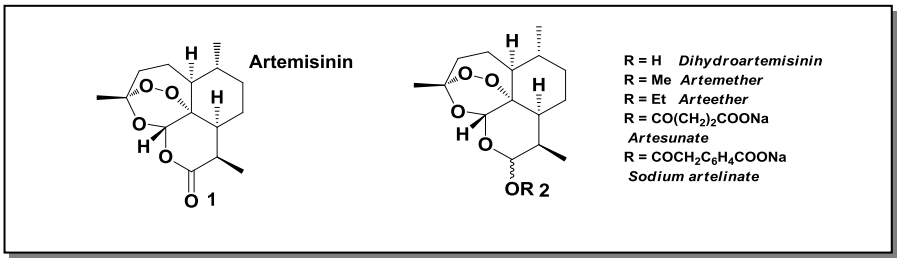


Fig. 7 *Artemisinin and its derivatives*

The schizonticidal activity of artemisinin and its derivatives is attributed to the reactivity of the endoperoxide bridge, a specific feature of this type of compounds. It is suggested an interference with haemoglobin catabolism in the FV of the parasite, inhibiting hemozoin formation and increasing hemozoin breakdown. This phenomenon leads to an accumulation of

toxic heme with subsequent membrane damage and further alkylation of parasite proteins by free radicals produced by artemisinin itself[46].

Artemisinin derivatives are fast acting substances, leading to a rapid clearance of the parasites from the blood. However, their short half-life precludes a long-lasting activity.

Therefore, artemisinins are preferably used not in monotherapy, but in combination with longer-acting drugs in the so called Artemisinin Combination Therapy (ACT) which represents the more effective treatment of uncomplicated malaria in areas of emerging high resistance to the most commonly used antimalarials.

About activity of artemisinin on gametocytes, it has been shown that gametocytes were sensitive to artemisinin at concentrations between 100 and 1000 ng/ml, while exposure to 10 ng/ml does not reduce the gametocytes level. It was furthermore shown that only younger stages of gametocytes were susceptible, while mature gametocytes were unaffected. However, there are few results indicating the possibility of a reduction in transmission of malaria in areas where artemisinin derivatives are extensively used[47].

1.6.3 Primaquine and other 8-aminoquinolines

Primaquine, an 8-aminoquinoline, was synthesized for the first time in 1946 in the United States by Elderfield and coworkers and for decades has been a significant drug in antimalarial therapy. Now, it is principally used in the radical cure of *P. vivax* infection in combination with chloroquine. In addition, it exhibits gametocytocidal activity against the mature sexual forms, reducing the prevalence of gametocytes circulating in the peripheral bloodstream. It has been also demonstrated a sporontocidal effect with the prevention of oocysts and sporozoites formation in mosquito. Infact, primaquine is actually the only antimalarial drug used in vivo as transmission blocking therapy[44].

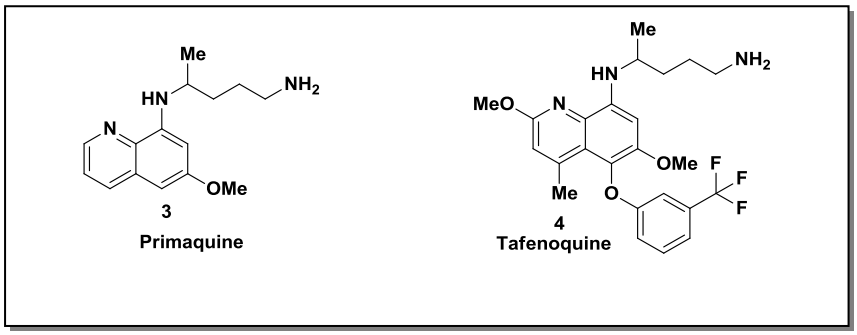


Fig.8 *Primaquine and its derivative tafenoquine*

About the mechanism of action, there are several hypothesis. Primaquine acts on parasite mitochondria, in particular on ubiquinone function as an electron carrier in the respiratory chain. Infact, from the third stage of gametocytogenesis, gametocytes begin to show a marked increase of cristate mitochondria. Furthermore, primaquine is involved in the haemoglobin metabolism during intra-erythrocytic stage, because of its hepatic conversion in metabolites able of causing oxidative stress. For both sexual and asexual activity, biotransformation seems to be necessary. In the human body, primaquine is rapidly metabolized with oxidative deamination at C-4' into carboxyprimaquine which has reduced antimalarial activity. Another metabolite is 5,6-dihydroxy-8-aminoquinoline which show intense inhibition of exoerythrocytic stage, while 6-hydroxy-8-aminoquinoline is even less active than carboxyprimaquine. Primaquine has a short half-life of about 7-8 h considered optimal to maintain the effect over a period sufficient to eliminate gametocytes from the bloodstream. The recommended regimen in adult is a single dose of 30-45 mg two weeks after ACT treatment[48].

Despite these promise, primaquine treatment involves some limitations. The major is represented by adverse interaction with *glucose-6-phosphate dehydrogenase* deficient (G6PDd)

individuals causing hemolytic anemia. This effect is related to a metabolite formation, 5-hydroxy-primaquine able to generate the oxidation of glutathione to glutathione disulfide and form insoluble aggregates of haemoglobin in red cells that are then removed from circulation by the spleen and liver.

Tafenoquine is an 8-aminoquinoline related to primaquine currently under clinical development. It has longer half-life than primaquine (14 days) and it's less toxic. Tafenoquine is active on all stages of *Plasmodium*, including liver schizonts.

1.6.4 Methylene Blue (Methylthioninium Chloride)

Methylene Blue (MB) is a heterocyclic aromatic compound used primarily to treat methemoglobinemia caused by cyanide poisoning. It was one of the first synthetic compounds used in malaria treatment.

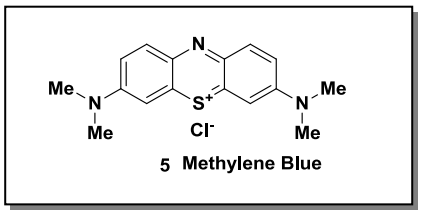


Fig.9 *Methylene blue*

About its mechanism of action, c methemoglobinemia can be considered a condition in which the heme ferrous ion is oxidized to ferric state. The reduced form of MB, acting as an electron donor, can reverse this oxidation. Against *Plasmodium*, this redox-cycling mechanism interferes with glutathione reductase, but another hypothesis suggests that MB can also interfere with hemozoin formation and its detoxification.

However, mechanisms of resistance to this drug are reported, infact it is recommended in combination with amodiaquine or artesunate exhibiting a strong gametocytocidal activity. It seems to act against both early and late stage gametocytes[49].

1.6.5 Toward ideal gametocytocidal drugs

The last of existing drugs easily selected for resistance, makes the research of gametocytocidal drugs or drug combinations with transmission blocking activity, an high priority for malaria control and elimination. This urgent research is compounded by the absence of an effective gametocytocidal drug: primaquine is the only compound used for this purpose, but its side effects limit its use in therapy.

Another important restriction involved in this research is ethical: a drug that theoretically only kills gametocytes or inhibits sporogony, would confer no direct benefit to a person infected with malaria; it would simply reduce the possibility that an infected person might pass on the infection to mosquito and thus, to other members of community[44]. Furthermore, gametocytocidal monotherapies and gametocytocides with low activity against asexual stages, are more likely to accelerate the resistance phenomenon but, if they are included as part of a combination therapy, might help slow the development of resistance. Thus, from a community perspective, an ideal transmission blocking drug should affect both phases of *Plasmodium* life-cycle, asexual and sexual, in particular the mature stage, in order to treat the clinical symptoms of the infected person, and at the same time, to prevent the disease transmission. Ideally, molecules showing different mode of action/targets in asexual and sexual parasites will be selected to avoid potential resistance to propagate to sexual stages and to be transmitted rapidly.

An ideal transmission blocking drug should also exhibit persistent and stable pharmacokinetic and pharmacodynamic characteristics to compensate for delays in parasite development, and release from sequestration. This follows, of course, after the array of other necessary and desirable properties that any drug should have, including safety, bioavailability, and lack of existing or easily selected for resistance[44].

Next to the priority of the development of new gametocytocidal compounds, there is a parallel need to identify valid throughput screening approaches (HTS assay) and valid pharmacological assay to evaluate the effective gametocytocidal activity. Unlike asexual parasite assays measuring parasite proliferation, greater variability in end-point readout exists between different gametocytocidal assays. This is compounded by difficulties in routinely producing viable, functional and stage-specific gametocyte populations.

CHAPTER 2

AIM OF WORK

2.1 From Malaria Box to MMV019918

In recent years, the advent of public-private partnerships has facilitated collaborative efforts between pharmaceutical companies with not-profit organizations and universities. In 2007, a renewed effort for malaria global eradication was proposed by the Bill and Melinda Gates Foundation (BMGF)[7] at Malaria Forum in Seattle. In 2011, scientists reconvened in Bethesda, to discuss the latest research and developments into the sexual reproduction of *Plasmodium* and the latest strategies for malaria control. One of the key deliverables of this meeting was the need for phenotypic assays for the identification of gametocytocidal and sporontocidal compounds, as transmission blocking drugs[50].

Over the last years, several methods of large-scale high throughput screening (HTS) have been performed to identify compounds with activity against the asexual blood stages of *Plasmodium falciparum*. The not-for-profit organization **Medicines for Malaria Venture** (MMV), established in 1999, supported HTS efforts of *St. Jude Children's Research Hospital*, *Novartis* and *GlaxoSmithKline* (GSK) to screen over 4 million compounds for in vitro antimalarial activity. Of these, over 25,000 compounds have been identified and deposited in the *ChEMBL neglected tropical diseases archive*, an Open Access screening repository that allows researchers from around the world to access this data free of charge. These compounds, active against the asexual stages of the malaria parasite life cycle, were "distilled" in a set of 400 diverse compounds that formed the basis of the so called **Malaria Box**, that was made available to various research groups for a screening work in order to identify molecules with dual activity against both the asexual and sexual stages[51].

Two hundred compounds of the MMV malaria box are considered to be drug-like and 200 probe-like. Of the 200 compounds classified as 'drug-like', shown in **Fig. 10**, 72 had IC50 values ≤1600 nM and of these, only 20 (10%) had activity against either early or late stage gametocytes. Of the 20 'drug-like' active compounds, 7 (35%) were active against both early and late stage gametocytes, 9 (45%) were active against late stage gametocytes only and 4 (20%) were classified as active against early gametocytes only[52]. Of particular note is **MMV019918** which has IC50 value < 1000 nM against all three stages tested. This compound falls into the "drug-like" classification given to the MMV malaria box compounds.

Having biological activity against the asexual blood stages, early and late stage gametocytes, in addition to its "drug-like" chemical characteristics, data confirmed in several screening campaigns performed in different laboratories using different methodologies, **MMV019918** was selected as a suitable hit compound for further investigation and optimization[50].

This promising compound **MMV019918**, is reported as active on both asexual and sexual stages (early and late) with nanomolar inhibition in all different assay formats, (IC50 ranging from 320-890 nM). Despite it falls into the drug-like classification given to MMV Malaria Box compounds, a certain cytotoxic profile has been demonstrated against polarized hepatocytes cell line (HepG2) and against human fibroblasts (MRC-5) cells; furthermore in terms of cardio-toxicity, a moderate activity on hERG channels was reported.

Its structure suggests a relatively versatile pathway for synthesis of a high number of analogues in order to improve the toxicological profile and potency. These results were decisive in the choice to start a new chemical and biological investigation of **MMV019918** .

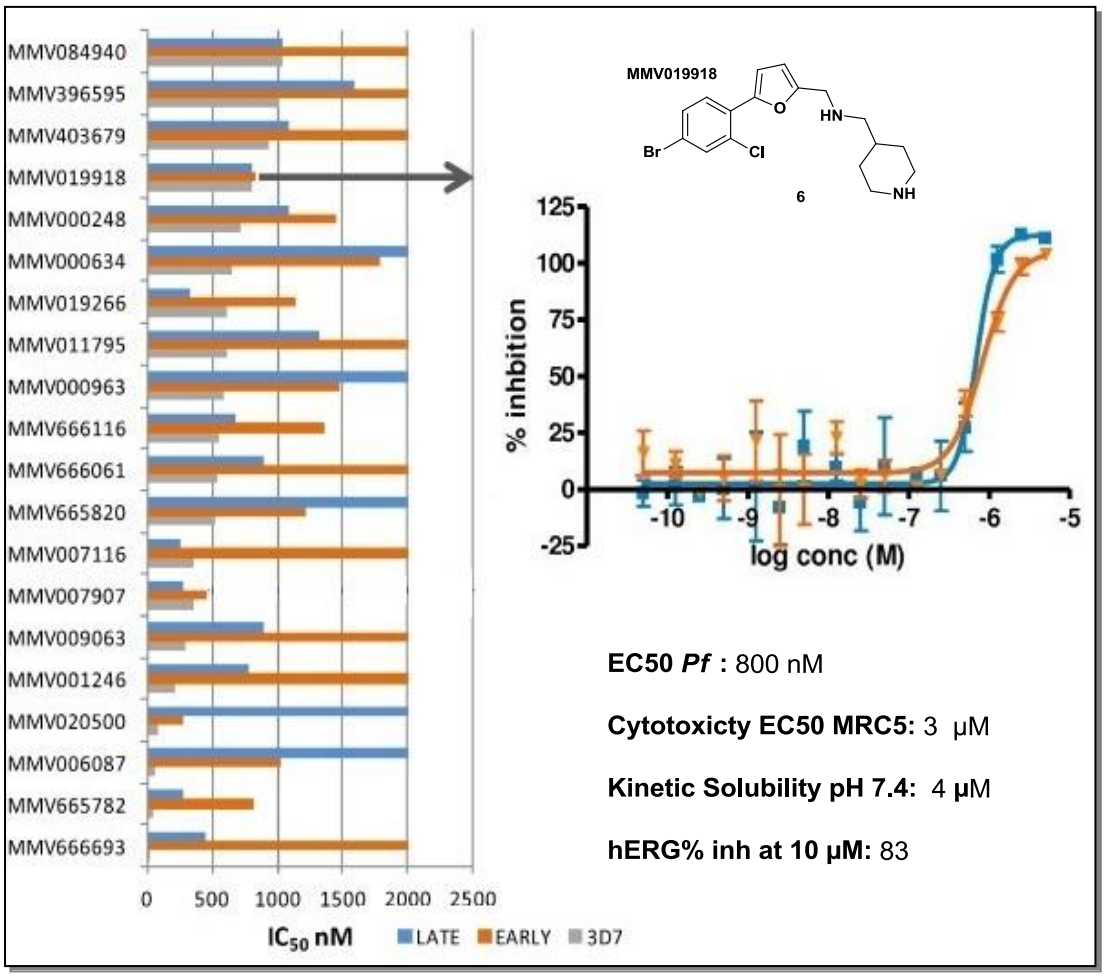


Fig 10.

Activity of compounds classified as "drug like" and MMV019918 IC50 evaluation

Orange triangles are indicative of the early (I-III) stage gametocyte activity whereas the blue squares represent the late (IV-V) stage gametocyte activity[51].

The project here described, involves the design and synthesis of analogues of **MMV019918** as a potent new class of transmission-blocking compounds. To achieve this goal the aim of work was divided into several landmarks:

- development of a versatile synthetic strategy of **MMV019918** and a rational design of derivatives considering two aspects: **potency** and **toxicity**
- evaluate and improve toxicological profile (cytotoxicity and cardiotox)

2.2 Design of MMV019918 derivatives

MMV019918 chemical structure can be generalized as a 5-aryl-furan scaffold in which three different moieties can be identified (**Fig. 11**). This molecular simplification is important first of all for the development of synthetic strategy, but also for the design of analogues.

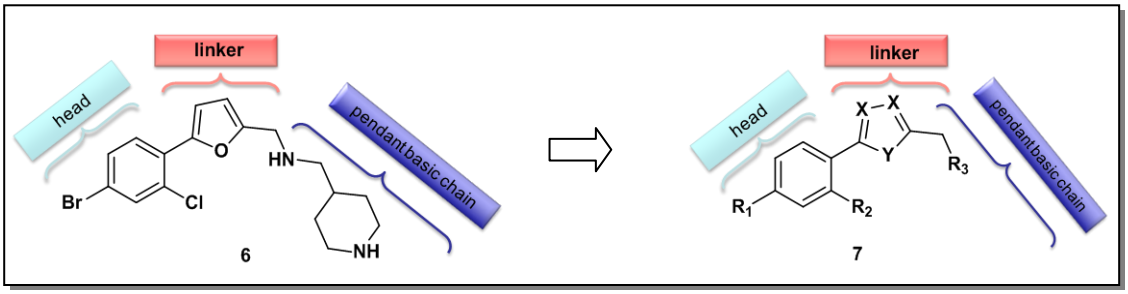


Fig 11. *MMV019918 molecular simplification*

The first moiety is an aromatic and hydrophobic **head**, decorated with electron-withdrawing substituents in *ortho* and *para* positions, chlorine and bromine respectively. These substituents, as well as the aromatic ring, were identified as points of structural investigation through the synthesis of analogues. The second moiety is an heterocyclic central **linker**, a furan ring linked directly to the aromatic head with a C-C bond at C-5, and functionalized at C-2. Also this part of the molecule can be subjected to modifications to evaluate the heterocyclic scaffold for both the biological and toxicological activity. In addition, of great importance is the functionalization on C-2 with the third moiety, a pendant **basic chain**, in particular a piperidinyl-methanamine which confers a certain basicity and water-solubility to the whole molecule. This lateral chain can be the subject of a considerable investigation since the reduction as well as the increase of basicity can give useful pieces of information about activity and toxicity.

2.2.1 Aromatic and hydrophobic head

Considering the proposed structural moieties of **MMV019918**, the first set of changes concerns the aromatic and hydrophobic head, to evaluate the real effect of substituents on the aromatic ring. Accordingly, the electronic effect of several substituents, would clearly have an effect on drug ionization and polarity and furthermore it would have an effect on how easily a drug can pass through the cell membrane or how strongly it can interact with a putative binding site. The design of the substituents to be introduced was based on the exploration of electron-releasing, electron-withdrawing, polar, hydrophobic and bulky substituents. In order to explore the lowest number of substituents with the maximum informative power, the *Craig plots* was taken as a guide for the selection. As shown in **Fig. 12**, it is a two-dimensional plot divided in four sections corresponding to the correlation of positive and negative values of hydrophobicity constant (π) and *Hammet* electronic constant (δ).

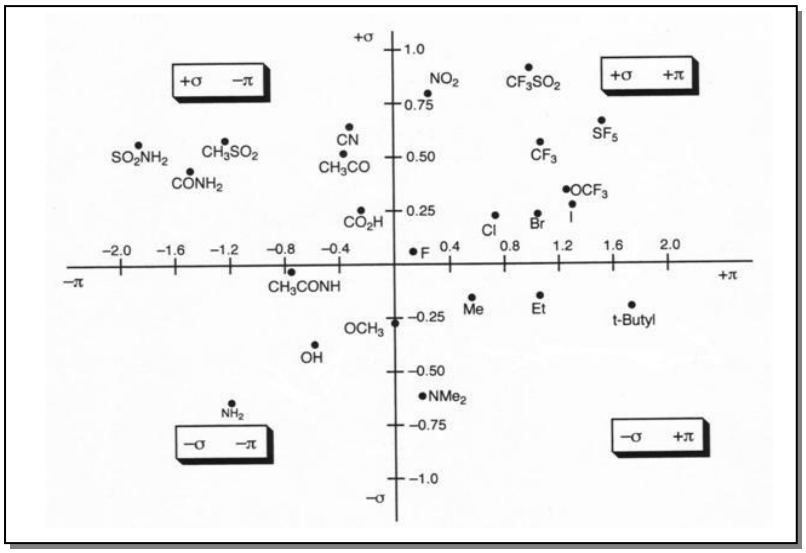


Fig. 12. *Craig plots*

According to these combinations of values, a first series of derivatives has been developed maintaining initially, the electron withdrawing effect and the hydrophobic character of substituents (+δ, +π), in mono or di-substituted molecules (Fig. 13 *derivatives* **8-13**)

Always keeping the electron withdrawing effect, was changed the hydrophobic character (+δ, -π) by the introduction of a nitrile functional group (Fig.13 derivative **14**). Subsequently, also an electron donating effect (-δ, -π) has been evaluated introducing polar functional groups such as methoxyl and hydroxyl (Fig. 13 derivatives **15** and **16**).

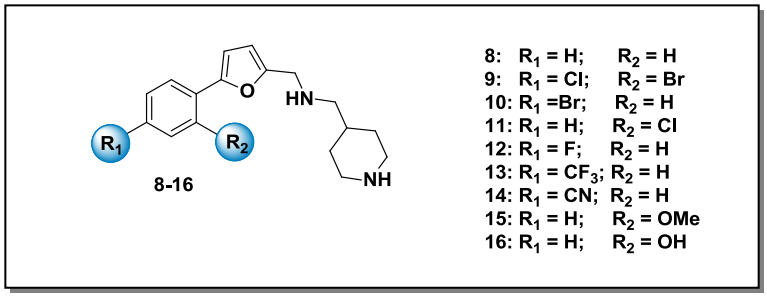


Fig. 13. *MMV019918 aromatic substitutions.*

Subsequently, the role of the steric hindrance, through introduction of bulky groups, such as an aromatic ring was evaluated. The bulk, size and shape of a drug would influence how easily it can approach and interact with the binding site. In particular, bulky substituents may help to properly orient a drug for maximum binding and increase activity, or may act like a shield hindering the ideal interaction between a drug and its binding site. The replacement with a more bulky substituent allowed to evaluate its real effect on bioactivity. For example, the biphenyl system constitutes the structural moiety of a wide range of compounds with great pharmacological activity. In biphenyl derivatives, two phenyl groups are joined by a single bond (sp^2-sp^2) but this system is not planar, one ring being slightly twisted or canted in relation to the other as a consequence of steric crowding. This hindrance results in a chiral conformation, having a dihedral angle of about 45º, equilibrates rapidly with its enantiomers by rotation around single bond. This is what happens in derivatives (Fig. 14, derivatives **17**, **18a-j**) where a phenyl group was introduced, creating a biphenyl system and maintaining at the same time the electron withdrawing and hydrophobic effects exercised by the chlorine atom. It was chosen chlorine as substituent, once in *ortho* and once in *para* position, for simplicity of synthesis, because of the considerable reactivity of bromine used as the coupling point to introduce the phenyl group.

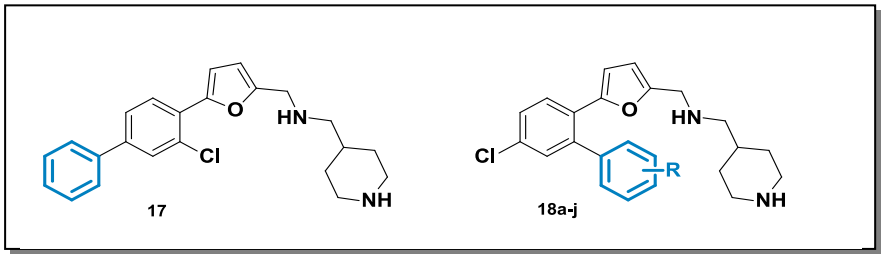


Fig. 14. *MMV019918 steric hindrance.*

2.2.2 Heterocyclic central linker

A second set of derivatives has been developed introducing changes on the heterocyclic central linker.

Furan derivatives have occupied a unique place in the field of medicinal chemistry and actually, the incorporation of the furan nucleus is an important synthetic strategy in drug discovery.

Furan is a planar five-membered heterocyclic ring . It is the most reactive compound of the five-membered heterocycles. It is a non polar aromatic compound and the presence of ether oxygen adds polarity as well as the potential for hydrogen bonding.

The designed modifications were developed according to the classical theories of *bioisosteric replacement*. Infact, the first classical bioisosteres introduced are a thiophene, a condensed benzothiophene, and a pyridine (Fig. 15 derivatives **19**,**20**,**23** and **24**). In this way it was maintained the electronegative effect of the furan oxygen. Moreover, other heterocycles were investigated, in particular the family of azoles to evaluate the different effect of the nitrogen such as a basic and aromatic 1,2,4-triazole and an 1,3,4-oxadiazole (Fig. 15 derivatives **21**,**22**).

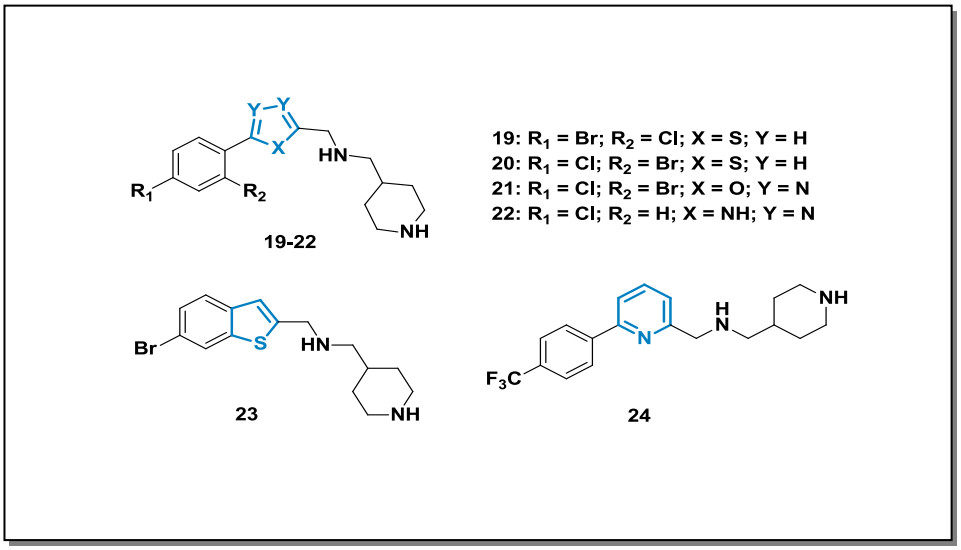


Fig. 15. *MMV019918 heterocyclic substitutions.*

2.2.3 Pendant lateral side chain

The last moiety in the hit compound subjected to structural investigation was the **pendant basic chain**. The amine functionality is the most common functional group found in drug molecules. Infact, the physicochemical property of primary importance in the drug chemistry of the amino group, is its basicity. This property is closely related to water solubility or solubility in other vehicles for drug administration. Furthermore, the protonated amino group present in many drug compounds provides a cationic center required for binding to many drug targets including receptors and enzymes.

In **MMV019918** the amino group is represented by a piperidinyl-methanamine. A first cyclic moiety was modified with an open-chain amine, a *N*,*N*[1]-dimethyl-1,3-propanediamine (Fig. 16 derivative **26**). This choice was also based due to the presence, in the Malaria Box, of a derivative having this chain, **MMV020505** (Fig. 16 derivative **25**).

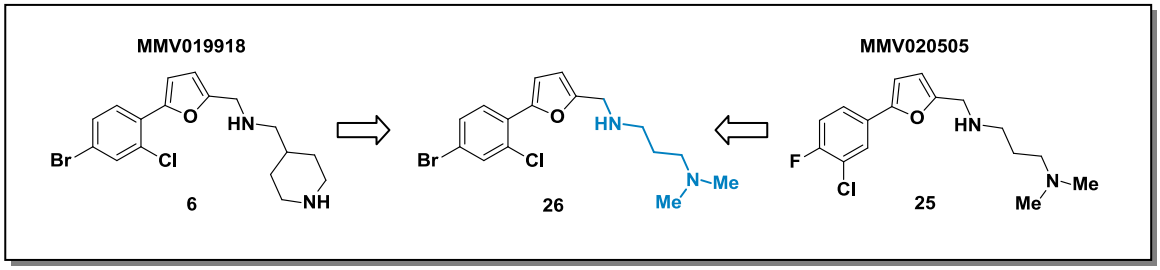


Fig. 16 *MMV019918 and MMV020505 hybridation approach.*

This chain was further investigated by preparing the corresponding quaternary ammonium salts, benzyl and methyl respectively (Fig. 17 derivatives **27** and **28**). This because it has been shown that molecules with one or two quaternary ammonium salts would be very efficient against *P. falciparum* blood stage[53], probably acting through the inhibition of choline transport.

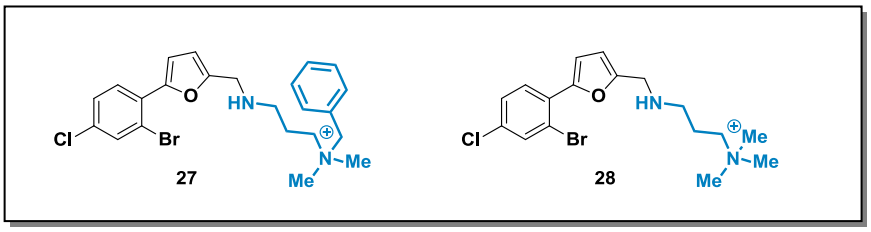


Fig. 17. *MMV019918 quaternary ammonium salts derivatives.*

At the same time, it was also decided to proceed with the design of a series of derivatives of the biphenyl compound **18a**, reasoning in terms of potency and toxicity. New derivatives were

identified with changes in the lateral chain. The piperidine ring was replaced by a piperazine and a *N*-methyl-piperazine (Fig. 18 derivatives **29** and **30**), and also several tertiary amines were designed and synthesized (Fig.18 derivatives **31**-**33**).

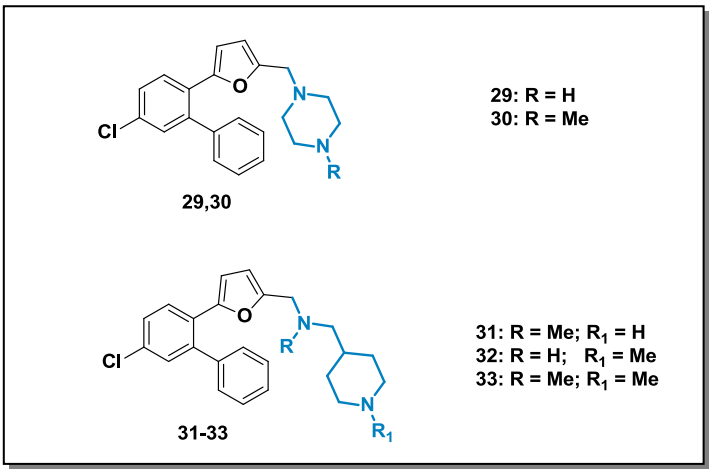


Fig. **18.** *Compound **18a** derivatives with lateral side chain substitutions.*

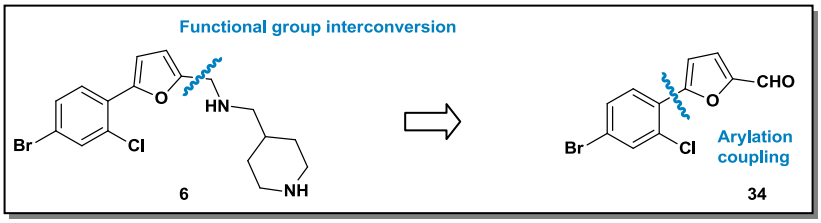
CHAPTER 3

CHEMISTRY

3.1 Development of MMV019918 synthesis

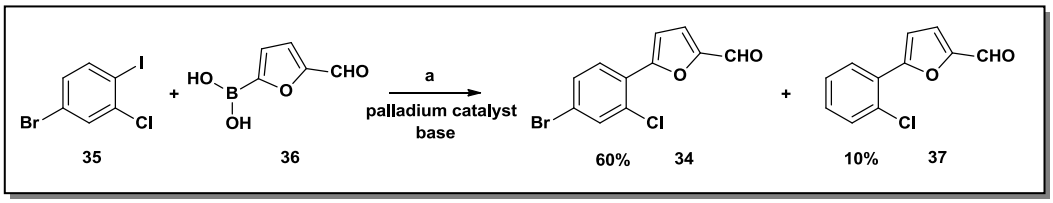
For a versatile synthesis of the designed compounds, the retrosynthetic analysis for the synthesis of **MMV019918** and derivatives is depicted in Scheme1.

The synthetic precursor chosen for the aliphatic side chain is a diamine that could be introduced in intermediate **34** through a classical functional group interconversion approach. The arylfuran-carboxaldehyde intermediate **34**, could be in turn introduced through an arylation of the furan moiety. Different arylation approaches were attempted and used in the reaction Schemes described below.



Scheme 1. *Retrosynthetic approach*

The first approach to the synthesis of 5-(4-bromo-2-chlorophenyl)furan-2-carbaldehyde **34** is represented by a Suzuki-Miyaura coupling (Scheme 2). It is a palladium-catalyzed cross coupling reaction between an organoboronic acid and an aromatic halide (See Box 1)[54].



Scheme.2 *Suzuki-Miyaura coupling general conditions*

Being commercially available (5-formylfuran-2-yl)boronic acid **36**, the starting substrate chosen to perform the coupling reaction was the 4-bromo-2-chloro-1-iodobenzene **35**. Using this aryl-halide substrate, the reactivity of the iodine prevailed over that of bromine and chlorine, and the reaction turned out to be exquisitely regioselective, conducing to the formation of the expected reaction product without the presence of by-products resulting from attack of the boronic acid in other positions of the aromatic ring. However, in addition to the desired product **34**, small quantities of the dehalogenated product **37** were identified and characterized, probably formed during the reductive amination step.

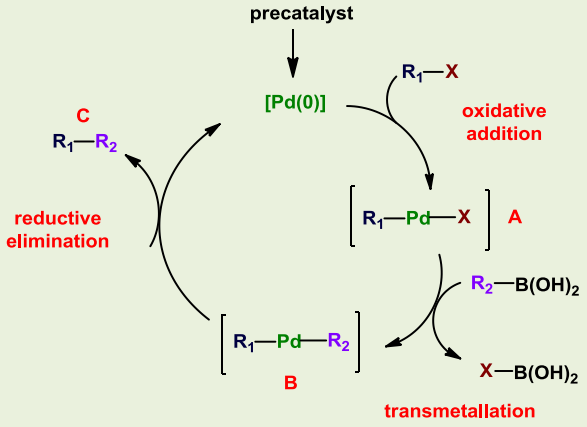
Several reaction conditions were then investigated in order to limit the formation of by-products and to improve the reaction yields. (Table 1). Because of the poor solubility of the boronic acid **36**, a mixture of dimethoxyethane and ethanol resulted to be the best solvent; furthermore bis(triphenylphophine)palladium(II)dichloride and sodium bicarbonate were chosen as catalyst and base.

Catalyst	Solvent	Base	Time	°C	Yield
Tetrakis(triphenylphosphine)palladium0	Tol./EtOH	Na_2CO_3	12 h	100	30%
Tetrakis(triphenylphosphine)palladium0	DMF	K_2CO_3	24 h	120	10%
Bis(triphenylphophine)palladium(II)dichloride	DME/EtOH	Na_2CO_3	12 h	70	60%

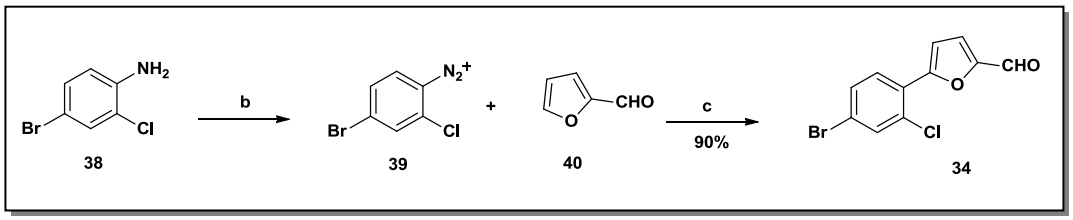
Table 1. *Suzuki-Miyaura coupling reaction conditions*

Box 1. Suzuki-Miyaura coupling reaction mechanism

It is a palladium-catalyzed cross coupling between an organoboronic acid and halide. About the reaction mechanism, three different steps can be identified. The first step is the *oxidative addition* of palladium to the halide to form an organopalladium species (step **A**). Reaction of this with a base gives an intermediate which, via *transmetalation* with the boronate-complex (produced by the activation with the base), forms a second organopalladium species (step **B**). *Reductive elimination* of the desired product (step **C**), restores the original palladium catalyst which completes the catalytic cycle.



A second approach that was explored to obtain the aldehyde **34**, was the Meerwein arylation, a convenient method for the synthesis of aryl-furans (Scheme 2, **Box 2**)[55].

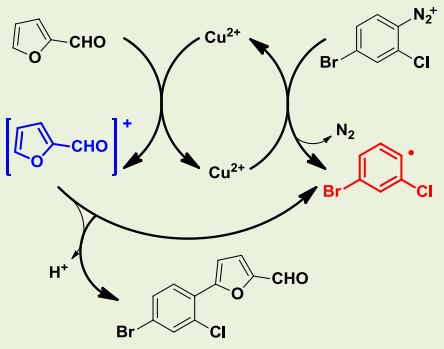


Scheme 2. *Meerwein radical arylation conditions:*
b) HCl 37%, $NaNO_2$, H_2O, 0 °C, 1 h; **c)** $CuCl_2$, acetone, 0-25 °C, 12 h.

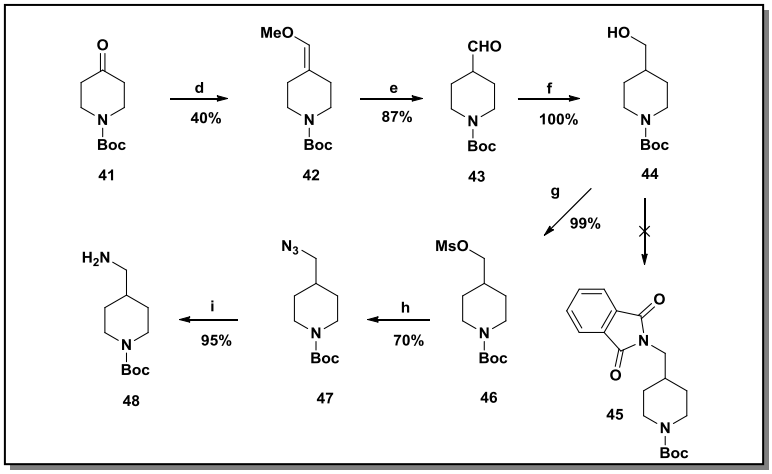
This reaction protocol, applied to aniline **38** and performed in a one-pot reaction in aqueous medium, allowed to obtain the desired product **34** with satisfactory yield (90%). Further advantages of the Meerwein arylation protocol are that is suitable for large scale preparations, the reaction conditions are milder and the aniline substrates used as starting materials, are less expensive than halides necessary for the Suzuki coupling.

Box 2. Meerwein radical arylation mechanism

Meerwein arylation is a copper-catalyzed arylation of furan derivatives with arenediazonium salts. This reaction involves a radical mechanism. The initial step is copper(II)-mediated electron transfer from the substrate molecule to diazonium ion, obtained from aniline. The catalytic system Cu+↔ Cu2+ mediates electron transfer from furaldehyde to diazonium ion. Aryl radical thus formed reacts with furaldehyde radical generated in the first cycle with formation of the desired product.

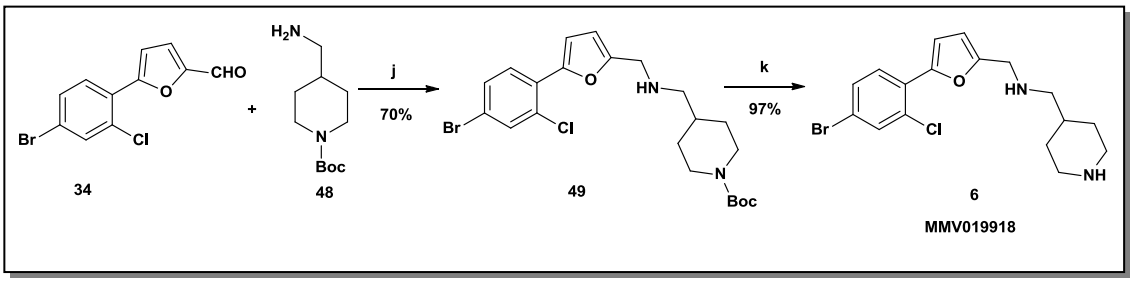


In Scheme 3 is shown the synthesis of the basic pendant chain **48**.



Scheme 3. *Synthesis of amine lateral side chain.* ***Reagents and conditions:***
d) (Methoxymethyl)triphenylphosphonium chloride, NaHDMs, dry THF, 0-25 °C, 12 h; **e)** $CeCl_3$, NaI, MeCN, 40 °C, 12 h; **f)** $NaBH_4$, MeOH, 25 °C, 1 h; **g)** MsCl, TEA, dry DCM, 0-25 °C, 12 h; **h)** NaN_3, dry DMF, 0-85 °C, 12 h; **i)** H_2, Pd/C (10%), MeOH, 25 °C, 3 h.

Starting from *N*-Boc-piperidone **41**, a Wittig reaction was performed using (methoxymethyl)triphenylphosphonium chloride and sodium bis(trimethylsilyl)amide as base. The resulting alkene **42** was subjected to hydrolysis performed with cerium chloride and sodium iodide. The hydrolysis of vinyl ether function involves the protonation of the vinyl group, followed by rapid hydration of the alkoxycarbocation thus formed and fast decomposition of the ensuing hemiacetal intermediate. The aldehyde **43**, was reduced to alcohol **44** using sodium borohydride as the reducing agent. At first, the synthetic strategy planned, at this point involved a Mitzunobu reaction to obtain a phtalimide derivative **45** and affording subsequently the amine **48** by hydrazinolysis. However, the Mitzunobu reaction has not been successful, leading to a complex reaction mixture. Employing a different synthetic strategy, the primary alcohol **44**, was converted into the corresponding methanesulfonate **46**, and then subjected to a nucleophilic substitution to form the azide **47**. A final catalytic hydrogenation, using palladium on carbon, led to the formation of the desired piperidinyl-methanamine **48** in satisfactory yield.
The synthons **34** and **48**, were then subjected to a reductive amination reaction (Scheme 4) to obtain the secondary amine **49**.



Scheme 4. *Functional group interconversion approach.* ***Reagents and conditions:***
j) 1. MeOH, 70 °C, 12 h; 2. $NaBH_4$, MeOH 0-25 °C, 1 h; **k)** AcCl, MeOH, 25 °C, 4 h.

The careful investigation and final identification of the appropriate reaction conditions led to optimum in order to increase yields and reduced the formation of by-products. The main by-product identified in the reaction was the corresponding tertiary amine, resulting from reaction of **48** with aldehyde **34** present in excess under the employed reaction conditions. To prevent the formation of this by-product, was necessary to constantly monitor the reaction progress, and the reaction was conducted in a diluted solution. Taking these precautions, once the substrates had reacted completely, it was possible to add the reducing agent in situ giving the amine **49** in good yield.

The final deprotection of the piperidinyl amine function from tert-butoxy-carbonyl group, performed with hydrochloridric acid formed in situ from acetyl chloride and methanol, gave the final compound **6**, **MMV019918** without further purification.

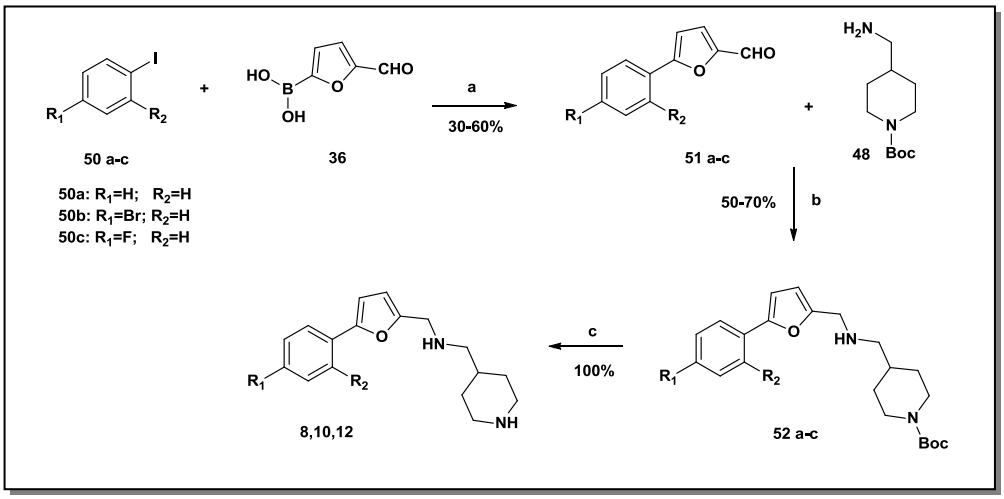
The versatility of the entire synthetic strategy for the preparation of **MMV019918**, allowed its application for the preparation of various analogues using less expensive and commercially available starting materials.

3.2 Synthesis of MMV019918 derivatives bearing different aryl moieties

The synthetic strategy designed for **MMV019918** was useful for the development of derivatives bearing substitutions in the three identified moieties.

Regarding the changes of the aromatic portion, the two main arylation methods, Suzuki-Miyaura coupling and Meerwein arylation, were applied according to the commercially available substrates.

The Suzuki-Miyaura coupling has been used for the synthesis of several derivatives **8**, **10** and **12**, in which the electron withdrawing effect and hydrophobic character are maintained. The synthesis is shown in Scheme 5. The aryl iodides **50a-c** were chosen as starting substrates and the aldehydes thus obtained **51a-c**, were subjected to the reductive amination with amine **48** using sodium borohydride, and to the subsequent deprotection with acetyl chloride and methanol affording the final desired products **8**, **10** and **12**.

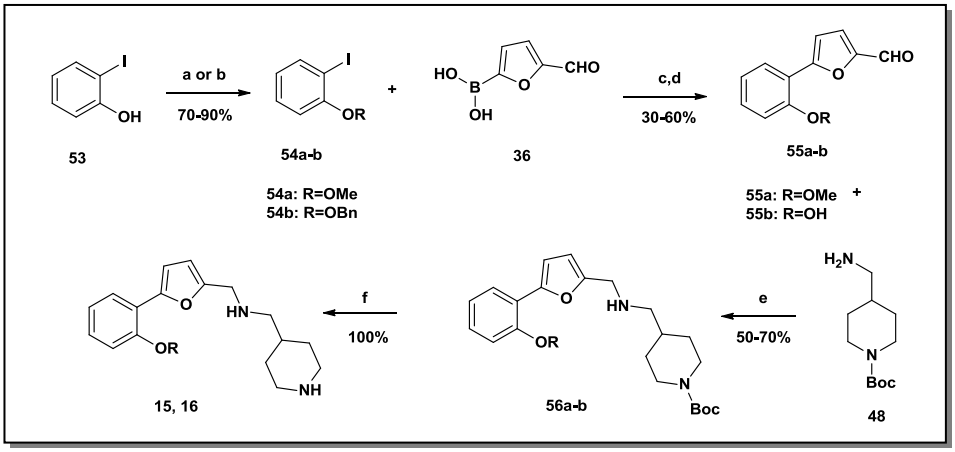


Scheme 5. *Synthesis of derivatives **8,10,12**.**Reagents and conditions:***

a) Bis(triphenylphophine)palladium(II)dichloride, Na_2CO_3, DME/EtOH 3:1, 70 °C, 12 h; **b)** 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 1 h; **c)** AcCl, MeOH, 25 °C, 4 h.

Also derivatives with an electrondonating effect were synthesized with this arylation approach. The synthesis of these compounds is shown in Scheme 6. The starting 2-iodophenol **53** was at first protected with a methyl- and benzyl- groups using methyl iodide and benzyl bromide respectively, in the presence of a base to obtain the Suzuki substrates **54a-b**. The synthetic protocol remains the same, with the exception of aldehyde **55b**, which was deprotected from the benzyl function. The deprotection reaction was performed with boron trichloride at low temperature in order to avoid the reduction of furan ring. From these aldehydes the classical

reductive amination was performed followed by amine deprotection with acetyl chloride and methanol to obtain the final compounds **15** and **16**.



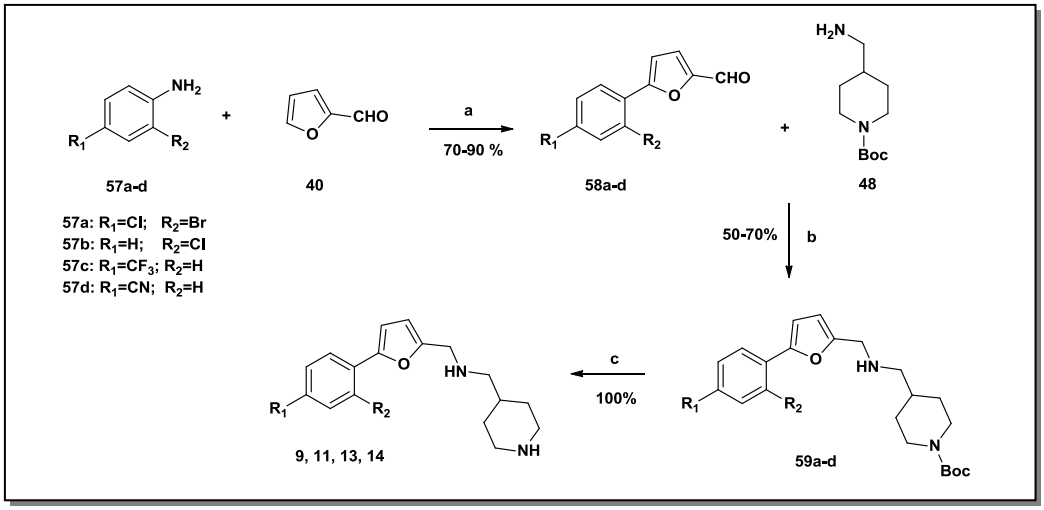
Scheme 6. *Synthesis of derivatives **15,16. Reagents and conditions:***

a) MeI, KOH, dry MeCN, 25 °C, 3 h; **b)** BnBr, K_2CO_3, dry THF, 60 °C, 12 h; **c)** Bis (triphenylphosphine)palladium (II)dichloride, Na_2CO_3, DME/EtOH 3:1, 70 °C, 12 h; **d)** BCl_3, dry DCM, -78 °C, 2 h; **e)** 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 1 h; **f)** AcCl, MeOH, 25 °C, 4 h.

The radical Meerwein arylation has been applied to the synthesis of derivatives **9**, **11**, **13** and **14**, for which anilines were commercially available as starting substrates. Their synthesis is shown in Scheme 7.

The first step is the diazotization of aromatic amines **57a-d**, with nitrous acid (generated in situ form sodium nitrite and a mineral acid) obtaining the corresponding diazonium salts stable at 0 °C in water solution. The addition to the reaction mixture of furaldehyde **40** in acetone and copper(II) chloride as catalyst, resulted in the radical reaction process affording aldehydes **58a-d** with satisfactory yields (70-90%). In several cases, precipitation of the aldehyde from the aqueous environment greatly facilitated the purification of the desired products, which were simply isolated by filtration and recrystallization.

At this point the aldehydes **58a-d** thus obtained were subjected to the same synthetic protocol of reductive amination with amine **48** and sodium borohydride to form the secondary amines **59a-d** and subsequent amine deprotection in acidic medium to obtain the desired final products **9**, **11**, **13** and **14**.

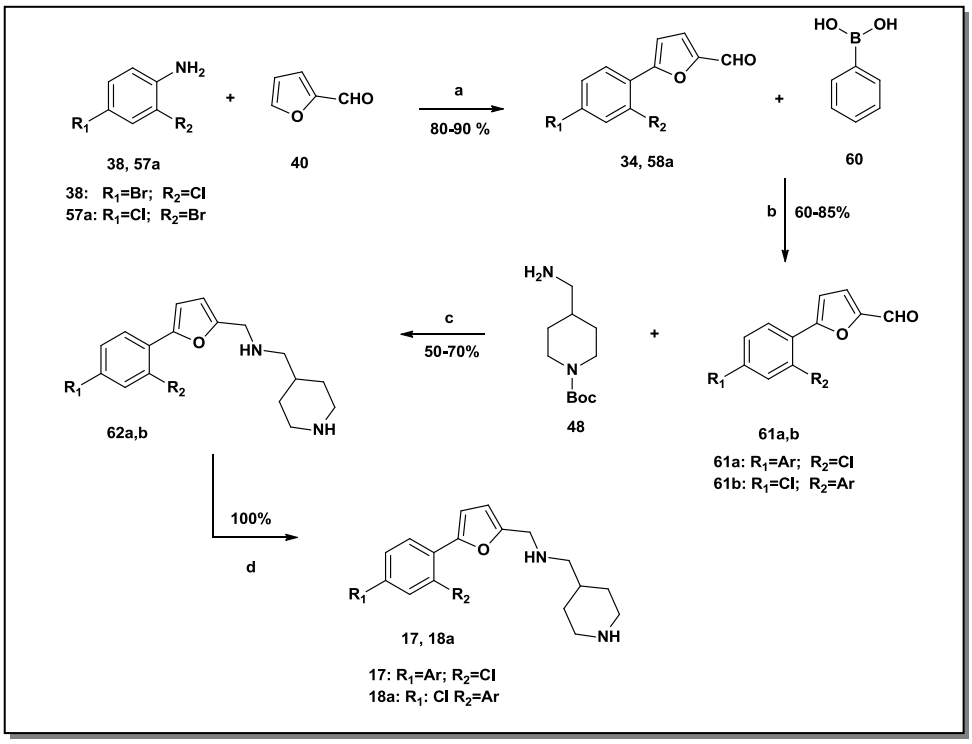


Scheme 7. *Synthesis of derivatives **9**, **11**, **13**, and **14**. **Reagents and conditions:***

a) $NaNO_2$, HCl, $CuCl_2$, acetone, water, 0-25 °C, 24 h; **b)** 1. MeOH, 70 °C, 12 h, 2. NaBH4, MeOH, 0-25 °C, 1 h; **c)** AcCl, MeOH, 25 °C, 4 h.

3.3 Synthesis of compound 18 and its derivatives

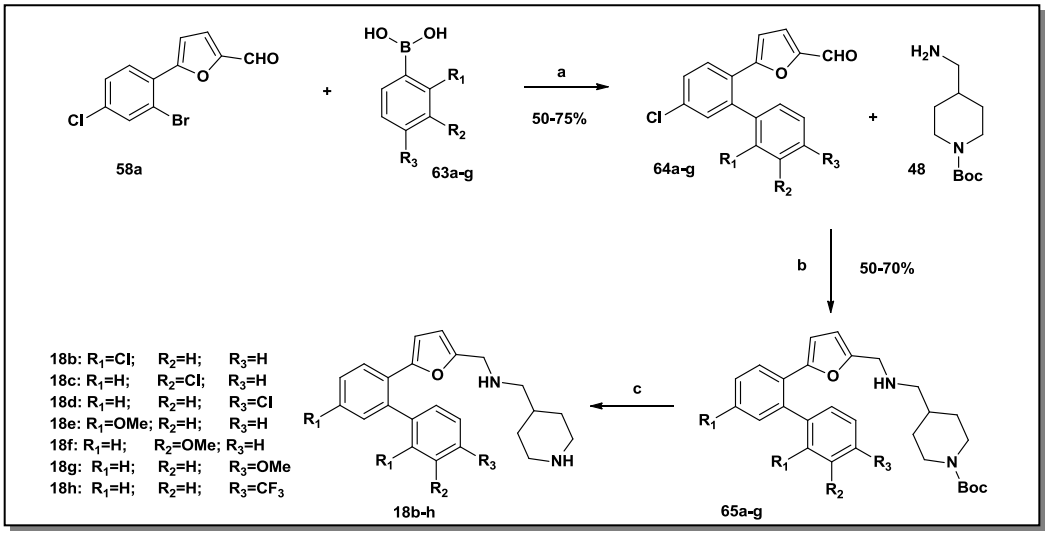
The two arylation methods here described were combined for the development of synthetic strategy used for the preparation of derivatives **17** and **18a**, where a phenyl group was introduced, creating a biphenyl system. The synthesis is shown in the Scheme 8. From anilines **38** and **57a**, chosen as starting materials, the aldehydes **34** and **58a** were obtained through the one-pot diazotization and radical Meerwein arylation with furaldehyde **40**. On these substrates, the higher reactivity of bromine than chlorine atom was exploited to introduce the second aromatic ring in *para* position for **61a**, and in *ortho* position for **61b**. The *Suzuki-Miyaura* coupling has been performed using phenyl boronic acid **60** and also in this case, several reaction conditions were investigated. Tetrakis(triphenylphosphine)palladium(0), (generated in situ with triphenylphosphine and palladium(II)acetate), was identified as the best catalyst, sodium carbonate was used as the base and toluene as the solvent. The reaction was complete within 12 h affording the products **61a-b** in satisfactory yields (60-85%).The subsequent reductive aminations and amine deprotections allowed to obtain the final compounds **17** and **18a**.



Scheme 8. *Synthesis of derivatives **17** and **18**. **Reagents and conditions:***

a) $NaNO_2$, HCl, $CuCl_2$, acetone, water, 0-25 °C, 24 h; **b)** Tetrakis(triphenylphosphine)palladium0, Na_2CO_3, dry toluene, 90 °C, 12 h; **c)** 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 1 h; **d)** AcCl, MeOH, 25 °C, 4 h.

This strategy resulted versatile also for the synthesis of some derivatives of compound **18** bearing substituents on the second aromatic ring of the biphenyl system. As shown in Scheme 9, starting from the previously described aldehyde **58a**, several phenyl boronic acids **63a-g** were used in the following Suzuki-Miyaura coupling, obtaining aldehydes **64a-g**. Electron withdrawing and electrondonating substituents were investigated in all *ortho*, *meta* and *para* positions of the aromatic ring, maintaining reaction yields in the range of 50-75%. The subsequent reductive amination and amine deprotection of the secondary amines **65a-g**, allowed to obtain the desired final products **18b-h**.

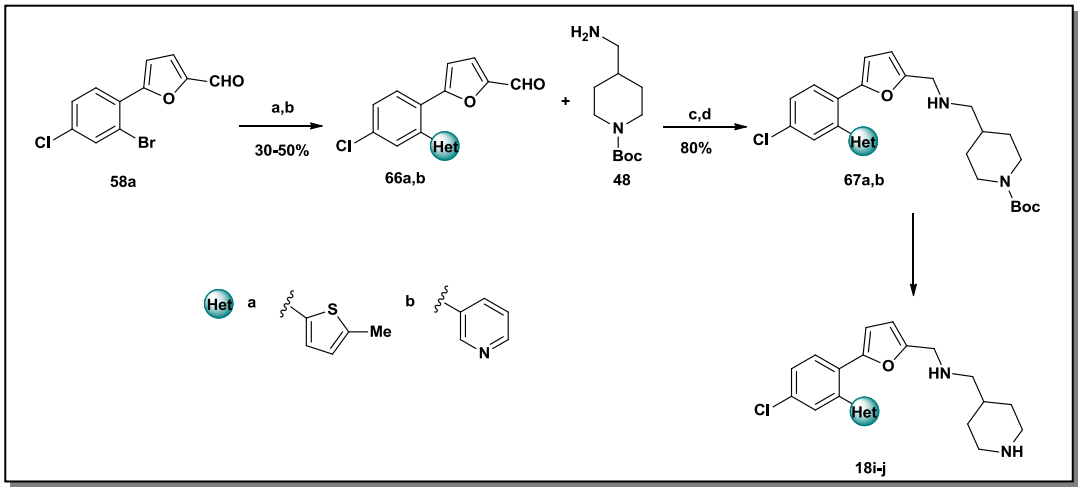


Scheme 9. *Synthesis of derivatives **18b-h**. **Reagents and conditions:***

a) Tetrakis(triphenylphosphine)palladium0, Na_2CO_3, dry toluene, 90 °C, 12 h; **b)** 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 1 h; **c)** AcCl, MeOH, 25 °C, 4 h.

The need to replace the biphenyl system with bioisosteric heterocycles, required the identification of new arylation strategies. Two principal bioisosteric replacements were investigated, the pyridine and the thiophene rings. Since we did not have access to the commercially available boronic acids, two different transition metal catalyzed arylations (**Box.3**) were chosen in order to exploit different synthetic intermediates.

Arylation of pyridines, is still an outstanding challenge because they are electron-poor and have a tendency to adopt a nonproductive N-bound coordination mode with metal centers. However, an interesting arylation was proposed by *Mengchun et al*. as the first C3-selective arylation



Scheme 10. *Synthesis of derivatives* ***18i-j. Reagents and conditions:***

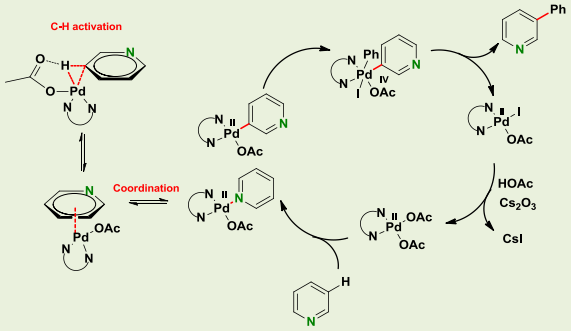
a) Pyridine, 1,10-phenantroline, CS_2CO_3, 160 °C, 12 h; **b)** 2-methyl-thiophene; PivOH, K_2CO_3, DMA, 130 °C, 12 h; **c)** 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 1 h; **d)** AcCl, MeOH, 25 °C, 4 h.

of pyridines using a catalyst generated in situ from palladium(II) acetate and 1,10-phenanthroline[56].

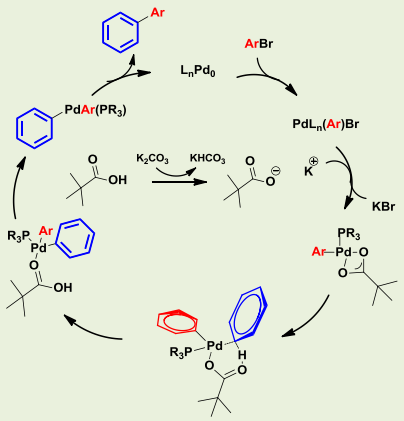
For the thiophene derivative, an arylation method based on metalation-deprotonation mechanism was chosen. Thiophenes are ideal substrates for direct arylation, due to the facile palladation through a concerted metalation-deprotonation pathway, which renders the reaction generally highly selective for the 2 and 5 position on thiophenes. *Fagnou et al*. described the development of a palladium-pivalic acid (PivOH) co-catalyst combination with unprecedented reactivity in direct arylation[57]. From this two arylated intermediates **66a,b**, the synthetic protocol, shown in Scheme 10, remained the same with the reductive amination and amine deprotection to obtain the final derivatives **18i-j**.

Box 3. Metal catalyzed arylations

A C3-selective arylation of pyridines was proposed by *Mengchun et al.* using a catalyst generated in situ from palladium(II) acetate and 1,10-phenanthroline. About the reaction mechanism, it has been proposed that the N-bound pyridine substrate dissociates from palladium, assisted by the ligand. Pyridine subsequently reorients itself to bind to palladium through the π system, which triggers C3-H activation to form the aryl-palladium(II) species. Oxidative addition of iodobenzene to this intermediate followed by reductive elimination furnishes the desired 3-phenylpyridine.



Also *Fagnou et al.* described the development of a palladium-pivalic acid (PivOH) co-catalyst combination to arylate thiophenes. The pivalate anion is a key component in C-H bond cleaving, lowering the energy of C-H bond cleavage and acting as a catalytic proton shuttle from substrate to the stoichiometric carbonate base. Infact, PivOH acts as promoter of phosphine dissociation from the palladium(II) intermediate, enabling the metalation-deprotonation transition state, and at the same time as a catalytic proton shuttle in this metalation-deprotonation mechanism. Polar aprotic solvents are generally the solvent of choice, in particular dimetylacetamide (DMA) allowed complete consumption of starting material.



3.4 Synthesis of MMV019918 derivatives with modified central linker.

Also the central heterocyclic linker of **MMV019918**, was subjected to structure-activity relationships.

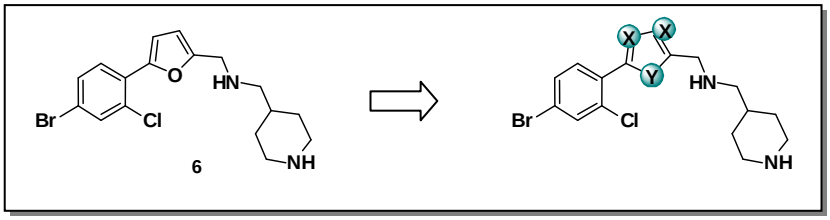
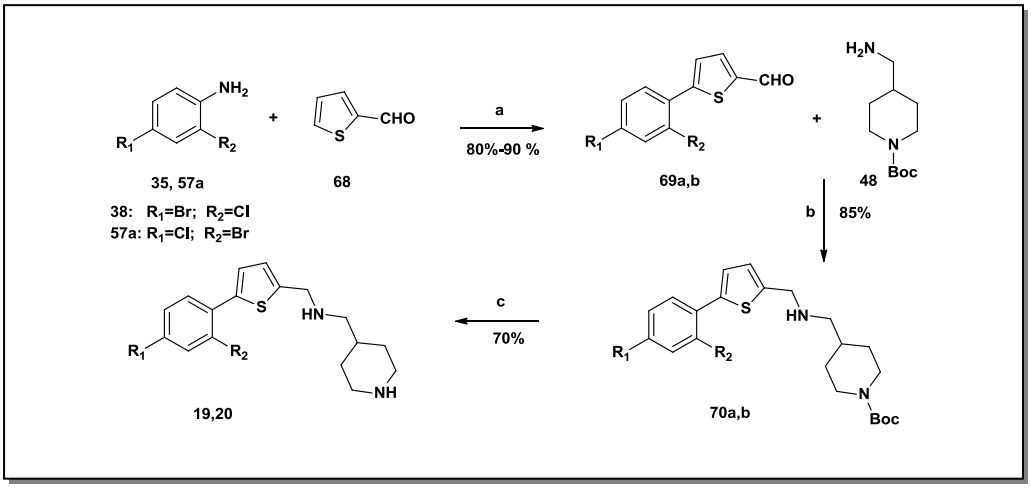


Fig.19. *Central linker substitutions development.*

The first classical bioisosteric replacement was represented by a thiophene ring (analogues **19** and **20**). Being commercially available 2-thiophene-carboxaldehyde **68**, the synthetic strategy shown in the Scheme 11, remains the same as those previously presented. Starting from anilines **38** and **57a** through a radical Meerwein arylation, aldehydes **69a,b**, were obtained. The subsequent reductive amination with amine **48** and Boc deprotection afforded the desired products **19** and **20**.

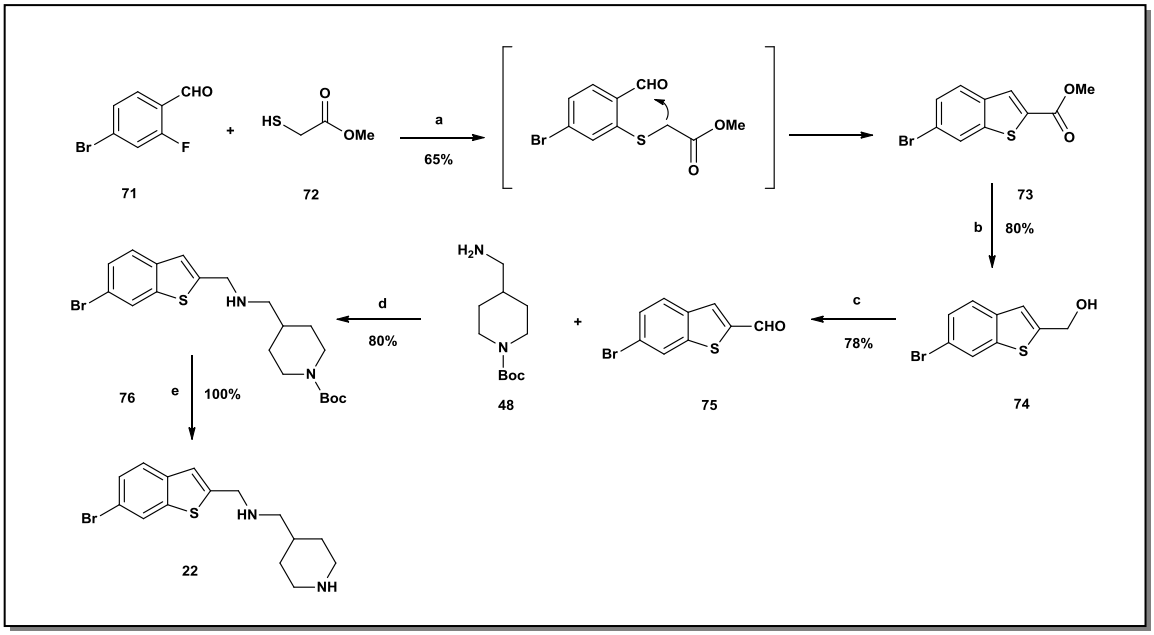


Scheme 11. *Synthesis of derivatives **19** and **20**. **Reagents and conditions:***

a) $NaNO_2$, HCl, $CuCl_2$, acetone, water, 0-25 °C, 24 h; **b)** 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 12 h; **c)** AcCl, MeOH, 25 °C, 4 h.

For the synthesis of the benzothiophene derivative **22** shown in the Scheme 12, a condensation of the methyl thioglycolate **72** with 4-bromo-2-fluorobenzaldehyde **71** under basic conditions was performed[58]. The methyl ester function of intermediate **73** was exposed to diisobutyl aluminum hydride (DIBAL) in order to afford the corresponding aldehyde **75**. Unfortunately over-reduction to alcohol **74** occurred. Starting from this latter compound, the desired aldehyde

75 was obtained by an oxidation reaction using pyridinium chlorocromate (PCC). This intermediate was then used in the next steps of the synthesis performing the reductive amination using amine **48** and sodium borohydride, and subsequent acidic Boc deprotection with the formation of desired final compound **22**.

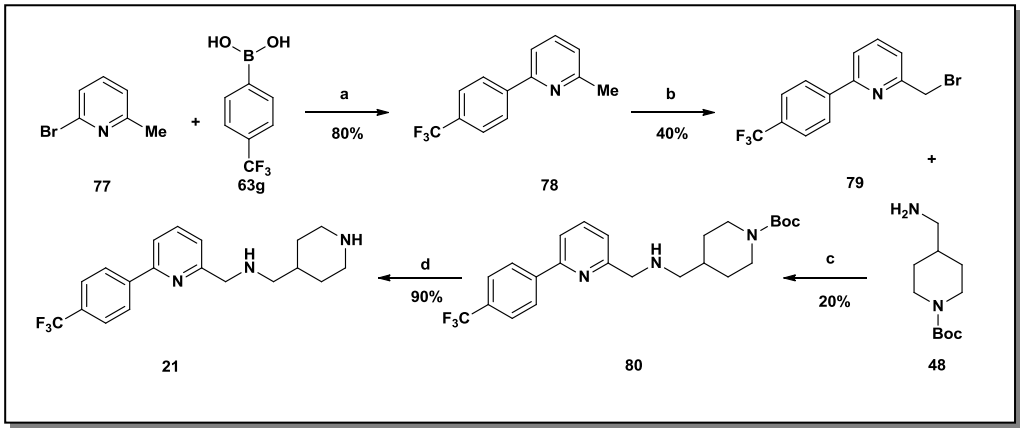


Scheme 12. *Synthesis of derivative **22**. **Reagents and conditions:***

a) K_2CO_3, dry DMF, 100 °C, 12 h; **b)** DIBAL, dry DCM, -78 °C, 2 h; **c)** PCC, dry DCM, 25 °C, 3h; **d)** 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 12 h; **e)** AcCl, MeOH, 25 °C, 4 h.

Another substitution of the central linker is represented by the pyridine derivative **21**. In this case (Scheme 13), it was possible to apply a C-2 selective Suzuki-Miyaura arylation, starting from the commercially available 2-bromo-6-methylpyridine **77** and 4-trifluoromethyl phenyl boronic acid **63g**. The best conditions to obtain the compound **78** in high yields were the use of the tetrakis(triphenylphosphine)palladium(0) as the catalyst in anhydrous *N*,*N*-dimethylformamide (DMF) and in the presence of potassium carbonate. The methyl group at C6 of the pyridine ring, was subjected to a bromination reaction using *N*-bromosuccinimide (NBS) and azobisisobutyronitrile AIBN, generally known as Wohl-Ziegler reaction[59]. This reaction involves a radical pathway in which NBS represents the source of bromine (Br_2) and hydrobromidic acid (HBr) and AIBN act as radical initiators, needed for the homolytic bond cleavage of Br_2. Despite the bromine concentration remained low, a small amount of di-brominated compound was observed. The desired mono-brominated intermediate **79**, was then used to alkylate amine **48** affording the secondary amine **80** as the main product and the

corresponding tertiary amine as by-product. The final Boc deprotection gave in high yield the final derivative **21**.

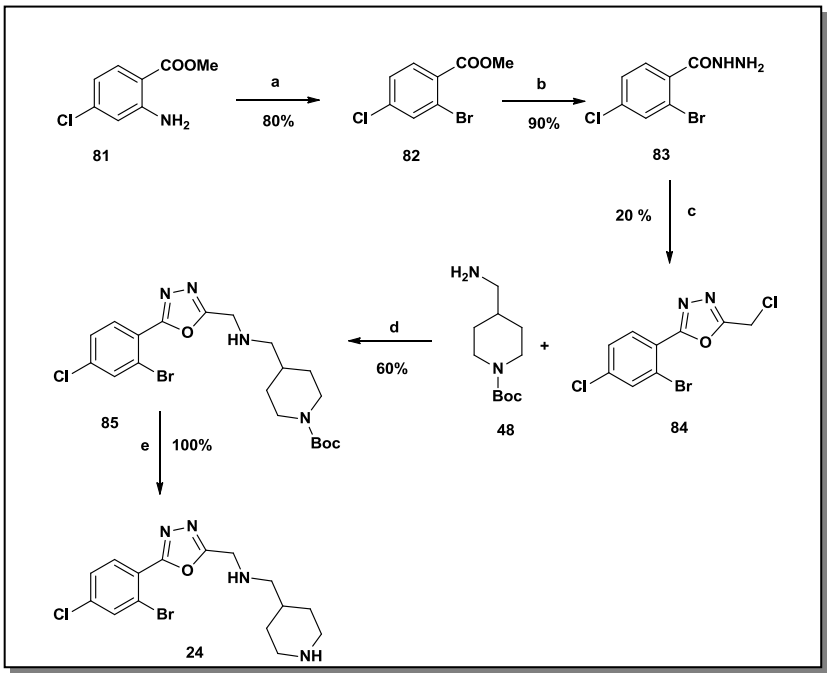


Scheme 13. *Synthesis of derivative **21**. **Reagents and conditions:***

a) tetrakis(triphenylphosphine)palladium0, K_2CO3, dry DMF, 120 °C, 12 h; **b)** NBS, AIBN, dry CCl_4, 60 °C 5 h; **c)** K_2CO_3, dry MeCN 0-25 °C 3 h; **d)** AcCl, MeOH, 25 °C, 4 h.

Regarding the triazole and oxadiazole derivatives **23** and **24**, the synthetic strategy, shown in Scheme 14, differs from the arylation methods previously reported because the appropriate heterocyclic boronic acids were not commercially available. As a consequence, two different cyclization pathways were exploited, starting from the common hydrazide intermediate **83** Accordingly, 2-amino-4-bromo-benzoic acid methyl ester **81** was subjected to a Sandmeyer reaction, to obtain **82**, that was converted into the corresponding benzoic hydrazide **83** using hydrazine monohydrate.

This intermediate **83** was cyclized into 5-aryl-2-(chloromethyl)-1,3,4-oxadiazoles **84**, prepared by the reaction with chloroacetic acid in the presence of phosphorous oxychloride ($POCl_3$) as a dehydrating agent. The chloride **84** thus obtained was used to alkylate amine **48** affording the secondary amine **85** without trace of tertiary amine. The subsequent amino group deprotection gave the desired final compound **24**.

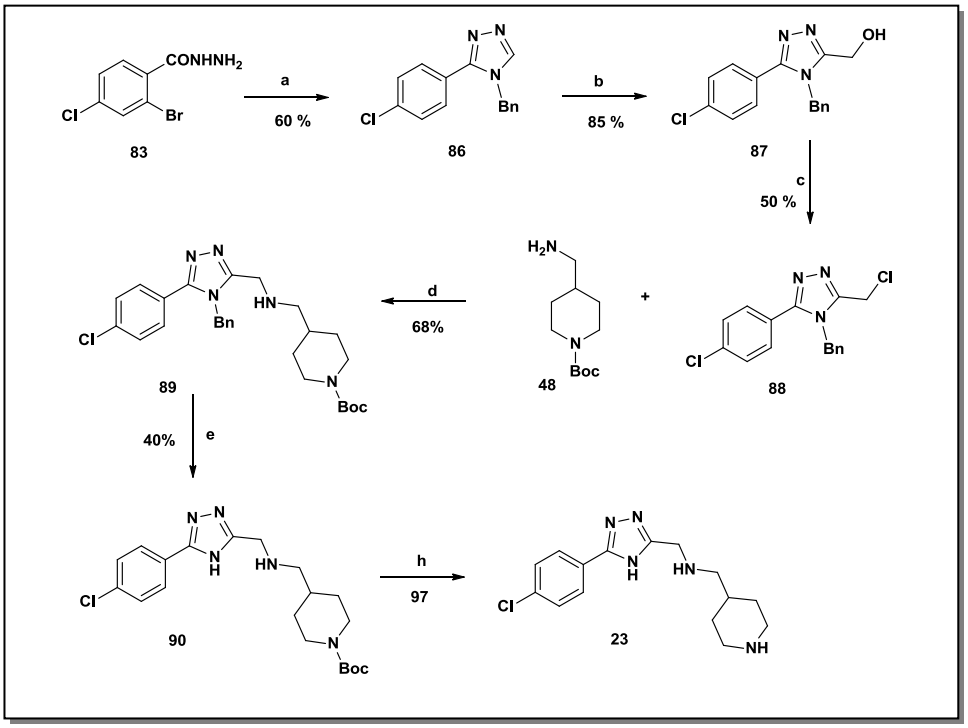


Scheme 14. *Synthesis of derivative **25**. **Reagents and conditions:***

a) $NaNO_2$, HCl, CuBr, TBAB, 0-25 °C, 1 h; **b)** hydrazine monohydrate, EtOH, 70 °C, 12 h; **c)** chloroacetic acid, $POCl_3$, 90 °C, 12 h; **d)** K_2CO_3, dry MeCN, 0-25 °C 3 h; **e)** AcCl, MeOH, 25 °C, 4 h.

The synthesis of the triazole **23**, is shown in the Scheme 15. A Pellizzari like reaction, a condensation between an acyl hydrazine with an amide, was chosen for the construction of the heterocyclic ring. The reaction mechanism, begins by the hydrazide nitrogen attack on the carbonyl carbon of the *N*,*N*-dimethylformamide. Benzyl amine represents the source of the third nitrogen which, acting on the carbonyl group, performs an intramolecular cyclization into the five membered ring. After a proton migration from the nitrogen to the oxygen, a water molecule and dimethyl amine are released to form the correspondent aryl-1,2,4-triazole. Unexplicably, only the bromo dehalogenated product **86** was obtained from this reaction.

The alcoholic function of **87** was introduced by a Prince electrophilic addition using formaldehyde. The alcoholic function was activated to the corresponding chloride **88**, and the subsequent alkylation step, furnished the secondary amine **89** as the main product. The most common method of benzyl deprotection is through hydrogenolysis (Pd–C). However, these conditions could not be used in this case due to the presence of an aryl halide that could also be displaced by hydrogenolysis.



Scheme 15. *Synthesis of derivative **23**. **Reagents and conditions:***

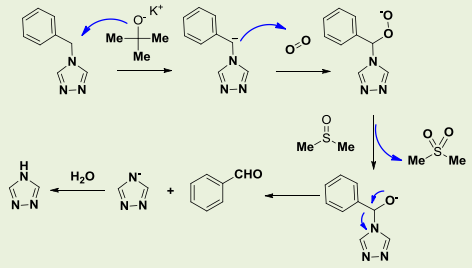
a) DMF-DMA; benzylamine, AcOH, MeCN, 120 °C, 12 h; **b)** HCHO, acq. sol. 37 %, 90 °C, 12 h; **c)** $SOCl_2$, Pyridine, dry DCM, 25 °C, 4 h; **d)** K_2CO_3, dry MeCN, 0-25 °C 3 h; **e)** KO-tBu, O_2, DMSO, 25 °C, 30 min; **f)** AcCl, MeOH, 25 °C, 4 h.

Gigg and Conant described the use of potassium *tert*-butoxide, dimethylsulfoxide and oxygen for a rapid oxidative *N*-debenzylation (**Box.4**)[60]. They also demonstrated that halogens are stable under these oxidative conditions.

The subsequent Boc-group deprotection in acidic medium gave the final compound **23**.

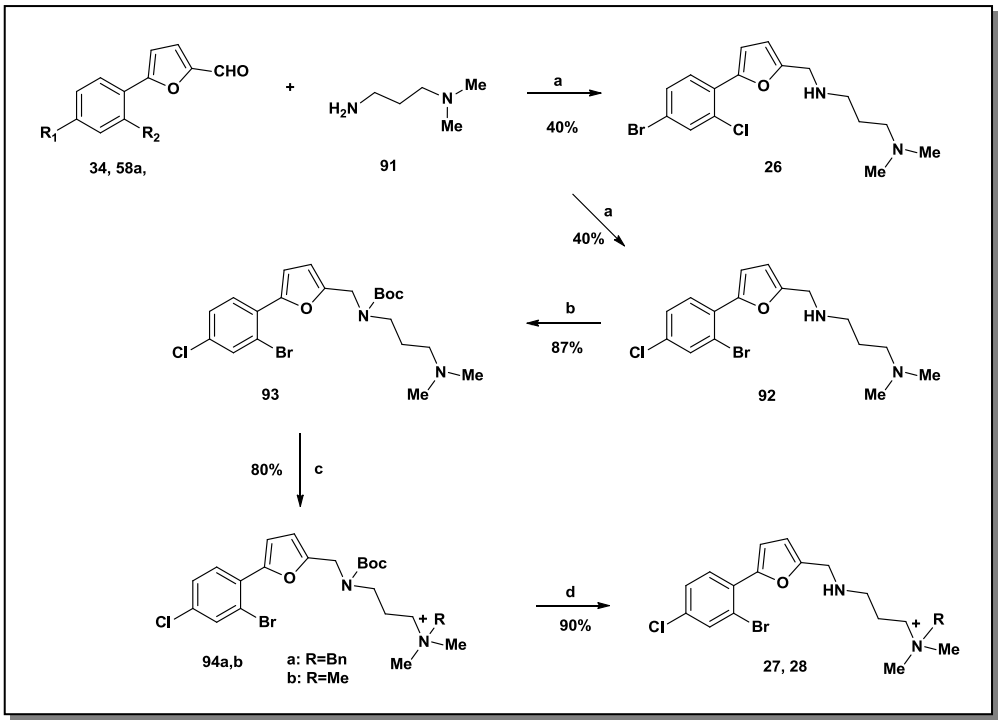
Box.4 Oxidative selective *N*-debenzylation

Gigg and Conant described the use of potassium *tert*-butoxide, dimethylsulfoxide and oxygen for a rapid oxidative *N*-debenzylation About the proposed mechanism, the base in this reaction is able to generate a benzylic anion which reacts with oxygen as it is being bubbled into the reaction mixture. The intermediate peroxy anion, is easily reduced in the presence of DMSO to afford the sulfone and an anion, which breaks down to afford benzaldehyde and the deprotected heterocycle.



3.5 Synthesis of MMV019918 derivatives bearing different basic pendant chains

In **MMV019918** the pendant basic chain is a piperidinyl-methanamine. The open-chain analogue **26**, and the corresponding quaternary ammonium salts, benzyl **27** and methyl **28** respectively, were designed and synthesized as described in Scheme 16.

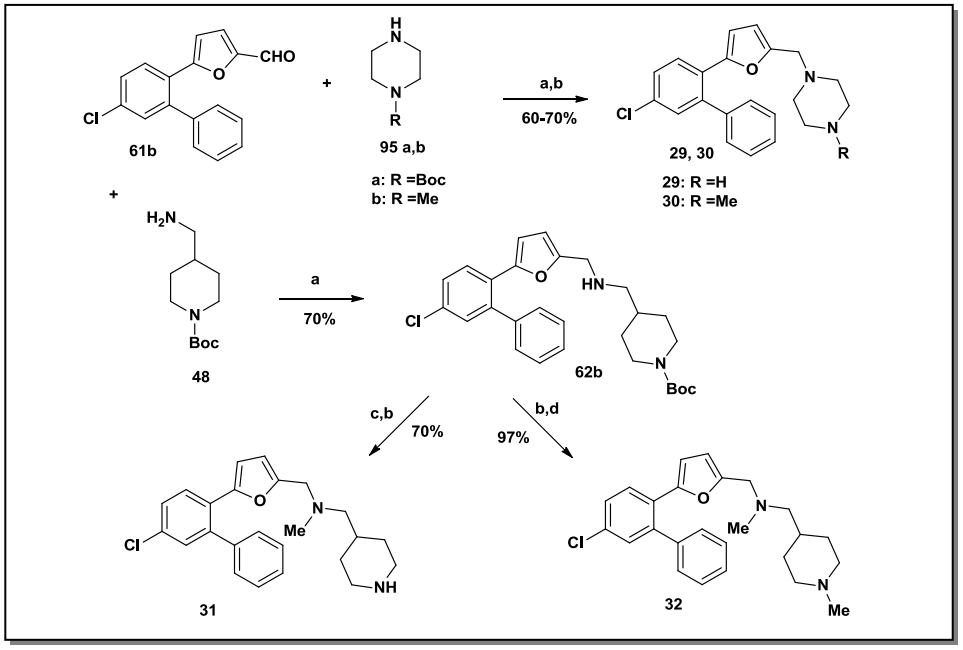


Scheme 16. *Synthesis of derivatives **26-28**. **Reagents and conditions:***

a) 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 1 h; **b)** Boc-anhydride, TEA, MeOH, 25 °C, 4 h; **c)** benzyl bromide or iodomethane, dry acetone, 60 °C, 48 h; **e)** AcCl, MeOH, 25 °C, 6 h.

The aldehydes **34** and **58a**, obtained as previously described, were used to perform a reductive amination reaction with the commercially available *N*,N^1-dimethyl-1,3-propanediamine **91**. The secondary amine **26** obtained, can be considered a final **MMV019918** derivative, and from the reverse intermediate **92** it has been possible to obtain the corresponding ammonium salts derivatives. Infact, the amine group of compound **92**, has been protected to afford compound **93** using di-tert-butyl dicarbonate (Boc_2O), and triethylamine (TEA) as base. Then, a Menshutkin reaction has been performed to convert the tertiary amine to a quaternary ammonium salts **94a,b** using benzyl bromide and iodomethane, respectively. The final Boc deprotection using acetyl chloride and methanol gave the two final derivatives **27** and **28** in satisfactory yields.

Modifications at the pendant basic chain were also performed on the biphenyl compound **18a**, (Scheme 17). Directly from compound **18a**, one or two methyl groups were introduced. Substitution of the piperidine ring with a piperazine and methyl piperazine was also performed.



Scheme 17. *Synthesis of derivatives **29-32**. **Reagents and conditions:***

a) 1. MeOH, 70 °C, 12 h, 2. $NaBH_4$, MeOH, 0-25 °C, 1 h; **b)** AcCl, MeOH, 25 °C, 4 h; **c)** $ZnCl_2$, $NaBH_4$, HCHO, dry DCM 25 °C, 12 h; **d)** HCHO acq. sol. 37%, AcOH, $NaCNBH_3$, MeCN, 25 °C, 12 h.

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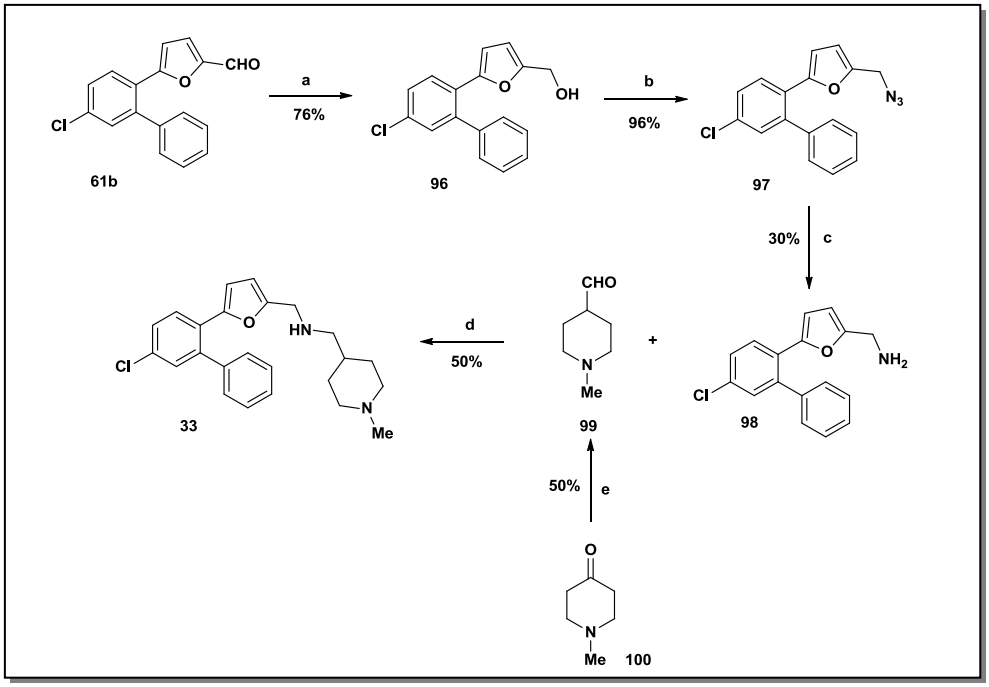
For the synthesis of the above-mentioned compounds, aldehyde **61b**, obtained as previously described, was used as key intermediate to perform the reductive amination with the commercially available Boc-piperazine **95a** and methyl piperazine **95b**, to obtain the final derivatives **29** and **30**. The same aldehyde **61b** was exploited for the synthesis of tertiary amines **30-31**. Two different reductive aminations with formaldehyde were conducted on both Boc-protected **62b** and deprotected derivative **18a**. In the first case the presence of an acid-sensitive functionality, has made necessary the use of zinc borohydride (generated in situ from zinc chloride and sodium borohydride) as a mild, safe and efficient one-pot reagent system and the subsequent Boc deprotection afforded the final desired compound **31**[61]. On the other hand, from the deprotected compound **18a** it has been possible to use a simple Eschweiler-Clarke reaction with an excess of formaldehyde in acidic environment and sodium ciano borohydride as the reducing agent to obtain the amine **32**.

For the synthesis of derivative **33**, (Scheme 18), aldehyde **61b** was converted in the corresponding primary amine **98**. The starting aldehyde was converted to alchol **96** using sodium borohydride , afterward a functional group interconversion to azide **97** using biphenyl-phosphoryl-azide (DPPA) and 1,8-diazabicycloundec-7-ene (DBU) was performed, followed by the final Staudinger reduction using triphenylphosphine and water. The reductive amination

reaction with 1-methylpiperidine-4-carbaldehyde **99**, obtained from a Wittig reaction and a subsequent acidic hydrolysis, gave the final desired amine derivative **33**.



Scheme 17. *Synthesis of derivative **33** **Reagents and conditions:***

a) $NaBH_4$, THF-MeOH, 0-25 °C, 1,5 h; **b)** DPPA, DBU, dry toluene, 0-25 °C, 12 h; **c)** PPh3, THF, H_2O, 60 °C, 12 h; **d)** 1: MeOH, 70 °C, 12 h, 2: NaBH4, MeOH, 0-25 °C, 1 h; **e)** 1. (Methoxymethyl)triphenylphosphonium chloride, tert-BuOOK, dry THF, -60-25 °C, 12 h; 2. HCl 1N, 0 °C 4 h..

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 The phenotypic screening in antimalarial drug discovery and development.

Screening strategies to identify leads fall into three categories: virtual screening, target-based high-throughput screening, and phenotypic screening.

Virtual screening for malaria is hindered by the paucity of well-validated targets, many of which also lack of structural informations, although structural genomic efforts exist. Target-based screening has dominated drug discovery in the past few decades. Small-molecule target-based screenings are designed to interact with specific proteins that are essential for parasite survival and are absent from, or structurally dissimilar to those occurring in the host. However, the main metabolic activity of the parasite is localized inside the FV, where the compounds must enter and remain active. Consequently, in order to retain full drug efficacy, the putative hit should be permeable enough to pass across several membranes as well as remain stable in an acidic environment. Moreover, many compounds designed against specific parasite targets usually present low selectivity. This fact shows that target exclusiveness in the parasite might be a necessary but not sufficient condition to validation[62].

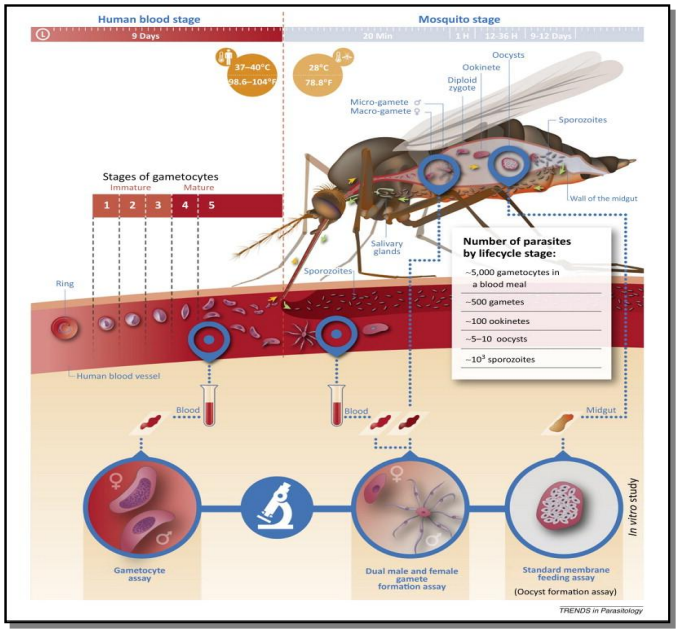


Fig.19 *Schematic representation of different antimalarial screening assays*[63].

Therefore, phenotypic screening stands out as the keystone assay for those studies preceding the preclinical drug discovery phase. A recent study showed that phenotype-based screens identified more first-in-class small molecules between 1999 and 2008 than other screening methods. Therefore, phenotypic screening seems a logical approach of choice for lead

identification efforts[62]. Additionally, inhibitors identified in phenotypic screening are guaranteed to act against their antimalarial target in its intracellular context, with physiologic concentrations of substrates and effectors. Determining the mode of action for novel hits remains a challenge, but new technologies, including genetics, chem-informatics, and proteomics are proving successful. A phenotypic screening that identify active compounds against asexual parasites, selected on their potency, physic and chemical properties, can similarly be profiled for transmission blocking potential. The most representative are *Lactate Dehydrogenase Assay* (pLDH), and *Luciferase-Reporter Anti-Gametocyte Assay* (GC-LUC). pLDH assay was successfully used in drug screening against asexual parasites, and as a reliable marker for Plasmodium falciparum gametocyte viability. This assay is robust and usable on any parasite strain, including field isolates. However, the assay requires purity of the gametocyte population, as pLDH is present also in asexual parasites, and a long assay time, as pLDH activity persists for 24 h after gametocyte death[64]. To achieve specificity for gametocytes, coupled with increased sensitivity and scalability, it was exploited the unsurpassed versatility of bioluminescent assays, based on luciferase reporters driven by gametocyte-specific promoters (GC-LUC). This provides a chemical susceptibility profile of parasite sexual blood stages, with good predictability of the transmission-blocking efficacy of compounds[65]. However, a filter assay is necessary to narrow-down those TB potential. Such a role can be played by a functional gametocytes assay, the *Standard Membrane Feeding Assay* (SMFA). Mosquito ingesting a blood meal containing gametocytes previously incubated with the compound of interest for at least 24 h. The potency of the compound to inhibit the development of sporogony in the mosquito is evaluated by quantifying the number of oocysts produced and mosquito infected[66].

4.2 Results and discussion

4.2.1 In vitro antimalarial activity and structure-activity relationships (SARs) of MMV019918.

All synthesized compounds were tested *in vitro* against of *P. falciparum* strains, namely CQ-S D10 and CQ-R W2 strains by the research group of Prof. Donatella Taramelli of University of Milano. The antimalarial activity (IC_{50}, µM) was quantified as inhibition of parasite growth, measured with the production of parasite lactate dehydrogenase (pLDH). Furthermore the gametocytocidal activity (IC_{50}, µM) was evaluated with a gametocytes luciferase assay (GC-LUC). In the GC-LUC assay, drug-treated gametocyte samples were transferred to 96-well black microplates and D-luciferin (1 mM in citrate buffer 0.1 M, pH 5.5) was added at a 1:1 volume ratio. Luminescence measurements were performed after 45 min with 500 ms integration time.

Effect of different aromatic substitution.

In order to identify the optimal transmission blocking requirements, the effect of different aromatic substituents on antiplasmodial activity was investigate, and the results are reported in Table 1.

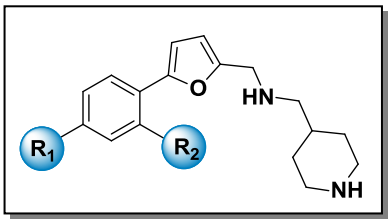


Table 1.

Cpd	R_1	R_3	IC_{50} (µM) [a]		GC-LUC (µM) [a]
			D10	W2	
6	Br	Cl	0.771	2.378	1.213
8	H	H	3.516	9.077	8.63
9	Cl	Br	0.509	1.864	1.125

10	Br	H	1.126	8.01	4.41
11	H	Cl	2.110	4.388	5.91
12	F	H	2.089	7.944	6.04
13	CF_3	H	0.408	1.55	1.9
14	CN	H	1.076	6.3	18.4
15	H	OMe	1.12	3.06	12.7
16	H	OH	11.3	17.4	8.3
17	Ar	Cl	1.02	3.00	1.38
18a	Cl	Ar	0.88	2.44	1.38

[a] IC_{50} values are the mean of at least three determinations in duplicate. Standard errors were all within 10%

The preliminary activity characterization, revealed that substituents at the hydrophobic head play a key role for in vitro activity against both asexual and sexual parasite stages as shown in Table 1. In particular, the presence of both halogens is important for activity since the unsubstituted analogue **8**, as well as the mono-halogenated derivatives **10-12** are less potent antiplasmodial and gametocytocidal agents than the hit compound **6**. Inversion of chlorine and bromine has not a high impact on potency as demonstrated by the regioisomeric derivative **9**. Among the other electron-withdrawing substituents investigated, the trifluoromethyl- derivative **13** maintained a potency similar to **6**, while for the cyano-analogue **14** a drop of potency was observed. Introduction of electron-donating substituents such as methoxy- or hydroxyl-functional groups **15** and **16** resulted in a dramatic drop of potency against gametocytes. Interestingly, introduction of an aromatic ring at the C2 or C4 as in compounds **17** and **18a** respectively, was tolerated and antiplasmodial and gametocytocidal activity similar to the hit compound **6** was observed.

Effect of different heterocycle substitution.

As mentioned in previous chapter, another set of analogues with substitutions on the heterocyclic central linker was investigated. The results are reported in Table 2.

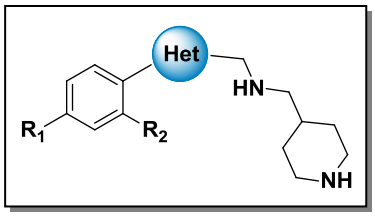
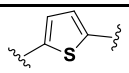
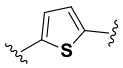
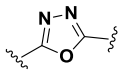
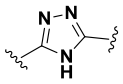
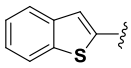
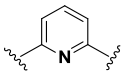


Table 2.

Cpd	Het	R_1	R_2	IC_{50} (µM) [a]		GC-LUC [a] (µM)
				D10	W2	
19		Br	Cl	0.729	2.220	0.853
20		Cl	Br	0.532	1.420	0.830
21		Cl	Br	>13	7.9	>26
22		Cl	H	>13	>13	>13
23		Br	-	1.00	1.50	1.40
24		CF_3	H	0.463	3.40	4.00

[a] IC_{50} values are the mean of at least three determinations in duplicate. Standard errors were all within 10%

The role of the heterocyclic ring on activity was evaluated through the synthesis of thiophene- **19**, **20**, benzothiophene- **23**, triazole- **22**, oxadiazole- **21** and pyridine- **24** derivatives (Table 2). While the sulfur-based heterocycles were well tolerated and in some cases led to a slight improvement of activity, the other heterocycles investigated led to a dramatic drop of potency.

Effect of the different protonable lateral chains.

The last moiety in the hit compound subjected to structural investigation was the pendant basic chain. The results are reported in Table 3.

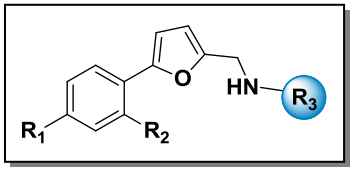
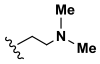
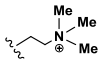
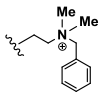


Table 3.

Cpd	R_1	R_2	R_3	IC_{50} (µM) [a]		GC-LUC [a] (µM)
				D10	W2	
26	Br	Cl		0.771	2.378	1.213
27	Cl	Br		3.516	9.077	8.63
28	Cl	Br		0.509	1.864	1.125

[a] IC_{50} values are the mean of at least three determinations in duplicate. Standard errors were all within 10%

The linear derivative **26** showed a potency comparable to **6**, while the ammonium quaternary salts **27** and **28** displayed a drop of potency against gametocytes. Interestingly, benzyl-derivative **28** maintained its potency against the asexual forms of the parasite.

4.2.2 In vitro toxicity.

The most interesting compounds resulted from the first screening assays, were subjected to cytotoxicity assays to investigate their effects on cell viability in vitro. IC_{50} values against human fibroblast (NIH3T3) were determined and the results are reported in Table 4.

Table 4.

Cpd	R_1	R_2	R_3	X	TC50 (µM)
6	Br	Cl	piperidine	O	3
9	Cl	Br	piperidine	O	<5
13	CF_3	H	piperidine	O	50
17	Ar	Cl	piperidine	O	5
18a	Cl	Ar	piperidine	O	20
19	Br	Cl	piperidine	S	5
20	Cl	Br	piperidine	S	20
26	Br	Cl	propanediamine	O	5

Compounds **13**, **18a** and **20** resulted the least toxic with an acceptable selectivity profile showing an improvement over the hit compound **6**.

Based on these results, analogue **18a** was selected for a second round of SAR studies, since it presented an improved toxicity profile and the extra aromatic ring offering a possibility for further scaffold decoration with the aim of improving potency.

4.2.3 In vitro antimalarial activity and structure-activity relationships (SARs) of 18a analogue.

The encouraging results obtained from in vitro antiplasmodial activity and the good safety profile of the compound **18a**, prompted us to design a set of derivatives in order to improve the potency profile. The compounds **18b-j**, bearing both electron withdrawing and electrondonating substituents in all *ortho*, *meta* and *para* positions and the aromatic ring replacement with other heterocycles, were tested to evaluate their antimalarial activity. Also the corresponding tertiary amines **29-33** were evaluate and the results are shown in Table 5.

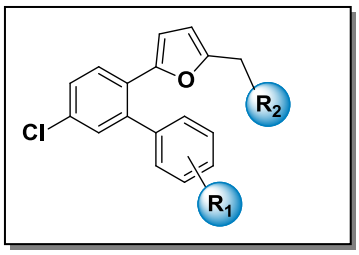


Table 5.

Cpd	R_1	Ar	R_2	IC_{50} (µM) [a]		GC-LUC [a] (µM)
				D10	W2	
18b	*o*-Cl	Ph	Piperidinyl-methanamine	0.74	1.7	2.0
18c	*m*-Cl	Ph	Piperidinyl-methanamine	0.48	1.2	1.47
18d	*p*-Cl	Ph	Piperidinyl-methanamine	0.40	0.75	1.2
18e	*o*-OMe	Ph	Piperidinyl-methanamine	NT	NT	NT
18f	*m*-OMe	Ph	Piperidinyl-methanamine	NT	NT	NT
18g	*p*-OMe	Ph	Piperidinyl-methanamine	0.41	0.84	1.1
18h	*p*-CF_3	Ph	Piperidinyl-methanamine	0.62	1.1	1.7

18i	H	Pyridine	Piperidinyl-methanamine	2.0	3.4	4.0
18j	H	Me-thiophene	Piperidinyl-methanamine	7.7	8.6	18
29	H	Ph	piperazine	>10	>10	>10
30	H	Ph	Me-piperazine	>10	>10	>10
31	H	Ph	*N*-Me-(piperidin-4-yl)methanamine	0.95	0.95	0.89
32	H	Ph	*N*-Me-(Me-piperidin4-yl) methanamine	0.89	0.76	2.97
33	H	Ph	1-Me-piperidin-4-yl)methanamine	0.47	0.58	2.02

[a] IC_{50} values are the mean of at least three determinations in duplicate. Standard errors were all within 10%

No relevant difference in potency were observed in compounds **18b-h**, bearing electron-donating (**18e-h**) or electron-withdrawing (**18b-d**) substituents at the three positions of the extra aromatic ring. However, replacement of the aromatic ring with a pyridine or a methyl-thiophene (**18i-j**) resulted in a relevant drop of potency. Also the introduction of one or two methyl groups on the piperidine ring (**31**-**33**) resulted in a significant decrease of activity, while substitution of piperidine moiety with a piperazine ring **29** or methyl-piperazine **31**, resulted in a complete loss of potency.

4.2.4 Standard membrane feeding assay (SMFA)

The standard membrane-feeding assay (SMFA) remains the assay of choice to assesses the capacity of compounds to inhibit the development of oocysts in the mosquito and thus reduce the mosquito's ability to transmit malaria parasites. In this assay, cultured gametocytes are mixed with blood and test compound, and this mix is fed to laboratory reared mosquitoes to determine the effect of the compound on the parasites establishment in the mosquito. As the outcome of the SMFA is mosquito infection and the timing and duration of compound exposure is controlled, the assay is easily adapted to assess effects active at any stage of parasite development from gametocyte inhibition, to inhibition of sporogony[65].

MMV019918 has been recently shown to have an EC_{50} in the SMFA assay of 0.07 µM. Evaluation of compound **18a** in the SMFA assay resulted in an IC_{50} of 0.21 µM. This result derived by a linear correlation between oocysts count by microscopy and the measurement of intensity of luminescence expressed as oocyst intensity relative light units in function of compound concentration.

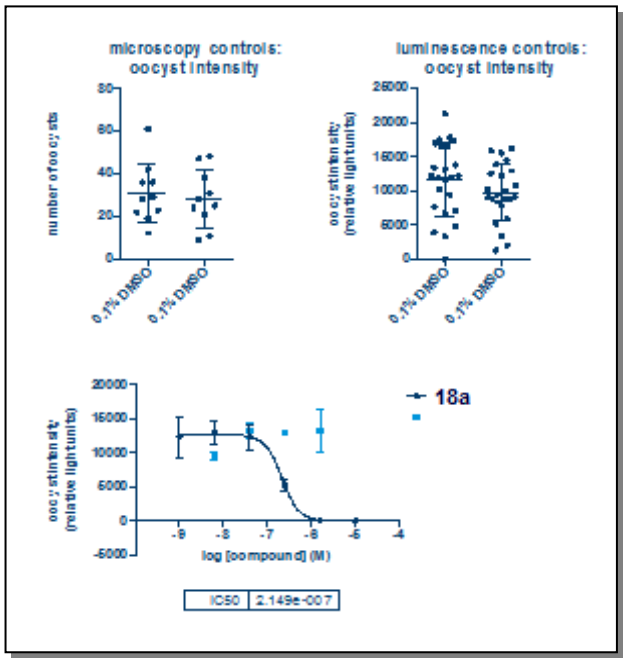


Fig.20. *SMFA results.*

The SMFA provide a direct method, based on the count of oocysts by microscopy after mosquito midgut sectioning. This is a labour intensive and subjective task, which imposes significant limits on the assay scalability. Furthermore the direct assay, in which the compound is added at the moment of the gametocyte activation to form a gamete, interrogates compound

action in the mosquito midgut. Pharmacokinetics in the mosquito are largely unknown, and detoxifying enzymes may limit the compound's action in the midgut, restricting the direct SMFA to monitoring effects on gametogenesis, ookinete formation and early sporogony.
Recently, a direct method was described. This SMFA format is based on the use of a parasite line expressing a fusion of the green fluorescent and firefly luciferase proteins (NF54HT-GFP-Luc). Gametocytes were incubated with compounds for 24 hours, and mosquitoes were fed a blood-meal containing this gametocyte culture/compound mix. The measurement of luciferase activity represents oocyst intensity and it is a simple linear function of luminescence intensity. Oocysts are an intermediate parasite life-stage which even when developing concurrently in a host mosquito may differ in size and sporozoites content. Whereas microscopy produces discrete data, luminescence measurements provide continuous data that reflect actual numbers of developing sporozoites, which may be more directly relatable to mosquito infectivity[65].

4.2.5. Evaluation of activity of 18a against hepatocyte parasite stages.

Compound **18a** was evaluated in a liver stage assay in collaboration with Reto Caldelari (Institute of Cell Biology, Bern). As a first step toxicity of **18a** in HepG2 tolerance was assayed and resulted between 0.5 and 1 μM (a value similar to that reported in the literature for the hit compound **6**). Compound **18a**, at dilutions just below the tolerance concentrations did not dramatically reduce the parasite size in HepG2 cells.

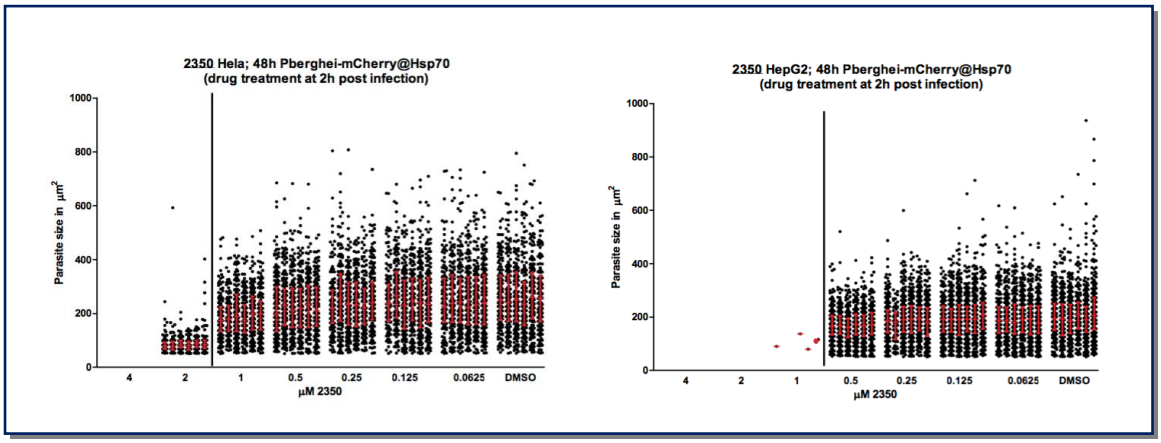


Fig.21. *Liver stage assay.*

There might still be a positive effect by the drug on parasite size when one would analyze it in non-dividing primary hepatocytes, as some drugs specially influencing mitosis will harm the cancer cells.

4.3 Conclusions

During my PhD program I was involved in the synthesis of a novel series of transmission blocking compounds structurally related to MMV019918 including in the Malaria Box.
In particular, a broad spectrum of analogues was characterized for its activity against asexual and sexual life cycle stages of *Plasmodium falciparum*. A selected set of compounds were also evaluated for toxicity in vitro and the analogue **18a** was submitted to a more in depth biological investigation. In particular, compound **18a** resulted active in the SMFA assay with potency comparable to the hit compound, while no activity against *P. berghei* hepatocytic life-cycle stage was observed. Based on our results, the tight SAR observed prevented the optimization of potency against the parasite. Even though the biological activity of **6** and **18a** against gametocytes and asexual *P. falciparum* stages remains interesting and worth of further investigation, exploitation of this scaffold will only be possible by identifying its target at the molecular level. This is a necessary pre-requisite for investigating if dissociation of in vitro toxicity from the specific gametocytocidal and schizonticidal activities is a feasible task.

CHAPTER 5

EXPERIMENTAL SECTION

Experimental methods

Starting materials and solvents were purchased from commercial suppliers and used without further purification. Reaction progress was monitored by TLC using silica gel 60 F254 (0.040-0.063 mm) with detection by UV. Silica gel 60 (0.040-0.063 mm) was used for column chromatography ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 300 MHz, or a Bruker 400 MHz spectrometer using the residual signal of the deuterated solvent as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz (Hz). Mass spectra were recorded utilizing electron spray ionization (ESI) Agilent 1100 Series LC/MSD spectrometer. Melting points were determined using a Büchi Melting point B-540. Yields refer to purified products and are not optimized. All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents.

5.1 Synthesis of compound 6.

5-(4-Bromo-2-chlorophenyl)furan-2-carbaldehyde (34).

Procedure A To a solution of **35** (150 mg, 0.47 mmol) in a mixture of dry DME (3 mL) and dry EtOH (3 mL), bis(triphenylphosphine)palladium(II) dichloride (17 mg, 0.02 mmol) was added. After 30 min, a solution of sodium carbonate (299 mg, 2.82 mmol) in H_2O (2 mL) and a solution of 5-formyl-2-furanylboronic acid **36** (93 mg, 0.66 mmol) in dry EtOH (1,5 mL) were added in this order. The reaction was heated under reflux for 12 h. After this time, H_2O (4 mL) was added at 25 °C and the organic phase was extracted with EtOAc (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% EtOAc in petroleum ether) to give **34** as orange solid (79 mg, 60%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 9.69 (s, 1H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.65 (s, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.33 (d, *J* = 3.8 Hz, 1H), 7.30 (d, *J* = 3.8 Hz, 1H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 177.6, 154.6, 151.8, 133.7, 132.4, 130.7, 130.2, 126.8, 123.6, 123.0, 113.6. MS (ESI) *m/z* 308 $[M + Na]^+$. mp (EtOAc/*n*-hexane) 141-144 °C.

Procedure B To a suspension of **38** (1.0 g, 4.8 mmol) in H_2O (20 mL), hydrochloridric acid (37%, 2 mL) was added. The resulting solution was cooled at 0 °C and a solution of sodium nitrite (397 mg, 5.76 mmol) in H_2O (2 mL) was added dropwise. After 1 h, a solution of **40** (402 µL, 4.8 mmol) in acetone (2 mL) and solid copper(II) chloride (128 mg, 0.96 mmol) were added. The mixture kept at 25 °C for 12 h. EtOAc was added (3 x 10 mL) and the organic phase was separated, dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% EtOAc in petroleum ether) to give **34** as orange solid (1.23 g, 90%).

tert-Butyl 4-(methoxymethylene)piperidine-1-carboxylate (42).

To a solution of (methoxymethyl)triphenylphosphonium chloride (6.3 g, 18.3 mmol) in dry THF (20 mL), sodium bis(trimethylsilyl)amide (1 M in dry THF, 45 mL) was added dropwise at 0 °C. After 1h, a solution of **41** (2.8 g, 14.1 mmol) in dry THF (20 mL) was slowly added at 0 °C and the mixture kept at 25 °C for 12 h. The solvent was removed in vacuo, H_2O and EtOAc were added and the organic phase was separated, washed with an aqueous solution of hydrochloridric acid 1 N (1 x 5 mL) and with a saturated solution of sodium bicarbonate (1 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% EtOAc in petroleum ether) to give **42** as yellow oil (1.28 g, 40%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 5.81 (s, 1H), 3.51 (s, 3H), 3.33 (t, *J* =

6.3 Hz, 4H), 2.20 (t, *J* = 6.0 Hz, 2H), 1.96 (t, *J* = 6.0 Hz, 2H), 1.42 (s, 9H). MS (ESI) *m/z* 250 $[M + Na]^+$.

***tert*-Butyl 4-formylpiperidine-1-carboxylate (43).**

To a solution of **42** (807 mg, 3.6 mmol) in CH_3CN (20 mL), cerium chloride (530 mg, 1.4 mmol) and sodium iodide (160 mg, 1.1 mmol) were added. The reaction mixture was heated under reflux for 12 h. The solvent was removed in vacuo and the crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **43** as yellow oil (667 mg, 87%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 9.57 (s, 1H), 3.88 (d, *J* = 13.3 Hz, 2H), 2.84 (t, *J* = 13.8, 10.9 Hz, 2H), 2.39 – 2.28 (m, 1H), 1.80 (d, *J* = 13.5, 2H), 1.49 – 1.41 (m, 2H), 1.36 (s, 9H). MS (ESI) *m/z* **268** $[M + MeOH + K]^+$.

***tert*-Butyl 4-(hydroxymethyl)piperidine-1-carboxylate (44).**

To a solution of **43** (660 mg, 3.1 mmol) in MeOH (10 mL), sodium borohydride (117 mg, 3.1 mmol) was added at 0 °C and the mixture kept at 25 °C for 1 h. The solvent was removed in vacuo, EtOAc was added (15 mL) and the organic phase was washed with a saturated solution of ammonium chloride (1 x 5mL), dried over sodium sulfate, filtered and evaporated in vacuo. The product **44** was obtained as yellow oil without further purification (666 mg, 100%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 4.03 – 3.90 (d, *J* = 12.6 Hz, 2H), 3.31 (d, *J* = 6.2 Hz, 2H), 2.56 (t, *J* = 12.8 Hz, 2H), 1.67 – 1.54 (m, 2H), 1.54 – 1.42 (m, 1H), 1.32 (s, 9H), 1.07 – 0.90 (m, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 154.9, 79.3, 67.6, 67.0, 43.7, 38.8, 38.7, 37.7, 28.4. MS (ESI) *m/z* 238 $[M + Na]^+$.

***tert*-Butyl 4-((methylsulfonyl)oxy-methyl)piperidine-1-carboxylate (46).**

To a solution of **44** (666 mg, 3.1 mmol) in dry DCM (30 mL), triethylamine (2,6 mL, 18.6 mmol) and methanesulfonyl chloride (959 µL, 12.4 mmol) were added dropwise at 0 °C. Reaction kept at 25 °C for 12 h. A saturated solution of sodium bicarbonate (5 mL) was added and the organic phase was extracted with DCM (3 x 10 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **46** as yellow oil (900 mg, 99%).

^{1}H NMR: (300 MHz, $CDCl_3$) δ 3.98-3.73 (m, 2H), 2.97 – 2.84 (m, 2H), 2.79 (s, 3H), 2.48 (t, *J* = 13.1 Hz, 2H), 1.67 (br s, 1H), 1.50 (d, *J* = 13.2 Hz, 2H), 1.21 (s, 9H), 1.05 – 0.92 (m, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 154.8, 79.6, 73.7, 43.3, 37.3, 36.0, 31.8, 28.5, 28.3. MS (ESI) *m/z* 294 $[M + H]^+$.

***tert*-Butyl 4-(azidomethyl)piperidine-1-carboxylate (47).**

To a solution of **46** (900 mg, 3.1 mmol) in dry DMF (10 mL), sodium azide (400 mg, 6.2 mmol) was added at 0 °C. The reaction was heated under reflux at 90 °C for 12 h. The solvent was removed in vacuo and the product **47** was obtained as yellow oil without further purification (521 mg, 70 %). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 4.01 (d, *J* = 12.1 Hz, 2H), 3.08 (d, *J* = 6.3 Hz, 2H), 2.57 (t, *J* = 12.8 Hz, 2H), 1.68 – 1.49 (m, 3H), 1.34 (s, 9H), 1.15-0.96 (m, 1H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 111.3, 79.7, 57.2, 43.4, 36.7, 29.8, 28.6. MS (ESI) *m/z* 263 $[M + Na]^+$.

***tert*-Butyl 4-(aminomethyl)piperidine-1-carboxylate (48).**

To a solution of **47** (520 mg, 2.1 mmol) in MeOH (15 mL), a catalytic amount of palladium on carbon 10 wt.% was added under argon atmosphere. The reaction environment was saturated with hydrogen gas. The mixture was stirred at 25 °C for 3 h. The suspension was filtered on paper and concentrated in vacuo giving **48** as uncolorless oil without further purification (427 mg, 95%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 3.88 (d, *J* = 13.1 Hz, 2H), 2.50 (d, *J* = 17.4 Hz, 4H), 2.45-2.33 (m, 2H), 1.50 (d, *J* = 13.0 Hz, 2H), 1.38-1.27 (m, 1H), 1.27 (s, 9H), 0.90-0.45 (m, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 155.0, 79.3, 48.1, 43.4, 39.7, 29.9, 28.6. MS (ESI) *m/z* 215 $[M + H]^+$.

5-(4-Bromo-2-chlorophenyl)-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl-piperidine (49).

A solution of **34** (90 mg, 0.31 mmol) and **48** (67 mg, 0.31 mmol) in MeOH (10 mL), was refluxed for 12 h. After this time, the reaction was cooled at 0 °C and sodium borohydride (12 mg, 0.31 mmol) was added. After 1h, the solvent was removed in vacuo, EtOAc was added (10 mL) and the organic phase was washed with a saturated solution of ammonium chloride (1 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **49** as yellow oil (90 mg, 60%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.6 Hz, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.37 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.02 (d, *J* = 3.3 Hz, 1H), 6.27 (d, *J* = 3.3 Hz, 1H), 4.06 (d, *J* = 9.6 Hz, 2H), 3.79 (s, 2H), 2.66 (dd, *J* = 22.1, 9.9 Hz, 2H), 2.50 (d, *J* = 6.6 Hz, 2H), 1.67 (d, *J* = 13.0 Hz, 2H), 1.61 – 1.49 (m, 1H), 1.47 – 1.36 (m, 9H), 1.08 (d, *J* = 16.2, Hz, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 155.1, 152.9, 148.9, 133.4, 130.6, 130.3, 128.6, 128.4, 120.6, 112.3, 111.0, 79.5, 59.6, 57.6, 50.8, 34.7, 30.6, 28.7. MS (ESI) *m/z* 506 $[M + Na]^+$.

[5-(4-Bromo-2-chlorophenyl)furanyl]-*N*-piperidiny-4-yllmethyl-methanamine (6).

A solution hydrochloridric acid 1 N was prepared using acetyl chloride (355 µL, 4.97 mmol) in MeOH (4.64 mL). This solution (540 µL, 0.54 mmol) was added to neat amine **49** (90 mg, 0.18 mmol) at 0 °C and the mixture kept at 25 °C for 4 h. The solvent was removed in vacuo, a saturated solution of sodium bicarbonate was added and the organic phase was extracted with EtOAc (3 x 4 mL), dried over sodium sulfate, filtered and evaporated in vacuo, giving the product **6** as yellow oil without further purification (68 mg, 100%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.88 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 1.8 Hz, 1H), 7.58 (d, *J* = 6.8 Hz, 1H), 7.19 (d, *J* = 3.3 Hz, 1H), 6.85 (d, *J* = 3.3 Hz, 1H), 4.42 (s, 2H), 3.44 (d, *J* = 12.7 Hz, 2H), 3.05 (dd, *J* = 17.7, 9.1 Hz, 2H), 2.17 (brs, 1H), 2.06 (d, *J* = 14.3 Hz, 2H), 1.55 (dd, *J* = 23.5, 11.6 Hz, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 150.57, 147.11, 133.06, 130.83, 130.42, 129.25, 127.77, 121.36, 113.67, 112.37, 51.90, 43.96, 43.37, 31.88, 26.45.MS (ESI) *m/z* 383 $[M + H]^+$.

5.2 Synthesis of compounds 8-16

5-Phenylfuran-2-carbaldehyde (51a).

Starting from **50a** (100 mg, 0.49 mmol) and boronic acid **36** (96 mg,0.68 mmol), the title compound was prepared following the procedure **A** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **51a** as a yellow oil (70 mg, 83%).**^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.61 (s, 1H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.38 (ddd, *J* = 14.4, 7.9, 4.3 Hz, 3H), 7.28 (d, *J* = 2.4 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H). MS (ESI) *m/z* 173 $[M + H]^+$, 195 $[M + Na]^+$.

5-(4-Bromophenyl)furan-2-carbaldehyde (51b).

Starting from **50b** (100 mg, 0.35 mmol) and boronic acid **36** (65 mg, 0.46 mmol), the title compound was prepared following the procedure **A** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **51b** as a yellow solid (60 mg, 68%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 7.63 (d, *J* = 6.8 Hz, 2H), 7.53 (d, *J* = 4.8 Hz, 2H), 7.27 (d, *J* = 3.6 Hz, 1H), 6.80 (d, *J* = 3.6 Hz, 1H). MS (ESI) *m/z* 252 $[M + H]^+$. mp (EtOAc/*n*-hexane) 152-155 °C.

5-(4-Fluorophenyl)furan-2-carbaldehyde (51c).

Starting from **50b** (100 mg, 0.45 mmol) and boronic acid **36** (88 mg, 0.63 mmol), the title compound was prepared following the procedure **A** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to

give **51c** as a yellow oil (70 mg, 82%).**^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.59 (s, 1H), 7.78 – 7.72 (m, 2H), 7.27 (d, *J* = 3.5 Hz, 1H), 7.14 – 7.04 (m, 2H), 6.74 (d, *J* = 3.4 Hz, 1H). MS (ESI) *m/z* 191 $[M + H]^+$, 213 $[M + Na]^+$.

5-Phenylfuran-2-yl-methylamino-methyl- *N-tert*-butoxycarbonylpiperidine (52a).

Starting from **51a** (30 mg, 0.17 mmol) and **48** (36 mg, 0.17 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **52a** as colorless oil (40 mg, 63%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.7 Hz, 2H), 7.34 (t, *J* = 7.7 Hz, 2H), 7.22 (dd, *J* = 13.3, 5.8 Hz, 1H), 6.55 (d, *J* = 3.2 Hz, 1H), 6.22 (d, *J* = 3.1 Hz, 1H), 4.06 (brs, 2H), 3.80 (s, 2H), 2.66 (t, *J* = 11.5 Hz, 2H), 2.52 (d, *J* = 6.6 Hz, 2H), 1.69 (d, *J* = 12.4 Hz, 2H), 1.57 (brs, 2H), 1.42 (s, 9H), 1.14 – 1.04 (m, 2H). MS (ESI) *m/z* 393 $[M + Na]^+$.

5-(4-Bromophenyl)-furan-2-yl-methylamino-methyl-*N-tert*-butoxycarbonylpiperidine (52b).

Starting from **51b** (64 mg, 0.25 mmol) and **48** (53 mg, 0.17 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **52b** as yellow oil (80 mg, 64%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.51 – 7.37 (m, 4H), 6.54 (d, *J* = 3.2 Hz, 1H), 6.21 (d, *J* = 3.1 Hz, 1H), 4.05 (brs, 2H), 3.78 (s, 2H), 2.65 (t, *J* = 11.9 Hz, 2H), 2.51 (d, *J* = 6.5 Hz, 2H), 1.67 (d, *J* = 12.7 Hz, 2H), 1.62 – 1.50 (m, 2H), 1.41 (s, 9H), 1.13 – 1.03 (m, 2H). MS (ESI) *m/z* 471 $[M + Na]^+$.

5-(4-Fluoro-phenyl)-furan-2-yl-methylamino-methyl-*N-tert*-butoxycarbonylpiperidine (52c).

Starting from **51c** (32 mg, 0.17 mmol) and **48** (36 mg, 0.17 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **52c** as uncolorless oil (46 mg, 70%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.03 (t, *J* = 8.6 Hz, 2H), 6.47 (d, *J* = 3.1 Hz, 1H), 6.21 (d, *J* = 3.0 Hz, 1H), 4.06 (brs, 2H), 3.79 (s, 2H), 2.66 (t, *J* = 12.0 Hz, 2H), 2.52 (d, *J* = 6.5 Hz, 2H), 1.68 (d, *J* = 13.0 Hz, 2H), 1.62 – 1.50 (m, 2H), 1.42 (s, 9H), 1.14 – 1.03 (m, 2H).MS (ESI) *m/z* 411 $[M + Na]^+$.

1-Iodo-2-methoxybenzene (54a).

To a solution of 2-iodophenol **53** (200 mg, 0.91 mmol) in dry CH_3CN (5 mL), potassium hydroxide (76 mg,1.36 mmol) and iodomethane (283 µL, 4.55 mmol) were added at 0 °C and the reaction kept at 25 °C for 3 h. The solvent was removed in vacuo. H_2O was added (5 mL) and the organic phase was extracted with hexane (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product **54a** was obtained as brown oil and was used in the next step without further purification (168 mg, 78%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.9, Hz, 1H), 7.33 – 7.21 (m, 1H), 6.92 – 6.76 (m, 2H), 3.87 (s, 3H). (ESI) *m/z* 257 $[M + Na]^+$.

1-(Benzyloxy)-2-iodobenzene (54b).

To a solution of 2-iodophenol **53** (200 mg, 0.91 mmol) in dry THF (8 mL), potassium carbonate (251 mg, 1.81 mmol) and benzyl bromide (130 µL, 1.09 mmol) were added. Reaction was heated under reflux for 12 h. The solvent was removed in vacuo. An aqueous solution of sodium bicarbonate (3 mL) was added and the organic phase was extracted with DCM (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **54b** as a yellow oil (250 mg, 88%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.49 – 7.44 (m, 2H), 7.32 – 7.25 (m, 5H), 6.88 (d, *J* = 2.5 Hz, 2H), 5.10 (s, 2H). (ESI) *m/z* 311 $[M + H]^+$.

5-(2-Methoxyphenyl)furan-2-carbaldehyde (55a).

Starting from **54a** (160 mg, 0.75 mmol) and boronic acid **36** (147 mg,1.05 mmol), the title compound was prepared following the procedure **A** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **55a** as a yellow oil (50 mg, 33%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.63 (s, 1H), 8.03 (d, *J* = 5.2 Hz, 1H), 7.40 – 7.34 (m, 1H), 7.32 (dd, *J* = 3.8, 1.2 Hz, 1H), 7.12 (dd, *J* = 3.7, 1.1 Hz, 1H), 7.08 – 6.96 (m, 2H), 3.93 (s, 3H). (ESI) *m/z* 225 $[M + Na]^+$.

5-(2-Hydroxyphenyl)furan-2-carbaldehyde (55b).

Starting from **54b** (250 mg, 0.80 mmol) and boronic acid **36** (158 mg,1.12 mmol), the title compound was prepared following the procedure **A** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give the product as a yellow oil (122 mg, 55%).**^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.58 (s, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.47 – 7.35 (m, 5H), 7.35 – 7.26 (m, 1H), 7.26 (d, *J* = 1.1 Hz, 1H), 7.08 – 7.01 (m, 3H), 5.14 (s, 2H). (ESI) m/z 301 $[M + Na]^+$. To a solution of the crude product (122 mg, 0.43 mmol), in dry DCM (7 mL), boron trichloride (395 µL, 4.38 mmol) was added at

-78 °C. After 3 h, the mixture warmed to 25 °C and the solvent was removed in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give the product **55b** as a yellow oil (97 mg, 55%).**^{1}H NMR** (75 MHz, $CDCl_3$) δ 9.63 (s, 1H), 7.60 (d, *J* = 6.5 Hz, 1H), 7.26 (d, *J* = 1.1 Hz, 1H), 7.13 – 7.08 (m, 1H), 6.92 – 6.84 (m, 2H), 6.76 (d, *J* = 3.3 Hz, 1H), 6.28 (d, *J* = 2.0 Hz, 1H). (ESI) *m/z* 189 $[M + H]^+$, 187 $[M – H]^+$.

5-(2-Methoxyphenyl)-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonylpiperidine (56a).

Starting from **55a** (50 mg, 0.25 mmol) and **48** (53 mg, 0.25 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **56a** as yellow oil (65 mg, 65%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 2.3 Hz, 1H), 7.27 – 7.17 (m, 1H), 7.04 – 6.91 (m, 2H), 6.86 (d, *J* = 3.0 Hz, 1H), 6.26 (d, *J* = 2.7 Hz, 1H), 4.08 (brs, 2H), 3.92 (s, 3H), 3.83 (s, 2H), 2.67 (t, *J* = 2.4 Hz, 2H), 2.53 (d, *J* = 6.6 Hz, 2H), 1.70 (d, *J* = 12.3 Hz, 2H), 1.58 (m, 1H), 1.44 (s, 9H), 1.17 – 1.03 (m,2H). MS (ESI) *m/z* 411 $[M + Na]^+$.

5-(2-Hydroxyphenyl)-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonylpiperidine (56b).

Starting from **55b** (80 mg, 0.42 mmol) and **48** (90 mg, 0.42 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **56b** as yellow oil (95 mg, 60%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.60 (d, *J* = 6.5 Hz, 1H), 7.13 – 7.08 (m, 1H), 6.94 – 6.85 (m, 2H), 6.76 (d, *J* = 3.3 Hz, 1H), 6.28 (d, *J* = 2.0 Hz, 1H), 4.67 (brs, 1H), 4.06 (brs, 2H), 3.84 (s, 2H), 2.66 (t, *J* = 12.3 Hz, 2H), 2.54 (d, *J* = 5.9 Hz, 2H), 1.69 (d, *J* = 13.7 Hz, 2H), 1.62 – 1.55 (m, 1H), 1.45 (s, 9H), 1.10 (m, 3.0 Hz, 2H). MS (ESI) *m/z* 387 $[M + H]^+$, 409 $[M + Na]^+$.

5-(4-Chloro-2-bromophenyl)furan-2-carbaldehyde (58a).

Starting from **57a** (2.0 g, 9.7 mmol) and **40** (802 µL, 9.7 mmol), the title compound was prepared following the procedure **B** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **58a** as an orange solid (2.5 g, 90%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.69 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 2.0 Hz, 1H), 7.40 (d, *J* = 8.6, Hz, 1H), 7.35 (q, *J* = 3.8 Hz, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 177.6, 154.6, 151.8, 133.7, 132.4, 130.7, 130.2, 126.8, 123.6, 123.0, 113.6. MS (ESI) *m/z* 308 $[M + Na]^+$. mp (EtOAc/*n*-hexane)141-144 °C.

5-(2-Chlorophenyl)furan-2-carbaldehyde (58b).

Starting from **57b** (100 mg, 0.78 mmol) and **40** (65 µL, 0.78 mmol), the title compound was prepared following the procedure **B** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (10 % EtOAc in petroleum ether) to give **58a** as an yellow solid (112 mg, 70%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 7.97 (d, *J* = 5.9 Hz, 1H), 7.44 (d, *J* = 6.5 Hz, 1H), 7.36 – 7.26 (m, 4H). MS (ESI) *m/z* 207 $[M + H]^+$, 228 $[M + Na]^+$. mp (EtOAc/*n*-hexane) 68-72 °C.

5-(4-(Trifluoromethyl)phenyl)furan-2-carbaldehyde (58c).

Starting from **57c** (300 mg, 1.86 mmol) and furaldehyde **40** (154 µL,1.86 mmol), the title compound was prepared following the procedure **B** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5 % EtOAc in petroleum ether) to give **58a** as an yellow solid (312 mg, 70%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 9.65 (s, 1H), 7.85 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 8.6 Hz, 2H), 7.30 (d, *J* = 3.8 Hz, 1H), 6.91 (d, *J* = 3.8 Hz, 1H). MS (ESI) *m/z* 263 $[M + Na]^+$. mp (EtOAc/*n*-hexane) 64-66 °C.

4-(5-Formylfuran-2-yl)benzonitrile (58d).

Starting from **57d** (600 mg, 5.1 mmol) and furaldehyde **40** (421 µL,1.86 mmol), the title compound was prepared following the procedure **B** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (15 % EtOAc in petroleum ether) to give **58d** as brown solid (753 mg, 75%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 9.71 (s, 1H), 7.92 (d, *J* = 8.6 Hz, 2H), 7.76 (d, *J* = 8.6 Hz, 2H), 7.34 (d, *J* = 3.4 Hz, 1H), 6.97 (d, *J* = 3.4 Hz, 1H). MS (ESI) *m/z* 220 $[M + Na]^+$. mp (EtOAc/*n*-hexane)145-150 °C.

5-(4-Chloro-2-bromophenyl)-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (59a).

Starting from **58a** (50 mg, 0.18 mmol) and **48** (38 mg, 0.18 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **59a** as yellow oil (60 mg, 70%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.6 Hz, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.37 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.02 (d, *J* = 3.3 Hz, 1H), 6.27 (d, *J* = 3.3 Hz, 1H), 4.06 (d, *J* = 9.6 Hz, 2H), 3.79 (s, 2H), 2.66 (dd, *J* = 22.1, 9.9 Hz, 2H), 2.50 (d, *J* = 6.6 Hz, 2H), 1.67 (d, *J* = 13.0 Hz, 2H), 1.61 – 1.49 (m, 1H), 1.47 – 1.36 (m, 9H), 1.08 (ddd, *J* = 16.2, 12.4, 4.3 Hz, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 155.1, 152.9, 148.9, 133.4, 130.6, 130.3, 128.6, 128.4, 120.6, 112.3, 111.0, 79.5, 59.6, 57.6, 50.8, 34.7, 30.6, 28.7. MS (ESI) *m/z* 506 $[M + Na]^+$.

5-(2-Chlorophenyl)-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonylpiperidine (59b).

Starting from **58b** (45 mg, 0.22 mmol) and **48** (47 mg, 0.22 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **59b** as yellow oil (60 mg, 68%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 1.5 Hz, 1H), 7.38 (d, *J* = 4.3 Hz, 1H), 7.28 – 7.23 (m, 1H), 7.16 – 7.11 (m, 1H), 7.01 (d, *J* = 3.3 Hz, 1H), 6.27 (d, *J* = 3.3 Hz, 1H), 4.04 (brs, 2H), 3.80 (s, 2H), 2.65 (t, *J* = 11.7 Hz, 2H), 2.51 (d, *J* = 6.7 Hz, 2H), 1.67 (d, *J* = 13.5 Hz, 2H), 1.60 – 1.51 (m, 2H), 1.41 (s, 9H), 1.15 – 1.01 (m, 2H). MS (ESI) *m/z* 427 $[M + Na]^+$.

5-(4-Trifluoromethylphenyl)-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (59c).

Starting from **58c** (100 mg, 0.41 mmol) and **48** (89 mg, 0.41 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **59c** as yellow oil (116 mg, 65%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.85 (d, *J* = 8.1 Hz, 2H), 7.66 (d, *J* = 7.6 Hz, 2H), 6.89 (d, *J* = 3.4 Hz, 1H), 6.42 (d, *J* = 3.5 Hz, 1H), 4.04 (d, *J* = 12.1 Hz, 2H), 3.84 (s, 2H), 2.74 (t, *J* = 12.7 Hz, 2H), 2.53 (d, *J* = 6.4 Hz, 2H), 1.74 (d, *J* = 11.4 Hz, 3H), 1.44 (s, 9H), 1.15-0.98 (m, 2H). MS (ESI) *m/z* 439 $[M + H]^+$, 461 $[M + Na]^+$.

5-(4-Cyanophenyl)-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonylpiperidine (59d).

Starting from **58d** (51 mg, 0.26 mmol) and **48** (55 mg, 0.41 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **59d** as yellow oil (68 mg, 67%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.82 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 3.4 Hz, 1H), 6.43 (d, *J* = 3.4 Hz, 1H), 4.04 (d, *J* = 13.5 Hz, 2H), 3.82 (s, 2H), 2.73 (t, *J* = 12.9 Hz, 2H), 2.51 (d, *J* = 6.3 Hz, 2H), 1.80 – 1.60 (m, 3H), 1.43 (s, 9H), 1.14-0.97 (m, 2H). MS (ESI) *m/z* 396 $[M + H]^+$, 418 $[M + Na]^+$.

1-(5-Phenylfuran-2-yl)-*N*-piperidin-4-ylmethyl-methanamine (8).

Starting from **52a** (40 mg, 0.1 mmol) and hydrochloridric acid 1 N (430 µL, 0.43 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and

evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **8** as yellow oil (27 mg, 100%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.22 (dd, *J* = 12.1, 4.9 Hz, 1H), 6.55 (d, *J* = 3.1 Hz, 1H), 6.23 (d, *J* = 3.1 Hz, 1H), 3.80 (s, 2H), 3.33 (d, *J* = 12.5 Hz, 2H), 2.78 (t, *J* = 11.6 Hz, 2H), 2.56 (d, *J* = 6.5 Hz, 2H), 1.90 (d, *J* = 13.5 Hz, 2H), 1.64 (d, *J* = 3.0 Hz, 1H), 1.54 – 1.40 (m, 2H).**^{13}C NMR** (75 MHz, $CDCl_3$) δ 154.02, 153.35, 131.14, 128.83, 127.33, 123.78, 109.18, 105.81, 55.70, 46.78, 36.78. MS (ESI) *m/z* 271 $[M + H]^+$.

[5-(4-Chloro-2-bromophenyl)furanyl]-*N*-piperidiny-4-yl-methyl-methanamine (9).

Starting from **59a** (50 mg, 0.1 mmol) and hydrochloridric acid 1 N (400 µL, 0.4 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **9** as yellow oil (38 mg, 100%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 1.2 Hz, 1H), 7.61 (s, 1H), 7.28 (d, *J* = 2.2 Hz, 1H), 7.05 (s, 1H), 6.27 (s, 1H), 3.78 (s, 2H), 3.30 (d, *J* = 11.1 Hz, 2H), 2.74 (t, *J* = 11.8 Hz, 2H), 2.53 (d, *J* = 5.2 Hz, 2H), 1.86 (d, *J* = 12.9 Hz, 2H), 1.61 (s, 1H), 1.48 – 1.30 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 153.71, 149.50, 133.15, 132.86, 129.82, 129.15, 127.52, 118.94, 111.53, 109.21, 54.28, 45.27, 44.72, 34.92, 29.21. MS (ESI) *m/z* 383 $[M + H]^+$.

1-[5-(4-Bromophenyl)furan-2-yl]-*N*-piperidin-4-ylmethyl-methanamine (10).

Starting from **52b** (44 mg, 0.1 mmol) and hydrochloridric acid 1 N (400 µL, 0.4 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **10** as yellow oil (35 mg, 100%). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.59 (d, *J* = 8.5 Hz, 2H), 7.48 (d, *J* = 8.6 Hz, 2H), 6.78 (d, *J* = 3.3 Hz, 1H), 6.68 (d, *J* = 3.2 Hz, 1H), 4.34 (s, 2H), 3.38 (d, *J* = 12.5 Hz, 2H), 3.02 (d, *J* = 6.8 Hz, 2H), 2.95 (d, *J* = 11.4 Hz, 2H), 2.11 (brs, 1H), 2.01 (d, *J* = 13.6 Hz, 2H), 1.51 (dd, *J* = 23.4, 11.3 Hz, 2H).**^{13}C NMR** (75 MHz, CD_3OD) δ 154.77, 144.89, 131.84, 129.27, 125.57, 121.63, 114.97, 106.83, 51.31, 43.68, 43.20, 31.31, 26.24. MS (ESI) *m/z* 349 $[M + H]^+$.

[5-(2-Chlorophenyl)furanyl]-*N*-piperidiny-4-yl-methyl-methanamine (11).

Starting from **59b** (60 mg, 0.1 mmol) and hydrochloridric acid 1 N (400 µL, 0.4 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **11** as yellow oil (34 mg, 90%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.91 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.51 – 7.47 (m, 1H), 7.41 – 7.26 (m, 2H), 7.12 (d, *J* = 3.5 Hz, 1H), 6.75 (d, *J* = 3.5 Hz, 1H), 4.32 (s, 2H), 3.40 (d, *J* = 2.4 Hz, 2H), 3.30 (dt, *J* = 2.3, 1.6 Hz, 2H), 3.04 – 2.94 (m, 2H), 2.15 – 2.07 (m, 1H), 2.02 (d, *J* = 14.3 Hz, 2H), 1.52 (td, *J* = 14.7, 4.0 Hz, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 151.66, 146.33, 130.67, 130.21, 128.95, 128.60, 128.21, 127.12, 113.77, 111.82, 51.74, 43.91, 43.35, 31.74, 26.37. MS (ESI) *m/z* 305 $[M + H]^+$.

1-[5-(4-Fluorophenyl)furan-2-yl]-*N*-piperidin-4-ylmethyl-methanamine (12).

Starting from **52c** (35 mg, 0.1 mmol) and hydrochloridric acid 1 N (400 µL, 0.4 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **12** as yellow oil (25 mg, 100%). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.76 (d, *J* = 5.6 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 2H), 6.79 (d, *J* = 3.2 Hz, 1H), 6.74 (d, *J* = 3.2 Hz, 1H), 4.40 (s, 2H), 3.44 (d, *J* = 12.1 Hz,2H), 3.07 (d, *J* = 6.6 Hz, 2H), 3.00 (d, *J* = 13.2 Hz, 2H), 2.15 (brs, 1H), 2.04 (d, *J* = 12.3 Hz, 2H), 1.54 (dd, *J* = 23.4, 11.3 Hz, 2H).**^{13}C NMR** (75 MHz, CD_3OD) δ 164.39, 161.12, 155.01, 144.47, 144.46, 126.78, 126.74, 126.01, 125.90, 115.69, 115.40, 114.92, 105.90, 105.89, 51.28, 43.71, 43.20, 31.31, 26.24. MS (ESI) *m/z* 289 $[M + H]^+$.

[5-(4-Trifluoromethylphenyl)furanyl]-*N*-piperidiny-4-yl-methyl-methanamine (13).

Starting from **59c** (20 mg, 0.04 mmol) and hydrochloridric acid 1 N (200 µL, 0.1 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 2 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **13** as yellow oil (10 mg, 65%). **^{1}H NMR** (300

MHz, CD_3OD) δ 7.86 (d, *J* = 8.1 Hz, 2H), 7.66 (d, *J* = 7.6 Hz, 2H), 6.89 (d, *J* = 3.3 Hz, 1H), 6.42 (d, *J* = 3.3 Hz, 1H), 3.83 (s, 2H), 3.16 (d, *J* = 11.7 Hz, 2H), 2.72 (t, *J* = 12.4 Hz, 2H), 2.53 (d, *J* = 6.3 Hz, 2H), 1.85 (d, *J* = 14.0 Hz, 2H), 1.70 (brs, 1H), 1.28 – 1.15 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 154.32, 151.61, 134.23, 128.49, 128.06, 125.38, 125.32, 125.27, 125.22, 123.31, 109.69, 107.82, 54.59, 45.35, 45.09, 35.38, 30.03.MS (ESI) *m/z* 338 $[M + H]^+$.

[5-(4-Cyanophenyl)furanyl]-*N*-piperidiny-4-yl-methyl-methanamine (14).

Starting from **59d** (50 mg, 0.1 mmol) and hydrochloridric acid 1 N (400 µL, 0.4 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (2 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **14** as yellow oil (37 mg, 100%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.88 (d, *J* = 8.6 Hz, 2H), 7.72 (d, *J* = 8.6 Hz, 2H), 7.03 (d, *J* = 3.5 Hz, 1H), 6.77 (d, *J* = 3.5 Hz, 1H), 4.41 (s, 2H), 3.47 – 3.37 (m, 2H), 3.08 (t, *J* = 7.7 Hz, 2H), 3.02 – 2.93 (m, 2H), 2.14 (brs, 1H), 2.08 – 2.00 (m, 2H), 1.51 (dd, *J* = 18.7, 8.6 Hz, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 153.68, 146.32, 134.25, 132.64, 131.93, 124.37, 118.54, 115.15, 110.60, 109.40, 51.41, 43.61, 43.19, 31.34, 26.21. MS (ESI) *m/z* 296 $[M + H]^+$.

1-[5-(2-Methoxyphenyl)furan-2-yl]-*N*-piperidin-4-ylmethyl-methanamine (15).

Starting from **56a** (60 mg, 0.1 mmol) and hydrochloridric acid 1 N (400 µL, 0.4 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **15** as yellow oil (30 mg, 100%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.81 (d, *J* = 7.8 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.07 – 6.94 (m, 2H), 6.84 (d, *J* = 3.2 Hz, 1H), 6.33 (d, *J* = 2.6 Hz, 1H), 3.92 (s, 3H), 3.80 (s, 2H), 3.05 (d, *J* = 12.4 Hz, 2H), 2.61 (t, *J* = 12.5 Hz, 2H), 2.50 (d, *J* = 6.6 Hz, 2H), 1.77 (d, *J* = 13.0 Hz, 2H), 1.65 (m, 1H), 1.21 – 1.08 (m, 2H). MS (ESI) *m/z* 301 $[M + H]^+$, 323 $[M + Na]^+$.

1-[5-(2-hydroxyphenyl)furan-2-yl]-*N*-piperidin-4-yl-methyl-methanamine (16).

Starting from **56b** (80 mg, 0.2 mmol) and hydrochloridric acid 1 N (800 µL, 0.8 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (2 mL) was added and the

organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **16** as yellow oil (60 mg, 90%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.75 – 7.71 (m, 1H), 7.09 – 7.02 (m, 1H), 6.91 – 6.81 (m, 3H), 6.32 (d, *J* = 2.7 Hz, 1H), 3.78 (s, 2H), 3.00 (d, *J* = 12.4 Hz, 2H), 2.57 (d, *J* = 12.2 Hz, 2H), 2.48 (d, *J* = 6.7 Hz, 2H), 1.72 (d, *J* = 13.2 Hz, 2H), 1.66 – 1.55 (m, 1H), 1.21 – 1.02 (m, 2H). MS (ESI) *m/z* 287 $[M + H]^+$.

5.3 Synthesis of compounds 17, 18a-j.

5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (61b).

Triphenylphosphine (220 mg, 0.84 mmol) and palladium (II) acetate (12 mg, 0.05 mmol) were dissolved in dry toluene (3 mL) under argon atmosphere. After 30 min, a solution of **58a** (300 mg, 1.05 mmol) in dry toluene (5 mL) was added. After further 30 min, a solution of sodium carbonate (701 mg, 6.6 mmol) in H_2O (3 mL) and powered phenyl boronic acid **60** (128 mg, 1.05 mmol) were added in this order. Reaction was heated under reflux for 12 h. The solvent was removed under vacuo. H_2O (2 mL) and EtOAc (8 mL) were added, the organic phase was separated, dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5 % EtOAc in petroleum ether) to give **61b** as yellow oil (254 mg, 85%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.93 (d, *J* = 5.3 Hz, 1H), 7.44 – 7.40 (m, 5H), 7.33 (d, *J* = 2.7 Hz, 2H), , 7.02 (d, *J* = 3.1 Hz, 1H), 5.62 (d, *J* = 3.7 Hz, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.62, 157.67, 151.50, 142.58, 140.05, 135.38, 131.11, 129.78, 129.39, 129.04, 128.96, 127.48, 127.35, 126.40, 120.41, 115.64, 112.35. MS (ESI) *m/z* 305 $[M + Na]^+$.

5-(3-Chloro-[1,1'-biphenyl]-4-yl)furan-2-carbaldehyde (61a).

Starting from **34** (80 mg, 0.28 mmol) and phenyl boronic acid **60** (35 mg, 0.28 mmol), the title compound was prepared following the procedure reported for compound **61b**. The crude product was purified by flash chromatography on silica gel (5 % EtOAc in petroleum ether) to give **61a** as yellow oil (50 mg, 63%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.08 (d, *J* = 1.5 Hz, 1H), 7.70 (d, *J* = 1.6 Hz,1H), 7.62 – 7.56 (m, 3H), 7.50 – 7.36 (m, 3H), 7.35 (d, *J* = 0.8 Hz, 2H). MS (ESI) m/z 305 $[M + Na]^+$. **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.62, 157.67, 151.50, 142.58, 140.05, 135.38, 131.11, 129.78, 129.39, 129.04, 128.96, 127.48, 127.35, 126.40, 120.41, 115.64, 112.35. MS (ESI) *m/z* 305 $[M + Na]^+$.

5-(2-Chloro-[1,1'-biphenyl])-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (62a).

Starting from **61a** (50 mg, 0.17 mmol) and **48** (38 mg, 0.17 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **62a** as yellow oil (60 mg, 74%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.3 Hz, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.72 (d, *J* = 1.9 Hz, 1H), 7.68 – 7.60 (m, 2H), 7.54 – 7.34 (m, 3H), 7.11 (d, *J* = 3.3 Hz, 1H), 6.44 (d, *J* = 3.3 Hz, 1H), 4.08 (brs, 2H), 3.66 (s, 2H), 2.67 (t, *J* = 12.3 Hz, 2H), 2.43 (d, *J* = 6.6 Hz, 2H), 1.66 (d, *J* = 12.6 Hz, 2H), 1.60 – 1.49 (m, 1H), 1.45 (s, 9H), 1.14 – 1.03 (m, 2H). (ESI) m/z 481 $[M + H]^+$, 504 $[M + Na]^+$. **^{13}C NMR** (75 MHz, $CDCl_3$) δ 155.08, 153.53, 151.34, 141.10, 140.83, 132.92, 130.85, 129.05, 128.58, 128.22, 128.16, 127.76, 110.09, 108.92, 79.51, 55.07, 46.60, 36.67, 30.54, 28.68. MS (ESI) *m/z* 481 $[M + H]^+$, 504 $[M + Na]^+$.

5-(4-Chloro-[1,1'-biphenyl])-furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (62b).

Starting from **61b** (50 mg, 0.17 mmol) and **48** (38 mg, 0.17 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **62b** as yellow oil (65 mg, 80%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.5 Hz, 1H), 7.41 – 7.31 (m, 4H), 7.28 – 7.20 (m, 3H), 6.00 (d, *J* = 3.2 Hz, 1H), 5.56 (d, *J* = 3.2 Hz, 1H), 4.08 (brs, 2H), 3.66 (s, 2H), 2.67 (t, *J* = 12.3 Hz, 2H), 2.43 (d, *J* = 6.6 Hz, 2H), 1.66 (d, *J* = 12.6 Hz, 2H), 1.60 – 1.49 (m, 1H), 1.45 (s, 9H), 1.14 – 1.03 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 155.08, 153.53, 151.34, 141.10, 140.83, 132.92, 130.85, 129.05, 128.58, 128.22, 128.16, 127.76, 110.09, 108.92, 79.51, 55.07, 46.60, 36.67, 30.54, 28.68. MS (ESI) *m/z* 481 $[M + H]^+$, 504 $[M + Na]^+$.

5-(2',5-Dichloro-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (64a).

Starting from **58a** (200 mg, 0.70 mmol) and 2-chloro-phenyl boronic acid **63a** (109 mg, 0.70 mmol), the title compound was prepared following the procedure reported for compound **61b**. The crude product was purified by flash chromatography on silica gel (5 % EtOAc in petroleum ether) to give **64a** as yellow oil (80 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 7.56 – 7.43 (m, 2H), 7.37 (m, 4H), 7.03 (d, *J* = 3.8 Hz, 1H), 5.57 (d, *J* = 3.7 Hz, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.52, 157.01, 151.85, 139.08, 138.75, 135.33, 134.20, 133.45, 131.01, 130.72, 130.12, 129.49, 128.92, 128.85, 128.32, 127.52, 111.15. MS (ESI) *m/z* 317 $[M + H]^+$.

5-(3',5-Dichloro-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (64b).

Starting from **58a** (204 mg, 0.72 mmol) and 3-chloro-phenyl boronic acid **63b** (112 mg, 0.72 mmol), the title compound was prepared following the procedure reported for compound **61b**. The crude product was purified by flash chromatography on silica gel (2 % EtOAc in petroleum ether) to give **64b** as yellow oil (100 mg, 44%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 7.47 – 7.42 (m, 1H), 7.39 – 7.32 (m, 2H), 7.28 (dd, *J* = 9.9, 3.7 Hz, 2H), 7.16 – 7.11 (m, 1H), 7.06 (d, *J* = 3.8 Hz, 1H), 5.73 (d, *J* = 3.8 Hz, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.52, 157.05, 151.75, 141.74, 141.42, 130.99, 130.57, 130.20, 129.58, 129.16, 128.73, 128.59, 128.40, 128.32, 126.74, 126.37, 112.34. MS (ESI) *m/z* 317 $[M + H]^+$.

5-(4',5-Dichloro-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (64c).

Starting from **58a** (128 mg, 0.45 mmol) and 4-chloro-phenyl boronic acid **63c** (156 mg, 0.45 mmol), the title compound was prepared following the procedure reported for compound **61b**. The crude product was purified by flash chromatography on silica gel (2 % EtOAc in petroleum ether) to give **64c** as yellow oil (72 mg, 50%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 2.2 Hz, 1H), 7.42 (d, *J* = 2.3 Hz, 1H), 7.41 – 7.36 (m, 2H), 7.29 (d, *J* = 2.2 Hz, 1H), 7.23 – 7.17 (m, 2H), 7.06 (d, *J* = 3.8 Hz, 1H), 5.72 (d, *J* = 3.8 Hz, 1H). **^{13}C NMR:** (300 MHz, $CDCl_3$) δ 177.47, 157.16, 151.75, 141.17, 138.45, 135.52, 134.64, 131.03, 130.48, 129.63, 129.20, 128.57, 126.44, 112,29, 77.69, 77.26, 76.84. MS (ESI) *m/z* 318 $[M + H]^+$.

5-(5-Chloro-2'-methoxy-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (64d).

Starting from **58a** (250 mg, 0.87 mmol) and 2-methoxy-phenyl boronic acid **63d** (133 mg, 0.87 mmol), the title compound was prepared following the procedure reported for compound **61b**. The crude product was purified by flash chromatography on silica gel (2 % EtOAc in petroleum ether) to give **64d** as yellow oil (180 mg, 66%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.52 (s, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 7.43 – 7.33 (m, 3H), 7.14 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.02 (dd, *J* = 8.3, 5.7 Hz, 2H), 6.91 (d, *J* = 8.3 Hz, 1H), 5.63 (d, *J* = 3.7 Hz, 1H), 3.56 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.42, 158.14, 156.56, 151.45, 138.94, 135.21, 131.47, 130.64, 129.46, 128.90, 128.73, 128.11, 127.38, 126.72, 121.31, 111.35, 110.71, 55.64. MS (ESI) *m/z* 313 $[M + H]^+$.

5-(5-Chloro-3'-methoxy-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (64e).

Starting from **58a** (250 mg, 0.87 mmol) and 3-methoxy-phenyl boronic acid **63e** (133 mg, 0.87 mmol), the title compound was prepared following the procedure reported for compound **61b**.

The crude product was purified by flash chromatography on silica gel (2 % EtOAc in petroleum ether) to give **64e** as yellow oil (130 mg, 48%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.52 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.39 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 2H), 7.03 (d, *J* = 3.8 Hz, 1H), 6.94 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.81 – 6.77 (m, 2H), 5.69 (d, *J* = 3.7 Hz, 1H), 3.77 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.62, 158.12, 156.56, 151.45, 138.74, 135.21, 131.47, 130.61, 129.46, 128.95, 128.73, 128.11, 127.38, 126.78, 121.31, 111.35, 110.71, 56.01. MS (ESI) *m/z* 313 $[M + H]^+$.

5-(5-Chloro-4'-methoxy-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (64f).

Starting from **58a** (200 mg, 0.70 mmol) and 4-methoxy-phenyl boronic acid **63f** (106 mg, 0.70 mmol), the title compound was prepared following the procedure reported for compound **61b**. The crude product was purified by flash chromatography on silica gel (5 % EtOAc in petroleum ether) to give **64f** as yellow oil (100 mg, 46%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 3.8 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.78 (s, 1H), 5.67 (d, *J* = 3.8 Hz, 1H), 3.86 (s, 3H). MS (ESI) *m/z* 313 $[M + H]^+$.

5-(5-Chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-2-yl)furan-2-carbaldehyde (64g).

Starting from **58a** (195 mg, 0.68 mmol) and 4-trifluoromethyl-phenyl boronic acid **63g** (129 mg, 0.68 mmol), the title compound was prepared following the procedure reported for compound **61b**. The crude product was purified by flash chromatography on silica gel (10 % EtOAc in petroleum ether) to give **64g** as yellow oil (155 mg, 65%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.53 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.66 (d, *J* = 5.2 Hz, 2H), 7.47 (d, *J* = 2.2 Hz, 1H), 7.38 (d, *J* = 4.1 Hz, 2H), 7.30 (d, *J* = 2.1 Hz, 1H), 7.05 (d, *J* = 3.8 Hz, 1H), 5.70 (d, *J* = 3.7 Hz, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.46, 156.90, 151.86, 140.85, 135.63, 134.17, 130.95, 130.86, 129.78, 129.59, 126.57, 126.00, 125.96, 125.91, 125.86, 125.81, 122.76, 112.32. MS (ESI) *m/z* 351 $[M + H]^+$.

5-(2',5-Dichloro-[1,1'-biphenyl)furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (65a).

Starting from **64a** (25 mg, 0.08 mmol) and **48** (17 mg, 0.08 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65a** as yellow oil (25 mg, 60%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.84 (d, *J* = 8.6 Hz, 1H), 7.51 – 7.34 (m, 5H), 7.29 – 7.24 (m, 1H), 6.10 (d, *J* = 3.3 Hz, 1H), 5.47 (d, *J* = 3.4 Hz, 1H), 4.08 (brs, 2H), 3.66 (s, 2H),

2.67 (t, *J* = 12.3 Hz, 2H), 2.43 (d, *J* = 6.6 Hz, 2H), 1.66 (d, *J* = 12.6 Hz, 2H), 1.60 – 1.49 (m, 1H), 1.45 (s, 9H), 1.14 – 1.03 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 155.07, 153.88, 150.98, 142.82, 139.22, 134.37, 133.12, 130.70, 129.82, 129.23, 128.50, 128.27, 127.89, 127.39, 110.29, 108.99, 79.49, 55.10, 46.60, 36.70, 30.54, 28.69. MS (ESI) *m/z* 538 $[M + Na]^+$.

5-(3',5-Dichloro-[1,1'-biphenyl)furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (65b).

Starting from **64b** (60 mg, 0.18 mmol) and **48** (40 mg, 0.18 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65b** as yellow oil (65 mg, 70%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.5 Hz, 1H), 7.39 – 7.22 (m, 5H), 7.15 – 7.11 (m, 1H), 6.04 (d, *J* = 3.2 Hz, 1H), 5.67 (d, *J* = 3.3 Hz, 1H), 4.09 (brs, *J* = 9.9 Hz, 2H), 3.67 (s, 2H), 2.68 (t, *J* = 12.3 Hz, 2H), 2.44 (d, *J* = 6.6 Hz, 2H), 1.67 (d, *J* = 11.7 Hz, 2H), 1.51 – 1.58 (m, 1H), 1.45 (s, 9H), 1.03 – 1.15 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 155.07, 153.88, 150.98, 142.82, 139.22, 134.37, 133.12, 130.70, 129.82, 129.23, 128.50, 128.27, 127.89, 127.39, 110.29, 108.99, 79.49, 55.10, 46.60, 36.70, 30.54, 28.69. MS (ESI) *m/z* 538 $[M + Na]^+$.

5-(4',5-Dichloro-[1,1'-biphenyl)furan-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (65c).

Starting from **64c** (72 mg, 0.23 mmol) and **48** (49 mg, 0.23 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65c** as yellow oil (75 mg, 64%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 8.6 Hz, 3H), 7.23 – 7.14 (m, 3H), 6.04 (d, *J* = 3.3 Hz, 1H), 5.67 (d, *J* = 3.3 Hz, 1H), 4.08 (brs,, 2H), 3.66 (s, 2H), 2.68 (t, *J* = 12.8 Hz, 2H), 2.43 (d, *J* = 6.6 Hz, 2H), 1.74 – 1.60 (m, 2H), 1.55 (m, 1H), 1.44 (s, 9H), 1.08 (m, 2H). **^{13}C NMR:** (300 MHz, $CDCl_3$) δ 155.06, 153.75, 151.16, 139.51, 133.84, 133.16, 130.75, 130.48, 128.81, 128.60, 128.16, 128.12, 110.21, 109.03, 79.50, 55.12, 46.58, 36.65, 30.56, 28.69, 1.23. MS (ESI) *m/z* 538 $[M + Na]^+$.

5-(5-Chloro-2'-methoxy-[1,1'-biphenyl)furan-2-yl-methylamino-methyl-*N*-*tert*-butoxy carbonyl piperidine (65d).

Starting from **64d** (30 mg, 0.09 mmol) and **48** (20 mg, 0.09 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65d** as yellow oil (31 mg, 67%). **^{1}H NMR** (300 MHz, DMSO) δ 7.75 (d, *J* = 8.5 Hz, 1H), 7.48 – 7.38 (m, 3H), 7.13 – 6.98

(m, 3H), 6.07 (d, *J* = 3.3 Hz, 1H), 5.42 (d, *J* = 3.3 Hz, 1H), 3.88 (brs, 2H), 3.67 (s, 2H), 3.55 (s, 3H), 2.68 (t, *J* = 12.3 Hz, 2H) 2.30 (d, *J* = 6.6 Hz, 2H), 1.63 (t, *J* = 12.4 Hz, 2H), 1.47 (s, 1H), 1.36 (s, 9H), 0.98 – 0.85 (m,2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 176.30, 155.07, 153.41, 151.21, 142.39, 140.63, 132.87, 130.73, 129.62, 128.15, 127.83, 121.47, 114.37, 113.62, 110.18, 109.08, 79.49, 55.48, 55.02, 46.57, 36.65, 30.53, 28.68. MS (ESI) *m/z* 534 $[M + Na]^+$

5-(5-Chloro-3'-methoxy-[1,1'-biphenyl)furan-2-yl-methylamino-methyl-*N*-*tert*-butoxy carbonylpiperidine (65e).

Starting from **64e** (30 mg, 0.09 mmol) and **48** (20 mg, 0.09 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65e** as yellow oil (25 mg, 55%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.5 Hz, 1H), 7.40 – 7.26 (m, 3H), 6.94 – 6.75 (m, 3H), 6.02 (d, *J* = 3.2 Hz, 1H), 5.62 (d, *J* = 3.3 Hz, 1H), 4.08 (brs, 2H), 3.77 (s, 3H), 3.68 (s, 2H), 2.67 (t, *J* = 12.4 Hz, 2H), 2.45 (d, *J* = 6.6 Hz, 2H), 1.67 (d, *J* = 11.9 Hz, 2H), 1.60 – 1.49 (m, 1H), 1.45 (s, 9H), 1.01 – 1.15 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 176.30, 155.07, 153.41, 151.21, 142.39, 140.63, 132.87, 130.73, 129.62, 128.15, 127.83, 121.47, 114.37, 113.62, 110.18, 109.08, 79.49, 55.48, 55.02, 46.57, 36.65, 30.53, 28.68. MS (ESI) *m/z* 534 $[M + Na]^+$.

5-(5-Chloro-4'-methoxy-[1,1'-biphenyl)furan-2-yl-methylamino-methyl-*N*-*tert*-butoxy carbonylpiperidine (65f).

Starting from **64f** (40 mg, 0.13 mmol) and **48** (27 mg, 0.13 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65f** as yellow oil (40 mg, 60%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.4 Hz, 1H), 7.31 (dd, *J* = 8.5, 2.1 Hz,2H), 7.16 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 6.02 (d, *J* = 3.1 Hz, 1H), 5.59 (d, *J* = 3.2 Hz, 1H), 4.08 (brs, 2H), 3.83 (s, 3H), 3.69 (s, 2H), 2.67 (t, *J* = 12.3 Hz, 2H), 2.45 (d, *J* = 6.6 Hz, 2H), 1.67 (d, *J* = 11.7 Hz, 2H), 1.58 – 1.50 (m, 1H), 1.44 (s, 9H), 1.13 – 0.99 (m, 2H). **^{13}C NMR** (300 MHz, $CDCl_3$) δ 176.30, 159.34, 155.06, 153.27, 151.51, 140.58, 133.36, 132.88, 131.01, 130.17, 128.32, 128.24, 127.51, 114.02, 110.11, 109.06, 79.49, 55.49, 55.06, 46.58, 36.61, 30.53, 28.68, 6.98. MS (ESI) *m/z* 534 $[M + Na]^+$.

5-(5-Chloro-4'-trifluoromethyl-[1,1'-biphenyl)furan-2-yl-methylamino-methyl-*N*-*tert*-butoxy carbonylpiperidine (65g).

Starting from **64g** (60 mg, 0.17 mmol) and **48** (37 mg, 0.13 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65g** as yellow oil (67 mg, 72%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.72 – 7.60 (m, 3H), 7.38 (d, *J* = 8.3 Hz, 3H), 7.27 – 7.23 (m, 1H), 6.04 (d, *J* = 3.2 Hz, 1H), 5.64 (d, *J* = 3.2 Hz, 1H), 4.06 (brs, 2H), 3.64 (s, 2H), 2.67 (t, *J* = 12.5 Hz, 2H), 2.43 (d, *J* = 6.6 Hz, 2H), 1.66 (d, *J* = 13.0 Hz, 2H), 1.59 – 1.47 (m, 1H), 1.45 (s, 9H), 1.01 – 1.14 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 155.077, 154.05, 150.89, 144.78, 139.21, 133.26, 130.66, 129.56, 129.35, 128.66, 128.47, 128.09, 125.48, 111.88, 110.34, 108.98, 79.49, 55.12, 46.56, 36.79, 36.63, 30.54, 28.68. MS (ESI) m/z 571 $[M + Na]^+$.

5-(4-Chloro-2-(5-methylthiophen-2-yl)phenyl)furan-2-carbaldehyde (66a).

To a solution of palladium (II) acetate (2 mg, 0.007 mmol) and tricyclohexylphosphine tetrafluoroborate (5 mg, 0.014 mmol) in dry DMA (3 mL) in a sealed tube, potassium carbonate (72 mg, 0.52 mmol) and pivalic acid (10 mg, 0.10 mmol) were added under argon atmosphere. After 30 min 5-(4-chloro-2-bromophenyl)furan-2-carbaldehyde **58a** (100 mg, 0.35 mmol) was added and after further 10 min, 2-methylthiophene (34µL, 0.35 mmol) was added. Reaction was heated at 100 °C for 24 h. EtOAc (10 mL) was added, the organic phase was washed with H_2O (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10 % EtOAc in petroleum ether) to give **66a** as yellow oil (52 mg, 50%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.66 (s, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.37 – 7.28 (m, 3H), 6.89 (d, *J* = 3.3 Hz, 1H), 6.71 (d, *J* = 2.4 Hz, 1H), 2.50 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.69, 153.28, 151.07, 141.43, 135.94, 130.53, 129.16, 128.82, 127.83, 127.27, 126.10, 124.81, 119.14, 15.54. MS (ESI) *m/z* 302 $[M + H]^+$.

5-(4-Chloro-2-(pyridin-3-yl)phenyl)furan-2-carbaldehyde (66b).

To a solution of 1,10-phenantroline (19 mg, 0.10 mmol), palladium (II) acetate (8 mg, 0.03 mmol) and cesium carbonate (684 mg, 2.1 mmol) in dry pyridine (5 mL) in a sealed tube, **58a** (200 mg, 0.70 mmol) was added under argon atmosphere. Reaction was heated at 140 °C for 24 h. The solvent was removed under vacuum. The crude product was purified by flash chromatography on silica gel (25 % EtOAc in petroleum ether) to give **66b** as yellow oil (59 mg, 30%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.50 (s, 1H), 8.66 (d, *J* = 4.3 Hz, 1H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.72 (t, *J* = 7.7, Hz, 1H), 7.48 (dd, *J* = 11.0, 2.5 Hz, 2H), 7.36 – 7.25 (m, 2H), 7.08 (d, *J* = 3.7 Hz, 1H), 5.86 (d, *J* = 3.7 Hz, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.54, 157.90, 157.52,

152.01, 149.91, 141.15, 136.86, 130.91, 129.96, 129.11, 126.47, 124.36, 123.33, 123.17, 122.48, 111.90. MS (ESI) *m/z* 284 $[M + H]^+$, 306 $[M + Na]^+$.

5-[4-Chloro-2-(5-methylthiophen-2-yl)phenyl]furan-2-yl-methylamino-methyl-*N*-tert-butoxy-carbonylpiperidine (67a).

Starting from **66a** (25 mg, 0.08 mmol) and **48** (18 mg, 0.08 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **67a** as yellow oil (32 mg, 80%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.6 Hz, 2H), 7.30 – 7.25 (m, 2H), 6.83 (d, *J* = 3.4 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 6.31 (s, 1H), 4.08 (brs, 2H), 3.81 (s, 2H), 2.68 (t, *J* = 12.2 Hz, 2H), 2.57 (d, *J* = 6.5 Hz, 2H), 2.48 (s, 3H), 1.81 – 1.67 (m, 2H), 1.66 – 1.61 (m, 1H), 1.45 (s, 9H), 1.22 – 1.03 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 155.07, 153.23, 147.15, 140.01, 133.61, 132.99, 129.58, 128.80, 127.83, 126.04, 125.77, 117.03, 111.93, 79.52, 55.13, 46.52, 36.72, 30.55, 28.68, 15.52. MS (ESI) *m/z* 523 $[M + Na]^+$.

5-[4-Chloro-2-(pyridin-3-yl)phenyl]furan-2-yl-methylamino-methyl-*N*-*tert*-butoxy-carbonylpiperidine (67b).

Starting from **66b** (36 mg, 0.13 mmol) and **48** (27 mg, 0.13 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **67b** as orange oil (50 mg, 80%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 4.7 Hz, 1H), 7.63 (dd, *J* = 12.3, 8.0 Hz, 2H), 7.44 (d, *J* = 2.0 Hz, 1H), 7.37 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.27 – 7.17 (m, 3H), 6.03 (d, *J* = 3.1 Hz, 1H), 5.72 (d, *J* = 3.2 Hz, 1H), 4.05 (brs, 2H), 3.60 (s, 2H), 2.64 (t, *J* = 12.1 Hz, 2H), 2.39 (d, *J* = 6.6 Hz, 2H), 1.80 (brs, 2H), 1.63 (d, *J* = 12.4 Hz, 2H), 1.42 (s, 9H), 1.09 – 0.99 (m, 2H). MS (ESI) *m/z* 504 $[M + Na]^+$.

1-(5-(3-Chloro-[1,1'-biphenyl]-4-yl)furan-2-yl)-*N*-piperidin-4-ylmethyl-methanamine (17).

Starting from **62a** (50 mg, 0.1 mmol) and hydrochloridric acid 1 N (500 µL, 0.5 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (2 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on alumina gel (2% MeOH, 0.5% NH_4OH, in DCM) to give **17** as yellow oil (30 mg, 80%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.97 (d, *J* = 8.3 Hz, 1H), 7.72 (d, *J* = 1.9 Hz, 1H), 7.68 – 7.60 (m, 3H), 7.54 – 7.34 (m, 3H), 7.11 (d, *J* = 3.3 Hz, 1H), 6.44 (d, *J* = 3.3 Hz, 1H), 3.84 (s, 2H), 3.06 (d, *J* = 12.2

Hz, 2H), 2.61 (t, *J* = 11.6 Hz, 2H), 2.52 (d, *J* = 6.9 Hz, 2H), 1.78 (d, *J* = 13.6 Hz, 2H), 1.66 (brs, 1H), 1.25 – 1.08 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 154.30, 149.44, 140.89, 139.37, 133.37, 130.37, 130.27, 129.30, 129.15, 128.81, 128.16, 128.12, 127.09, 125.61, 112.00, 109.40, 55.71, 46.77, 46.73, 36.80, 31.59. MS (ESI) *m/z* 381 $[M + H]^+$.

1-(5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-piperidin-4-ylmethyl-methanamine (18a).

Starting from **62b** (60 mg, 0.1 mmol) and hydrochloridric acid 1 N (400 µL, 0.4 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18a** as yellow oil (38 mg, 100%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.76 (d, *J* = 8.5 Hz, 1H), 7.43 – 7.35 (m, 4H), 7.27 – 7.21 (m, 3H), 6.10 (d, *J* = 3.1 Hz, 1H), 5.60 (d, *J* = 3.3 Hz, 1H), 3.63 (s, 2H), 3.05 (d, *J* = 12.4 Hz, 2H), 2.60 (t, *J* = 11.7 Hz, 2H), 2.39 (d, *J* = 6.7 Hz, 2H), 1.72 (d, *J* = 13.0 Hz, 2H), 1.64 – 1.53 (m, 1H), 1.20 – 1.04 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 171.79, 152.73, 151.41, 141.10, 141.07, 132.74, 130.30, 128.71, 128.39, 128.18, 127.60, 127.51, 109.98, 109.25, 53.67, 45.34, 44.03, 33.88, 27.44. MS (ESI) *m/z* 381 $[M + H]^+$.

1-(5-(2',5-Dichloro-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-(piperidin-4-ylmethyl)methanamine (18b).

Starting from **65a** (20 mg, 0.04 mmol) and hydrochloridric acid 1 N (150 µL, 0.15 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18b** as yellow oil (13 mg, 82%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.91 (d, *J* = 8.5 Hz, 1H), 7.74 – 7.41 (m, 4H), 7.30 (d, *J* = 6.4 Hz, 1H), 7.24 (s, 1H), 6.45 (d, *J* = 3.1 Hz, 1H), 5.45 (d, *J* = 3.1 Hz, 1H), 4.23 (s, 2H), 3.42 (d, *J* = 13.4 Hz, 2H), 3.06 – 2.82 (m, 4H), 2.00 (d, *J* = 13.7 Hz, 2H), 1.50 – 1.28 (m, 3H). **^{13}C NMR** (75 MHz, CD_3OD) δ 152.94, 144.78, 138.92, 137.89, 133.23, 132.77, 130.69, 130.15, 129.66, 129.46, 128.42, 128.10, 127.48, 127.23, 113.96, 109.36, 51.23, 43.03, 38.75, 31.24, 28.70, 26.12, 23.53.MS (ESI) *m/z* 416 $[M + H]^+$.

1-(5-(3',5-dichloro-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-(piperidin-4-ylmethyl)methanamine (18c).

Starting from **65b** (40 mg, 0.07 mmol) and hydrochloridric acid 1 N (300 µL, 0.31 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18c** as yellow oil (25 mg, 86%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.75 (d, *J* = 8.5 Hz, 1H), 7.45 – 7.38 (m, 3H), 7.24 (d, *J* = 10.1 Hz, 2H), 7.18 – 7.13 (m, 1H), 6.25 (d, *J* = 3.2 Hz, 1H), 3.79 (s, 2H), 3.38 (d, *J* = 12.7 Hz, 2H), 2.96 (t, *J* = 12.8 Hz, 2H), 2.57 (d, *J* = 6.7 Hz, 2H), 1.95 (d, *J* = 13.7 Hz, 2H), 1.86 – 1.77 (m, 1H), 1.46 – 1.31 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 151.68, 151.13, 142.79, 139.58, 134.09, 133.23, 130.20, 130.02, 128.79, 128.69, 128.10, 127.98, 127.71, 127.30, 110.50, 110.25, 53.04, 44.91, 43.71, 33.14, 26.89. MS (ESI) *m/z* 416 $[M + H]^+$.

1-(5-(4',5-Dichloro-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-(piperidin-4-ylmethyl)methanamine (18d).

Starting from **65c** (52 mg, 0.97 mmol) and hydrochloridric acid 1 N (492 µL, 3.88 mmol) the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (3 mL) was added and the organic phase was extracted with EtOAc (3 x 5mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18d** as yellow oil (50 mg, 99%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.71 (d, *J* = 8.5 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.26 – 7.15 (m, 3H), 6.17 (d, *J* = 3.3 Hz, 1H), 5.75 (d, *J* = 3.3 Hz, 1H), 3.66 (s, 2H), 3.38 (brs, 2H), 2.93 (d, *J* = 12.8, 2H), 2.45 (d, *J* = 6.7 Hz, 2H), 1.93 (d, *J* = 12.8 Hz, 2H),1.77 – 1.70 (m, 1H), 1.44 – 1.24 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 153.02, 150.97, 139.49, 139.42, 133.34, 132.72, 130.17, 130.01, 128.43, 128.25, 128.01, 127.63, 109.81, 108.91, 54.75, 45.36, 45.19, 35.62, 30.59.MS (ESI) *m/z* 416 $[M + H]^+$.

1-(5-(5-Chloro-2'-methoxy-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-(piperidin-4-ylmethyl-methanamine (18e).

Starting from **65d** (30 mg, 0.05 mmol) and hydrochloridric acid 1 N (230 µL, 0.23 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the

organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18e** as yellow oil (18 mg, 90%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.78 (d, *J* = 8.5 Hz, 1H), 7.39 (ddd, *J* = 10.8, 8.1, 2.1 Hz, 2H), 7.18 – 6.97 (m, 4H), 6.07 (d, *J* = 3.3 Hz, 1H), 5.49 (d, *J* = 3.3 Hz, 1H), 3.66 (s, 2H), 3.60 (s, 3H), 3.11 (d, *J* = 12.6 Hz, 2H), 2.66 (t, *J* = 11.5 Hz, 2H), 2.41 (d, *J* = 6.7 Hz, 2H), 1.77 (d, *J* = 13.1 Hz, 2H), 1.68 – 1.55 (m, 1H), 1.23 – 1.07 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 156.94, 152.47, 151.78, 137.48, 132.16, 130.71, 130.20, 129.98, 129.48, 129.10, 127.26, 127.12, 120.72, 111.14, 109.18, 108.58, 54.75, 54.43, 45.40, 45.00, 35.10, 29.55. MS (ESI) *m/z* 412 $[M + H]^+$.

1-(5-(5-Chloro-3'-methoxy-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-(piperidin-4-ylmethyl-methanamine (18f).

Starting from **65e** (25 mg, 0.04 mmol) and hydrochloridric acid 1 N (160 µL, 0.16 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18f** as yellow oil (19 mg, 100%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.75 (d, *J* = 8.5 Hz, H), 7.42 – 7.24 (m, 3H), 6.95 (d, *J* = 7.9, Hz, 1H), 6.79 (dd, *J* = 9.0, 4.9 Hz, 2H), 6.13 (d, *J* = 3.3 Hz, 1H), 5.68 (d, *J* = 3.3 Hz, 1H), 3.76 (s, 3H), 3.65 (s, 2H), 3.18 (d, *J* = 12.6 Hz, 2H), 2.74 (t, *J* = 12.6, Hz, 2H), 2.42 (d, *J* = 6.7 Hz, 2H), 1.81 (d, *J* = 13.6 Hz, 2H), 1.70 – 1.54 (m, 1H), 1.30 – 1.11 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 160.01, 152.88, 151.31, 142.34, 140.95, 132.69, 130.17, 129.45, 128.18, 127.54, 120.99, 114.22, 113.20, 109.93, 109.19, 54.56, 54.26, 45.38, 44.77, 34.82, 29.02. MS (ESI) *m/z* 412 $[M + H]^+$.

1-(5-(5-Chloro-4'-methoxy-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-(piperidin-4-ylmethyl-methanamine (18g).

Starting from **65f** (40 mg, 0.08 mmol) and hydrochloridric acid 1 N (310 µL, 0.31 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18g** as yellow oil (28 mg, 85%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.72 (d, *J* = 8.5 Hz, 1H), 7.35 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.22 (d, *J* = 2.1 Hz,

1H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.12 (d, *J* = 3.3 Hz, 1H), 5.65 (d, *J* = 3.3 Hz, 1H), 3.82 (s, 3H), 3.65 (s, 2H), 3.15 (d, *J* = 12.5 Hz, 2H), 2.70 (t, *J* = 12.5 Hz, 2H), 2.41 (d, *J* = 6.7 Hz, 2H), 1.78 (d, *J* = 12.9 Hz, 2H), 1.70 – 1.53 (m, 1H), 1.36 – 1.08 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 159.64, 152.77, 151.59, 140.92, 133.20, 132.69, 130.48, 129.84, 128.40, 128.28, 127.20, 113.77, 109.88, 109.17, 54.61, 54.40, 45.43, 44.87, 34.94, 29.25. MS (ESI) *m/z* 412 $[M + H]^+$.

1-(5-(5-Chloro-4'-trifluoromethyl-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-(piperidin-4-ylmethyl-methanamine (18h).

Starting from **65g** (40 mg, 0.07 mmol) and hydrochloridric acid 1 N (280 µL, 0.28 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18h** as yellow oil (30 mg, 93%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.79 (d, *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.51 – 7.42 (m, 3H), 7.33 (d, *J* = 2.2 Hz, 1H), 6.30 (d, *J* = 3.4 Hz, 1H), 5.72 (d, *J* = 3.4 Hz, 1H), 3.86 (s, 2H), 3.39 (d, *J* = 12.7 Hz, 2H), 2.95 (t, *J* = 12.9, 2H), 2.64 (d, *J* = 6.7 Hz, 2H), 1.96 (d, *J* = 14.6 Hz, 2H), 1.85 (m, 1H), 1.48 – 1.28 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 151.99, 149.74, 144.82, 139.72, 133.50, 133.43, 130.23, 129.60, 128.89, 128.31, 127.82, 125.33, 125.28, 111.38, 110.42, 63.20, 52.65, 44.56, 43.58, 32.67, 26.73. MS (ESI) *m/z* 450 $[M + H]^+$.

1-(5-(4-Chloro-2-(5-methylthiophen-2-yl)phenyl)furan-2-yl)-*N*-(piperidin-4-ylmethyl) methanamine. (18i).

Starting from **67a** (20 mg, 0.04 mmol) and hydrochloridric acid 1 N (160 µL, 0.16 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18i** as yellow oil (15 mg, 96%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.58 (d, *J* = 6.8 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 3.5 Hz, 1H), 6.71 (d, *J* = 3.4, Hz, 1H), 6.43 (s, 1H), 5.48 (s, 1H), 3.81 (s, 2H), 3.24 (brs, 2H), 2.88 – 2.78 (m, 2H), 2.56 (d, *J* = 6.8 Hz, 2H), 2.46 (s, 3H), 1.92 (d, *J* = 15.8 Hz, 2H), 1.75 (m, 1H), 1.38 – 1.21 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 152.92, 147.06, 139.98, 133.42, 132.62, 129.63,

128.45, 127.58, 127.21, 125.56, 117.16, 112.09, 53.94, 45.40, 44.22, 34.64, 27.80, 13.95. MS (ESI) *m/z* 401 $[M + H]^+$.

1-(5-(4-Chloro-2-(pyridin-3-yl)phenyl)furan-2-yl)-*N*-(piperidin-4-ylmethyl)methanamine (18j).

Starting from **67b** (15 mg, 0.03 mmol) and hydrochloridric acid 1 N (120 µL, 0.12 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **18j** as yellow oil (11 mg, 100%). **^{1}H NMR** (400 MHz, CD_3OD) δ 8.57 (s, 1H), 7.84 (t, *J* = 7.4 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.53 – 7.36 (m, 4H), 7.33 (d, *J* = 7.7 Hz, 1H), 6.14 (s, 1H), 5.74 (d, *J* = 2.6 Hz, 1H), 3.56 (s, 2H), 3.02 (d, *J* = 11.7 Hz, 2H), 2.56 (t, *J* = 12.1 Hz, 2H), 2.34 (d, *J* = 6.4 Hz, 2H), 1.68 (d, *J* = 12.6 Hz, 2H), 1.54 (brs, 1H), 1.13 – 1.01 (m, 2H). MS (ESI) *m/z* 382 $[M + H]^+$.

5.4 Synthesis of compounds 19-24.

5-(4-Bromo-2-chlorophenyl)thiophene-2-carbaldehyde (69a).

Starting from **38** (300 mg, 1.5 mmol) and **68** (135 µL,1.5 mmol), the title compound was prepared following the procedure **B** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (10 % EtOAc in petroleum ether) to give **69a** as yellow oil (360 mg, 80%). **^{1}H NMR**: (300 MHz, CDCl3) δ 9.93 (s, 1H), 7.75 (d, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 2.0 Hz, 1H), 7.41 (s, 1H), 7.38 – 7.35 (m, 2H). **^{13}C NMR**: (75 MHz, CDCl3) δ 183.1, 148.7, 144.2, 136.3, 133.5, 133.5, 132.4, 131.1, 130.7, 129.1, 123.5 MS (ESI) *m/z* 302 $[M + H]^+$.

5-(4-Chloro-2-bromophenyl)thiophene-2-carbaldehyde (69b).

Starting from **57a** (200 mg, 0.97 mmol) and **68** (87 µL,0.97 mmol), the title compound was prepared following the procedure **B** reported for compound **34**. The crude product was purified by flash chromatography on silica gel (10 % EtOAc in petroleum ether) to give **69b** as yellow oil (227 mg, 78%). **^{1}H NMR**: (300 MHz, CDCl3) δ 9.93 (s, 1H), 7.75 (d, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 2.0 Hz, 1H), 7.41 (s, 1H), 7.38 – 7.35 (m, 2H). **^{13}C NMR**: (75 MHz, CDCl3) δ 183.1, 148.7, 144.2, 136.3, 133.5, 133.5, 132.4, 131.1, 130.7, 129.1, 123.5. MS (ESI) *m/z* 302 $[M + H]^+$.

5-(4-Bromo-2-chlorophenyl)-thiophene-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (70a).

Starting from **69a** (88 mg, 0.4 mmol) and **48** (85 mg, 0.4 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **70a** as uncolorless oil (143 mg, 72%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.67 (s, 1H), 7.51 – 7.41 (m, 2H), 7.23 (d, *J* = 3.7 Hz, 1H), 6.99 (d, *J* = 3.7 Hz, 1H), 4.06 (d, *J* = 12.8 Hz, 2H), 3.96 (s, 2H), 2.73 (t, *J* = 12.8 Hz, 2H), 2.51 (d, *J* = 6.3 Hz, 2H), 1.76 – 1.66 (m, 3H), 1.43 (d, *J* = 0.9 Hz, 13H), 1.15 - 0.99 (m, 2H). **^{13}C NMR**: (75 MHz, CD_3OD) δ 155.3, 144.5, 139.6, 133.9, 132.9, 132.5, 127.8, 127.7, 125.8, 122.6, 112.3, 79.7, 54.2, 43.3, 35.9, 30.2, 27.5. MS (ESI) *m/z* 500 $[M + H]^+$, 522 $[M + Na]^+$.

5-(4-Chloro-2-bromophenyl)-thiophene-2-yl-methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (70b).

Starting from **69b** (48 mg, 0.2 mmol) and **48** (43 mg, 0.2 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **70b** as uncolorless oil (69 mg, 70%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.73 (s, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 3.6 Hz, 1H), 6.99 (d, *J* = 3.6 Hz, 1H), 4.05 (d, *J* = 13 Hz, 2H), 3.97 (s, 2H), 2.74 (t, *J* = 12.9 Hz, 2H), 2.53 (d, *J* = 6.3 Hz, 2H), 1.74 (d, *J* = 12.1 Hz, 2H), 1.72-1.53 (m, 1H) 1.44 (s, 9H), 1.07 (d, *J* = 11.6 Hz, 2H). **^{13}C NMR**: (75 MHz, CD3OD) δ 155.3, 144.5, 139.6, 133.9, 132.9, 132.5, 127.8, 127.7, 125.8, 122.6, 112.3, 79.7, 54.2, 43.3, 35.9, 30.2, 27.5. MS (ESI) *m/z* 500 $[M + H]^+$, 522 $[M + Na]^+$.

Ethyl 6-bromobenzothiophene-2-carboxylate (73).

To a solution of **71** (150 mg, 0.7 mmol) in dry DMF (4 mL), potassium carbonate was added (150 mg, 1.1 mmol). The mixture was cooled to 0 °C and **72** (88 µL, 0.8 mmol) was added. The reaction mixture was heated under reflux for 12 h. H_2O (3 mL) was added and the organic phase was extracted with EtOAc (3 x 4mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **73** as white solid (160 mg, 85%). **^{1}H NMR**: (300 MHz, CDCl3) δ 7.90 (s, 2H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H). mp (EtOAc/*n*-hexane) 46-49 °C.

(6-Bromobenzothiophen-2-yl)methanol (74).

To a solution of **73** (160 mg, 0.59 mmol) in dry DCM (5 mL), diisobutyl-aluminum hydride (2,4 mL, sol. 1.0 M in dry DCM) was added at -78 °C. After 3 h, H_2O (3 mL) was added and the organic phase was extracted with DCM (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo giving the product **74** as a colorless oil without further purification (86 mg, 60%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.43 (s, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 4.88 (s, 2H), 2.27 (brs, 1H). MS (ESI) *m/z* 242 $[M + H]^+$.

6-Bromobenzothiophene-2-carbaldehyde (75).

A solution of **74** (86 mg, 0.4 mmol) in dry DCM (5 mL) was added to a suspension of pyridinium chlorochromate (114 mg, 0.5 mmol) in dry DCM (4 mL). After 2 h the mixture was filtered on celite and the filtrate was concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (10% EtOAc in petroleum ether) to give **75** as white solid (83 mg, 87%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 10.10 (s, 1H), 8.06 (s, 1H), 7.98 (s, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H). MS (ESI) m/z 264 $[M + Na]^+$. mp (EtOAc/*n*-hexane) 92-95 °C.

(6-Bromobenzothiophen-2-yl)methylamino-methyl-N-tert-butoxycarbonylpiperidine (76).

Starting from **75** (30 mg, 0.1 mmol) and **48** (30 mg, 0.1 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **76** as colorless oil (22 mg, 50%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.97 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.20 (s,1H), 4.13 – 4.05 (m, 2H), 4.02 (s, 2H), 2.73 (t, *J* = 12.7 Hz, 2H), 2.50 (d, *J* = 6.4 Hz, 2H), 1.73 (d, *J* = 12.0 Hz, 2H), 1.43 (s, 9H), 1.27 (s, 1H), 1.14 – 0.98 (m, 2H). MS (ESI) *m/z* 440 $[M + H]^+$, 462 $[M + Na]^+$.

2-Methyl-6-(4-(trifluoromethyl)phenyl)pyridine (78).

Triphenylphosphine (366 mg, 1.4 mmol) and palladium (II) acetate (20 mg, 0.1 mmol) were dissolved in a mixture of dry DMF (7 mL) and H_2O (3 mL) under argon atmosphere. After 30 min, **77** (198 µL, 1.7 mmol) was added. After further 30 min, potassium carbonate (721 mg, 5.2 mmol) and powered 4-trifluoromethyl-phenyl boronic acid **63g** (331 mg, 1.7 mmol) were added in this order. The reaction mixture was heated at reflux for 12 h. The solvent was removed under vacuo. H_2O (2 mL) and EtOAc (8 mL) were added, the organic phase was separated, dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2 % EtOAc in petroleum ether) to give **78** as white solid

(336 mg, 81%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 8.13 – 8.01 (d, *J* = 7,83 Hz, 2H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.56 (t, *J* = 7.73 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 2.61 (s, 3H). MS (ESI) *m/z* 238 $[M + H]^+$.

2-(Bromomethyl)-6-(4-trifluoromethyl)phenyl-pyridine (79).

To a solution of **78** (336 mg, 1.4 mmol) in CCl_4 (14 mL), N-bromosuccinimide (904 mg, 5.1 mmol) and azobisisobutyronitrile (98 mg, 0.6 mmol) were added portionwise. The reaction was heated under reflux for 12 h. The mixture was filtered on paper and the filtrate was concentrated under vacuo. The crude product was purified by flash chromatography on silica gel (20 % DCM in petroleum ether) to give **79** as white solid (132 mg, 30%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.00 Hz, 2H), 7.81 (t, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 8.12 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 4.63 (s, 2H). MS (ESI) *m/z* 317 $[M + H]^+$.

6-[4-(Trifluoromethyl)phenyl-pyridin-2-yl]methylamino-methyl-*N*-*tert*-butoxycarbonyl piperidine (80).

To a solution of **48** (66 mg, 0.3 mmol) in dry MeCN (1 mL), was slowly added a solution of **79** (49 mg, 0.2 mmol) in dry MeCN (3 mL)and powered potassium carbonate (171 mg, 1.2 mmol). After 12 h, the solvent was removed in vacuo. H_2O (1 mL) and EtOAc (5 mL) were added and the organic phase was washed with an aqueous solution NaOH (1 N, 2 mL) dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2 % MeOH in DCM) to give **80** as colorless oil. (18 mg, 20%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 8.25 (d, *J* = 8.1 Hz, 2H), 7.87-7.82 (m, 2H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 7.0 Hz, 1H), 4.06 (d, *J* = 13.5 Hz, 2H), 3.99 (s, 2H), 2.76 (t, *J* = 12.7 Hz, 2H), 2.60 (d, *J* = 6.2 Hz, 2H), 1.78 (d, *J* = 12.0 Hz, 2H), 1.44 (s, 9H), 1.28 (s, 1H), 1.17 – 1.04 (m, 2H). MS (ESI) *m/z* 450 $[M + H]^+$, 472 $[M + Na]^+$.

Methyl 2-Bromo-4-chlorobenzoate (82).

To a suspension of methyl 2-amino-4-chlorobenzoate **81** (300 mg, 1.6) in MeCN (15 mL), *p*-toluenesulfonic acid (370 mg, 1.94 mmol) was added and a yellow precipitate was formed. *tert*-butyl nitrite (230 µL, 1.94 mmol) was added at 0 °C followed by tetra-*n*-butylammonium bromide (1.03 g, 3.22 mmol) and a catalytic amount of cooper(II)bromide. After 1 h the solvent was removed in vacuo. H_2O (3 mL) was added and the organic phase was extracted with DCM (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5 % EtOAc in petroleum ether) to give **82** as

yellow oil. (302 mg, 80%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 3.3 Hz, 1H), 7.56 (s, 1H), 7.24 (d, *J* = 4.0 Hz, 1H), 3.84 (s, 3H). MS (ESI) *m/z* 250 $[M + H]^+$.

2-Bromo-4-chlorobenzohydrazide (83).

To a solution of **82** (300 mg, 1.2 mmol) in EtOH (4 mL), hydrazine monohydrate (376 µL, 12 mmol) was added. The reaction mixture was heated at reflux for 12 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **83** as yellow oil (239 mg, 80%). **^{1}H NMR** (300 MHz, Acetone) δ 7.88 (d, *J* = 8.5 Hz, 1H), 7.66 (d, *J* = 1.9 Hz, 1H), 7.51 – 7.44 (m, 1H), 2.84 (s, 3H). MS (ESI) *m/z* 272 $[M + Na]^+$.

2-(2-Bromo-4-chlorophenyl)-5-(chloromethyl)-1,3,4-oxadiazole (84).

To a solution of **83** (200 mg, 0.8 mmol) in phosphoryl chloride (2 mL), chloroacetic aid (76 mg, 0.8 mmol) was added. The reaction mixture was heated under reflux for 12 h. After this time the mixture was cooled at 0 °C, and an aqueous solution of sodium bicarbonate (2 mL) was slowly added. The organic phase was extracted with EtOAc (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (15% EtOAc in petroleum ether) to give **84** as yellow oil (49 mg, 20%). **^{1}H NMR** (300 MHz, CD_3OD) δ 8.04 (d, *J* = 8.7 Hz, 1H), 7.94 – 7.89 (m, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 4.96 (s, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 139.13, 134.11, 132.61, 131.86, 130.09, 129.56, 128.36, 122.05, 32.49. MS (ESI) *m/z* 308 $[M + H]^+$, 330 $[M + Na]^+$.

[5-(2-Bromo-4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methylamino-methyl-*N*-*tert*-butoxy carbonyl-piperidine (85).

To a solution of **48** (12 mg, 0.06) in dry MeCN (1 mL), a solution of **84** (20 mg, 0.065 mmol) in dry MeCN (2 mL), potassium carbonate (134 mg, 0.97 mmol) and a catalytic amount of potassium iodide were slowly added. The reaction mixture was heated under reflux for 1 h. After this time, the solvent was removed in vacuo. H_2O (1 mL) and EtOAc (5 mL) were added and the organic phase was separated, dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **85** as yellow oil (25 mg, 81%). **^{1}H NMR** (300 MHz, CD_3OD) δ 8.02 (d, *J* = 8.6 Hz, 1H), 7.89 (d, *J* = 5.0 Hz, 1H), 7.59 (dd, *J* = 8.5, 1.9 Hz, 1H), 4.08 (d, *J* = 5.8 Hz, 2H), 4.03 (s, 2H), 2.74 (s, 2H), 2.57 (s, 2H), 1.74 (d, *J* = 13.9 Hz, 2H), 1.69 – 1.61 (m, 1H), 1,44 (s, 9H) 1.17 – 0.99 (m, 2H). MS (ESI) *m/z* 508 $[M + H]^+$.

4-Benzyl-3-(4-chlorophenyl)-4*H*-1,2,4-triazole (86).

To a solution of **83** (239, 0.95 mmol) in MeCN (10 mL), *N*,*N*-dimethylformamide dimethyl acetal (128 µL , 0.95 mmol) was added. The reaction was heated at 50 °C for 1 h. After this time, benzyl amine (101 µL, 0.85 mmol) and acetic acid (2 mL) were added and reaction was heated at 120 °C for 12 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in EtOAc) to give **86** as yellow oil. (198 mg, 60%)**.** **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.46 (dd, *J* = 8.5, 2.1 Hz, 2H), 7.41 – 7.33 (m,2H), 7.30 – 7.23 (m, 3H), 7.06 – 6.96 (m, 1H), 5.17 (s, 2H). MS (ESI) *m/z* 350 $[M + H]^+$.

[4-Benzyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-yl]methanol (87).

A solution of **86** (100 mg, 0.29) in formaldehyde (aq. sol. 37%, 2,8 mL) was heated under reflux for 12 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in EtOAc) to give **87** as yellow oil. **^{1}H NMR** (300 MHz, CD_3OD) δ 7.55 – 7.44 (m, 4H), 7.32 – 7.26 (m, 2H), 7.03 – 6.97 (m, 2H), 5.45 (s, 2H), 4.81 (d, *J* = 1.2 Hz, 2H). (43 mg, 50%). MS (ESI) *m/z* 300 $[M + H]^+$.

4-Benzyl-3-(chloromethyl)-5-(4-chlorophenyl)-4*H*-1,2,4-triazole (88).

To a solution of **87** (40 mg, 0.13 mmol) in dry DCM (4 mL), pyridine (12 µL, 0.14 mmol) and thionyl chloride (13 µL, 0.16 mmol) were added at 0 °C. After 3 h an aqueous solution of sodium bicarbonate was added and the organic phase was extracted with DCM, dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **88** as yellow oil (20 mg, 50%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.55 – 7.44 (m, 4H), 7.32 – 7.26 (m, 2H), 7.03 – 6.97 (m, 2H), 5.43 (s, 2H), 4.92 (s, 2H). (43 mg, 50%). MS (ESI) *m/z* 319 $[M + H]^+$.

4-[4-Benzyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-yl]methylamino-*N*-*tert*-butoxy carbonylpiperidine (89).

Starting from **88** (20 mg, 0.06 mmol) and **48** (12 mg, 0.05 mmol), the title compound was prepared following the procedure reported for compound **85**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **89** as uncolorless oil (21 mg, 72%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.55 – 7.44 (m, 5H), 7.34 – 7.26 (m, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 5.47 (s, 2H), 4.02 (d, *J* = 13.4 Hz, 2H), 3.91 (s, 2H), 2.69 (s, 2H), 2.49 (s, 2H), 1.65 (d, *J* = 13.5 Hz, 2H), 1.61 – 1.50 (m, 1H), 1.44 (s, 9H), 1.11 – 0.94 (m, 2H). MS (ESI) *m/z* 497 $[M + H]^+$, 535 $[M + K]^+$.

[5-(4-Chlorophenyl)-4*H*-1,2,4-triazol-3-yl]methylamino-methyl-*N*-*tert*-butoxy-carbonyl piperidine (90).

To a solution of **89** (20 mg, 0.04 mmol) in DMSO (30 µL), a solution of potassium *tert*-butoxide (34 mg, 0.30 mmol) in dry THF (1 mL) was added. The reaction environment was saturated with oxygen. After 2 h an aqueous solution of ammonium chloride (0.5 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **90** as yellow oil (14 mg, 87%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.97 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 7.9 Hz, 2H), 4.07 (d, *J* = 13.2, 2H), 3.96 (s,2H), 2.77 (d, *J* = 12.0 Hz, 2H), 2.58 (d, *J* = 6.0 Hz, 2H), 1.76 (d, *J* = 12.4 Hz, 2H), 1.44 (s, 9H), 1.31 – 1.21 (m, 2H), 1.15 – 1.04 (m, 1H). MS (ESI) *m/z* 407 $[M + H]^+$.

[5-(4-Bromo-2-chlorophenyl)thiophenyl]-*N*-piperidiny-4-yllmethyl-methanamine (19).

Starting from **70a** (36 mg, 0.07 mmol) and hydrochloridric acid 1 N (280 µL, 0.28 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **19** as yellow oil (27 mg, 100%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.38 (d, *J* = 1.6 Hz, 1H), 7.26 (d, *J* = 0.9 Hz, 1H), 7.20 (d, *J* = 2.4 Hz, 1H), 6.90 (d, *J* = 2.7 Hz, 1H), 3.98 (s, 2H), 3.08 (d, *J* = 12.2 Hz, 2H), 2.57 (d, *J* = 6.5 Hz, 2H), 1.74 (m, 4H), 1.61 (brs, 2H), 1.29 – 1.07 (m, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 207.16, 146.37, 137.93, 133.20, 132.64, 132.29, 130.33, 127.77, 125.01, 121.38, 55.63, 49.01, 46.26, 36.60, 31.13. MS (ESI) *m/z* 400 $[M + H]^+$.

[5-(4-chloro-2-bromophenyl)thiophenyl]-*N*-piperidiny-4-yllmethyl-methanamine (20).

Starting from **70b** (20 mg, 0.04 mmol) and hydrochloridric acid 1 N (160 µL, 0.16 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **20** as yellow oil (14 mg, 90%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.73 (s, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.40 – 7.37 (m, 1H), 7.14 (d, *J* = 1.5 Hz, 1H), 6.98 (d, *J* = 2.7 Hz, 1H), 3.95 (s, 2H), 3.06 (d, *J* = 12.8 Hz, 2H), 2.62 (t, *J* = 13.3, Hz, 2H), 2.51 (d, *J* = 6.6 Hz, 2H), 1.78 (d, *J* = 13.2 Hz, 2H), 1.65 (m, 1H), 1.28 – 1.08 (m, 2H). **^{13}C**

NMR (75 MHz, $CDCl_3$) δ 207.16, 146.37, 137.93, 133.20, 132.64, 132.29, 130.33, 127.77, 125.01, 121.38, 55.63, 49.01, 46.26, 36.60, 31.13. MS (ESI) *m/z* 400 $[M + H]^+$.

1-(Piperidin-4-yl)-*N*-((6-(4-(trifluoromethyl)phenyl)pyridin-2-yl)methyl)methanamine (21).

Starting from **80** (30 mg, 0.06 mmol) and hydrochloridric acid 1 N (240 µL, 0.24 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **21** as yellow oil (20 mg, 98%). **^{1}H NMR** (300 MHz, CD_3OD) δ 8.25 (d, *J* = 8.2 Hz, 2H), 7.87 (d, *J* = 7.3 Hz, 2H), 7.78 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 7.1 Hz, 1H), 3.96 (s, 2H), 3.21 (d, *J* = 12.4 Hz, 2H), 2.78 (t, *J* = 11.4 Hz, 2H), 2.58 (d, *J* = 6.6 Hz, 2H), 1.91 (d, *J* = 13.4 Hz, 2H), 1.80 (brs, 1H), 1.27 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 162.06, 159.15, 155.29, 149.12, 142.99, 137.99, 130.83, 130.39, 127.37, 126.34, 125.47, 125.42, 119.50, 54.66, 54.10, 44.74, 34.96, 29.56, 28.97, 21.78, 17.59, 13.17, 7.13, -9.33. MS (ESI) *m/z* 350 $[M + H]^+$.

1-(5-Bromobenzothiophen-2-yl)-*N*-(piperidin-4-ylmethyl)methanamine (22).

Starting from **76** (22 mg, 0.05 mmol) and hydrochloridric acid 1 N (200 µL, 0.2 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **22** as yellow oil (17 mg, 100%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.97 (s, 1H), 7.61 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 4.2, Hz, 1H), 7.20 (s, 1H), 4.01 (s, 2H), 3.03 (d, *J* = 12.4 Hz, 2H), 2.58 (t, *J* = 12.4, Hz, 2H), 2.49 (d, *J* = 6.7 Hz, 2H), 1.75 (d, *J* = 13.2 Hz, 2H), 1.71 – 1.56 (m, 1H), 1.13 (m, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 145.93, 141.58, 138.97, 127.40, 124.54, 124.32, 121.48, 117.32, 54.76, 45.44, 35.89, 30.45. MS (ESI) *m/z* 362 $[M + Na]^+$.

1-(5-(4-Chlorophenyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(piperidin-4-ylmethyl)methanamine (23).

Starting from **90** (15 mg, 0.03 mmol) and hydrochloridric acid 1 N (120 µL, 0.12 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the

organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo giving the product **23** as yellow oil without further purification (11 mg, 84%). **^{1}H NMR** (300 MHz, CD_3OD) δ 8.03 (d, *J* = 8.2 Hz, 2H), 7.63 (d, *J* = 8.1 Hz, 2H), 4.62 (s, 2H), 3.46 (d, *J* = 12.1 Hz, 2H), 3.11 (t, *J* = 11.8 Hz, 2H), 2.83 (brs, 2H), 2.32 (brs, 1H), 2.14 (d, *J* = 11.9 Hz, 2H), 1.68 (d, *J* = 11.1 Hz, 2H). MS (ESI) *m/z* 397 $[M + H]^+$.

1-(5-(2-Bromo-4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-*N*-(piperidin-4-ylmethyl)methan amine (24).

Starting from **85** (20 mg, 0.04 mmol) and hydrochloridric acid 1 N (160 µL, 0.16 mmol), the title compound was prepared following the procedure reported for compound **6**. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo.

The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **24** as yellow oil (13 mg, 82%). **^{1}H NMR** (300 MHz, CD_3OD) δ 8.03 (d, *J* = 8.3 Hz, 1H), 7.91 (d, *J* = 4.9, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 5.48 (s, 2H), 3.39 (d, *J* = 12.5 Hz, 2H), 3.32 – 3.27 (m, 2H), 2.97 (t, *J* = 12.4 Hz, 2H), 2.07 – 1.98 (m, 2H), 1.90 – 1.82 (m, 1H), 1.50 – 1.24 (m, 2H). MS (ESI) *m/z* 386 $[M + H]^+$.

5.5 Synthesis of compounds 26-33.

***N*^{1}-[(5-(4-Bromo-2-chlorophenyl)furan-2-yl-methyl]-*N*^{3},*N*^{3}-dimethylpropane-1,3-diamine (26).**

To a solution of *N*^{1},*N*^{1}-dimethylpropane-1,3-diamine **91** (22 µL, 0.17 mmol) in dry DCM (2 mL), was added a solution of aldehyde **34** (45 mg 0.16 mmol) in dry DCM (5 mL). After 1 h sodium triacetoxyborohydride (51 mg, 0.24 mmol) was added at 0 °C and the mixture kept at 25 °C for 12 h. After this time, sodium cyanoborohydride (20 mg, 0.32 mmol) was added and the solution was maintained at the same temperature for further 1 h. A saturated solution of sodium bicarbonate (3 mL) was added and the organic phase was extracted with DCM (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **26** as yellow oil (50 mg, 85%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.82 (d, *J* = 6.1 Hz, 1H), 7.66 (s, 1H), 7.51 (d, *J* = 6.5 Hz, 1H), 7.09 (d, *J* = 3.2 Hz, 1H), 6.43 (d, *J* = 3.1 Hz, 1H), 3.83 (d, *J* = 2.1 Hz, 2H), 2.66 (t, *J* = 6.1 Hz, 2H), 2.43 – 2.36 (m, 2H), 2.26 (s, 6H), 1.74 – 1.70 (m, 2H). **^{13}C NMR** (75 MHz,

CD_3OD) δ 153.71, 148.71, 132.95, 130.35, 130.29, 128.84, 128.37, 120.44, 112.25, 109.78, 57.44, 46.86, 45.24, 44.13, 25.59. MS (ESI) *m/z* 394 $[M + Na]^+$.

N^1-[(5-(4-chloro-2-bromophenyl)furan-2-yl-methyl]-N^3,N^3-dimethylpropane-1,3-diamine (92).

Starting from **91** (65 µL, 0.5 mmol) and **58a** (130 mg, 0.5 mmol), the title compound was prepared following the procedure reported for compound **26**. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **92** as yellow oil (92 mg, 50%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.80 (d, *J* = 8.6 Hz, 1H), 7.68 (s, 1H), 7.43 (d, *J* = 9.0 Hz, 1H), 7.12 (d, *J* = 3.4 Hz, 1H), 6.41 (d, *J* = 3.4 Hz, 1H), 3.87 (s, 2H), 2.64 (t, *J* = 7.3 Hz, 2H), 2.41 – 2.26 (m, 2H), 2.22 (s, 6H), 1.80 (t *J* = 6.0 Hz, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 153.71, 148.71, 132.95, 130.35, 130.29, 128.84, 128.37, 120.44, 112.25, 109.78, 57.44, 46.86, 45.24, 44.13, 25.59. MS (ESI) *m/z* 394 $[M + Na]^+$.

***tert*-Butyl[(5-(4-chloro-2-bromophenyl)furan-2-yl-methyl]-3-dimethylamino-propyl-carbamate (93).**

To a solution of **92** (57 mg, 0.2 mmol) in dry MeOH (4 mL), triethylamine (63 µL, 0.5 mmol) and di-*tert*-butyl dicarbonate (49 mg, 0.2 mmol) were added. The reaction mixture was kept at 25 for 4 h. After this time, the solvent was removed in vacuo. H_2O (2 mL) was added and the organic phase was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **93** as yellow oil (82 mg, 87%). **^{1}H NMR**: (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.5 Hz, 1H), 7.60 (s, 1H), 7.27 (d, *J* = 8.5,1H), 7.06 (s, 1H), 6,28 (br s, 1H), 4.42 (br s, 2H), 3.29 (br s, 2H), 2.19 (s, 6H), 1.67 (brs, 4H), 1.44 (s, 9H). MS (ESI) *m/z* 394 $[M + H]^+$.

***N*-Benzyl-3-(((5-(2-bromo-4-chlorophenyl)furan-2-yl)methyl)(*tert*-butoxycarbonyl)amino)-*N*,*N*-dimethylpropan-1-aminium (94a).**

To a solution of **93** (30 mg, 0.1 mmol) in dry acetone (2 mL), benzyl bromide (38 µL, 0.3 mmol) was added. The reaction mixture was heated under reflux for 72 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **94a** as yellow oil (32 mg, 58%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.80 – 7.62 (m, 2H), 7.57 – 7.45 (m, 5H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.09 (d, *J* = 3.5 Hz, 1H), 6.47 (d, *J* = 3.3 Hz, 1H), 4.54 (s, 2H), 4.49 (s, 2H), 3.46 (t, *J* = 6.7 Hz, 1H), 3.38 – 3.21 (m, 2H), 3.00 (s, 6H), 2.14 (t, *J* = 9.0 Hz, 2H), 1.49 (s, 9H). MS (ESI) *m/z* 562 $[M + H]^+$.

(5-(2-Bromo-4-chlorophenyl)furan-2-yl)methyl)(*tert*-butoxycarbonyl)amino)-*N*,*N*,*N*-trimethylpropan-1-aminium (94b).

To a solution of **93** (30 mg, 0.1 mmol) in dry acetone (2 mL), iodomethane (16 µL, 0.3 mmol) was added. Reaction refluxed for 72 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on alumina gel (33% MeCN in DCM) to give **94b** as yellow oil (29 mg, 60%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.78 – 7.72 (m, 2H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.12 (d, *J* = 3.4 Hz, 1H), 6.49 (d, *J* = 3.4 Hz, 1H), 4.54 (s, 2H), 3.43 (t, *J* = 6.9 Hz, 2H), 3.30 (s, 2H), 3.10 (s, 9H), 2.03 (br s, 2H), 1.50 (s, 9H). MS (ESI) *m/z* 485 $[M + H]^+$.

***N*-Benzyl-3-[(5-(4-chloro-2-bromophenyl)furan-2-yl-methyl-amino]-*N*,*N*-dimethylpropan-1-aminium. (27).**

To a solution of **94a** (21 mg, 0.04 mmol) in MeOH (2 mL), a solution of hydrochloridric acid 1 N (222 µL), prepared using acetyl chloride (355 µL, 4.97 mmol) in MeOH (4.64 mL) was added at 0 °C. The reaction mixture kept at 25 °C for 4 h. The solvent was removed under vacuo to give the **28** as yellow oil without further purification (17 mg, 94%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.90 (d, *J* = 7.7 Hz, 1H), 7.73 (s, 1H), 7.65-7.42 (m, 6H), 7,20 (s, 1H), 6.85 (s, 1H), 4.60 (s, 2H), 4.45 (s, 2H), 3.51 (br s, 2H), 3.22 (br s, 2H), 3.09 (s, 6H), 2.39 (br s, 2H). MS (ESI) *m/z* 462 $[M + H]^+$.

3-[(5-(4-Chloro-2-bromophenylfuran-2-yl-methyl-amino]-*N*,*N*,*N*-trimethylpropan-1-aminium (28).

To a solution of **94b** (10 mg, 0.02 mmol) in MeOH (2 mL), a solution of hydrochloridric acid 1 N (110 µL), prepared using acetyl chloride (355 µL, 4.97 mmol) in MeOH (4.64 mL) was added at 0 °C. The reaction mixture kept at 25 °C for 4 h. The solvent was removed under vacuo to give the **29** as colorless oil without further purification (7 mg, 95%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.89 (d, *J* = 7,01 1H), 7.48 (s,1H), 7.21 (d, *J* = 3.4 Hz, 1H), 6.85 (d, *J* = 3.4 Hz, 1H), 4.46 (s, 2H), 3.53 (br s, 4H), 3.20 (s, 9H), 2.28 (br s, 2H). (ESI) *m/z* 385 $[M + H]^+$.

1-((5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)methyl)piperazine (29).

Starting from **51b** (80 mg, 0.28 mmol) and **95a** (52 mg, 0.28 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give a yellow oil (70 mg, 55%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.4 Hz, 1H), 7.38 – 7.30 (m, 4H), 7.24 (m, 3H), 6.04 (d, *J* = 3.3 Hz, 1H), 5.60 (d, *J* = 3.3 Hz, 1H), 3.48 (s, 2H), 3.41 – 3.34 (m, 4H), 2.37 – 2.26 (m, 4H), 1.45 (s, 9H). MS (ESI) *m/z* 475 $[M + Na]^+$. The crude product (70 mg, 0.19 mmol) was dissolved in a

solution of HCl 1N (380 µL, 0.38 mmol), prepared using acetyl chloride (355 µL, 4.97 mmol) in MeOH (4.64 mL) at 0 °C. The reaction mixture kept at 25 °C for 4 h. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (2 mL) was added and the organic phase was extracted with EtOAc (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **30** as yellow oil (63 mg, 95%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.72 (d, *J* = 8.5 Hz, 1H), 7.47 – 7.33 (m, 4H), 7.29 – 7.17 (m, 3H), 6.18 (d, *J* = 3.3 Hz, 1H), 5.68 (d, *J* = 3.3 Hz, 1H), 3.53 (s, 2H), 3.07 – 2.97 (m, 4H), 2.59 – 2.48 (m, 4H). **^{13}C NMR** (75 MHz, CD_3OD) δ 152.07, 150.29, 141.32, 141.00, 132.99, 130.36, 128.69, 128.55, 128.41, 128.13, 127.61, 127.50, 111.20, 109.93, 53.90, 50.28, 44.00. MS (ESI) *m/z* 353 $[M + H]^+$.

1-((5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)methyl-4-methylpiperazine (30).

Starting from **61b** (60 mg, 0.21 mmol) and 1-methylpiperazine **95b** (23 µL, 0.21 mmol), the title compound was prepared following the procedure reported for compound **49**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **31** as yellow oil (53 mg, 70%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.4 Hz, 1H), 7.34 (m, 4H), 7.31 – 7.20 (m, 3H), 6.03 (d, *J* = 3.2 Hz, 1H), 5.51 (s, 1H), 3.49 (s, 2H), 2.41 (brs, 8H), 2.28 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 151.60, 151.17, 141.03, 140.90, 132.90, 130.79, 129.06, 128.62, 128.47, 128.17, 127.80, 127.72, 110.93, 110.24, 55.18, 54.81, 52.62, 46.13. MS (ESI) *m/z* 389 $[M + Na]^+$.

1-(5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-methyl-*N*-(piperidin-4-ylmethyl-methanamine (31).

To a solution of **62b** (15 mg, 0.03 mmol) in dry DCM (3 mL), zinc(II)chloride (8 mg, 0.06 mmol) and paraformaldheyde (2 mg, 0.06 mmol) were added and the mixture kept at 25 °C for 12 h. After this time sodium borohydride (2 mg, 0.06 mmol) was added at 0 °C. After 1h, H_2O (1 mL) was added and the organic phase was extracted with DCM (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give a yellow oil (12 mg, 85%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.5 Hz, 1H), 7.43 – 7.33 (m, 4H), 7.25 (m, 3H), 5.99 (d, *J* = 3.3 Hz, 1H), 5.50 (d, *J* = 3.3 Hz, 1H), 4.04 (brs, 2H), 3.47 (s, 2H), 2.68 (t, *J* = 12.3 Hz, 2H), 2.19 (s, 3H), 2.15 (d, *J* = 7.1 Hz, 2H), 1.70 (d, *J* = 12.6 Hz, 2H), 1.59 (brs, 1H), 1.45 (s, 9H), 1.05 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 155.11, 152.03, 151.37, 141.06, 140.83, 132.85, 132.32, 130.82, 129.05, 128.64, 128.22, 127.78, 110.54, 110.17, 80.54, 62.75, 54.18, 42.95,

34.45, 30.79, 29.91, 28.69. MS (ESI) *m/z* 496 $[M + H]^+$, 518 $[M + Na]^+$. The crude product (12 mg, 0.02 mmol) was dissolved in a solution of hydrochloridric acid 1 N (50 µL, 0.05 mmol), prepared using acetyl chloride (355 µL, 4.97 mmol) in MeOH (4.64 mL) at 0 °C. The reaction mixture kept at 25 °C for 4 h. The solvent was removed in vacuo. A saturated solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with EtOAc (3 x 2 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **31** as yellow oil (6 mg, 87%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.4 Hz, 1H), 7.35 (m, 3H), 7.25 (m, 4H), 5.99 (d, *J* = 3.2 Hz, 1H), 5.49 (d, *J* = 3.2 Hz, 1H), 3.47 (s, 2H), 3.09 (d, *J* = 10.4 Hz, 2H), 2.60 (t, 2H), 2.18 (s, 3H), 2.15 (d, *J* = 7.1 Hz, 2H), 2.01 (s, 2H), 1.74 (d, *J* = 11.8 Hz, 2H), 1.08 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 152.14, 151.29, 141.08, 140.79, 132.78, 130.80, 129.06, 128.65, 128.20, 127.78, 110.48, 110.17, 63.31, 54.14, 46.48, 42.97, 34.41, 31.66, 1.24. MS (ESI) *m/z* 396 $[M + H]^+$.

1-(5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-*methyl*-*N*-((1-methylpiperidin-4-yl) methyl)methanamine (23).

To a solution of **18a** (10 mg, 0.03 mmol) in dry MeCN (2 mL) , acetic acid (1 mL) and formaldehyde (aq. sol. 37%, 1 mL) were added. The reaction kept at 25 °C, for 12 h. After this time, sodium cyanoborohydride (3 mg, 0.05 mmol) was added. After further 1 h, the solvent was removed in vacuo. A saturated solution of sodium bicarbonate was added and the organic phase was extracted with EtOAc (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **32** as yellow oil (8 mg, 76%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.4 Hz, 1H), 7.36 – 7.31 (m,, 4H), 7.32 – 7.20 (m, 3H), 5.97 (d, *J* = 3.2 Hz, 1H), 5.47 (d, *J* = 3.2 Hz, 1H), 3.45 (s, 2H), 2.91 (d, *J* = 10.8 Hz, 2H), 2.32 (s, 3H), 2.16 (s, 5H), 2.01 (dd, *J* = 13.2, 9.7 Hz, 2H), 1.75 (d, *J* = 12.7 Hz, 2H), 1.54 – 1.41 (m, 1H), 1.38 – 1.21 (m, 2H). MS (ESI) *m/z* 409 $[M + H]^+$, 204 [M / 2].

(5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)methanol (96).

To a solution of **61b** (100 mg, 0.35 mmol) in a mixture of THF (2 mL) and MeOH (1 mL), sodium borohydride (13 mg, 0.35 mmol) was added at 0 °C. The mixture kept at 25 °C for 1 h. After this time, the solvent was removed in vacuo. H_2O was added and the organic phase was extracted with EtOAc (3 x 5 mL),), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **96** as yellow oil (76 mg, 76%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.69 – 7.64 (m,

1H), 7.39 – 7.30 (m, 4H), 7.23 (dd, *J* = 7.3, 2.1 Hz, 3H), 5.94 (d, *J* = 2.5 Hz, 1H), 5.56 (d, *J* = 2.4 Hz, 1H), 3.68 (s, 2H). MS (ESI) *m/z* 285 $[M + H]^+$.

2-(Azidomethyl)-5-(5-chloro-[1,1'-biphenyl]-2-yl)furan (97).

To a solution of **96** (76 mg, 0.27 mmol) in dry toluene (6 mL), diphenyl phosphoryl azide (70 µL, 0.32 mmol) and 1,8-diazabicycloundec-7ene (50 µL, 0.32) mmol were added at 0 °C. The mixture kept at 25 °C for 12 h. After this time, the solvent was removed in vacuo. H_2O (2 mL) was added and the organic phase was extracted with EtOAc (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo giving **97** as yellow oil without further purification (80 mg. 96%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.69 – 7.64 (m, 1H), 7.39 – 7.30 (m, 4H), 7.23 (dd, *J* = 7.3, 2.1 Hz, 3H), 5.94 (d, *J* = 2.5 Hz, 1H), 5.56 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 2H). MS (ESI) *m/z* 310 $[M + H]^+$.

(5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)methanamine (98).

To a solution of **107** (80 mg, 0.26 mmol) in dry THF (5 mL), triphenylphosphine (135 mg, 0.51 mmol) was added. The mixture was heated under reflux for 12 h. After this time H_2O was added and the mixture refluxed for further 2 h. The solvent was removed in vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **98** as yellow oil (22 mg, 30%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.69 – 7.64 (m, 1H), 7.39 – 7.30 (m, 3H), 7.23 (dd, *J* = 7.3, 2.1 Hz, 4H), 5.94 (d, *J* = 2.5 Hz, 1H), 5.56 (d, *J* = 2.4 Hz, 1H), 3.68 (s, 2H), 1.54 (brs, 2H).. MS (ESI) *m/z* 284 $[M + H]^+$.

1-Methylpiperidine-4-carbaldehyde (99)

To a solution of (methoxymethyl) triphenylphosphonium chloride (1.8 g, 5.3 mmol) in dry THF (10 mL), a solution of potassium tert-butoxide (493 mg, 4.4 mmol) in dry THF (10 mL) was added at -20 °C. After 90 min, a solution of **100** (543 µL, 0.92 mmol) in dry THF (10 mL) was added at -60 °C. After 2 h the temperature was allowed to rise and stirring at 25 °C for 16 h. After this time hydrochloridric acid (aq. sol. 37%, 5 mL) was added at 0 °C. After 2 h, Et_2O (20 mL) was added, the aqueous layer was basified with NaOH (aq. sol. 4 N, 10 mL) and the organic layer was extracted with Et_2O (3 x 15 mL), dried over sodium sulfate, filtered and evaporated in vacuo giving **99** as yellow oil without further purification (280 mg. 50%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9,57 (s, 1H), 2.47 (t, *J* = 6.1 Hz, 2H), 2.22 (t, *J* = 6.1 Hz, 2H), 2.12 (d, *J* = 5.3 Hz, 3H), 2.09 (s, 3H), 1.84 (t, *J* = 5.4 Hz, 2H).

1-(5-(5-Chloro-[1,1'-biphenyl]-2-yl)furan-2-yl)-*N*-((1-methylpiperidin-4-yl-methyl-methanamine (33).

Starting from **98** (20 mg, 0.07 mmol) and **99** (10 mg, 0.07 mmol), the title compound was prepared following the procedure reported for compound 4**9**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **33** as yellow oil (13 mg, 50%). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.74 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 7.9 Hz, 4H), 7.22 (dd, *J* = 8.2, 3.0 Hz, 3H), 6.11 (d, *J* = 3.0 Hz, 1H), 5.60 (d, *J* = 3.1 Hz, 1H), 3.65 (s, 2H), 2.97 (d, *J* = 11.3 Hz, 2H), 2.41 (d, *J* = 6.7 Hz, 2H), 2.35 (s, 3H), 2.18 (t, *J* = 13.0 Hz, 2H), 1.78 (t, *J* = 14.0 Hz, 2H), 1.54 – 1.45 (m, 1H), 1.26 (d, *J* = 8.4 Hz, 2H). MS (ESI) *m/z* 395 $[M + H]^+$.

CHAPTER 6

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Part 2

Investigation of 5-Aryl-Heterocycles As Potential Inhibitors of Metallo β-Lactamase Enzymes

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CHAPTER 1

INTRODUCTION

1.1 Background: from penicillin to the *multidrug* resistance phenomenon

It is more than sixty years since ***β*-lactam antibiotics** were first introduced into clinical practice, but their therapeutic role remains extremely high and they account for half of total antibiotic prescriptions. This increasing therapeutic importance and the discovery of significant new members through synthetic chemical or fermentation screening programs, has led to an exponential growth of research in this area during the last decade.

The clinical success of the first *β*-lactam antibiotic, **penicillin G**, discovered by Alexander Fleming in 1928, prompted the search for and development of additional derivatives giving rise to the *β*-lactam antibiotics in clinical use today.

Given the rapid replication rates of bacteria, it is not surprising that the phenomenon of resistance appeared and it continues to evolve and spread among pathogenic bacteria[1]. Many of the bacterial pathogens associated with epidemics of human diseases, have evolved into multidrug-resistant (MDR) forms, subsequent to a wide and indiscriminate antibiotic use, as shown in **Fig.1**.

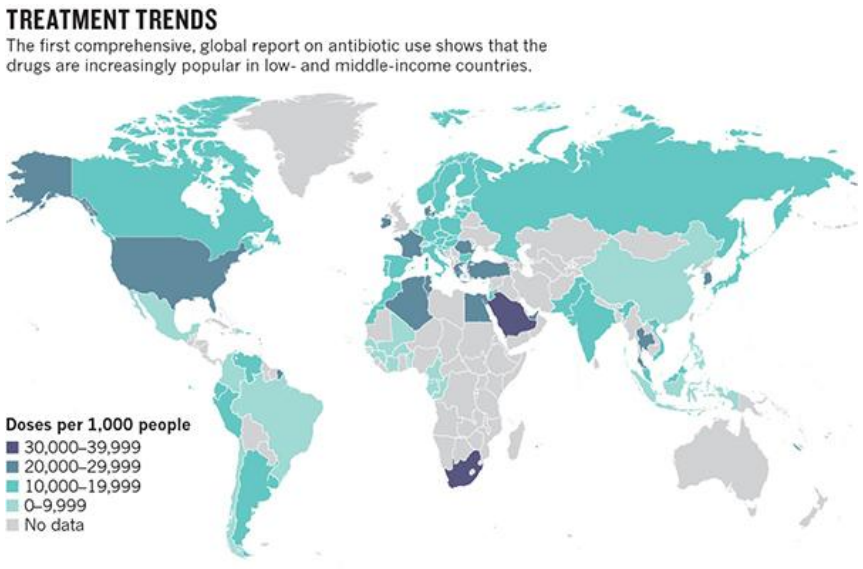


Fig.1. *Global antibiotic consumption grew by 30% between 2000 and 2010*[2].

Although there are several mechanisms of *β*-lactam resistance, including modification of the target, reduction in permeability, and efflux of the antibiotic, the major reason for resistance is antibiotic inactivation by the action of a variety of enzymes known as *β*-lactamases. These enzymes are found in a large number of clinically important Gram-negative pathogens, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Klebsiella pneumoniae*,

which cause a variety of diseases in humans and animals. A strong correlation between β-lactam antibiotics used in the treatment of these infections and β-lactamases-related resistance development, has been observed over the past half-century. At this time, several groups and classes have been identified, comprising up to 1,000 resistance-related β-lactamases.

Within the last 12 years potent β-lactamase inhibitors such as *clavulanic acid* and *sulbactam* have become available, and are used in combination with a penicillin. However, many factors can influence the activity and pharmacodynamics of these combinations, including potency of both agents, pharmacokinetics of the inhibitor, type and quantity of β-lactamases produced by the target bacterium.

1.2 *β*-Lactam antibiotics

Antibiotics are molecules mostly produced by the metabolism of microorganisms able to inhibit the growth of other microorganisms. This definition includes all substances with antibiotic activity of natural, semi-synthetic or totally synthetic origin. Antibiotic are defined as agents that are "selectively" toxic for bacteria, killing them (bactericidal) or inhibiting their growth (bacteriostatic), without harm for the patient, acting on structures found in bacteria, but not in the host.

One major class of antibiotics is the ***β*-lactams**, which includes penicillins, cephalosporins, carbapenemes and monobactams. The common feature of this class is the presence of a *β*-lactam ring system, a highly strained and reactive cyclic amide. There are five relevant ring systems which appear in the active *β*-lactams, including *penam*, *penem*, *carbapenem*, *cefem* and *monobactam* ring structures (**Fig. 2**)[3].

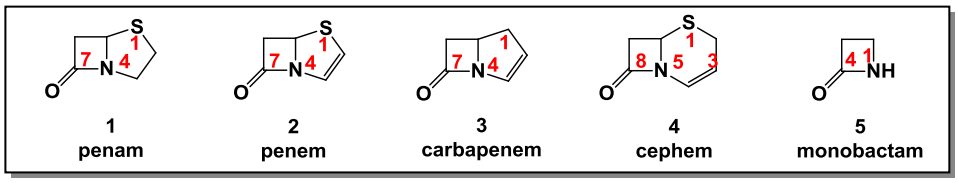


Fig.2 *Ring systems in the active β-lactams.*

The original penicillins were produced by fermentation, such as penicillins G and V. The availability of *6-aminopenicillanic acid* (6-APA) has allowed the creation of hundreds of synthetic and semisynthetic penicillins. The reactive nature of this ring makes penicillins susceptible to a variety of degradative processes, as shown in **Fig.3**: reaction with hydroxide ion, with ring opening to produce an inactive penicilloic acid which in turn undergoes to a spontaneous loss of carbon dioxide forming the corresponding penilloic acid[4].

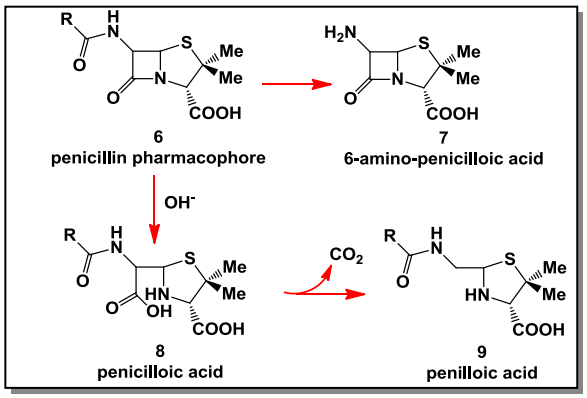


Fig.3 *Inactivation of penicillin system.*

Unlike penicillins, where the initial agent in the series was marketed with little SAR development, the cephalosporin structure required a great deal of refinement before a clinically useful agent was discovered. From the original *Cephalosporium acremonium* culture, was isolated *cephalosporin C*, with some activity against penicillin-resistant cultures. Chemical removal of the cephalosporin side-chain gave *7-aminocephalosporanic acid* (7-ACA), which like its cogener 6-APA was used as a synthetic starting point for most of the cephalosporins available today (5 generations, **Fig.4**)[4]. Because the *β*-lactam structure in cephalosporins is a 6 membered ring, the system is less strained and hence less reactive. However, much of this loss of strain is made up for by the olefinic linkage in the 6-membered ring and the acetoxy group appended to the molecule, which provides a suitable leaving group.

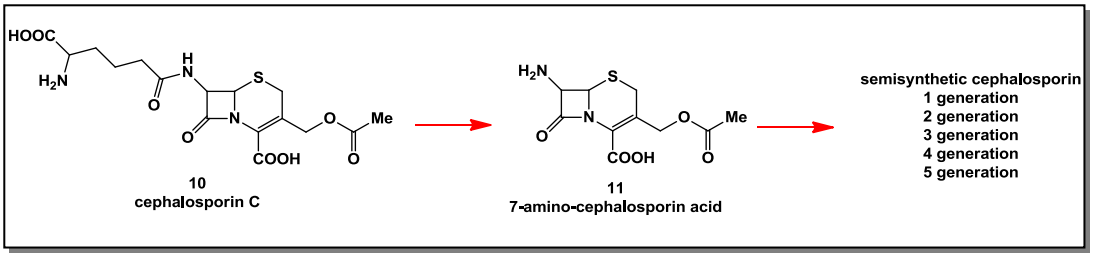


Fig.4 *Development of cephalosporins generation*

1.3 Mechanism of action

β-Lactam antibiotics work by blocking the synthesis of the bacterial cell wall. In particular they inhibit enzymes responsible for the synthesis of peptidoglycan, the polymer responsible of the cell stiffness.

Osmotic stability is normally preserved by a rigid cell wall comprising of alternating *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) units, linked by transglycosidases. A pentapeptide is attached to each NAM unit, and the cross-linking of two D-alanine-D-alanine dimer of this pentapeptide is catalyzed by some transpeptidases known as Penicillium Binding Proteins (PBPs)[5]. This cross-linking of adjacent glycan strands confers the rigidity of the cell wall (**Fig.5**).

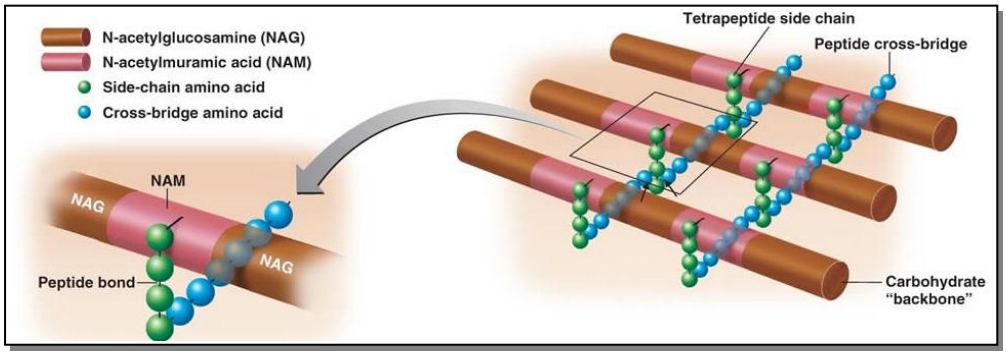


Fig.5. *Structure of peptidoglycan*[6].

The *β*-lactam ring is sterically similar to the D-alanine-D-alanine dimer of the NAM pentapeptide, so the PBPs "mistakenly" use the *β*-lactam as a "building block" during the cell wall synthesis. This results in the acylation of PBPs, unable to catalyze further transpeptidation reactions. The integrity of the bacterial cell wall is essential to maintaining cell shape in a hypertonic and hostile environment so, once cell wall synthesis is inhibited, enzymatic autolysis of the cell can occur. Thus, these antibiotics are generally bactericidal[7].

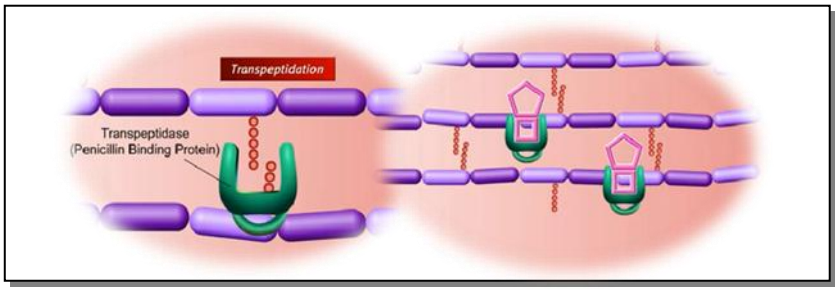


Fig.6 *Mechanism of action of β-lactam antibiotic*[6].

1.4 Antibiotics resistance

As it happens for the treatment of various types of infections, even for bacterial infections, the use of any therapeutic agent is compromised by the potential development of tolerance or resistance. In the specific case of antibiotics, the complexity of the processes that contribute to the emergence and dissemination of resistance has made this problem the most costly in terms of morbidity and mortality.

Until a few years ago, the attention of microbiologists was focused on Gram-positive bacteria, particularly meticillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus spp*[8]. But now, they agree that multidrug-resistant Gram-negative bacteria, represent the greatest risk to public health. The increase of Gram-negative bacteria resistance, is mainly due to mobile genes on plasmids that can readily spread through bacterial populations.

First of all, there are two main types of bacterial resistance: the intrinsic resistance, typical of all strains of a bacterial species to a particular drug; and the acquired resistance, which is the most worrying, caused by spontaneous and sporadic genetic mutations or by acquisition of foreign genetic material[9]. These genetic mutations lead to various fundamental events (**Fig.7**) in the acquisition of resistance:

- permeability changes in the bacterial cell wall which restricts antimicrobial access to target sites;
- active efflux of the antibiotic from the microbial cell by efflux pumps, which are able to export a wide range of substrates from periplasm to the surrounding environment;
- over-expression or modification of the target: changes in the active site of PBPs with lower the affinity for β-lactam antibiotics and subsequently increase resistance to these agents;
- acquisition of alternative metabolic pathways to those inhibited by the drug;
- enzymatic modification of the antibiotic and inactivation of the antimicrobial agent[10].

Today, the most worrying resistance mechanism remains the synthesis by bacteria itself of enzymes able to inactivate the antibiotic (**Fig.8**). Among these enzymes there are acylases, that act on side chains of penicillins, cephalosporins and related antibiotics to produce the intermediates 6-APA and 7-ACA respectively. Acylases are of minor importance in antibiotic resistance, but are commercially exploited in the enzymatic cleavage of penicillins and cephalosporins in the production of semisynthetic derivatives.

Another enzymatic degradation involves the removal of the acetyl group by cephalosporins esterases and this cleavage produces compounds of reduced antimicrobial activity. The presence of esterases in mammalian tissue are exploited for cleaving microbiologically inactive penicillin esters (pro-drugs) to release the active compounds.

However, the most widespread group of enzymes in Gram-negative and in some species of Gram-positive bacteria is β-lactamases. These enzymes hydrolyze the cyclic amide bond of susceptible β-lactam ring to give inactive products. In the case of penicillins, the product of hydrolysis are penicilloates which are stable and easily detectable. With cephalosporins, the corresponding cephalosporoates are usually unstable because the resulting fragmentation pattern depends on the nature of C-3 substituent. Thus the detection of cephalosporin hydrolyzed products is often complicated. In Gram-negative bacteria , β-lactamases can be both inducible and constitutive, located inside the periplasm, between the outer membrane and the peptidoglycan layer. On the other hand, in Gram-positive bacteria, β-lactamases are only inducible, produced only in the presence of the antibiotic and are excreted in the extracellular medium[11].

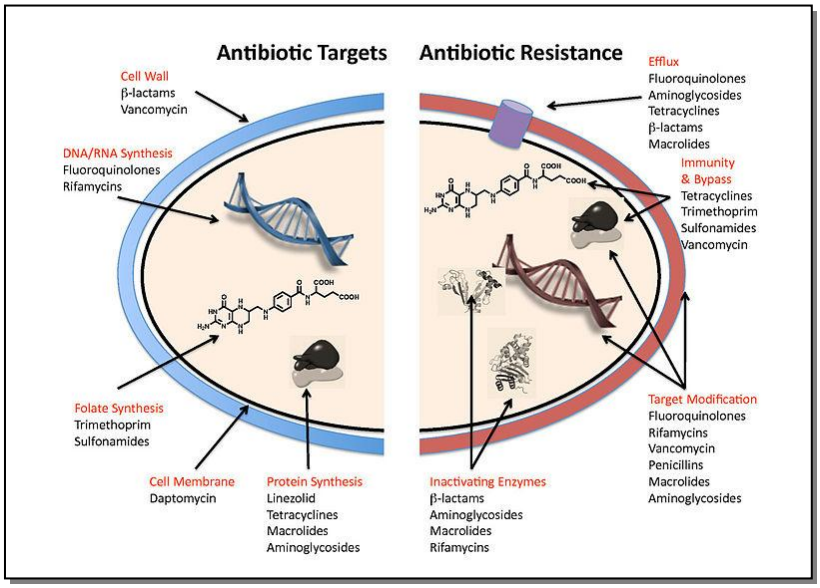


Fig.7.

Mechanism used by common antibiotics to deal with bacteria and ways by which bacteria become resistant to them[12].

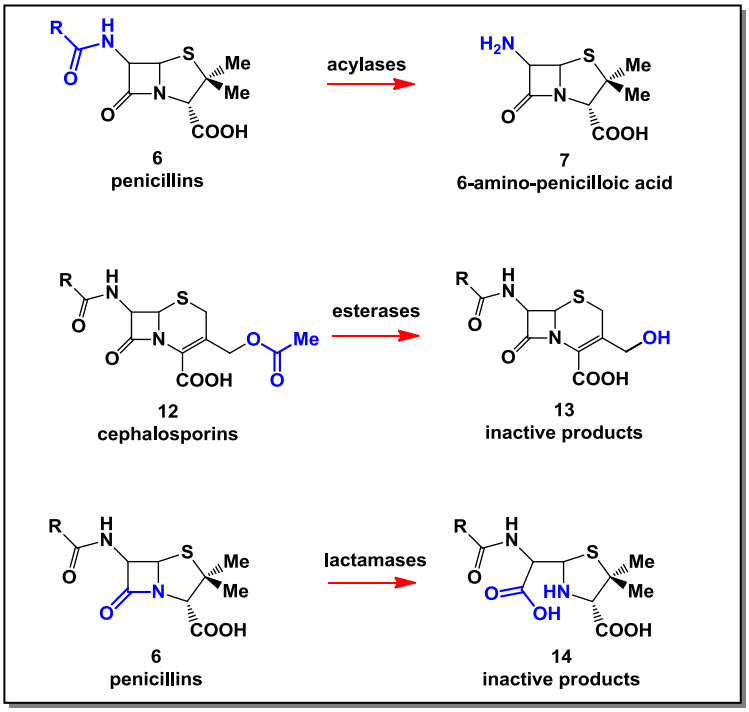


Fig.8. *The main antibiotic inactivation enzyme catalyzed.*

1.4.1. *β*-Lactamases

The first *β*-lactamase enzyme was identified in *Escherichia coli* before the clinical use of penicillin, described as "penicillinase" by E. P. Abraham and E. Chain. After the isolation of these "penicillin inactivators" from *Staphylococcus aureus*, the emergence of a significant clinical problem appeared soon[13]. The growing number of *β*-lactam antibiotics has since increased the selective pressure on bacteria, promoting the survival of organisms with multiple *β*-lactamases. Currently, more than 800 *β*-lactamases are identified[14].

Two classification schemes for *β*-lactamases are currently in use, the molecular method, based on amino acid sequences, proposed by Ambler and the functional classification scheme, proposed by Bush, Jacobi and Medeiros, based on substrate and inhibitor profiles. The most widely used is the Ambler classification that divides *β*-lactamases into two broad categories: serine enzymes and metallo enzymes[15]. The formers have a serine residue in the active site and the catalytic mechanism involves the formation of an acyl-enzyme intermediate. The metallo enzymes appear to involve coordination of the substrate and intermediates to the active site metal ion. On the basis of their amino acid sequences, the **serine *β*-lactamases** are subdivided into three classes: A, C, and D, whereas the class B or **metallo *β*-lactamases** are subdivided in B1, B2 and B3 and they will be described in more detail in the following sections[16].

1.4.2 Serine *β*-lactamases

Serine *β*-lactamases (SBLs), employ a serine active site to catalyze hydrolysis. They act acylating *β*-lactam antibiotic much like PBPs and then use a water molecule to hydrolyze the acylated *β*-lactam. So these enzymes are regenerated and can inactivate other *β*-lactam antibiotic molecules.

- **Class A**

Enzymes of this class are found in Gram-negative and Gram-positive organisms, cell-bound, periplasmic or secreted, and derived from plasmid or chromosomal genes. The activity of SBLs of class A, shown in **Fig.9**, starts with a nucleophilic attack of Ser70 residue on the carbonyl of the *β*-lactam antibiotic that results in a high energy tetrahedral acylated intermediate. This acylation mechanism is unclear but the most reliable view maintains that Lys73 residue serves as the general base to deprotonate Ser70 which proceeds in the nucleophilic attack on carbonyl. The acylated intermediate transits into a lower-energy covalent acyl-enzyme followed by nitrogen protonation and cleavage of the C-N bond. At this point an activated water molecule

attacks this covalent complex and lead to a high energy tetrahedral deacylated intermediate. The deacylation mechanism involve Glu166 as the activating base of the hydrolytic water. The subsequent hydrolysis of the bond between the *β*-lactam carbonyl and the oxygen of serine regenerates the active enzyme and releases the inactive *β*-lactam[17].

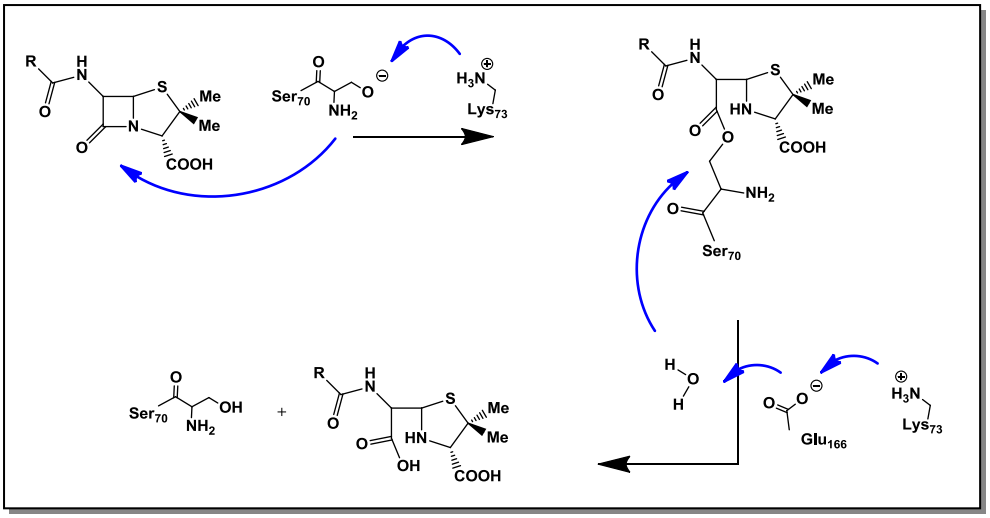


Fig.9. *Mechanism of action of class A SBLs.*

- **Class C**

AmpC *β*-*l*actamases are typically encoded on chromosomes of many Gram-negative bacteria as *Citrobacter*, *Serratia* and *Enterobacter* species where its expression is usually inducible, but these type of *β*-lactamases may also be carried on plasmids. The class C hydrolytic mechanism involves Tyr150 as base able to increase the nucleophilicity of Ser64 for acylation. While in the deacylation process seems to be the *β*-lactam nitrogen to act as base accepting the proton from the hydrolytic water and the Tyr150 proton helps to stabilize the water's developing negative charge[18].

- **Class D**

This class were initially classified as "oxacillinases" because of their ability to hydrolyze oxacillin. This class of enzymes is unique because of the direct role of carboxylation of the Lys70 in the active site. The carbamic acid on Lys70 can ionize to form a carbamate which may serve as general base to activating Ser67 for acylation and the hydrolytic water for deacylation[19].

1.4.3 Extended spectrum *β-lactamases* (ESBLs).

A separate discussion, it should be done for another class of *β*-lactamases, considered according to the Ambler classification system, as an evolution of class A, extended spectrum *β*-lactamases (ESBLs).

ESBLs were identified for the first time in 1983, when *β*-lactamases able to hydrolyze extended broad spectrum cephalosporins were documented in strains of *Klebsiella pneumoniae* from Germany. The spread was very rapid and currently, over 200 ESBLs have been described in a worldwide distribution[20].

ESBLs, which have been isolated from a wide variety of Enterobacteriaceae, as well as *Pseudomonas aeruginosa* and *Capnocytophaga ochracea*, are actually defined as *β*-lactamases capable of hydrolyzing penicillins, broad and extended spectrum cephalosporins, and monobactams and are inhibited by clavulanic acid. These phenotypic characteristics differentiate ESBLs from AmpC type *β*-lactamases, which are not inhibited by clavulanic acid or other β-lactamase inhibitors[21].

Most ESBLs are derivatives of TEM or SHV enzymes, characterized by a few point mutations at selected loci within the gene giving rise to the extended-spectrum phenotype. TEM-1 (isolated from a blood culture from a patient named Temoniera) is the most commonly encountered *β*-lactamase in Gram-negative bacteria responsible of ampicillin resistance in *E. coli* and in *H. influenzae* and *N. gonorrhoeae*. TEM-3, originally reported in 1989, was the first TEM-type -lactamase that displayed the ESBL phenotype and in the years since its first report, over 90 additional TEM derivatives have been described[22].

The native SHV-1 (sulphydryl variable) *β-l*actamase, found primarily in *K pneumoniae,* is a plasmid or chromosomally encoded-enzyme that confers resistance to penicillins and cephalosporins of first-generation. Unlike the TEM-type *β*-lactamases, there are relatively few derivatives of SHV-1. The majority of SHV variants possessing an ESBL phenotype, are characterized by the substitution of a serine to glycine at position 238. The serine residue at position 238 is critical for the efficient hydrolysis of ceftazidime, and the lysine residue is critical for the efficient hydrolysis of cefotaxime[22]. The OXA-type enzymes are another growing family of ESBLs. These *β*-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D. ESBLs evolution and facile dissemination will continue to plague us in the future, with risks being also exacerbated.

1.5 Metallo *β*-lactamases (MBLs)

Belonging to the class B of *β-lactamases* are metallo *β*-lactamases (MBLs), metallo enzymes requiring one or two zinc ions for their activity. MBLs can hydrolyze and inactivate all classes of *β*-lactams except monobactams and are well known for their efficient activity on carbapenems, the antibiotics with the broadest spectrum of activity.

The first MBL enzyme, the *Bacillus cereus* metallo *β*-lactamase, was identified in 1966 and during the two following decades remained the only known example of MBL. The situation changed in 1980 when various zinc enzymes have been discovered in an increasing number of nosocomial strains. The emergence and spread of acquired metallo *β*-lactamases, encoded by genes carried on mobile DNA elements among major Gram-negative bacteria, made the situation even more worrying. MBLs are now regarded a therapeutic challenge as presently there are no clinically useful inhibitors[23].

MBLs are classified in three subclasses B1, B2 and B3 on the basis of the aminoacidic sequences, substrate profile, and metal ion requirement. There is a relatively low sequence identity between the subclasses, but within a subclass the sequence identity is higher and this, with distinctive structural characteristics and spectrum profile, is the basis for the establishment of the three subclasses.

The subclass B1 is the largest and includes several well-studied *β*-lactamases: BCII from *Bacillus cereus*, CcrA from *Bacteroides fragilis*, IMP-1, SPM-1 from *Pseudomonas aeruginosa,* and BlaB from *Cryseobacterium meningosepticum*. These enzymes efficiently catalyze the hydrolysis of a wide range of substrates such as penicillins, cephalosporins, and carbapenems[24].

The subclass B2 hydrolyze only carbapenems and show a poor activity against penicillin and cephalosporins. The most common representatives are CphA from *Aeromonas hydrophila* and ImiS from *Aeromonas veronii*. Finally, subclass B3 includes a tetrameric zinc *β*-lactamase, the L1 enzyme from *Stenotrophomonas maltophilia,* and the monomeric FEZ-1 from *Legionella gormanii*. Both enzymes hydrolyze a wide range of *β*-lactam antibiotics from penicillins to carbapenems[25].

1.5.1 Metallo *β*-lactamases active site

Despite the little sequence identity, as a family, MBLs share a similar and characteristic structure. Infact they all show a similar αβ/βα sandwich structure, which was discovered in MBLs but is also recognized in other enzymes with diverse biological functions[26].

As shown in **Fig. 10**, the active site is located at the interface between two domains, in particular at the bottom of a wide shallow groove between two β-sheets. It has been indicated that the most important determinant in the substrate binding is the Zn(II) ion, while the conserved amino acid residues and flexible loops may play only minor roles in stabilizing the substrate-enzyme complex. Within the active site, there are two potential zinc-ion binding sites, often named sites 1 and 2. The zinc ligands in the two sites are not the same and are not fully conserved between different subclasses. These zinc binding motifs around the active site can distinguish MBL subclasses B1, B2 and B3. Infact the diverged amino acid sequences of the subclasses are reflected in somewhat different catalytic properties of the enzymes. For example B1 enzymes are active with both one or two zinc ions bound in the active site; B2 enzymes, are mononuclear enzymes and the binding of the second zinc inhibits catalysis; and B3 enzymes are active only in binuclear forms with two zinc ions bound[27].

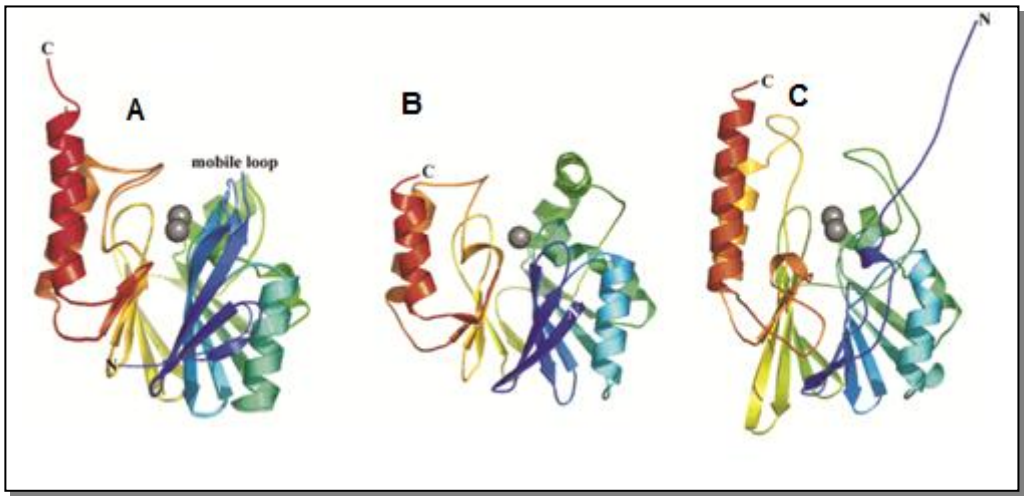


Fig.10. *αβ/βα sandwich structure of MBLs*[26]*:*

A) *CcrA of subclass B1;* ***B)*** *CphA of subclass B2;* ***C)*** *L1 of subclass B3*

In enzymes of subclass B1 the sandwich fold presents two β-sheets in the inner side, and with α-helices on each external face. As shown in **Fig. 11**, the zinc binding site 1 is located between this two domains. The zinc binding site 1 is also known as histidine site (His_3), because contains three histidine residues (His116, His118, His196) which, with their imidazole groups and with a water molecule, tetracoordinate the zinc ion. In the binding site 2, also known as cysteine site,

the metal is pentacoordinated by His263, Asp120, Cys221, and two water molecule, one of which forms a bridge between two metal ions. Interestingly, enzymes belonging to this subclass exist in both mononuclear an bi-nuclear Zn(II) forms. For example, the initial studies on the crystal structure of BcII enzyme at 2.5 Å resolution indicate the formation of a mononuclear enzyme. However, at 1.85 Å, the crystal structure reveals the presence of two zinc sites with different affinity for zinc ions. The catalytic activity of both mono and bi-nuclear enzyme forms suggests that the substrate at the binding sites of both species may be identical[28].

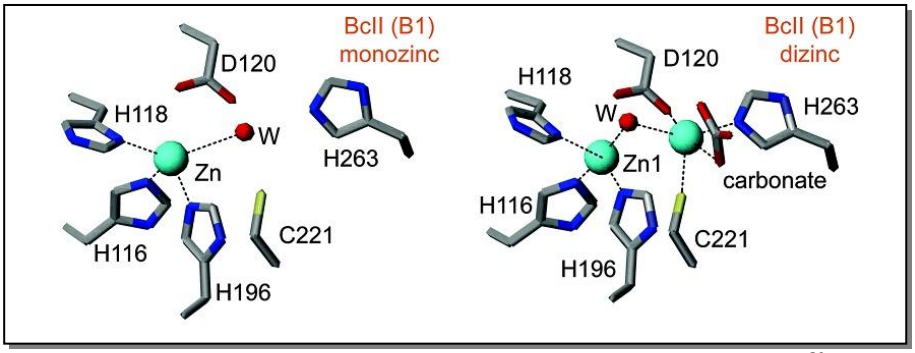


Fig.11. *The active site of BcII of subclass B1 in mono and di-nuclear form*[29].

In contrast to the B1 enzymes, the subclass B2 has a much narrower spectrum profile, hydrolyzing only carbapenems. As shown in **Fig. 12**, these enzymes are active only in mononuclear form. Furthermore, the introduction of a second zinc ion in the active site has been shown to inhibit the catalytic activity of the enzyme. For example, the crystal structure of CphA reveals a characteristic enzymatic activity: the enzyme presents a single Zn(II) in the active site, but located in site 2 and not in site 1. So, the ligands in site 1 do not interact with any Zn(II) ions even in presence of large excess of Zn(II)[29].

The enzymes belonging to subclass B3, contain a different metal binding motif than others MBLs, and require two zinc ions for their hydrolytic activity.

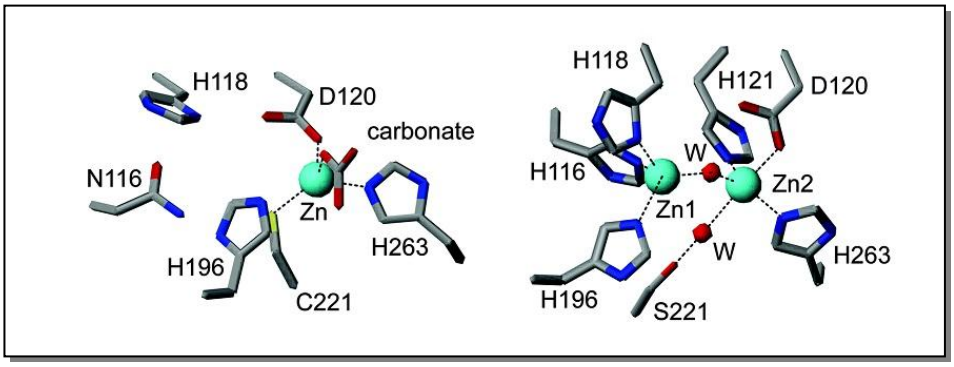


Fig.12. *The active sites of CphA of subclass B2 and L1 of subclass B3*[29].

The L1 enzyme lacks a number of amino acid residues proposed as important for MBLs activity. For example Cys184, considered essential for Zn(II) coordination, is absent. Also Lys187 and Asp163, critical for substrate binding and zinc orientation, are absent or replaced by other residues. The L1 crystal structure reveals that the two zinc binding sites, 1 and 2, are closely and tightly located in the active site with tetrahedral and trigonal bipyramidal coordination respectively. The crystal structure of another B3 enzyme, FEZ-1 shows that the active site is identical to L1 and both enzymes are active only in binuclear forms. Only recently, a third enzyme belonging to this subclass, GOB, has been identified[28].

Subclasses B1 and B3 of MBLs also contain a loop structure near the active site, loop L3 (residues 61-65). This is thought to undergo a conformational change and to interact with substrate or inhibitor molecules which have hydrophobic side chains. This loop is very flexible and when substrate or inhibitor diffuses into the active site, the loop moves to block it. This movement is caused by the interaction with the side chain of a tryptophan residue (W64), with the hydrophobic side chain of substrate. The deletion of this loop seriously influences the enzymatic activity, weakening the formation of complex substrate-enzyme.

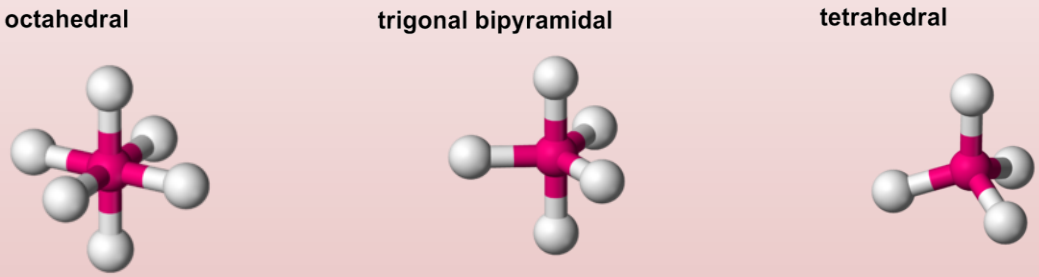
This L3 loop is absent in B2 subclass enzymes of MBLs, but they have an elongated α-helix (residues Arg140-Leu161) close to the active site groove. Thanks to the residue W150, this helix is able to follow the surface of protein and provides a hydrophobic face that contribute to the binding of substrates. This very well defined active site is responsible of the very narrow spectrum activity profile of this class of enzymes[30].

Furthermore, the distance between the zinc ion and other residues is crucial for enzymatic activity. A short distance allows the formation of a bi-dentate complex, while a longer distance contributes to greater stability, flexibility and orientation of the zinc in the active site.

Box 1. The role of zinc ion

Zinc is a fundamental trace element and is one of the most abundant transition metals in biology. Its role in several catalysis reactions is related to its ability to participate and at the same time to be exchangeable, all thanks to its exceptional flexibility of its coordination number and geometry. In addition, in contrast to iron, copper and other transition metals, zinc shows no redox properties facilitating its evolution in living systems without the risk of oxidative damage. Not less important is its ability to bind a variety of atoms, such as nitrogen, oxygen, and sulphur as ligands. All these properties make zinc an ideal metal cofactor for many enzymes in particular all zinc-hydrolases which requires a redox-stable catalytic center[31].

About MBLs, and more in general about metallo-proteases, zinc has three principal roles: structural, catalytic and co-catalytic roles. The first consists in maintaining the tertiary and sometimes quaternary structure of proteins. The catalytic role is essential for protein activity, often expressed trough the involvement of a water molecule. Catalytic zinc ions participate directly in the bond-making or breaking step in many reactions and their removal from enzyme active site by chelating agents completely abolished activity. The co-catalytic role occurs in enzymes which requires two or more zinc atoms in close proximity to one another for their activity, and they together operate as a catalytic unit[32].



As shown, inside the active site, zinc can exist as tetra-, penta- or exa-coordinated forms with amino acid residues. The geometries identified for zinc ions are three: octahedral, when the coordination number is six, trigonal bipyramidal, when the number is five and tetrahedral when the number is four. The most common is the tetrahedral geometry, in which the zinc ion is coordinated by three amino acid residues and a water molecule to complete the coordination sphere. The most frequently amino acid residues involved in coordination are histidine (through the imidazolyl nitrogen), glutamic or aspartic acids (through the carboxyl and carbonyl oxygens)and cysteine (through the sulphydryl group) as well as activated water molecule

1.5.2 Metallo *β*-lactamases mechanism of action

Metallo *β*-lactamases catalyze the hydrolysis of amide bond in *β*-lactam rings to generate inactive products. They require one or two Zn(II) ions for activity and catalysis does not proceed via a covalent intermediate, but rather through a direct nucleophilic attack of a species that is stabilized by the zinc in the active site.

In general, the nucleophilic element is represented by an hydroxide ion from a water molecule bridged between two metal ions. This occurs in binuclear enzymes, while for the mononuclear ones, the nucleophilic element is generally a water molecule. The nucleophilic attack by hydroxide group creates a tetrahedral species which rapidly interconverts into an intermediate in which the *β*-lactam nitrogen is in anionic form. The protonation of this nitrogen leads to the bond cleavage and to the product formation. The source of proton is not certain, it may come from a residue Asp120 or from a water molecule.

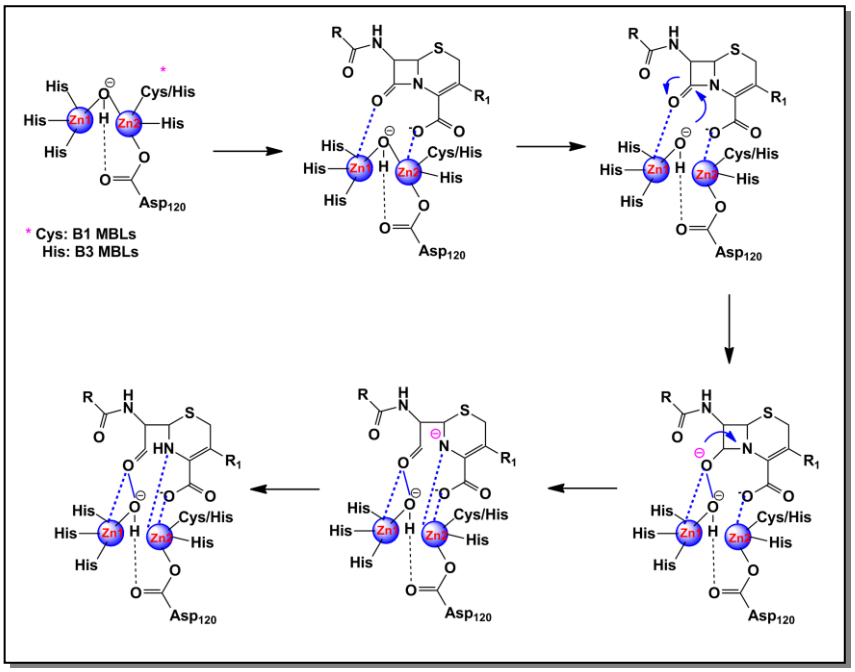


Fig.13. *Mechanism of action of binuclear MBLs of subclass B1 and B3.*

For B1 enzymes, which can act in both mono and bi-nuclear forms, different mechanisms have been proposed. For mono-zinc enzymes, the zinc ion in the active site interacts with His116, His118, His196 and a water molecule. The zinc ion acts as a Lewis acid decreasing the p*K*a of water molecule and so it can exist as hydroxide ion at neutral pH. In this form it can attack the carbonyl carbon of the *β*-lactam, with the formation of a tetrahedral intermediate stabilized by

the interaction of zinc ion. The residue Asp120 represents the general base which deprotonates the hydroxyl group to generate a second dianionic tetrahedral intermediate, this latter intermediate also stabilized by zinc ion. In a following step, Asp120 gives protonates the nitrogen of β-lactam to catalyze the amide bond cleavage. For B1 di-nuclear enzymes, the originally proposed mechanism (**Fig. 13**) involved zinc coordination directly to the β-lactam nitrogen, but this was not accepted because of its relatively low electron density due to amide-resonance. The catalysis starts with interaction of the β-lactam carbonyl oxygen with Zn1 site, and the interaction of carboxylate of six or five-membered ring with Zn2 site. Now the hydroxide ion bridged between two zinc ions is responsible for the nucleophilic attack which results in a negatively charged intermediate stabilized in this case by the oxyanion hole of the enzyme. This apical water molecule bound to zinc is optimally positioned to donate a proton to the leaving nitrogen. And subsequently, the newly formed hydroxide ion moves to promote dissociation of the product from the enzyme active site[33]. For B3 subclass enzymes the mechanism proposed (**Fig.13**) is similar to B1 mononuclear mechanism. Infact, the tetrahedral arrangement of the Zn1 site is achieved by the histidine triad 116, 118 and 196 and a bridging water. However, the Zn2 site is characterized by the presence of Asp120 and His263 and another additional His121. The other two coordinating ligands are found to be the bridging water and an additional water molecule[34].

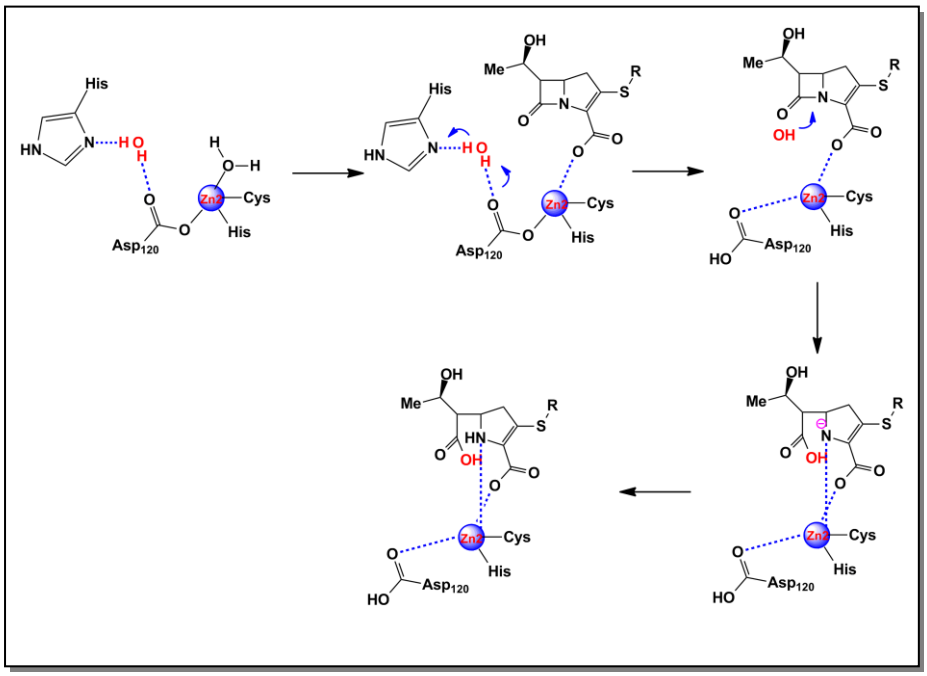


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Fig.14. *Mechanism of action of mononuclear MBLs of subclass B2.*

A different reaction mechanism has been proposed for mono-zinc subclass B2 of enzymes. There is just one interaction between the carboxylate in the Zn2 site. The nucleophilic hydroxide is generated by Asp120 and His118 residues and attacks the *β*-lactam carbonyl group, in turn activated by His196, to generate a tetrahedral intermediate. At this point the following nitrogen protonation and the amide bond cleavage do not take place with an hydroxide ion, but with a water molecule activated again by Asp120[35].

1.6 Carbapenemases

Carbapenemases represent the most versatile family of *β*-lactamases, with a breadth of spectrum unrivaled by other β-lactam-hydrolyzing enzymes. Although known as "carbapenemases," many of these enzymes recognize almost all hydrolyzable *β*-lactams, and most are resilient against inhibition by all commercially viable inhibitors[36]. The clinically relevant carbapenemases are encoded in mobile genetic elements and include VIMs (Verona Integron-encoded Metallo *β-lactamase*s), IMPs (Imipenemases), and the more recently emerged NDMs (New Delhi Metallo *β-lactamase*s) all belonging to subclass B1.

1.6.1 VIMs: Verona Integron-encoded Metallo *β*-lactamases

VIM-1 was identified in 1999, in an Italian clinical isolate of *P. aeruginosa* resistant to carbapenems. Soon after, the variant VIM-2 was reported, and since then, 40 different VIM variants emerged worldwide. Among them, VIM-2 is by far the most prevalent clinical variant as well as the one with the largest geographical spread. During evolution, the substrate profile of VIM enzymes is largely determined by the synergistic effect of several mutations.

The most relevant mutations found along the VIM family are located in a loop flanking the active site, named L10. This is a long loop that extends from residues 221 to 241, where in other B1 enzymes, a conserved Lys residue is found at position 224, to guide substrate binding with the carboxylate moiety of the antibiotics and with the Zn2 site. As shown in **Fig.15**, VIM enzymes display His (VIM-1, VIM-4, and VIM-7), Tyr (VIM-2) or Leu (VIM-13) residues at position 224. Furthermore, an Arg residue is mostly found in the nearby position 228 in 70% of the VIM isoforms, which is supposed to replace Lys224 on this interaction. Furthermore, Arg228 seems to be responsible for the substrate profile: the narrower cavity of variants displaying Arg228 would explain their higher affinity towards some substrates and at the same time the exclusion of substrates with bulky, containing a positively charged cyclic substituent, like cefepime and ceftazidime[37].

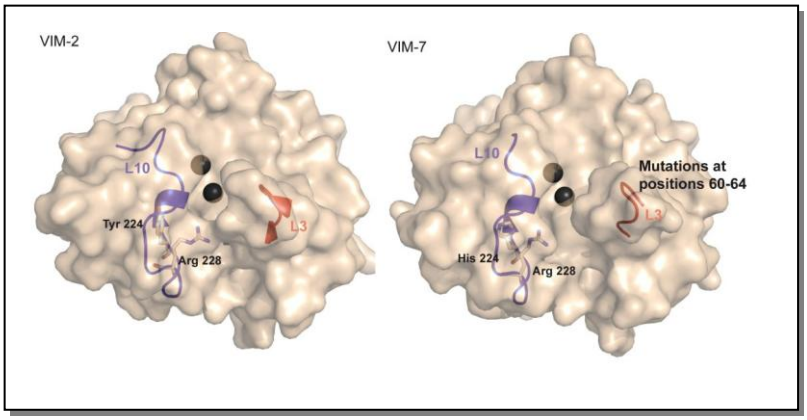


Fig. 15. *VIM-2 and VIM-7 isoform principal mutations*[38].

The substrate specificity is also influenced by mutations in L3. This loop is usually flexible, and can close upon ligand binding, providing hydrophobic contacts with the substrate through one aromatic residue, generally Phe61. Substitutions on this loop may collectively alter the dynamics of this region and the replacement of Pro68Ser at this position may increase the flexibility of the loop contributing to the altered activity profile[39].

1.6.2 IMPs: Imipenemases

IMP-1 was identified as the first transferable metallo *β*-lactamase, in 1988 from a clinical isolate case of *Pseudomonas aeruginosa* in Japan[40]. Soon, IMP family enzymes were found in *Enterobacteriaceae* and distributed along Asia and the rest of the world[41].

Several isoforms can be distinguished among IMP family, based on the identity of the residue at position 262, which has been considered as responsible for the substrate profile. IMP-1 presents a Ser residue at position 262, while IMP-6 a Gly residue. IMP-6 displays similar catalytic efficiencies to those shown by IMP-1 towards cefalotin, cefotaxime, cefoxitin, meropenem, and doripenem. However, IMP-1 is more efficient than IMP-6 towards penicillins (in particular penicillin G and ampicillin), ceftazidime, cephaloridine, and imipenem[42]. Thus, it was hypothesized that this mutation would be an adaptation towards newer carbapenems, in detriment of penicillinase activity.

Regarding residues located in the loops that might participate in interactions with the substrates, in contrast to VIM enzymes, all IMP variants possess a Lys residue at position 224 in loop L10

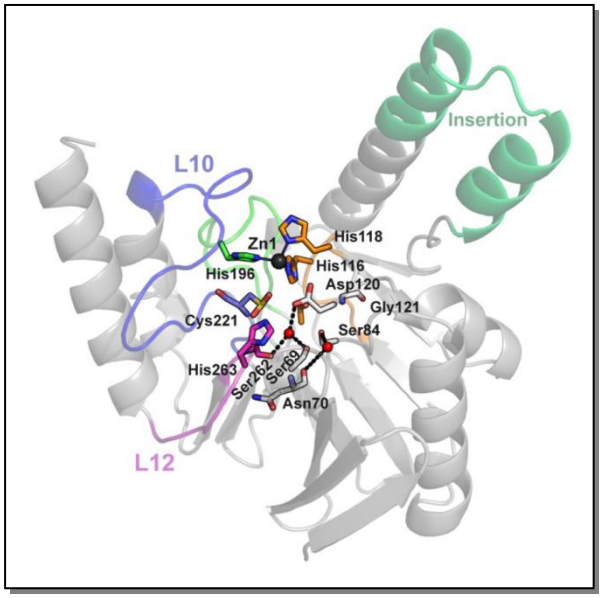


Fig. 16. *IMP-1 isoform active site*[43].

to interact with the substrate carboxylate. Other residues located in L10, supposed to be involved in substrate binding and catalysis, are Asn233 and Gly235. For example, in IMP-25 the formation of a hydrogen bond between Asn233 and Ser235 can alter the affinity for some substrates. In loop L3, the side chains of residues Val61 and Val67 contribute to the formation of a hydrophobic pocket to bind the substituent of substrates. Phe87 is also part of this cleft, and accepted a narrower variety of hydrophobic residues. Other related positions essential for hydrolysis of one or more substrates, are Lys69, Asp84 and Trp64.

1.6.3 NDMs: New Delhi Metallo *β*-lactamases

NDMs are a new class of carbapenemases, which can hydrolyze almost all known *β*-lactam antibiotics (except aztreonam), including the last resort carbapenems.

NDM-1 was first identified in 2008, from *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from a patient that acquired an infection in New Delhi in India. The spread of this enzymes has a complex epidemiology involving all seven continents (**Fig.17**), except Antarctica.

The Indian subcontinent and China are the major reservoirs. Balkan states, such as Serbia, Montenegro and Bosnia-Herzegovina may be considered as a 'secondary' reservoir area, while the Middle East (Marocco, Algeria, Libya, Egypt, Iraq, and Afghanistan), Southeast Asia (South Korea, Indonesia, Vietnam and Thailand) and parts of Europe (France, Italy) may be additional reservoir areas[44].

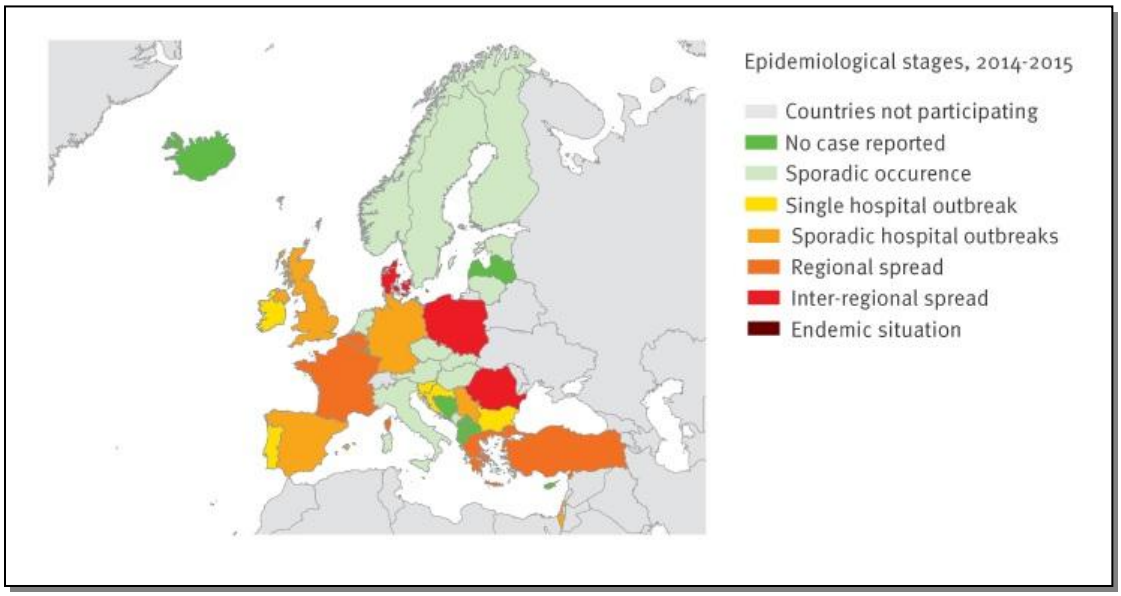


Fig. 17. *Epidemiological status of NDM-1 spread*[45].

The gene that encodes for NDM-1 is called $\boldsymbol{bla_{NDM\text{-}1}}$ and has been identified on bacterial chromosomes and plasmids. Plasmids carrying the $bla_{NDM\text{-}1}$ gene also carry a number of other genes conferring resistance to all aminoglycosides, macrolides, and sulphamethoxazole, thus making these isolates multidrug resistant.

As shown in **Fig.18**, the left domain of the NDM-1 molecule consists of two α-helices and seven anti-parallel β -strands, while the right domain is formed by the remaining two α -helices (α3 and α4) and four anti-paralleled β-strands (β–β1). It has been shown that the NDM-1 possesses additional residues from Phe163 to Asn166 located at the loop region which connect

left and right domains, and are not observed in other reported MBLs. The two catalytic zinc ions, coordinated by His120, His122, Asp124, His189, Cys208 and His250, are located at the external edge of the β/β sandwich, and are bridged by the side chain of Asp124. The loop region containing Met67–Gly71, connecting β2-β3 plays a crucial role in substrate and inhibitor binding. Moreover, the loop regions connecting β5-α2 and β10-α4 close this active site, acting as the "ceiling" and the "floor" respectively[46].

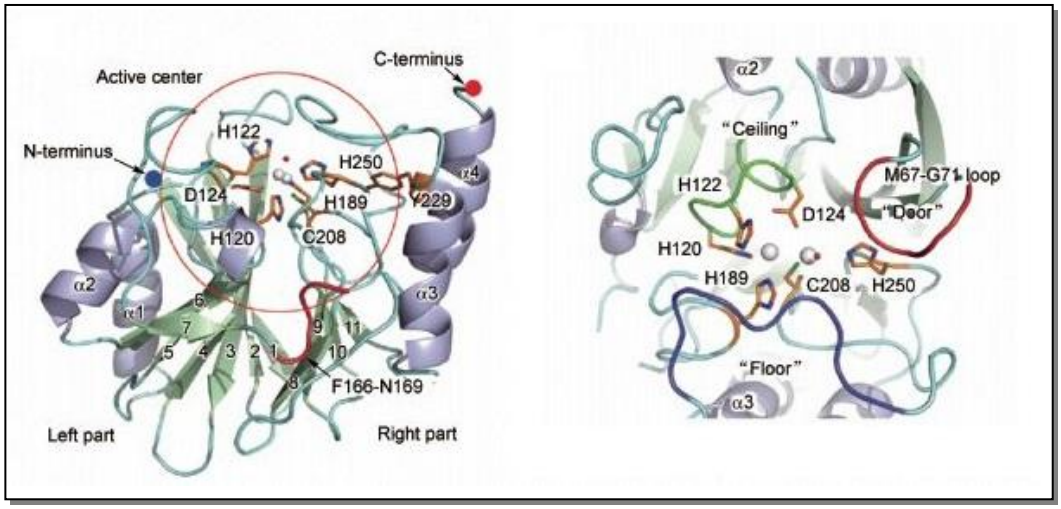


Fig.18. *Crystal structure of NDM-1 and active site*[46].

NDM-1 shows a broad substrate profile, being able to hydrolyze penicillins, carbapenems, and cephalosporins (with lower activity towards cefoxitin and ceftazidime)[47]. The NDM variants isolated are less diversified than VIM and IMP variants, with only one or two mutations between them.

1.7 Actual status of *β*-lactamases inhibitors

Two strategies have been developed to combat the problem of resistance. The first approach has been the synthesis/production of *β-lactamase* resistant antibiotics. But after few exposure to pathogens these antibiotics also become susceptible to ESBLs produced by MDR pathogens.

The second approach was to use *β*-lactamase inhibitors in combination with *β*-lactam antibiotics. These enzyme inhibitors act to permanently inactivate the *β*-lactamases in the periplasmic space so that, the partner antibiotics can reach their target. Broad spectrum *β-lactam* antibiotics plus *β-lactamase* inhibitors combination have found good safety records and clinical efficacies. For example, a combination of amoxicillin and clavulanic acid, works well for most of the bacterial infections, since clavulanic acid is known to inhibit SBLs. However, due to the structural diversity, it was difficult to design a potential inhibitor for MBLs. All known inhibitors of SBLs are inefficient towards MBLs for which, clinically approved inhibitors are not yet available. Furthermore, levels of MBLs produced by pathogens, can resist to various kind of clinically significant drugs and the diversity within the same enzymatic subclasses (B1, B2, and B3), have hindered the discovery of effective inhibitors.

Most MBLs inhibitors can be grouped into a small set of categories. These groups are mostly defined by different zinc-binding groups (ZBG), although some exceptions include covalent inhibitors and compounds without a known mechanism of action[48].

However, the selectivity is a very important parameter for the identification of a potent MBLs inhibitor. Infact, an indiscriminated inhibition or metal ions chelation could have untoward effects on natural or endogenous metalloenzymes. In particular, similarities between the active-site architectures of MBLs and essential mammalian enzymes have complicated the design of inhibitors that would be safe for human use.

1.7.1 MBLs inhibitors with zinc-binding sulfur atoms

The most extended class of MBLs inhibitors is represented by compounds containing a zinc-binding sulfur atom. Due to the high affinity of sulfur for zinc, compounds having a thiol group showed promising inhibition against MBLs. However, these types of inhibitors are very specific to their target enzymes, and therefore are not promising as broad-spectrum inhibitors.
Simple thiols, mercaptotriazoles, and peptides containing cysteine or homocysteine, are reported as inhibitors with prominent examples including 2-mercaptoacetic acid **17**, and thiomandelic acid **15**,**16**[49]. These compounds act forming a disulfide linkage with a cysteine residue in the active site of the enzyme. The thiolate anion can bind between both active site zincs, displacing the bridging hydroxide ion.
Thiomandelic acid was identified as a broad spectrum inhibitor with a sub-micromolar activity for nine MBLs and in particular the *K*i values of *S* and *R* enantiomers **15** and **16** for *B. cereus* enzyme were found 0.09 and 1.28 μM, respectively. Further studies identified mercaptoacetic acid **17** and 1,2-benzenedimethanethiol **18** as classical inhibitors of MBLs with IC_{50} value of 1.3 μM and of 2.3 μM, respectively. D-Captopril **19** was also reported as a broad spectrum potent inhibitor of subclass B1 and B3, but with a lower potency against subclass B2. It binds the active site with a similar replacement of the thiol group between both zinc ions, but its D-configuration allows an additional interaction between the carboxylate and an active-site lysine residue[50].

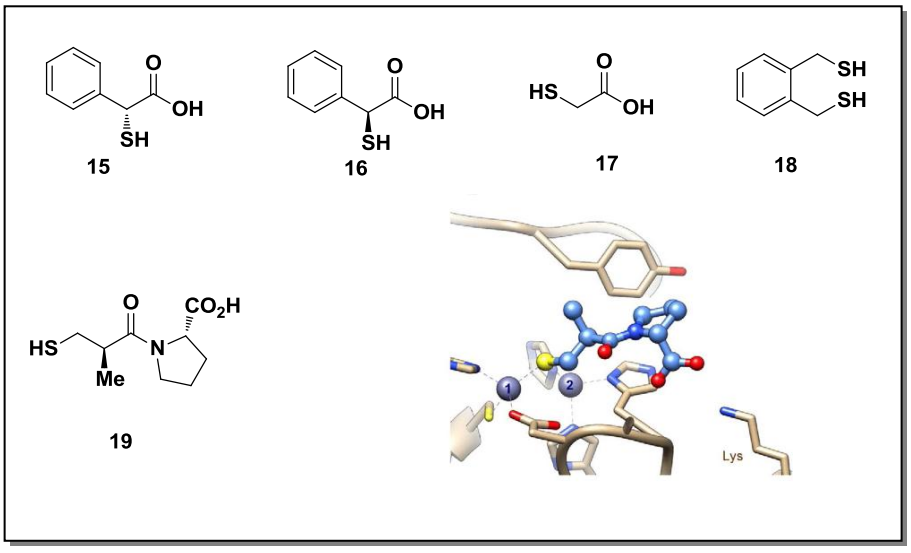


Fig.19. *Chemical structure of thiomandelic acid enantiomers **15**,**16**; mercaptoacetic acid, **17**; 1,2-benzenedimethanethiol **18**; and D-captopril **19** bound to a dinuclear zinc MBL*[48].

1.7.2 Dicarboxylate inhibitors

Dicarboxylates comprise the second most common class of MBLs inhibitors. Very recently, succinic acid derivatives with aromatic substituents have been shown to be potent inhibitors of the IMP-1 enzyme[48]. Their activity is related to binding the dinuclear metal center by using one carboxylate to form a mono-dentate bridge between both zinc ions and the second carboxylate to bridge between Zn2 and a conserved Lys residue. Several scaffolds have been used to constrain the two carboxylates, including alkenes **20** and several ring structures such as furan **21** and pyridine **22**.

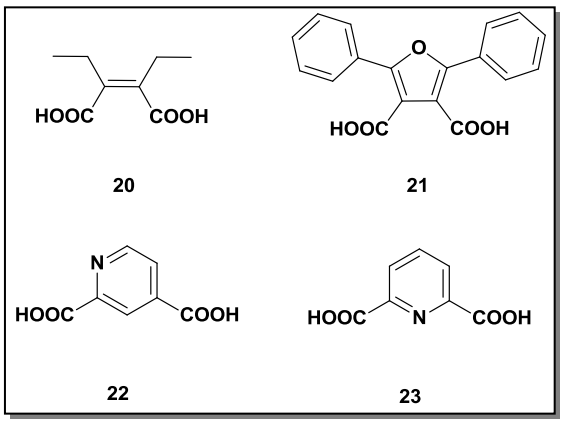


Fig.20. *Succinic acid derivatives **20-23**.*

Dipicolinic acid 2,6-PDCA **23** is a well known zinc chelator which has been used in the identification and characterization of metalloenzymes, including metallo β-lactamases. Several PDCA derivatives were screened for inhibitory activity against the enzyme subclasses B1, B2 and B3, revealing them as competitive inhibitors of CphA enzyme. They bind the catalytic zinc ion, act as an NO-chelating ligands, and interact through the enzyme active site with both electrostatic and hydrophobic contacts[51].

1.7.3 Zinc chelators

Chelation is a particular type of bonding of ions and molecules to metal ions. It involves the formation or presence of two or more separate coordinate bonds between ligand and a single central atom. Ethylenediaminetetraacetic acid (EDTA) was the first very potent zinc-chelating agent that is actually used ubiquitously in protein interaction studies and in molecular biology in general.

From this event, the search for MBLs inhibitors, employed screening techniques as well as the use of the crystal structure of the enzyme for molecular docking of candidate chelating agents. These approaches have led to the identification of phenazines. Phenazines comprise a large group of nitrogen-containing heterocyclic compounds that differ in their chemical and physical properties, based on the type and position of functional groups. More than 100 different phenazine structural derivatives have been identified in nature, and over 6,000 have been synthesized. Gilpin *et al*., in 1995 isolated two novel phenazines, **24** and **25**, from a S*treptomyces* and screened them against three MBLs [52].

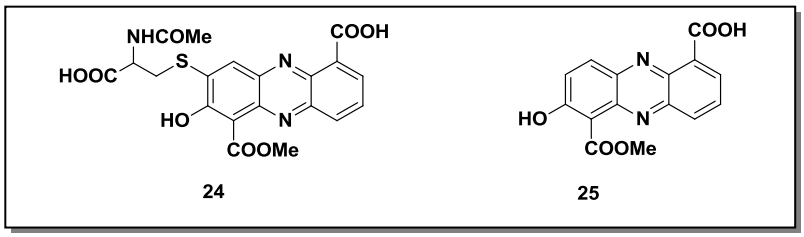


Fig.21 *Phenazine derivatives **24** and **25**.*

Another important group of zinc chelating agents are biphenyl tetrazoles (BPTs), identified as potent competitive inhibitors. The structure-activity relationships of biphenyl tetrazoles have showed that the ortho position of the tetrazole group, relative to the biphenyl ring system, is important in enzyme inhibition. Furthermore, modeling studies have suggested that the biphenyl tetrazole system, could bind with the tetrazole replacing the axial water as a ligand to Zn2 and placing the biphenyl group in contact with a hydrophobic region of a flexible loop.

The activity of the biphenyl tetrazole system was developed from a high-throughput screening of Merck chemical collection. To further explore the potency of biphenyl tetrazoles as MBLs inhibitors, in 1999, Toney *et al*., screened a series of compounds containing 3-*n*-butyl-1-phenylpyrazole-5-carboxylate against *B. fragilis* and IMP-1 metallo-β-lactamases. At first, the compound **26** was found to be a weak inhibitor against *B. fragilis* MBL, while it was found to be inactive against IMP-1. However, the substitution upon the phenyl ring and the esterification of the carboxylic group **27**, **28**, increased inhibitory activity for both MBLs[53].

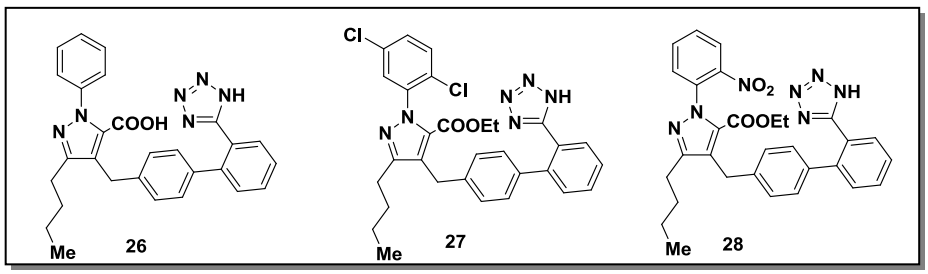


Fig.22. *Biphenyl tetrazole derivatives **26-28**.*

Another potential chelating molecule is one that contains an hydroxamic acid functional group, which is known to bind Zn ions, and therefore presents an inhibitory potential. *Walter et al.* synthesized amino acid-derived hydroxamates and screened them against different MBLs for inhibitory activity and found several compounds as inhibitors of clinically relevant enzymes[54]. In 2006, *Liénard* and his colleagues synthesized a series of derivatives of benzo-hydroxamic acid **29** and tested them against FEZ-1, IMP-1, BcII, CphA, and L1 MBLs for inhibitory activity. Their study resulted in the identification of selective inhibitors of FEZ-1 metallo-β-lactamase. The most potent selective inhibitor of FEZ-1 from this series was identified as 2,5-disubstituted benzophenone hydroxamic acid **30** with a *Ki* value of 6.1 ± 0.7 μM[55].

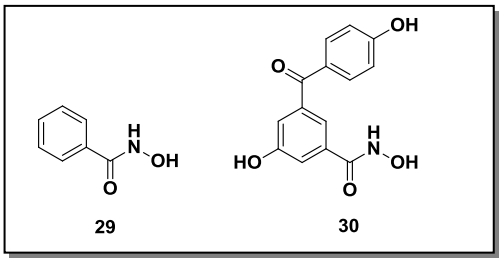


Fig.23. *Hydroxamic acid derivatives **29** and **30**.*

CHAPTER 2

AIM OF WORK

2.1 Aim of work

The increasing number of antibiotic resistant bacteria expressing metallo β-lactamases, represents a major threat to global public health. Although the MBLs of the B1 subfamily have sequence similarities as low as 40%, all of them have a similar catalytic mechanism and a similar substrate specificity. Hence, it has been speculated that it should be possible to design broad-spectrum inhibitors for B1 MBLs. Although such inhibitors would be of great clinical use, the design has been difficult so far.

The project here described, involves a preliminary investigation of a new potential class of MBL inhibitors. To achieve this goal the aim of work was divided into different tasks:

- the identification and synthesis of a new hit compound with potential MBLs inhibitory activity
- the SAR evaluation to improve the MBLs inhibitory potency
- evaluation of selectivity on several MBL isoforms (including VIM, IMP, NDM).

2.2 Identification of a new hit compound

High throughput screening (HTS) and virtual screening (VS) are the two main methods to identify novel scaffolds for drug discovery. Indeed, HTS has successfully identified a number of MBLs inhibitors, however structure-based drug design and virtual screening have not been widely applied in MBLs inhibitors development.

From a screening carried out on chemical library of Professor Campiani research group, it has been identified compound **31** (**Fig. 25**) as potential MBLs inhibitor. Inspection of the structure allowed to identify an arrangement of three different structural moieties that could be compatible with a general pharmacophore elaborated for MBLs inhibitors, shown in **Fig.24**. The pharmacophore consists of a zinc binding group (ZBG) able to chelate the catalytic zinc ion, a backbone binding on the surface of the active site pocket, and a linker between the ZBG and backbone. It is generally thought that the ZBG is a significant responsible for the potency and sometimes the selectivity of MBLs inhibitors.

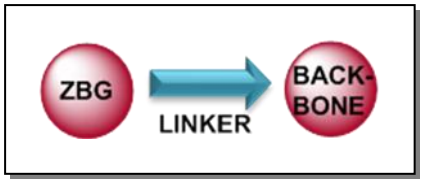


Fig 24. *General pharmacophore structure.*

It is known in the literature that phenol group is a potential ZBG, and also that several aryl-heterocycles play an important role in the ligand flexibility, allowing the rotation of the aromatic ring to accommodate the hydrophobic pockets in the binding site of different subclasses of MBLs. Furthermore, the proximity of the ZBG and the furan oxygen could form a hydrogen bond with the carboxyl group in the active site. These hypothesis have laid the foundations for the design and development of a new hit compound **32** (**Fig. 25**) with a different decoration pattern to improve the inhibitory activity against MBLs. Infact, the presence of positively charged residues in the substrates-binding loop of MBLs prompted us to replace the basic side chain with a neutral and hydrophobic one, resembling the side chain of common antibiotics.

The novel chemical scaffold features an *o*-hydroxyphenyl group that is directly connected to a furan aromatic linker to produce a cation interacting element. An amide function could be able to interact with zinc, thus generating a bidentate ZBG and representing at the same time the unit function with the backbone. The introduction of an aromatic backbone could generate an interaction with the hydrophobic amino acid residues in a flexible flap of the enzyme.

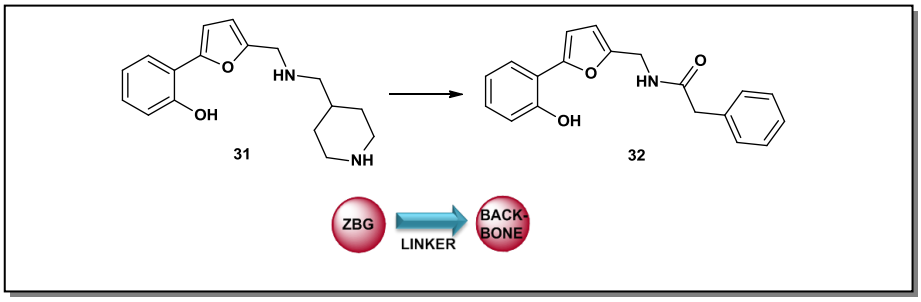


Fig.25. *Development of the hit compounds **31** and **32**.*

2.3 Structure activity relationships (SARs).

The next step was the synthesis of analogues to be tested in order to allow the study of the SARs and the creation of a robust model of interaction. The synthesized analogues were designed based on the investigation of three portion of the hit compound :

- the “metal binding moiety”, which consists of evaluating the different contribute of several ZBGs in the activity and selectivity modulation;
- the “ring moiety”, which consists of exploring a variety of ring system (aromatic or heteroaromatic) to which ZBG is attached;
- the “tail element”, which consists of attaching different tails to the heteroaromatic amide scaffold to modulate the enzyme binding.

2.3.1 “Metal binding moiety”

The main type of structural variation was the nature of the anchor groups able to coordinate the zinc ions. This ZBGs can be directly linked to an aromatic and heterocyclic moiety that can interact inside the enzyme cavity with hydrophilic or hydrophobic residues.

The development of the main ZBGs was one of the main purposes of the metal-ligand interaction study. The most frequently found ZBGs are carboxylate, sulfonamide and hydroxamate groups. It is possible that ZBGs contain multiple heteroatoms that interact with zinc, resulting in a bidentate interaction. The distances between the zinc and the two neighboring heteroatoms is crucial because carboxylates, for example, can form both monodentate and bidentate interactions, sulfonamides form predominantly monodentate interactions, while hydroxamates prefer bidentate coordination. The most common ZBG used in the discovery of metallo enzyme inhibitors is the hydroxamic acid group.

Sulfonamide group acts as ZBG most likely in its deprotonated state, binding in a tetrahedral geometry the Zn2 ion and leading to a strong electrostatic interaction, responsible for its good potency. So, the first two derivatives have been designed with hydroxamic and sulfonamide function respectively.

Recently, carboxylate and carboxylic acids (the synthetic precursors of most hydroxamic acids) have emerged as novel ZBGs. Their activity is related to the bound of the dinuclear metal center with the carbonyl oxygen and deprotonated hydroxyl group to form a mono-dentate bridge between both zinc ions. Simple thiols, thioethers, sulfoxides and sulfones are known as good ZBGs and therefore inhibit MBLs.

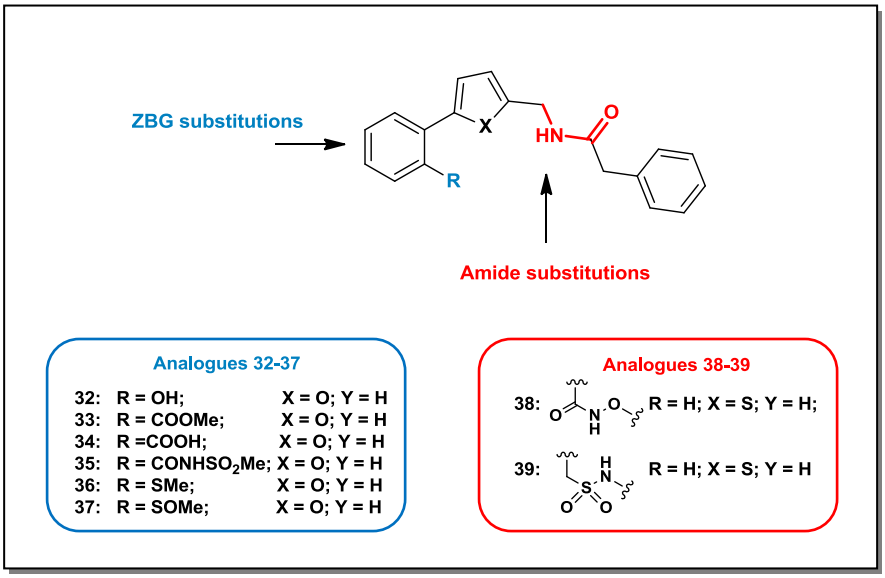


Fig.26. *Development of analogues **33-39**.*

All these functions are introduced on the aromatic ring of the designed scaffold with the synthesis of analogues **33-37**, and also the amide backbone was substituted obtaining derivatives **38** and **39**.

2.3.2 The "ring moiety"

The initial ZBG study has been subsequently expanded towards the exploration of different heterocyclic moieties.

The use of heterocyclic ring systems as bioisosteres for amide bonds represents a good chance to attenuate pharmacokinetic properties, enhance binding affinity, and provide novel chemical scaffolds to combat drug-resistant strains.

In the field of MBL inhibitors development, a triazole ring was reported in literature as a good system. Christopeit *et al.* identified the triazole fragment **40** as a VIM-2 inhibitor. They showed as this fragment directly interacts with the two Zn ions in the active site, whereby Zn1 was coordinated by the triazole ring and Zn2 by the thiol group, concluding that the thiol group is crucial for fragment binding, whereas the N-methyl group and the trifluoromethyl group did not influence the affinity[56].

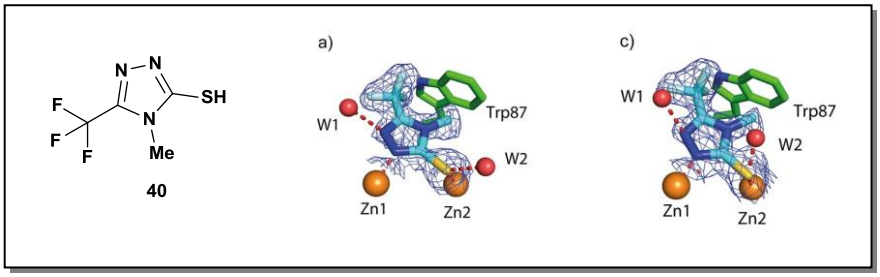


Fig.27.

X-ray structure of fragment 40 bound to the active site of VIM-2. The complexes crystallized with two copies in the asymmetric unit. Fragment carbon atoms are depicted in blue and VIM-2 carbon atoms in green. Water molecules are shown in red, Zn2+ and ions in orange[56].

Furthermore, Zahang *et al.* identified another triazole derivative **41** as IMP-1 and NDM-1 inhibitor showing that the triazole coordinates Zn2, just like the nitrogen atom derived from *β*-lactam hydrolysis and that a phenol group and an amide carbonyl are able to interacted with a Lys and Asn residues. The former interaction is reminiscent of that made by the C3/C4 carboxylate in penicillins and carbapenems, while the latter is reminiscent of the interaction between the *β* -lactam carbonyl group and Asn[57].

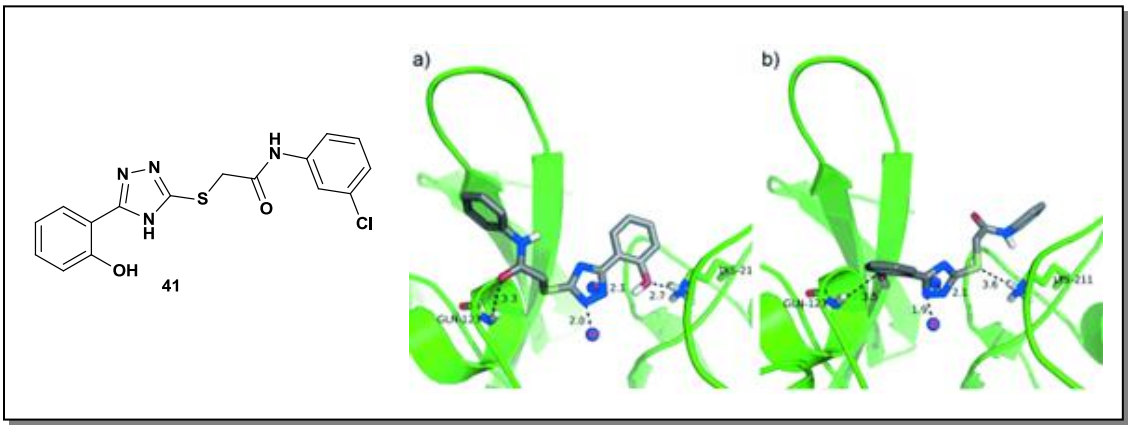


Fig.28. *Fragment **41** in the active site of NDM-1.*

The enzyme backbone is shown as cartoon in green, and selected residue side chains are shown as sticks colored by atom (C, cyan; N, blue; O, red; S, yellow). The Zn(II) ions are shown as purple spheres[57].

Looking at the medicinal importance of 1,2,4-triazole, 1,3,4-oxadiazole and thiophene moieties, it has been designed and synthesized a new series of heterocyclic derivatives (**42-44**). The furan ring was also replaced with a tetrahydrofuran ring (**45** and **46**).

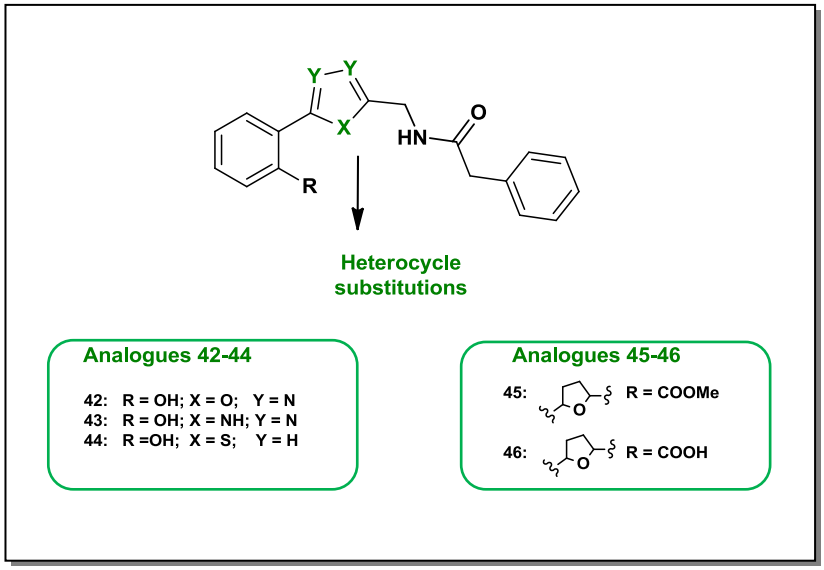


Fig.29 *Development of analogues **42-46** with heterocycle substitutions.*

2.3.3 “Tail element”

In the selective class of identified inhibitors, the backbone moieties have been hypothesized to interact with amino acid residues near the entrance of the active site differently between the MBLs classes. The surface-recognition backbones enjoy the widest range of chemotype tolerance, and have been the topic of extensive study in attempts to toggle potency, biodistribution, isoform selectivity, cardiotoxicity and, more recently, tissue targeting.

The backbone can take many forms, reflective of the diversity in enzyme active sites. It consists of assorted substituted aliphatic chains, aromatic rings, and heterocycles. So, in this work it was decided first of all, to investigate the inversion of the amide backbone with the synthesis of derivative **47**. It was also interesting to evaluate aromatic and aliphatic backbone, the length of this chain and in addition, the complete removal of backbone itself with the synthesis of analogues **48-50**.

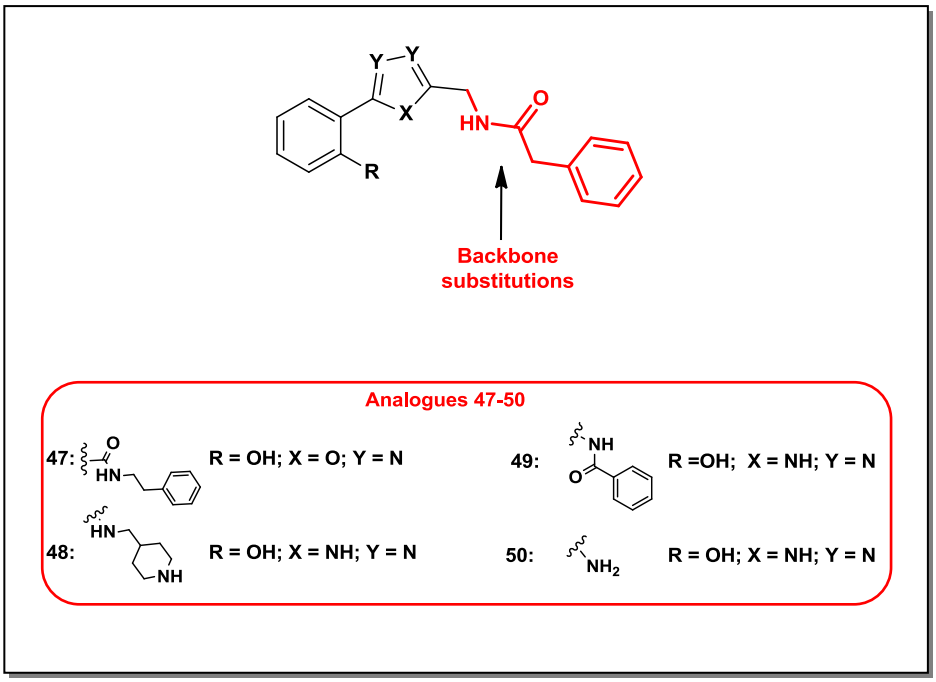


Fig.30. *Development of derivatives **47-50** with backbone substitutions.*

CHAPTER 3

CHEMISTRY

3.1 Synthesis of new hit compound with potential MBLs inhibitory activity.

The retrosynthetic approach for the designed compound is depicted in **Fig.31**. Three main disconnection points of the molecule were identified: the amide group, the heterocyclic functionalization and the C-C bond between the building block rings.

For the first fragment it was possible to apply an amide coupling reaction between a carboxylic acid and a key amine intermediate. The amine derived from a functional group interconversion of a carbonyl intermediate. which was obtained with an arylation coupling between a suitable protected aryl halide and a boronic acid.

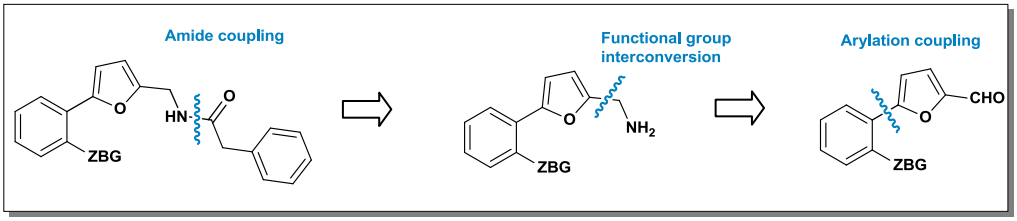
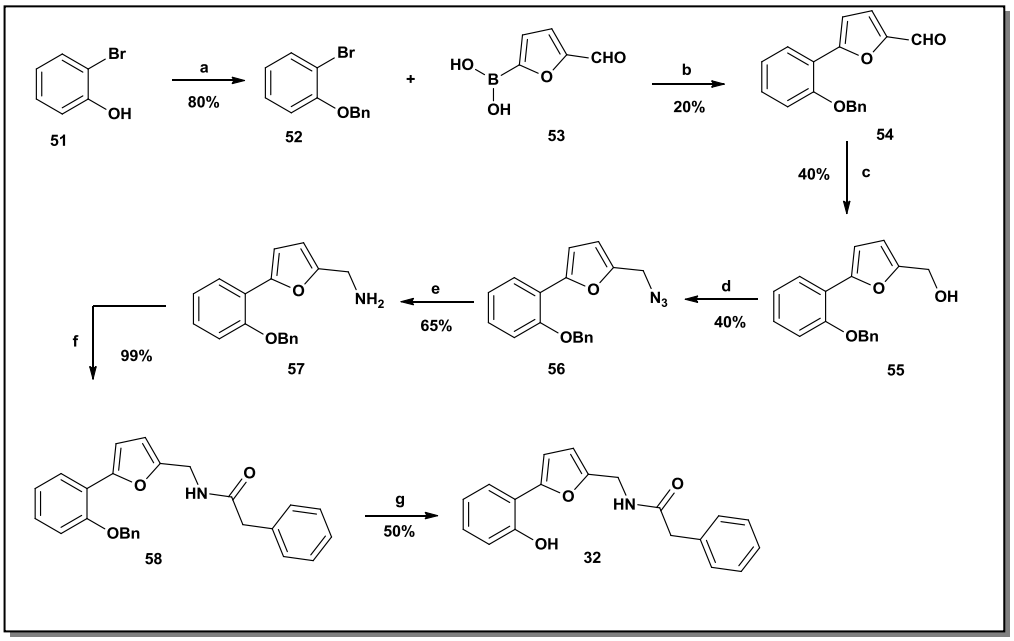


Fig.31. *Retrosynthetic approach.*

In Scheme 1 is shown the synthesis of the amide hit compound **32** with potential inhibitory activity against MBLs. The key intermediate of the synthetic strategy is the amine **57**. The synthesis started with a protection of alcoholic function of 2-bromophenol **51** with a benzyl group using benzyl bromide and potassium carbonate as base. The protected bromide **52** thus obtained, was subjected to a Suzuki-Miyaura coupling reaction with 2-formylfuran boronic acid **53** to introduce a C-C bond obtaining the aldehyde **54**[58]. The aldehyde was reduced to alchol **55** using sodium borohydride, afterward a functional group interconversion to azide **56** using diphenylphosphorylazide (DPPA) and 1,8-diazabicycloundec-7-ene (DBU) was performed, followed by a Staudinger reduction using triphenylphosphine and water to obtain the key amine **57**[59]. A coupling reaction between the amine and phenyl acetic acid was performed using 1-hydroxibenzotriazole (HOBt), *N*-(3-dimethylaminnopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) and triehtyl amine (TEA) as coupling reagents[60]. The final deprotection of benzyl group with boron trichloride (BCl_3) at low temperature, gave the desired compound **32** in satisfactory yield.



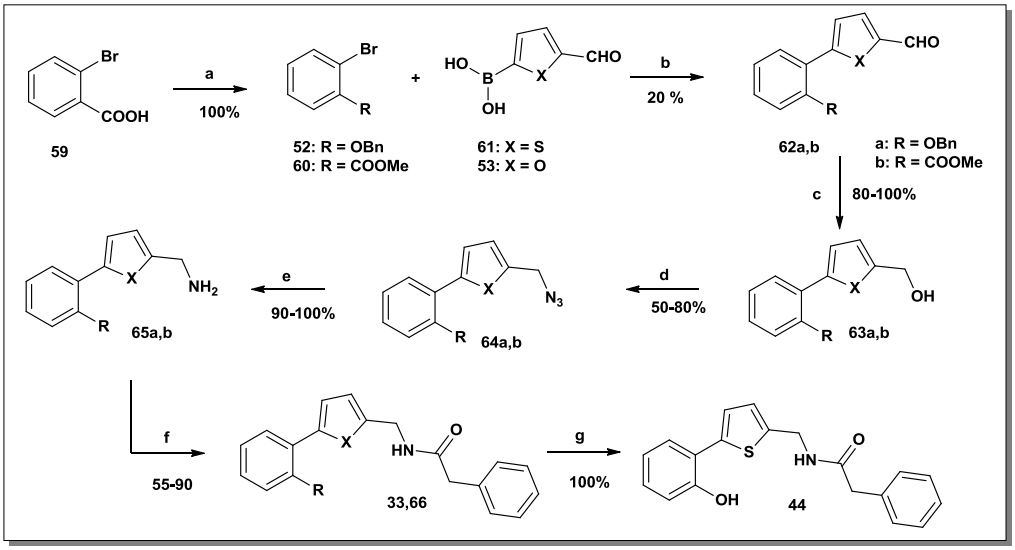
Scheme 1. Reagents and conditions:

a) benzylbromide, K_2CO_3, dry THF, 60 °C, 12 h; **b)** Tetrakis(triphenylphosphine)palladium0, K_2CO_3, dry toluene, 85 °C, 12 h; c) NaBH4, dry THF, 0-25 °C, 2 h; **d)** DPPA, DBU, dry toluene, 25 °C, 12 h; **e)** 1. PPh_3, dry THF, 60 °C, 12 h; 2. H_2O, 60 °C, 4 h; **f)** phenylacetic acid, HOBt, EDC, TEA, dry DCM, 0-25 °C, 12 h; **g)** BCl_3, dry DCM, -78 °C, 3 h.

3.2 Rational structure activity relationships (SARs).

The synthetic strategy previous described in Scheme 1, was successfully applied on the synthesis of the other derivatives. In the Scheme 2 is shown the synthesis of the thiophene analogue **44** and the compound **33** bearing a methyl ester function as ZBG.

Intermediate **52** was prepared as previously described while, ester **60**, was prepared by Fisher esterification on 2-bromobenzoic acid **59**. Suzuki-Miyaura arylation coupling with arylboronic acids **61** and **53** afforded aldehydes **62a,b**. These latter compounds were first reduced to **63a,b** using sodium borohydride, and the alcohols were converted to azides **64a,b** using DPPA and DBU in dry toluene. A Staudinger reduction using triphenylphosphine and water was performed obtaining the amines **65a,b**. The amide coupling reactions were conducted with HOBt, EDC and TEA to obtain **33** as the final compound. Derivative **66** was also subjected to benzyl deprotection using BCl_3 giving the desired compound **44** in satisfied yield.

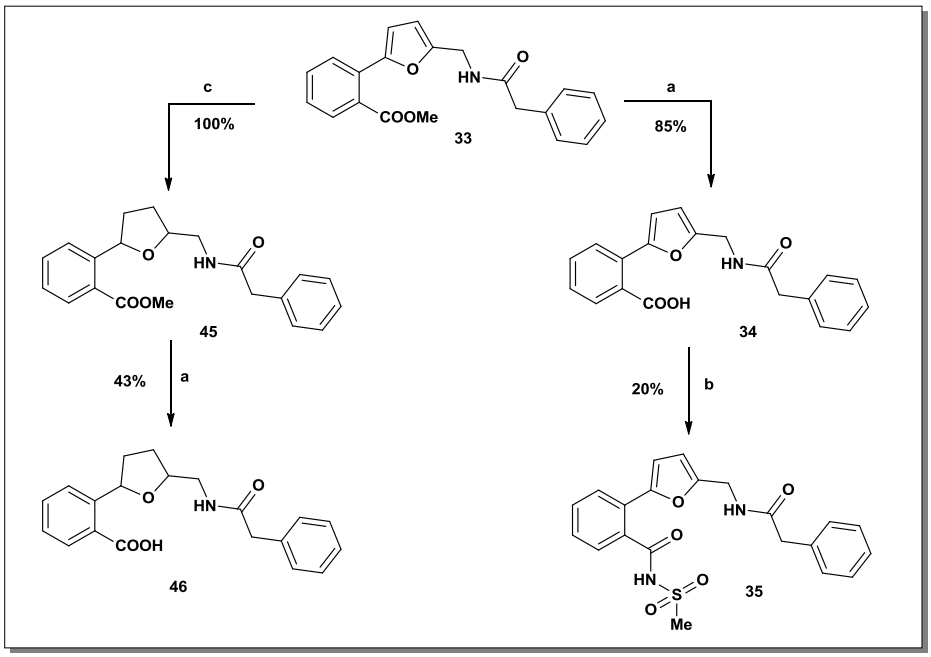


Scheme 2. Reagents and conditions:

a) MeOH, H_2SO_4, 70 °C, 12 h; **b)** Tetrakis(triphenylphosphine)palladium0, K_2CO_3, dry toluene, EtOH, 85 °C, 12 h; **c)** $NaBH_4$, dry THF, 0-25 °C, 2 h; **d)** DPPA, DBU, dry toluene, 25 °C, 12 h; **e)** 1. PPh_3, dry THF, 60 °C, 12 h; 2. H_2O, 60 °C, 4 h; **f)** phenylacetic acid, HOBt, EDC, TEA, dry DCM, 0-25 °C, 12 h; **g)** BCl_3, dry DCM, -78 °C, 3 h.

The final compound **33**, was also used as intermediate to obtain analogues **34** and **35**, with a carboxylic and methanesulfonamide functions respectively, and analogues **45** and **46** with tetrahydrofuran replacement (Scheme 3). The methyl ester function of compound **33** was hydrolyzed to carboxylic acid **34**, using an aqueous solution of sodium hydroxide. This analogue was also functionalized into the corresponding methanesulfonamde derivative **35**, through a coupling reaction with methanesulfonamide using EDC and dimethyl aminopyridine

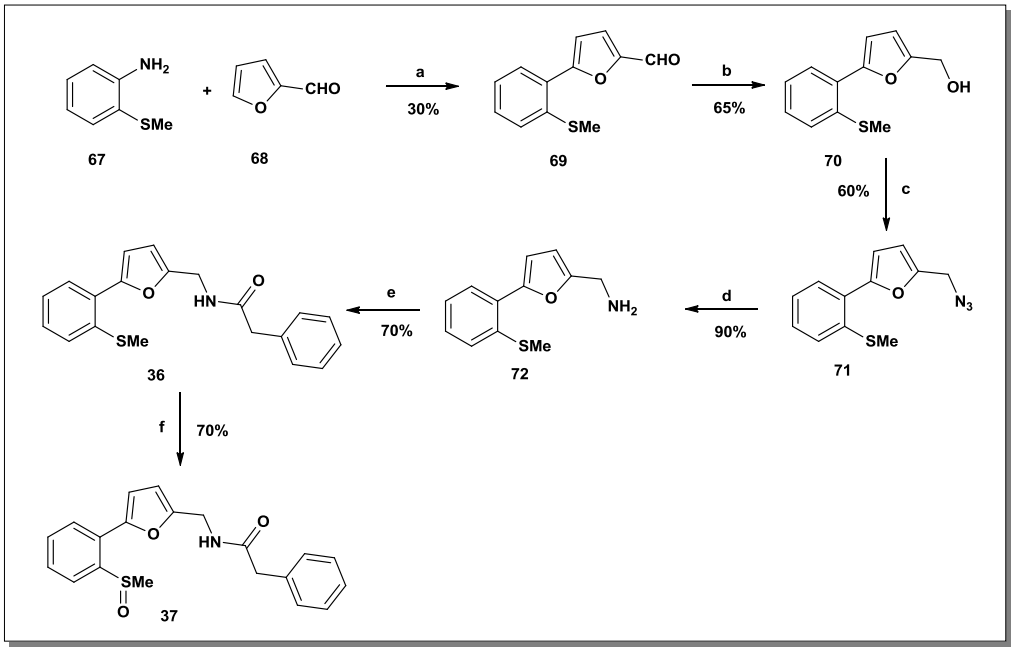
(DMAP) as coupling reagents. On the other hand, the same methyl ester analogue **33** was reduced to the corresponding tetrahydrofuran derivative **45**, through a palladium-catalyzed hydrogenation reaction, followed by hydrolysis of the methyl ester group to **46**.



Scheme 3. Reagents and conditions:

a) NaOH 1N, MeOH, 70 °C, 12h; **b)** methanesulfonamide, EDC, DMAP, dry DCM, 0-25 °C, 12 h. **c)** H_2, Pd/C, MeOH, 25 °C, 5 h.

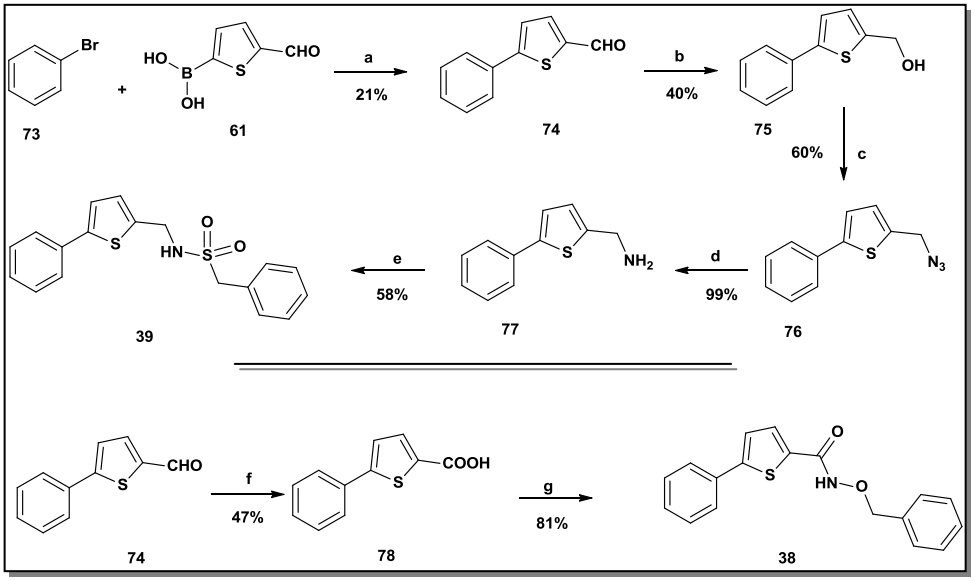
Since 2-methylthioaniline **67** was commercially available the synthesis of derivatives **36** and **37,** described in the Scheme 4, followed a different synthetic approach based on the Meerwein arylation protocol[61]. Accordingly, furaldehyde **68** was reacted with the diazonium salt derived from **67** in the presence of catalytic copper(II) chloride, to obtain the aldehyde **69**. The aldehyde was reduced to alchol **70**, converted at first to azide **71**, and after to amine **72** with the same approach previously described. A classical coupling reaction allowed to obtain the desired methylthio-derivative **36** in satisfactory yield. Furthermore, oxidation using *meta*-chloroperbenzoic acid, gave a second analogue **37** bearing a methylsulfoxide function.



Scheme 4. Reagents and conditions:

a) HCl, $NaNO_2$, $CuCl_2$, H_2O, acetone, 0-25 °C, 12 h; **b)** $NaBH_4$, dry THF, 0-25 °C, 2 h; **c)** DPPA, DBU, dry toluene, 25 °C, 12 h; **d)** 1. PPh_3, dry THF, 60 °C, 12 h; 2. H_2O, 60 °C, 4 h; **e)** phenylacetic acid, HOBt, EDC, TEA, dry DCM, 0-25 °C, 12 h; **f)** m-ClPBA, dry DCM, 0 °C, 0,5 h.

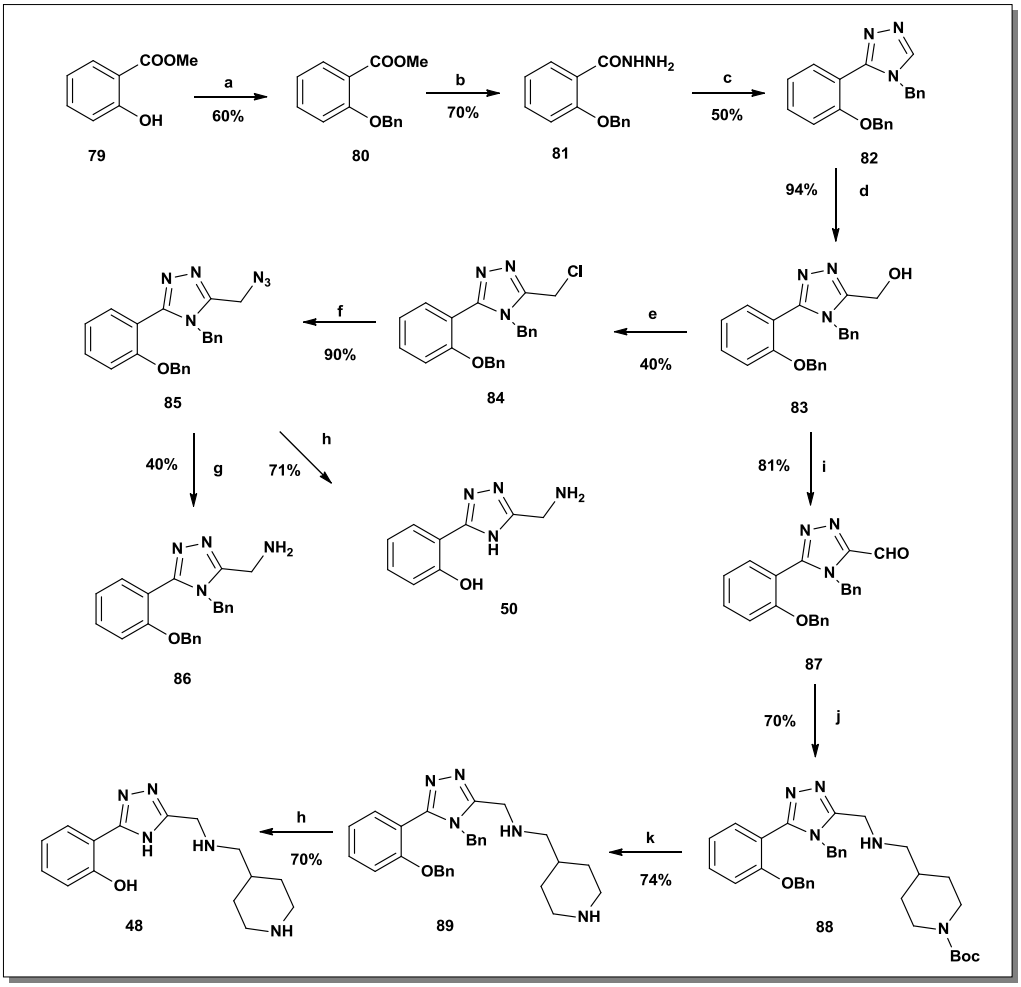
In Scheme 5 is reported the synthetic strategy used for the synthesis of analogues **38** and **39** bearing modifications of the amide function. The Suzuki-Miyaura reaction was chosen as arylation method starting from bromobenzene **73** and 2-thiophenyl boronic acid **61**. The aldehyde **74** thus obtained, was reduced to alchol **75**, converted to azide **76** and to amine **77** with subsequent nucleophilic substitution and Staudinger reduction. On the other hand, the same aldehyde **74** was oxidized to carboxylic acid **78** using silver oxide. Two different coupling reactions were performed: the first, between amine **77** and benzenesulfonyl chloride; and the second, between carboxylic acid **78** and O-benzyl-hydroxylamine to obtain the desired compounds **39** and **38**, respectively.



Scheme 5. Reagents and conditions:

a) Tetrakis(triphenylphosphine)palladium0, K_2CO_3, dry toluene, 85 °C, 12 h; **b)** $NaBH_4$, dry THF, 0-25 °C, 2 h; **c)** DPPA, DBU, dry toluene, 25 °C, 12 h; **d)** 1. PPh_3, dry THF, 60 °C, 12 h; 2. H_2O, 60 °C, 4 h; **e)** benezenesulfonyl chloride, TEA, dry DCM, 0-25 °C, 12 h; **f)** Ag_2O, H_2O, MeOH, 0-25 °C, 6 h. **g)** O-benzyl-hydroxylamine, HOBt, EDC, NMM, dry, DMF, 0-25 °C, 12 h.

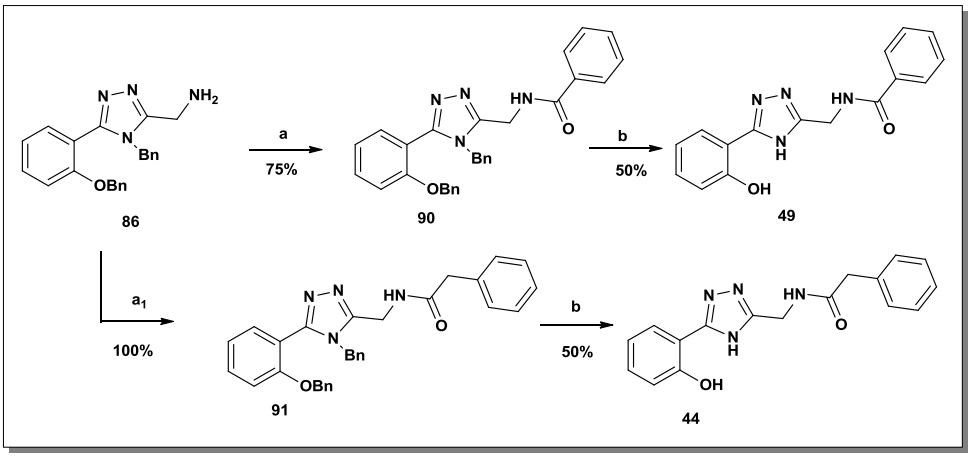
Regarding the triazole derivatives **43**, **48**- **50**, a key intermediate was identified in the amine **86**, which synthesis is shown in Scheme 6. It was prepared starting from methyl salicylate **79** which was protected as benzyl ether **80** using benzyl bromide and potassium carbonate, afterward the ester group was converted into the hydrazide **81** by treatment with hydrazine monohydrate. This hydrazide was subjected to a Pellizzari-like reaction using *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) and benzyl amine in presence of acetic acid to obtain the triazole intermediate **82**. A Prins reaction was performed using formaldehyde at reflux obtaining the alchol **83** which was activated to chloride **84**, in turn converted to azide **85** which was reduced to amine **86**. The azide intermediate was also useful to obtain the analogue **50**. Furthermore, the alchol **83** was oxidized to aldehyde **87**. Several oxidizing reagents were investigated. Both potassium permanganate and pyridinium chlorocromate (PCC) resulted inefficient because the isolated product was the decarboxylated triazole. So, a milder reagent, namely Dess-Martin periodinane (DMP) was used and the aldehyde was isolated in satisfactory yield. The carbonyl product was subjected to reductive amination with *N*-Boc-piperidine methanamine to obtain the secondary amine **98**, which was deprotected at first, from benzylic functions and after from the Boc group to obtain the desired compound **48**.



Scheme 6. Reagents and conditions:

a) benzylbromide, K_2CO_3, dry THF, 60 °C, 12 h; **b)** hydrazine monohydrate, EtOH, 70 °C, 12 h; **c)** DMF-DMA, benzylamine, AcOH, 50-120 °C, 12 h; **d)** formaldehyde aq.sol. 37 %; **e)** $SOCl_2$, Pyr., dry DCM, 0-25 °C, 4 h; **f)** NaN_3, dry DMF, 0-85 °C, 12 h; **g)** 1. PPh_3, dry THF, 60 °C, 12 h, 2. H_2O, 60 °C, 3 h; **h)** H_2, Pd/C 10 %, MeOH, 25 °C, 5 h; **i)** DMP, dry DCM, 0-25 °C, 1 h; **j)** 1. N-Boc-piperidine methanamine, MeOH, 70 °C, 12 h; 2. $NaBH_4$, MeOH, 0-25 °C, 1 h, **k)** TFA, dry DCM, 0-25 °C, 12 h.

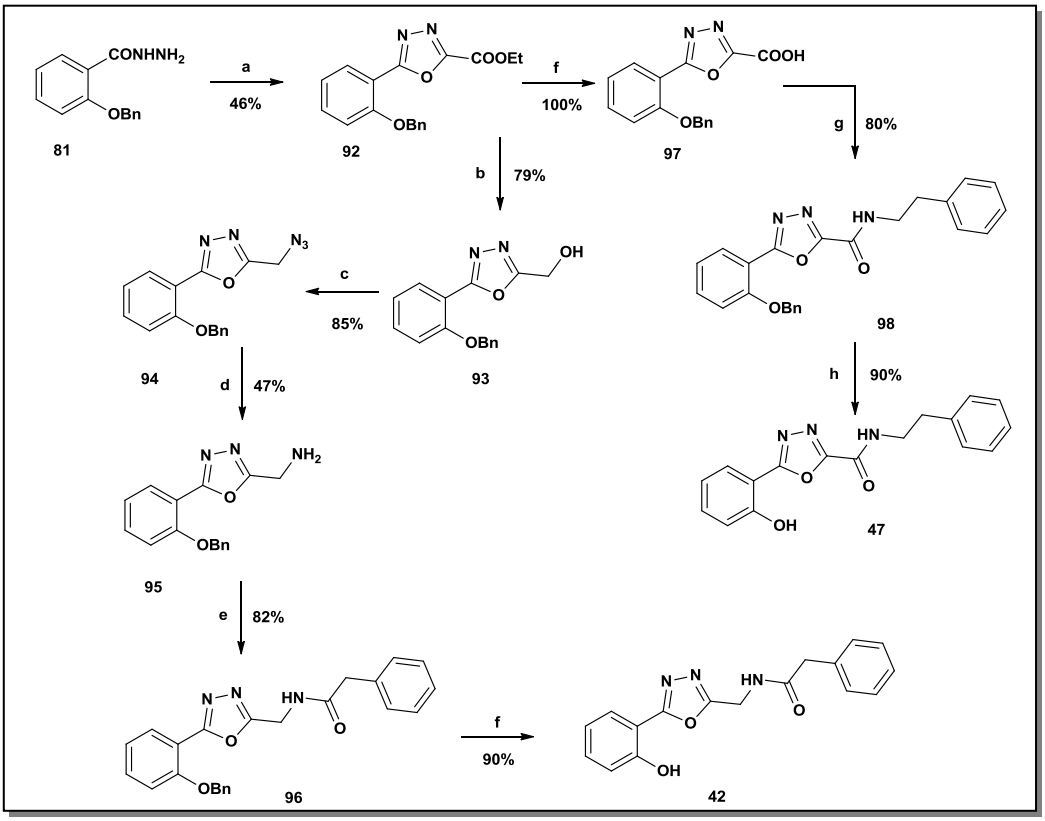
In the Scheme 7 is depicted the synthesis of derivative **44** and **49**. Starting from the amine intermediate **86**, synthesized as shown in Scheme 6, two coupling reactions were performed. One, using phenyl acetic acid, HOBt, EDC and TEA, and the second using benzoic acid with the same coupling reagents and conditions. Both amides **90** and **91** thus obtained were subjected to hydrogenolysis giving the final desired analogues **44** and **49** respectively.



Scheme 7. Reagents and conditions:.

a) benzoic acid, HOBt, EDC, TEA, 0-25 °c, 12 h, **a_1)** phenylacetic acid, HOBt, EDC, TEA, 0-25 °c, 12 h, **b)** H_2, Pd/C 10 %, 25 °C, 5 h.

Regarding the oxadiazole derivatives **42** and **47**, the synthesis is shown in the Scheme 8. The intermediate **81**, prepared as described in the Scheme 7, was cyclized into the oxadiazole ethyl ester **92** using ethylchlorooxo acetate and a catalytic amount of tosyl chloride. The ester thus obtained was subjected to the subsequent functional group interconversions previously described to finally obtain the amine **95**. Coupling reaction performed using phenyl acetic acid, HOBt, EDC and TEA as coupling reagents afforded amide **96**. The same ethyl ester **92** was also hydrolyzed to carboxylic acid **97**. Several hydrolysis conditions were investigated. All common hydrolysis reaction based on the use of inorganic bases revealed unsatisfactory because the only isolated product was the decarboxylated oxadiazole, formed during the acid work-up. A milder hydrolysis was performed using lithium bromide and TEA and the desired product **97**, isolated in good yield, was subjected to coupling reaction with 2-phenyl ethanamine using HOBt, EDC and TEA. Both amide **96** and **98** were finally deprotected from the benzylic function with boron trichloride at low temperature giving the desired final compounds **42** and **47** respectively.



Scheme 8. Reagents and conditions:

a) ethylchlorooxo acetate, dry DCM, 12 h; **b)** $NaBH_4$, MeOH, 0-25 °C, 1 h; **c)** DPPA, DBU, dry toluene, 25 °C, 12 h; **d)** 1. PPh_3, dry THF, 60 °C, 12 h, 2. H_2O, 60 °C, 3 h; **e)** phenylacetic acid, HOBt, EDC, TEA, dry DCM, 0-25 °C, 12 h; **f)** LiBr, TEA, dry THF, 25 °C, 4 h; **g)** 2-phenylethylamine, HOBt, EDC, TEA, dry DCM, 0-25 °C, 12 h; **h)** BCl_3, dry DCM, -78 °C, 2 h; **g)** LiBr, TEA, dry THF, 25 °C, 4 h.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Results and discussion

The identification of the hit compound **31** represents a promising starting point. To further optimize the inhibitor, all synthesized compounds were tested on three clinically relevant MBLs, NDM-1, IMP-1 and VIM-2, in collaboration with Prof. Doquier Jean-Denis of University of Siena.

Purified IMP-1, VIM-2 and NDM-1 MBL enzymes were produced using the *E. coli*-based expression system and purified. Compounds were dissolved in DMSO at the concentration of 100mM and subsequently diluted in 50 mM HEPES, 50 mM ZnSO4 (pH¼7.5), at a final concentration of 2–5 mM. For poorly soluble compounds, up to 10% DMSO was added to the buffer (the maximum DMSO concentration used in the assay did not affect the enzyme activity). The inhibitory activity of the various compounds on purified MBLs was investigated by measuring the variation of the initial rate of hydrolysis of 90 mM imipenem or 80 mM cefotaxime, used as the reporter substrate, at 30 °C in 50mM HEPES buffer (pH 7.5) supplemented with 50 mM ZnSO4, in the presence of various inhibitor concentrations. The MBL final concentration in the assays ranged 2.5–40 nM. The enzyme activity in % was calculated from the initial velocity compared to a control without tested compound[62]. The results are reported in Table 1.

Table 1.

Cpd	VIM-2	IMP-1	NDM-1
	% Inh. (50µM)	% Inh. (50µM)	% Inh. (50µM)
31	**60**	≤10	≤10
33	≤10	75	64
34	≤10	≤10	55
35	≤10	62	**69**
36	34	**80**	**72**
37	≤10	**>90**	37
38	≤10	**>90**	28

39	13	48	33
42	11	**>90**	61
43	≤10	66	**76**
44	≤10	**>90**	**74**
45	≤10	**>90**	15
47	≤10	≤10	13
49	≤10	17	63

These preliminary data revealed a good potential activity of several compounds (**36**, **37**, **38**, **32**, **44**, **45**) on IMP-1 considered one of the most difficult isoforms to inhibit. Other analogues (**35**, **36**, **43**, **44**) showed a certain potency towards NDM-1. Interesting are the activities of the thiophenyl and thiomethyl derivatives **44** and **36** on both isoforms, which represent a possible starting point of a broad spectrum inhibitors development.

The most interesting compounds resulted from this first screening assay, are at moment under further biological evaluation. The *K*i value will be determined using a competitive model of inhibition.

4.2 Conclusions

During my PhD program I was involved in the synthesis of a novel series of potential MBLs inhibitors. A previous screening performed on a library of compounds, led to the identification of a general molecular scaffold in which three main moieties can be identified: a ZBG, able to chelate one or two zinc ions in the enzyme active site, a linker which acts as a spacer linked to a terminal hydrophobic backbone. All moieties were largely replaced in order to obtain a detailed SAR evaluation. All synthesized compounds are tested on three clinically relevant MBL isoforms, VIM-2, IMP-1 and NDM-1. First satisfactory results were obtained evaluating the percentage isoforms inhibition. The subsequent determination of *K*i values, will allow us to deepen the potency and the selectivity of the best derivatives, object of this work.

CHAPTER 5

EXPERIMENTAL SECTION

Experimental methods

Starting materials and solvents were purchased from commercial suppliers and used without further purification. Reaction progress was monitored by TLC using silica gel 60 F254 (0.040-0.063 mm) with detection by UV. Silica gel 60 (0.040-0.063 mm) was used for column chromatography ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 300 MHz, or a Bruker 400 MHz spectrometer using the residual signal of the deuterated solvent as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz (Hz). Mass spectra were recorded utilizing electron spray ionization (ESI) Agilent 1100 Series LC/MSD spectrometer. Melting points were determined using a Büchi Melting point B-540. Yields refer to purified products and are not optimized. All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents.

5.1 Synthesis of compound 32.

1-(Benzyloxy)-2-bromobenzene (52).

To a solution of compound **51** (1.8 mL, 17.3 mmol) in dry THF (40 mL), potassium carbonate (4.79 mg, 35 mmol) and benzyl bromide (3.3 mL, 35 mmol) were added and the mixture was heated under reflux for 12 h. After this time, the mixture was filtered on celite and the solvent was removed under vacuum. The crude product was purified by flash chromatography on silica gel (100% petroleum ether) to give **52** as colorless oil (3.6 g, 80%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.49 (d, *J* = 7.3 Hz, 2H), 7.36 (dt, *J* = 21.6, 7.0 Hz, 3H), 7.27 – 7.20 (m, 1H), 6.94 (d, *J* = 7.2 Hz, 1H), 6.85 (t, *J* = 7.0 Hz, 1H), 5.17 (s, 2H).

5-(2-(Benzyloxy)phenyl)furan-2-carbaldehyde (54).

To a solution of triphenylphosphine (1.2 g, 4.5 mmol) and palladium(II)acetate (205 mg, 0.90 mmol) in a mixture of dry toluene (6 mL) and dry EtOH (6 mL), a solution of **52** (12 mL) in dry toluene was added. After 1 h, **53** and an aqueous solution of potassium carbonate (1.8 g, 13,6 mmol) were added and the mixture was heated under reflux for 12 h. The solvent was removed under vacuum. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **54** as yellow oil (250 mg, 20%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 9.63 (s, 1H), 8.07 (d, *J* = 7.9 Hz, 1H), 7.53 – 7.32 (m, 6H), 7.25 (d, *J* = 2.2 Hz, 2H), 7.10 – 7.03 (m, 3H), 5.21 (s, 2H).

(5-(2-(Benzyloxy)phenyl)furan-2-yl)methanol (55).

To a solution of **54** (126 mg, 0.45 mmol), in dry THF (8 mL), sodium borohydride (15 mg, 0.40 mmol) was added at 0 °C. The mixture was warmed to 25 °C. After 2 h, the solvent was removed under vacuum, EtOAc (6 mL) was added and the organic phase was washed with a saturated solution of ammonium chloride, dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (100% CH_3Cl) to give **55** as colorless oil (50 mg, 40%). **^{1}H NMR**: (300 MHz, Chloroform-*d*) δ 7.93 – 7.88 (m, 1H), 7.48 (d, *J* = 7.3 Hz, 2H), 7.40 (td, *J* = 9.2, 8.6, 4.1 Hz, 3H), 7.27 – 7.18 (m, 1H), 7.03 (t, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 3.3 Hz, 1H), 6.34 (d, *J* = 3.2 Hz, 1H), 5.18 (s, 2H), 4.66 (s, 2H).

2-(Azidomethyl)-5-(2-(benzyloxy)phenyl)furan (56).

To a solution of **55** (50 mg, 0.17 mmol) in dry toluene (6 mL), diphenyl phosphoryl azide (45 µL, 0.28 mmol) and 1,8-diazabicyclo[5.4.0] undec-7-ene (30 µL, 0.28 mmol) were added at 0 °C and the reaction mixture warmed at 25 °C. After 12 h, an aqueous solution of

hydrochloridric acid (1 mL, 1 N) was added and the organic layer was extracted with EtOAc (3 x5 mL), dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (1% EtOAc in petroleum ether) to give **56** as colorless oil (21 mg, 40%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.90 (d, *J* = 6.2 Hz, 1H), 7.51 – 7.36 (m, 5H), 7.28 – 7.20 (m, 1H), 7.09 – 6.99 (m, 2H), 6.87 (d, *J* = 3.3 Hz, 1H), 6.38 (d, *J* = 3.2 Hz, 1H), 5.19 (s, 2H), 4.34 (s, 2H). MS (ESI) *m/z* 328 $[M + Na]^+$.

(5-(2-(Benzyloxy)phenyl)furan-2-yl)methanamine (57).

To a solution of **56** (20 mg, 0.07 mmol) in dry THF (2 mL), triphenylphosphine (35 mg, 0.13 mmol) was added and the mixture was heated under reflux for 12 h. After this time, water (0.2 mL) was added and the reaction was refluxed for 3 h. The solvent was removed under vacuum and The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **57** as colorless oil (13 mg, 65%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.87 (d, *J* = 7.4 Hz, 1H), 7.49 – 7.35 (m, 5H), 7.21 (dd, *J* = 16.2, 8.9 Hz, 1H), 7.02 (t, *J* = 8.2 Hz, 2H), 6.83 (d, 3.3 Hz, 1H), 6.20 (d, *J* = 3.2 Hz, 1H), 5.17 (s, 2H), 3.88 (s, 2H). MS (ESI) *m/z* 280 $[M + H]^+$.

***N*-((5-(2-(benzyloxy)phenyl)furan-2-yl)methyl)-2-phenylacetamide (58).**

To a solution of phenyl acetic acid (3 mg, 0.02 mmol) in dry DCM (3 mL), hydroxy-benzotriazole (14 mg, 0.11 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (21 mg, 0.11 mmol) and triehtyl amine (18 µL, 0.13 mmol) were added at 0 °C. After 1 h, a solution of **57** (12 mg, 0.04 mmol) in dry DCM (3 mL) was added at 0 °C. The mixture warmed at 25 °C. After 12 h, the organic layer was washed with an aqueous solution of hydrochloridric acid (1 mL, 1 N), and with a saturated solution of sodium bicarbonate (1 x 1 mL), dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **58** as amorphous solid (16 mg, 99%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.6 Hz, 1H), 7.17 – 7.26 (m, 10 H), 7.20 (d, *J* = 7.4 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 2H), 6.79 (d, *J* = 3.2 Hz, 1H), 6.18 (d, *J* = 3.2 Hz, 1H), 5.85 (brs, 1H), 5.16 (s, 2H), 4.46 (d, *J* = 5.5 Hz, 2H), 3.61 (s, 2H). MS (ESI) *m/z* 420 $[M + Na]^+$.

***N*-((5-(2-Hydroxyphenyl)furan-2-yl)methyl)-2-phenylacetamide (32).**

To a solution of **58** (16 mg, 0.04 mmol) in dry DCM (4 mL), a solution 1 M of boron trichloride in DCM (50 µL, 0.57 mmol) was added at -78 °C and the reaction mixture was maintained at the same temperature for 2 h. After this time, an aqueous solution of sodium bicarbonate (1 mL) was added. After warming at 25 °C, the organic layer was extracted with DCM (3 x 3 mL),

dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (1% MeOH in DCM) to give **32** as yellow oil (6 mg, 50%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.36 – 7.21 (m, 7H), 7.16 (d, *J* = 6.9 Hz, 2H), 7.01 (d, *J* = 7.7 Hz, 1H), 6.91 (t, *J* = 7.1 Hz, 1H), 6.68 (s, 1H), 5.85 (brs, 1H), 4.38 (d, *J* = 5.7 Hz, 2H), 3.60 (s, 2H). **^{13}C NMR**: (75 MHz, CD_3OD) δ 172.53, 153.66, 140.38, 140.01, 135.66, 128.95, 128.36, 127.84, 126.69, 125.47, 124.47, 121.48, 119.55, 115.88, 42.62, 38.05, 31.88, 29.51, 22.54. MS (ESI) *m/z* 330 $[M + Na]^+$.

5.2 Synthesis of compounds 33-35 and 44-46.

Methyl 2-bromobenzoate (60).

To a solution of **59** (3.0 g; 15 mmol) in MeOH (20 mL), 5 drops of sulfuric acid were added and the mixture was heated under reflux for 24 h. The solvent was removed under vacuum. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **61** as colorless oil (3.2 g, 100%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.79 (dd, *J* = 7.3, 2.2 Hz, 1H), 7.68 – 7.64 (m, 1H), 7.40 – 7.28 (m, 2H), 7.26 (s, 1H), 3.93 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.70, 134.48, 132.76, 132.29, 131.47, 127.35, 121.78, 52.62. MS (ESI) *m/z* 215 $[M + H]^+$, 238 $[M + Na]^+$.

5-(2-(Benzyloxy)phenyl)thiophene-2-carbaldehyde (62a).

Starting from **52** (1.2 g, 4.5 mmol), the title compound was prepared following the procedure reported for compound **54**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **62a** as yellow oil (265 mg, 20%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.75 – 7.69 (m, 2H), 7.60 (d, *J* = 4.0 Hz, 2H), 7.48 – 7.28 (m, 5H), 7.03 (d, *J* = 15.1 Hz, 2H), 5.25 (s, 2H).

Methyl 2-(5-formylfuran-2-yl)benzoate (62b).

Starting from **61** (2.0 g, 9.3 mmol), the title compound was prepared following the procedure reported for compound **54**. The crude product was purified by flash chromatography on silica gel (10% EtOAc in petroleum ether) to give **63b** as yellow oil (428 mg, 20%). **^{1}H NMR**: (300 MHz, Chloroform-*d*) δ 9.62 (s, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.67 (d, *J* = 7.6 Hz, 1H), 7.54 (d, *J* = 7.3 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.29 (d, *J* = 3.6 Hz, 1H), 6.72 (d, *J* = 3.6 Hz, 1H), 3.84 (s, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.57, 176.30, 168.65, 158.15, 152.63, 131.45, 131.05, 129.90, 129.74, 129.35, 122.90, 110.99, 52.83. MS (ESI) *m/z* 253 $[M + Na]^+$, 269 $[M + K]^+$.

(5-(2-(Benzyloxy)phenyl)thiophen-2-yl)methanol (63a).

Starting from **62a** (105 mg, 0.36 mmol), the title compound was prepared following the procedure reported for compound **55**. The crude product was purified by flash chromatography on silica gel (100% $CHCl_3$) to give **63a** as colorless oil (53 mg, 50%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.4 Hz, 1H), 7.47 (d, *J* = 7.0 Hz, 2H), 7.37 (dd, *J* = 9.8, 3.6 Hz, 3H), 7.26 – 7.19 (m, 2H), 6.99 (dd, *J* = 13.7, 5.8 Hz, 3H), 5.21 (s, 2H), 4.80 (s, 2H). MS (ESI) *m/z* 319 $[M + Na]^+$, 335 $[M + K]^+$.

Methyl 2-(5-(hydroxymethyl)furan-2-yl)benzoate (63b).

Starting from **63b** (293 mg, 1.3 mmol), the title compound was prepared following the procedure reported for compound **55**. The crude product was purified by flash chromatography on silica gel (25% EtOAc in petroleum ether) to give **63b** as yellow oil (241 mg, 80%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.63 – 7.50 (m, 2H), 7.47 – 7.38 (m, 1H), 7.34 – 7.26 (m, 1H), 6.47 (d, *J* = 3.2 Hz, 1H), 6.31 (d, *J* = 3.1 Hz, 1H), 4.55 (s, 2H), 3.80 (s, 3H), 3.23 (brs, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.89, 154.60, 152.50, 131.10, 130.20, 129.80, 129.34, 128.18, 127.90, 109.90, 108.90, 57.72, 52.68. MS (ESI) *m/z* 233 $[M + H]^+$, 255 $[M + Na]^+$.

2-(Azidomethyl)-5-(2-(benzyloxy)phenyl)thiophene (64a).

Starting from **63a** (41 mg, 0.14 mmol), the title compound was prepared following the procedure reported for compound **56**. The crude product was purified by flash chromatography on silica gel (2% EtOAc in petroleum ether) to give **64a** as colorless oil (22 mg, 50%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 6.5 Hz, 1H), 7.48 (d, *J* = 7.0 Hz, 2H), 7.42 – 7.34 (m, 4H), 7.27 – 7.22 (m, 2H), 7.05 – 7.00 (m, 2H), 5.22 (s, 2H), 4.48 (s, 2H). MS (ESI) *m/z* 344 $[M + Na]^+$, 360 $[M + K]^+$.

Methyl 2-(5-(azidomethyl)furan-2-yl)benzoate (64b).

Starting from **63b** (230 mg, 1.04 mmol), the title compound was prepared following the procedure reported for compound **56**. The crude product was purified by flash chromatography on silica gel (10% EtOAc in petroleum ether) to give **64b** as colorless oil (213 mg, 80%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.67 (d, *J* = 6.4 Hz, 1H), 7.60 (d, *J* = 7.4 Hz, 1H), 7.52 – 7.44 (m, 1H), 7.40 – 7.32 (m, 1H), 6.53 (d, *J* = 3.3 Hz, 1H), 6.42 (d, *J* = 3.3 Hz, 1H), 4.30 (s, 2H), 3.85 (s, 3H). MS (ESI) m/z 258 $[M + H]^+$.

(5-(2-(Benzyloxy)phenyl)thiophene-2-yl)methanamine (65a).

Starting from **64a** (22 mg, 0.07 mmol), the title compound was prepared following the procedure reported for compound **57**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **65a** as colorless oil (21 mg, 100%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 7.2 Hz, 2H), 7.35 (dd, *J* = 12.3, 6.2 Hz, 4H), 7.26 – 7.17 (m, 1H), 7.02 – 6.96 (m, 2H), 6.87 (d, *J* = 3.5 Hz, 1H), 5.21 (s, 2H), 4.04 (s, 2H), 1.73 (s, 2H). MS (ESI) *m/z* 318 $[M + Na]^+$.

Methyl 2-(5-(aminomethyl)furan-2-yl)benzoate (65b).

Starting from **64b** (195 mg, 0.75 mmol), the title compound was prepared following the procedure reported for compound **57**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **65b** as colorless oil (145 mg, 90%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.62 – 7.52 (m, 2H), 7.41 (t, *J* = 7.3 Hz, 1H), 7.27 (t, *J* = 7.4 Hz, 1H), 6.45 (d, *J* = 3.1 Hz, 1H), 6.17 (d, *J* = 2.9 Hz, 1H), 3.79 (s, 6H), 1.64 (s, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.84, 131.05, 130.95, 129.97, 129.30, 128.14, 127.96, 127.65, 127.60, 108.91, 107.34, 52.57, 39.69. MS (ESI) *m/z* 231 $[M + H]^+$.

***N*-((5-(2-(Benzyloxy)phenyl)thiophen-2-yl)methyl)-2-phenylacetamide (66).**

Starting from **66a** (22 mg, 0.07 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **67** as colorless oil (25 mg, 90%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.59 (d, *J* = 6.5 Hz, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.39 – 7.30 (m, 7H), 7.27 – 7.22 (m, 3H), 6.98 (t, *J* = 7.6 Hz, 2H), 6.82 (d, *J* = 3.6 Hz, 1H), 5.73 (s, 1H), 5.19 (brs, 1H), 4.56 (d, *J* = 5.6 Hz, 2H), 3.60 (s, 2H). MS (ESI) *m/z* 436 $[M + Na]^+$.

Methyl 2-(5-((2-phenylacetamido)methyl)furan-2-yl)benzoate (33).

Starting from **66b** (145 mg, 0.63 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **67a** as colorless oil (121 mg, 55%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.7 Hz, 1H), 7.53 (d, *J* = 7.6 Hz, 1H), 7.49 – 7.41 (m, 1H), 7.30 (dt, *J* = 10.8, 5.8 Hz, 6H), 6.46 (d, *J* = 3.2 Hz, 1H), 6.21 (d, *J* = 3.1 Hz, 1H), 6.02 (brs, 1H), 4.41 (d, *J* = 5.5 Hz, 2H), 3.78 (s, 3H), 3.57 (s, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 170.93, 151.58, 151.49, 134.88, 131.12, 131.03, 129.97, 129.63, 129.39, 129.22, 128.22, 128.05, 127.89, 127.84, 127.57, 109.55, 109.49, 109.10, 52.58, 43.90, 37.07. MS (ESI) *m/z* 372 $[M + Na]^+$.

2-(5-((2-Phenylacetamido)methyl)furan-2-yl)benzoic acid (34).

To a solution of **33** (78 mg, 0.22 mmol) in MeOH (8 mL), an aqueous solution of sodium hydroxide (1 N, 3 mL) was added. The mixture was heated under reflux at 70 °C for 3 h. The solvent was removed under vacuum. An aqueous solution of hydrochloridric acid (1 N, 3 mL) was added and the organic layer was extracted with EtOAc (3 x 5 mL), dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (15% MeOH in DCM) to give **34** as colorless oil (63 mg, 85%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 10.19 (brs, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.49 (q, *J* = 7.6 Hz, 2H), 7.33 – 7.15 (m, 6H), 6.51 (d, *J* = 2.8 Hz, 1H), 6.35 (brs, 1H), 6.19 (d, *J* = 2.6 Hz, 1H), 4.35 (d, *J* = 5.3 Hz, 2H), 3.54 (s, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 172.08, 152.23, 151.57, 134.85, 131.04, 129.74, 129.49, 129.10, 127.88, 127.45, 109.45, 108.78, 43.46, 36.89, 29.91. MS (ESI) m/z 375 $[M + K]^+$, 334 $[M - H]^+$.

***N*-(Methylsulfonyl)-2-(5-((2-phenylacetamido)methyl)furan-2-yl)benzamide (35).**

To a solution of **33** (39 mg, 0.12 mmol) in dry DCM (13 mL), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (42 mg, 0.22 mmol), 4-(dimethylamino)pyridine (27 mg, 0.22 mmol) and methanesulfonamide (21 mg, 0.22 mmol) were added. After 12 h, water was added (3 mL) and the organic layer was extracted with DCM (3 x 5 mL), dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **35** as colorless oil (10 mg, 20%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.67 (d, *J* = 7.7 Hz, 1H), 7.54 – 7.45 (m, 2H), 7.37 (t, *J* = 7.1 Hz, 1H), 7.32 – 7.23 (m, 5H), 6.70 (d, *J* = 3.3 Hz, 1H), 6.31 (d, *J* = 3.3 Hz, 1H), 4.40 (s, 2H), 3.54 (s, 2H), 3.34 (s, 3H). **^{13}C NMR**: (75 MHz, CD_3OD) δ 152.58, 151.34, 135.65, 130.38, 128.98, 128.35, 128.14, 127.71, 127.36, 126.68, 126.64, 109.23, 109.00, 78.27, 42.60, 40.35, 36.14, 31.88, 29.56, 22.54. MS (ESI) *m/z* 435 $[M + Na]^+$, 451 $[M + K]^+$.

***N*-((5-(2-Hydroxyphenyl)thiophen-2-yl)methyl)-2-phenylacetamide (44).**

Starting from **66** (12 mg, 0.03 mmol), the title compound was prepared following the procedure reported for compound **32**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **44** as colorless oil (9 mg, 100%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.46 – 7.24 (m, 5H), 7.24 – 7.09 (m, 2H), 7.00 – 6.83 (m, 2H), 5.95 – 5.82 (m, 2H), 4.59 (d, *J* = 5.6 Hz, 2H), 3.63 (s, 2H). **^{13}C NMR**: (75 MHz, CD_3OD) δ 172.53, 153.66, 140.38, 140.01, 135.66, 128.95, 128.36, 127.84, 126.69, 125.47, 124.47, 121.48, 119.55, 115.88, 42.62, 38.05, 31.88, 29.51, 22.54. MS (ESI) *m/z* 346 $[M + Na]^+$.

Methyl 2-(5-((2-phenylacetamido)methyl)tetrahydrofuran-2-yl)benzoate (45).

To a solution of **33** (27 mg, 0.08 mmol) in MeOH (10 mL), a catalytic amount of palladium on carbon 10 wt.% was added under argon atmosphere. The reaction environment was saturated with hydrogen gas. The mixture was stirred at 25 °C for 3 h. The suspension was filtered on paper and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **45** as colorless oil (30 mg, 100%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.87 (t, *J* = 6.7 Hz, 1H), 7.57 – 7.37 (m, 2H), 7.36 – 7.20 (m, 6H), 5.91 (brs, 1H), 5.63 (t, *J* = 14.4, 7.0 Hz, 1H), 4.35 – 4.02 (m, 1H), 3.60 (s, 2H), 3.46 – 3.23 (m, 2H), 2.60 – 2.42 (m, 2H), 1.71 – 1.34 (m, 2H). MS (ESI) *m/z* 376 $[M + Na]^+$.

2-(5-((2-Phenylacetamido)methyl)tetrahydrofuran-2-yl)benzoic acid (46).

Starting from **46** (15 mg, 0.04 mmol), the title compound was prepared following the procedure reported for compound **34**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **46** as yellow oil (6 mg, 43%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.87 (t, *J* = 6.7 Hz, 1H), 7.57 – 7.37 (m, 2H), 7.36 – 7.20 (m, 6H), 5.91 (brs, 1H), 5.63 (t, *J* = 14.4, 7.0 Hz, 1H), 4.35 – 4.02 (m, 1H), 3.60 (s, 2H), 3.46 – 3.23 (m, 2H), 2.60 – 2.42 (m, 2H), 1.71 – 1.34 (m, 2H). MS (ESI) *m/z* 363 $[M + Na]^+$, 339 $[M - H]^+$.

5.3 Synthesis of compounds 36-37.

5-(2-(Methylthio)phenyl)furan-2-carbaldehyde (69).

To a suspension of **67** (1.2 g, 8.61 mmol) in H_2O (20 mL), hydrochloridric acid (37 %, 3.6 mL) was added. The resulting solution was cooled at 0 °C and a solution of sodium nitrite (714 mg, 10.4 mmol) in H_2O (10 mL) was added dropwise. After 1 h, a solution of **68** (714 µL, 8.61 mmol) in acetone (3 mL) and solid copper(II) chloride (231 mg; 1.71 mmol) were added. The mixture kept at 25 °C for 12 h. EtOAc was added (3 x 15 mL) and the organic phase was separated, dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **69** as orange oil (563 mg, 30%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 9.68 (s, 1H), 7.87 (d, *J* = 6.5 Hz, 2H), 7.40 – 7.31 (m, 3H), 7.28 – 7.22 (m, 2H), 7.18 (d, *J* = 3.8 Hz, 1H), 2.52 (s, 3H). MS (ESI) *m/z* 241 $[M + Na]^+$.

(5-(2-(Methylthio)phenyl)furan-2-yl)methanol (70).

Starting from **69** (433 mg, 2.0 mmol), the title compound was prepared following the procedure reported for compound **55**. The crude product was purified by flash chromatography on silica

gel (10% EtOAc in petroleum ether) to give **70** as colorless oil (271 mg, 65%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.72 (d, *J* = 7.9 Hz, 1H), 7.27 (dd, *J* = 4.4, 3.1 Hz, 2H), 7.24 – 7.17 (m, 1H), 6.88 (d, *J* = 3.3 Hz, 1H), 6.43 (d, *J* = 3.3 Hz, 1H), 4.68 (d, *J* = 5.5 Hz, 2H), 2.49 (s, 3H). MS (ESI) *m/z* 243 $[M + Na]^+$.

2-(Azidomethyl)-5-(2-(methylthio)phenyl)furan (71).

Starting from **70** (271 mg, 1.23 mmol), the title compound was prepared following the procedure reported for compound **56**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **71** as colorless oil (168 mg, 60%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 1H), 7.32 – 7.20 (m, 3H), 6.97 (d, *J* = 3.4 Hz, 1H), 6.49 (d, *J* = 3.4 Hz, 1H), 4.36 (s, 2H), 2.50 (s, 3H). MS (ESI) *m/z* 246 $[M + H]^+$.

(5-(2-(Methylthio)phenyl)furan-2-yl)methanamine (72).

Starting from **71** (168 mg, 0.68 mmol), the title compound was prepared following the procedure reported for compound **57**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **72** as colorless oil (100 mg, 90%). **^{1}H NMR**: (300 MHz, CD_3Cl) δ 7.68 (d, *J* = 7.1 Hz, 1H), 7.26 – 7.12 (m, 3H), 6.84 (d, *J* = 3.3 Hz, 1H), 6.23 (d, *J* = 3.2 Hz, 1H), 3.84 (s, 2H), 2.44 (s, 3H). MS (ESI) *m/z* 242 $[M + Na]^+$.

***N*-((5-(2-(Methylthio)phenyl)furan-2-yl)methyl)-2-phenylacetamide (36).**

Starting from **72** (100 mg, 0.46 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **36** as yellow oil (108 mg, 70%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.6 Hz, 1H), 7.37 – 7.23 (m, 7H), 7.18 (dd, *J* = 7.8, 4.3 Hz, 1H), 6.82 (d, *J* = 3.3 Hz, 1H), 6.26 (d, *J* = 3.3 Hz, 1H), 6.00 (brs, 1H), 4.46 (d, *J* = 5.5 Hz, 2H), 3.59 (s, 2H), 2.46 (s, 3H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 170.96, 151.43, 150.66, 135.78, 134.95, 129.68, 129.24, 129.17, 129.07, 128.10, 127.78, 127.58, 125.90, 124.93, 111.25, 109.35, 43.93, 43.70, 37.06, 16.25.MS (ESI) *m/z* 360 $[M + Na]^+$.

***N*-((5-(2-(Methylsulfinyl)phenyl)furan-2-yl)methyl)-2-phenylacetamide (37).**

To a solution of **36** (25 mg, 0.07 mmol) in $CHCl_3$ (3 mL), *m*-chloroperbenzoic acid (13 mg, 0.07 mmol) was added at 0 °C. After 30 min, water (1 mL) was added and the organic layer was extracted with $CHCl_3$ (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **37** as yellow oil (17 mg, 70%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 8.16 (d, *J* = 7.7, 1.3 Hz,

1H), 7.60 – 7.44 (m, 3H), 7.42 – 7.24 (m, 5H), 6.57 (d, *J* = 3.4 Hz, 1H), 6.27 (d, *J* = 3.4 Hz, 1H), 5.95 (brs, 1H), 4.52 (dd, *J* = 15.7, 5.9 Hz, 1H), 4.42 (dd, *J* = 15.8, 5.6 Hz, 1H), 3.66 (s, 2H), 2.64 (s, 3H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 171.08, 152.44, 151.09, 143.28, 134.73, 130.87, 129.69, 129.39, 129.07, 127.79, 127.29, 126.90, 123.97, 110.29, 109.90, 43.95, 43.43, 36.83, 31.15, 1.24. MS (ESI) *m/z* 376 $[M + Na]^+$.

5.4 Synthesis of compounds 38-39.

5-Phenylthiophene-2-carbaldehyde (74).

Starting from **73** (600 mg, 3.8 mmol), the title compound was prepared following the procedure reported for compound **54**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **74** as yellow oil (350 mg, 21%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.93 (s, 1H), 7.79 – 7.70 (m, 3H), 7.65 (d, *J* = 7.2 Hz, 1H), 7.47 – 7.37 (m, 2H), 7.21 – 7.18 (m, 1H). MS (ESI) *m/z* 189 $[M + H]^+$.

(5-Phenylthiophen-2-yl)methanol (75).

Starting from **74** (70 mg, 0.37 mmol), the title compound was prepared following the procedure reported for compound **55**. The crude product was purified by flash chromatography on silica gel (10% EtOAc in petroleum ether) to give **75** as yellow oil (40 mg, 60%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.60 – 7.54 (m, 2H), 7.39 – 7.32 (m, 2H), 7.30 – 7.22 (m, 1H), 7.15 (dd, *J* = 3.3, 2.4 Hz, 1H), 6.95 (d, *J* = 2.6 Hz, 1H), 4.79 (s, 2H), 2.06 (brs, 1H). MS (ESI) *m/z* 191 $[M + H]^+$.

2-(Azidomethyl)-5-phenylthiophene (76).

Starting from **75** (40 mg, 0.21 mmol), the title compound was prepared following the procedure reported for compound **56**. The crude product was purified by flash chromatography on silica gel (5% EtOAc in petroleum ether) to give **76** as colorless oil (30 mg, 60%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.9 Hz, 2H), 7.40 – 7.34 (m, 2H), 7.31 – 7.22 (m, 1H), 7.18 (d, *J* = 1.9 Hz, 1H), 7.00 (d, *J* = 1.4 Hz, 1H), 4.47 (s, 2H). MS (ESI) *m/z* 238 $[M + Na]^+$.

(5-Phenylthiophen-2-yl)methanamine (77).

Starting from **76** (30 mg, 0.14 mmol), the title compound was prepared following the procedure reported for compound **57**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **77** as colorless oil (26 mg, 99%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 6.6 Hz, 2H), 7.33 (d, *J* = 6.8 Hz, 2H), 7.24 (t, *J* = 6.8 Hz, 1H), 7.12 (s, 1H), 6.85 (s, 1H), 4.03 (s, 2H), 1.87 (brs, 2H). MS (ESI) *m/z* 190 $[M + H]^+$.

5-Phenylthiophene-2-carboxylic acid (78).

To a solution of **74** (50 mg, 0.26 mmol) in a mixture of H_2O (4 mL) and MeOH (1 mL), an aqueous solution of sodium hydroxide (1 N, 0.5 mL) and powered silver oxide (150 mg, 0.65 mmol) were added. The mixture was heated under reflux for 5 h. The solvent was removed under vacuum. An aqueous solution of hydrochloridric acid (1 N, 1 mL) was added and the organic layer was extracted with EtOAc (3 x 5 mL), dried over sodium sulfate, filtered and evaporated in vacuo giving **78** as yellow oil without further purification (25 mg, 47%). **^{1}H NMR**: (400 MHz, $CDCl_3$) δ 10.92 (brs, 1H), 7.88 (d, *J* = 3.1 Hz, 1H), 7.84 (d, *J* = 3.8 Hz, 1H), 7.66 – 7.58 (m, 2H), 7.48 – 7.20 (m, 2H), 7.16 – 7.09 (m, 1H). MS (ESI) *m/z* 203 $[M - H]^+$.

***N*-(Benzyloxy)-5-phenylthiophene-2-carboxamide (38).**

Starting from **78** (25 mg, 0.12 mmol) and benzylhydroxylamine (37 mg, 0.24 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **38** as amorphous solid (30 mg, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.73 (brs, 1H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.49 (s, 1H), 7.46 – 7.29 (m, 7H), 7.25 – 7.20 (m, 1H), 7.04 (s, 1H), 4.97 (s, 2H). MS (ESI) *m/z* 332 $[M + Na]^+$.

1-Phenyl-*N*-((5-phenylthiophen-2-yl)methyl)methanesulfonamide (39).

To a solution of **77** (30 mg, 0.15 mmol) in dry DCM (8 mL), triethylamine (23µL, 0.16 mmol) and benzene sulfonyl chloride (30 mg, 0.15 mmol) were added at 0 °C and the mixture warmed to 25 °C. After this time, water (2 mL) was added and the organic layer was extracted with DCM (3 x 3 mL), dried over sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **39** as amorphous solid (30 mg, 58%). **^{1}H NMR** (400 MHz, Acetone) δ 7.61 (d, *J* = 7.4 Hz, 2H), 7.41 – 7.19 (m, 9H), 7.00 (d, *J* = 3.4 Hz, 1H), 6.64 (brs, 1H), 4.38 (d, *J* = 5.4 Hz, 2H), 4.32 (s, 2H). (ESI) m/z 366 $[M + Na]^+$.

5.5 Synthesis of compounds 43, 48-50

Methyl 2-(benzyloxy)benzoate (80).

Starting from **79** (256 µL, 1.9 mmol), the title compound was prepared following the procedure reported for compound **52**. The crude product was purified by flash chromatography on silica gel (2% EtOAc in petroleum ether) to give **80** as uncolorless oil (275 mg, 60%). **^{1}H NMR**: (300

MHz, $CDCl_3$) δ 7.85 (d, *J* = 5.6 Hz, 1H), 7.51 (d, *J* = 6.3 Hz, 2H), 7.45 – 7.28 (m, 4H), 7.01 (d, *J* = 7.9 Hz, 2H), 5.18 (s, 2H), 3.90 (s, 3H). (ESI) m/z 265 $[M + Na]^+$.

2-(Benzyloxy)benzohydrazide (81).

To a solution of **80** (140 mg, 0.5 mmol) in EtOH (4 mL), hydrazine monohydrate (314 µL, 10 mmol) was added. Reaction refluxed for 12 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **81** as yellow oil (85 mg, 70%). **^{1}H NMR**: (300 MHz, Acetone-d_6) δ 8.93 (brs, 1H), 8.08 – 8.00 (m, 1H), 7.61 – 7.50 (m, 2H), 7.49 – 7.27 (m, 5H), 7.26 – 7.03 (m, 1H), 5.32 (s, 2H), 4.32 (brs, 2H). (ESI) m/z 265 $[M + H]^+$.

4-Benzyl-3-(2-(benzyloxy)phenyl)-4*H*-1,2,4-triazole (82).

To a solution of **81** (1.2 g, 0.95 mmol) in MeCN (50 mL), *N*,*N*-dimethylformamide dimethyl acetal (685 µL , 5.12 mmol) was added. Reaction was heated at 50 °C for 1 h. After this time, benzyl amine (548 µL, 4.60 mmol) and acetic acid (4 mL) were added and reaction refluxed at 120 °C for 12 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in EtOAc) to give **82** as yellow oil (873 mg, 50%). **^{1}H NMR** (300 MHz, Acetone-d_6) δ 8.36 (s, 1H), 7.55 – 7.46 (m, 1H), 7.41 – 7.29 (m, 5H), 7.28 – 7.18 (m, 5H), 7.10 – 6.94 (m, 3H), 5.19 (s, 2H), 5.16 (s, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 156.93, 152.83, 136.66, 135.28, 132.56, 131.84, 128.66, 128.54, 128.43, 128.16, 127.95, 127.66, 127.34, 127.20, 126.89, 121.15, 115.72, 113.04, 70.40, 48.77. (ESI) m/z 342 $[M + H]^+$.

(4-Benzyl-5-(2-(benzyloxy)phenyl)-4*H*-1,2,4-triazol-3-yl)methanol (83).

A solution of **82** (100 mg, 0.29) in formaldehyde (aq. sol. 37%, 2,8 mL) refluxed for 12 h. The solvent was removed under vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH in DCM) to give **83** as uncolorless oil (100 mg, 94%). **^{1}H NMR**: (300 MHz, CD_3OD) δ 7.47 (m, 1H), 7.35 – 7.21 (m, 6H), 7.20 – 7.08 (m, 4H), 7.01 (d, *J* = 5.8 Hz, 1H), 6.89 (m, 2H), 5.21 (s, 2H), 5.06 (s, 2H), 4.64 (s, 1H). **^{13}C NMR**: (75 MHz, CD_3OD) δ 156.91, 136.74, 135.33, 132.57, 131.89, 128.76, 128.57, 128.51, 128.25, 128.04, 127.90, 127.74, 127.40, 127.09, 121.17, 116.10, 113.10, 70.19, 54.45, 47.41. (ESI) m/z 372 $[M + H]^+$.

4-Benzyl-3-(2-(benzyloxy)phenyl)-5-(chloromethyl)-4*H*-1,2,4-triazole (84).

To a solution of **83** (100 mg, 0.27 mmol) in dry DCM (4 mL), pyridine (23 µL, 0.29 mmol) and thionyl chloride (25 µL, 0.29 mmol) were added at 0 °C. After 3 h an aqueous solution of sodium bicarbonate (1 mL) was added and the organic phase was extracted with DCM (3 x 5

mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (5% MeOH in EtOAc) to give **84** as yellow oil (20 mg, 40%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.55 – 7.47 (m, 1H), 7.35 – 7.27 (m, 5H), 7.25 – 7.14 (m, 4H), 7.09 – 7.01 (m, 1H), 6.94 – 6.88 (m, 3H), 5.18 (s, 2H), 5.11 (s, 2H), 4.69 (s, 2 H). **^{13}C NMR** (75 MHz, CD_3OD) δ 156.90, 154.95, 152.07, 136.63, 134.65, 132.75, 131.75, 128.68, 128.43, 128.10, 127.87, 127.08, 126.92, 121.19, 115.75, 113.01, 70.27, 33.49. (ESI) m/z 390 $[M + H]^+$, 412 $[M + Na]^+$.

3-(Azidomethyl)-4-benzyl-5-(2-(benzyloxy)phenyl)-4*H*-1,2,4-triazole (85).

To a solution of **84** (20 mg, 0.05 mmol) in dry DMF (4 mL), sodium azide (7 mg, 0.10 mmol) was added at 0 °C. Reaction refluxed at 90 °C for 12 h. The solvent was removed under vacuum giving the product **85** as yellow oil without further purification (30 mg, 90 %). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.53 – 7.41 (m, 2H), 7.32 (m, 3H), 7.22 (dd, *J* = 17.6, 6.3 Hz, 5H), 7.13 – 7.03 (m, 2H), 6.89 (d, *J* = 7.7 Hz, 2H), 5.06 (s, 4H), 4.31 (s, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 156.72, 150.36, 136.36, 134.83, 132.75, 132.50, 129.14, 128.88, 128.48, 128.33, 127.18, 126.96, 121.85, 116.86, 113.16, 111.36, 70.86, 48.32, 45.38. (ESI) m/z 397 $[M + H]^+$, 419 $[M + Na]^+$.

(4-Benzyl-5-(2-(benzyloxy)phenyl)-4*H*-1,2,4-triazol-3-yl)methanamine (86).

Starting from **85** (30 mg, 0.07 mmol), the title compound was prepared following the procedure reported for compound **57**. The crude product was purified by flash chromatography on silica gel (10% MeOH in DCM) to give **86** as uncolorless oil (10 mg, 40%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.50 – 7.43 (m, 2H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 7.0 Hz, 1H), 7.24 (d, *J* = 7.8 Hz, 3H), 7.17 (d, *J* = 6.6 Hz, 3H), 7.08 – 6.98 (m, 2H), 6.90 (d, *J* = 5.7 Hz, 2H), 5.05 (d, *J* = 9.0 Hz, 4H), 3.79 (s, 2H), 1.59 (brs, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 156.79, 136.56, 135.56, 132.82, 132.19, 129.24, 129.04, 128.87, 128.43, 128.23, 127.21, 126.86, 125.50, 121.77, 117.37, 113.24, 70.83, 68.18, 47.82, 37.81. (ESI) m/z 371 $[M + H]^+$.

2-(5-(Aminomethyl)-4*H*-1,2,4-triazol-3-yl)phenol (50).

To a solution of **85** (18 mg, 0.04 mmol) in MeOH (6 mL), a catalytic amount of palladium on carbon 10 wt.% was added under argon atmosphere. The reaction environment was saturated with hydrogen gas. The mixture was stirred at 25 °C for 12 h. The suspension was filtered on paper and concentrated in vacuo giving **50** as uncolorless oil without further purification (5 mg, 71%). **^{1}H NMR** (300 MHz, Acetone) δ 8.04 (d, *J* = 7.7 Hz, 1H), 7.40 – 7.19 (m, 1H), 7.03 – 6.82 (m, 2H), 4.70 (s, 2H), 4.51 (s, 1H), 1.29 (s, 2H). **^{13}C NMR** (75 MHz, Acetone) δ 157.04,

131.85, 130.88, 126.77, 126.62, 119.29, 117.24, 116.97, 47.30. (ESI) m/z 191 $[M + H]^+$, 213 $[M + Na]^+$.

5-(2-(Benzyloxy)phenyl)-4*H*-1,2,4-triazole-3-carbaldehyde (87).

To a solution of **83** (50 mg, 0.13 mmol) in dry DCM (5 mL) Des Martin periodinane (57 mg, 0.13 mmol) was added at 0 °C. The reaction mixture warmed to 25 °C. After 2 h, a saturated solution of sodium thiosulfate (3 mL) was added and the organic layer was extracted with DCM (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (50% EtOAc in petroleum ether) to give **87** as uncolorless oil (40 mg, 81%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 10.09 (s, 1H), 7.53 – 7.02 (m, 12H), 6.83 (d, *J* = 6.5 Hz, 2H), 5.44 (s, 2H), 5.05 (s, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 182.13, 156.65, 156.47, 151.40, 136.17, 135.43, 133.08, 132.63, 128.92, 128.81, 128.42, 128.32, 127.46, 127.21, 121.82, 115.67, 113.34, 70.92, 48.77. (ESI) m/z 280 $[M + H]^+$.

Tert-butyl4-((((5-(2-(benzyloxy)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)methyl)piperidine-1-carboxylate (88).

A solution of aldehyde **87** (40 mg, 0.10 mmol) and Boc-piperidine methanamine (25 mg, 0.12 mmol) in MeOH (5 mL), refluxed for 12 h. After this time, the reaction was cooled at 0 °C and sodium borohydride (4 mg, 0.10 mmol) was added. After 1h, the solvent was removed in vacuo, EtOAc was added (10 mL) and the organic phase was washed with a saturated solution of ammonium chloride (1 x 2 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **91** as yellow oil (40 mg, 71%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.55 – 7.46 (m, 2H), 7.39 – 7.00 (m, 10H), 6.91 (d, *J* = 6.1 Hz, 2H), 5.23 (s, 2H), 5.10 (s, 2H), 4.00 (d, *J* = 13.6 Hz, 2H), 3.81 (brs, 2H), 2.67 (brs, 2H), 2.35 (s, 2H), 1.59 (d, *J* = 12.2 Hz, 2H), 1.01 – 0.82 (m, 3H). **^{13}C NMR** (75 MHz, CD_3OD) δ 157.03, 155.28, 136.77, 135.57, 132.55, 131.88, 128.64, 128.45, 127.93, 127.85, 127.27, 126.77, 121.13, 116.08, 113.03, 79.68, 70.31, 54.60, 43.64, 36.27, 36.13, 30.12, 27.53. (ESI) m/z 568 $[M + H]^+$.

Tert-butyl4-((((5-(2-hydroxyphenyl)-4*H*-1,2,4-triazol-3-yl)methyl)amino)methyl)piperidine -1-carboxylate (89).

Starting from **88** (40 mg, 0.07 mmol), the title compound was prepared following the procedure reported for compound **50**. The crude product was purified by flash chromatography on silica gel (10% MeOH in DCM) to give **89** as yellow oil (20 mg, 74%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.91 (d, *J* = 6.3 Hz, 1H), 7.34 – 7.26 (m, 1H), 6.95 (dd, *J* = 14.1, 7.4 Hz, 2H), 4.05 (d, *J* = 15.8

Hz, 2H), 3.96 (s, 2H), 2.74 (brs, 2H), 2.58 (d, *J* = 6.3 Hz, 2H), 1.75 (d, *J* = 11.9 Hz, 3H), 1.43 (d, *J* = 1.3 Hz, 9H), 1.09 (d, *J* = 9.4 Hz, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 172.37, 158.15, 156.60, 155.49, 131.34, 126.82, 119.39, 116.70, 79.73, 54.59, 45.01, 35.82, 30.12, 27.49. (ESI) m/z 388 $[M + H]^+$.

2-(5-(((Piperidin-4-ylmethyl)amino)methyl)-4*H*-1,2,4-triazol-3-yl)phenol (48).

To a solution of **89** (10 mg, 0.02 mmol) in dry DCM (1 mL), trifluoroacetic acid (1 mL) was added at 0 °C. The reaction was warmed to 25 °C. After 12 h, the solvent was removed in vacuum. A saturated solution of sodium bicarbonate (1 mL) was added and the organic layer was extract with DCM (3 x 3 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (10% MeOH, 1% NH_4OH, in DCM) to give **48** as uncolorless oil (5 mg, 70%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.92 (d, *J* = 6.3 Hz, 1H), 7.33 (t, *J* = 7.0 Hz, 1H), 6.97 (dd, *J* = 13.6, 7.4 Hz, 2H), 4.03 (s, 2H), 3.39 (d, *J* = 12.6 Hz, 2H), 2.98 (t, *J* = 12.9 Hz, 2H), 2.69 (d, *J* = 6.7 Hz, 2H), 2.03 (d, *J* = 13.4 Hz, 2H), 1.89 – 1.82 (m, 1H), 1.42 (d, *J* = 13.4 Hz, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 156.55, 131.58, 126.80, 120.32, 116.71, 53.58, 43.81, 33.40, 29.56, 26.92. (ESI) m/z 288 $[M + H]^+$.

***N*-((4-Benzyl-5-(2-(benzyloxy)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)benzamide (90).**

Starting from **86** (30 mg, 0.08 mmol) and benzoic acid (5 mg, 0.04 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **90** as uncolorless oil (28 mg, 75%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.2 Hz, 3H), 7.50 – 7.41 (m, 3H), 7.41 – 7.33 (m, 3H), 7.22 (s, 4H), 7.17 – 7.11 (m, 3H), 7.03 (t, *J* = 8.0 Hz, 2H), 6.92 (dd, *J* = 6.3, 2.6 Hz, 2H), 5.19 (s, 2H), 5.02 (s, 2H), 4.65 (d, *J* = 5.2 Hz, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 167.61, 156.78, 136.36, 135.50, 133.67, 132.80, 132.35, 131.86, 129.03, 128.83, 128.64, 128.24, 128.16, 127.56, 127.17, 126.84, 121.70, 117.01, 113.19, 70.80, 47.94, 35.08. (ESI) m/z 475 $[M + H]^+$.

***N*-((4-Benzyl-5-(2-(benzyloxy)phenyl)-4*H*-1,2,4-triazol-3-yl)methyl)-2-phenylacetamide (91).**

Starting from **86** (10 mg, 0.02 mmol) and phenyl acetic acid (2 mg, 0.01 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **91** as uncolorless oil (10 mg, 100%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.40 (m, 2H), 7.32 – 1.16 (m, 12H), 7.09 – 6.97 (m, 4H), 6.85 (d, *J* = 5.6 Hz, 2H), 5.13 (s, 2H), 5.01 (s, 2H), 4.41 (d, *J* = 5.4 Hz, 2H), 3.47 (s, 2H). **^{13}C NMR**: (75 MHz, $CDCl_3$) δ 171.38, 156.75, 154.13, 152.74, 136.42,

135.55, 134.75, 132.76, 132.32, 129.60, 129.14, 129.06, 129.00, 128.88, 128.29, 128.12, 127.44, 127.25, 127.21, 126.72, 121.69, 116.98, 113.21, 70.81, 53.66, 47.79, 43.40, 34.58, 29.92. (ESI) m/z 511 $[M + Na]^+$.

***N*-((5-(2-Hydroxyphenyl)-4*H*-1,2,4-triazol-3-yl)methyl)benzamide (49).**

Starting from **90** (28 mg, 0.06 mmol), the title compound was prepared following the procedure reported for compound **50**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **49** as uncolorless oil (9 mg, 50%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 11.55 (brs, 1H), 7.87 – 7.71 (m, 3H), 7.51 – 7.38 (m, 2H), 7.38 – 7.29 (m, 2H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.88 (t, *J* = 7.4 Hz, 1H), 4.76 (d, *J* = 5.4 Hz, 2H). **^{13}C NMR**: (75 MHz, CD_3OD) δ 169.26, 156.61, 134.03, 131.70, 131.41, 128.39, 127.30, 126.65, 119.43, 116.72, 78.27, 47.53, 47.24, 46.96, 36.49. (ESI) m/z 295 $[M + H]^+$, 317 $[M + Na]^+$.

***N*-((5-(2-Hydroxyphenyl)-4*H*-1,2,4-triazol-3-yl)methyl)-2-phenylacetamide (43).**

Starting from **91** (16 mg, 0.03 mmol), the title compound was prepared following the procedure reported for compound **50**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **43** as uncolorless oil (5 mg, 50%). **^{1}H NMR**: (300 MHz, Methanol-d_4) δ 7.89 (d, *J* = 7.5 Hz, 1H), 7.35 – 7.26 (m, 4H), 7.26 – 7.19 (m, 2H), 7.00 – 6.90 (m, 2H), 4.54 (s, 2H), 3.60 (s, 2H). **^{13}C NMR**: (75 MHz, CD_3OD) δ 173.11, 156.63, 135.48, 131.46, 129.01, 128.39, 126.72, 126.62, 119.41, 116.73, 65.71, 42.46, 36.12, 29.57, 22.20, 14.24, 13.17. (ESI) m/z 309 $[M + H]^+$, 331 $[M + Na]^+$.

5.6 Synthesis of compounds 42 and 47.

Ethyl 5-(2-(benzyloxy)phenyl)-1,3,4-oxadiazole-2-carboxylate (92).

To a solution of **81** (200 mg, 0.82 mmol) in dry DCM (10 mL), triethylamine (346 µL, 2.46 mmol) and ethyl chlorooxoacetate (92 µL, 0.82 mmol) were added dropwise at 0 °C and the reaction mixture warmed to 25 °C. After 5 h, tosyl chloride (155 mg, 0.82 mmol) was added and the mixture refluxed for 12 h. The organic layer was washed with water (1 x 2 mL), a saturated solution of sodium bicarbonate (1 x 2mL) and brine (1 x 2 mL), dried over sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (25% EtOAc, in petroleum ether) to give **92** as amorphous solid (65 mg, 25%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.63 – 7.48 (m, 3H), 7.44 – 7.25 (m, 3H), 7.15 – 7.06 (m, 2H), 5.26 (s, 2H), 4.53 (q, *J* = 7.1 Hz, 2H), 1.45 (t, *J* = 7.1 Hz, 3H). **^{13}C**

NMR (75 MHz, $CDCl_3$) δ 165.76, 157.40, 156.73, 154.77, 136.34, 134.34, 131.38, 128.81, 128.11, 127.06, 121.41, 119.84, 113.67, 112.62, 70.73, 63.52, 14.36. (ESI) m/z 347 $[M + Na]^+$.

(5-(2-(Benzyloxy)phenyl)-1,3,4-oxadiazol-2-yl)methanol (93).

Starting from **92** (20 mg, 0.06 mmol), the title compound was prepared following the procedure reported for compound **55**. The crude product was purified by flash chromatography on silica gel (50% EtOAc in petroleum ether) to give **93** as uncolorless oil (18 mg, 100%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.5 Hz, 1H), 7.47 (dd, *J* = 13.5, 7.7 Hz, 3H), 7.40 – 7.22 (m, 3H), 7.06 (t, *J* = 7.9 Hz, 2H), 5.22 (s, 2H), 4.90 (d, *J* = 6.5 Hz, 2H), 1.83 (brs, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 157.03, 136.60, 133.42, 130.88, 128.77, 128.14, 127.08, 121.37, 113.80, 113.52, 70.80, 55.58. (ESI) m/z 305 $[M + Na]^+$.

2-(Azidomethyl)-5-(2-(benzyloxy)phenyl)-1,3,4-oxadiazole (94).

Starting from **93** (60 mg, 0.21 mmol), the title compound was prepared following the procedure reported for compound **56**. The crude product was purified by flash chromatography on silica gel (10% EtOAc in petroleum ether) to give **94** as uncolorless oil (50 mg, 78%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 8.4 Hz, 2H), 7.42 – 7.13 (m, 4H), 7.07 (t, *J* = 7.7 Hz, 2H), 5.23 (s, 2H), 4.57 (s, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 176.30, 136.53, 133.70, 130.96, 130.07, 129.23, 128.78, 128.19, 127.13, 121.39, 120.54, 120.36, 120.29, 113.79, 70.80, 44.62. (ESI) m/z 308 $[M + H]^+$.

(5-(2-(Benzyloxy)phenyl)-1,3,4-oxadiazol-2-yl)methanamine (95).

Starting from **94** (50 mg, 0.16 mmol), the title compound was prepared following the procedure reported for compound **57**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **95** as uncolorless oil (15 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 – 7.92 (m, 1H), 7.62 – 7.41 (m, 3H), 7.35 (dt, *J* = 19.5, 7.0 Hz, 3H), 7.08 (t, *J* = 7.7 Hz, 2H), 5.22 (s, 2H), 4.09 (s, 2H), 1.83 (s, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 176.30, 136.53, 133.70, 130.96, 130.07, 129.23, 128.78, 128.19, 127.13, 121.39, 120.54, 120.36, 120.29, 113.79, 70.80, 44.62. (ESI) m/z 282 $[M + H]^+$.

***N*-((5-(2-(Benzyloxy)phenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-phenylacetamide (96).**

Starting from **95** (10 mg, 0.03 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (5% MeOH in DCM) to give **96** as yellow oil (11 mg, 78%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.90 (d, *J* = 7.1 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 3H), 7.41 – 7.21 (m, 8H), 7.06 (t, *J* = 7.4 Hz, 2H), 6.25 (brs, 1H), 5.22 (s, 2H), 4.69 (d, *J* = 5.6 Hz, 2H), 3.63 (s, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$)

δ 171.28, 164.43, 163.27, 157.01, 136.57, 134.41, 133.41, 130.88, 129.67, 129.29, 128.83, 128.19, 127.73, 127.10, 121.36, 113.81, 113.47, 70.82, 43.62, 34.98. (ESI) m/z 399 $[M + H]^+$.

N-((5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)-2-phenylacetamide (42).

Starting from **96** (11 mg, 0.02 mmol), the title compound was prepared following the procedure reported for compound **32**. The crude product was purified by flash chromatography on silica gel (1% MeOH in DCM) to give **42** as yellow oil (4 mg, 70%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.91 (s, 1H), 7.68 (dd, *J* = 7.9, 1.5 Hz, 1H), , 7.50 – 7.21 (m, 6H), 7.10 (d, *J* = 8.4 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 6.14 (brs, 1H), 4.73 (d, *J* = 5.7 Hz, 2H), 3.69 (s, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.43, 165.12, 162.24, 157.75, 134.21, 134.14, 129.69, 129.44, 127.92, 126.87, 120.18, 117.81, 107.92, 43.67, 34.74, 29.91. (ESI) m/z 309 $[M + H]^+$.

5-(2-(Benzyloxy)phenyl)-1,3,4-oxadiazole-2-carboxylic acid (97).

To a solution of **92** (10 mg, 0.03 mmol) in a mixture of MeCN (1 mL) and water (0.02 mL), lithium bromide (86 mg, 0.9) and triehtyl amine (21 µL, 0.15 mmol) were added. After 5 h, water (0.5 mL) was added and the organic layer was extracted with EtOAc (3 x 3 mL), dried over sodium sulfate, filtered and concentrated giving **97** as yellow oil without further purification (10 mg, 100%). **^{1}H NMR** (300 MHz, CD_3OD) δ 7.94 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.52 (dd, *J* = 11.4, 4.5 Hz, 3H), 7.34 (t, *J* = 7.3 Hz, 2H), 7.25 (t, *J* = 7.6 Hz, 2H), 7.11 (t, *J* = 7.6 Hz, 1H), 5.32 (s, 2H). **^{13}C NMR** (75 MHz, CD_3OD) δ 164.07, 161.55, 158.42, 157.32, 136.92, 133.63, 130.41, 128.33, 128.27, 127.54, 126.92, 121.01, 114.07, 113.05, 70.26.

5-(2-(Benzyloxy)phenyl)-*N*-phenethyl-1,3,4-oxadiazole-2-carboxamide (98).

Starting from **97** (33 mg, 0.11 mmol), the title compound was prepared following the procedure reported for compound **58**. The crude product was purified by flash chromatography on silica gel (2% MeOH in DCM) to give **99** as yellow oil (10 mg, 26%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.8 Hz, 1H), 7.61 – 7.45 (m, 5H), 7.44 – 7.27 (m, 7H), 7.11 (q, *J* = 8.8 Hz, 3H), 5.28 (s, 2H), 3.75 (dd, *J* = 13.5, 6.9 Hz, 2H), 2.95 (t, *J* = 7.1 Hz, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.35, 169.68, 165.57, 138.29, 136.57, 136.41, 134.09, 133.46, 131.20, 131.10, 130.88, 129.02, 128.93, 128.88, 128.83, 128.16, 128.12, 127.11, 127.06, 121.37, 113.81, 113.76, 112.79, 60.60, 41.22, 35.72. (ESI) m/z 400 $[M + H]^+$.

5-(2-Hydroxyphenyl)-*N*-phenethyl-1,3,4-oxadiazole-2-carboxamide (47).

Starting from **98** (10 mg, 0.02 mmol), the title compound was prepared following the procedure reported for compound **32**. The crude product was purified by flash chromatography on silica gel (1% MeOH in DCM) to give **47** as yellow oil (4 mg, 70%). **^{1}H NMR** (300 MHz, CD_3OD) δ

7.94 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.52 (dd, *J* = 11.4, 4.5 Hz, 3H), 7.34 (t, *J* = 7.3 Hz, 2H), 7.25 (t, *J* = 7.6 Hz, 2H), 7.11 (t, *J* = 7.6 Hz, 1H), 3.75 (dd, *J* = 13.5, 6.9 Hz, 2H), 2.95 (t, *J* = 7.1 Hz, 2H).

CHAPTER 6

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