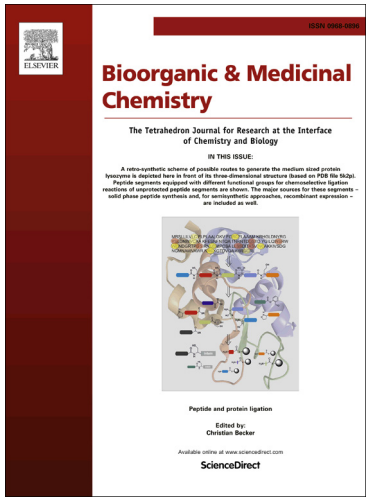
# New 5-HT1A, 5HT2Aand 5HT2C receptor ligands containing a picolinic nucleus: Synthesis, in vitro and in vivo pharmacological evaluation

# Accepted Manuscript




New 5-$HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$ receptor ligands containing a picolinic nucleus: Synthesis, *in vitro* and *in vivo* pharmacological evaluation

Ferdinando Fiorino, Elisa Magli, Ewa Kędzierska, Antonio Ciano, Angela Corvino, Beatrice Severino, Elisa Perissutti, Francesco Frecentese, Paola Di Vaio, Irene Saccone, Angelo A. Izzo, Raffaele Capasso, Paola Massarelli, Ilaria Rossi, Jolanta Orzelska-Gòrka, Jolanta Helena Kotlińska, Vincenzo Santagada, Giuseppe Caliendo

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# New 5-$HT_{1A}$, 5$HT_{2A}$ and 5$HT_{2C}$ receptor ligands containing a picolinic nucleus: Synthesis, *in vitro* and *in vivo* pharmacological evaluation

Ferdinando Fiorino[a*], Elisa Magli[a], Ewa Kędzierska[c], Antonio Ciano[a], Angela Corvino[a], Beatrice Severino[a], Elisa Perissutti[a], Francesco Frecentese[a], Paola Di Vaio[a], Irene Saccone[a] Angelo A. Izzo[a], Raffaele Capasso[d], Paola Massarelli[b], Ilaria Rossi[b], Jolanta Orzelska-Gòrka[c] Jolanta Helena Kotlińska[c], Vincenzo Santagada[a] and Giuseppe Caliendo[a]

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**ABSTRACT**

Picolinamide derivatives, linked to an arylpiperazine moiety, were prepared and their affinity to 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors was evaluated. The combination of structural elements (heterocyclic nucleus, alkyl chain and 4-substituted piperazine), known to play critical roles in affinity for serotoninergic receptors, and the proper selection of substituents led to compounds with high specificity and affinity towards serotoninergic receptors. In binding studies, several molecules showed high affinity in nanomolar and subnanomolar range at 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors and moderate or no affinity for other relevant receptors ($D_1$, $D_2$, $\alpha_1$ and $\alpha_2$). N-(2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethyl)picolinamide (**3o**) with Ki = 0.046 nM, was the most affine and selective derivative for the 5-$HT_{1A}$ receptor compared to other serotoninergic dopaminergic and adrenergic receptors. N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)picolinamide (**3b**), instead, showed a subnanomolar affinity towards 5-$HT_{2A}$ with Ki = 0.0224 nM, whereas N-(2-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)ethyl)picolinamide (**3s**) presented an attractive 5-$HT_{2C}$ affinity with $K_i$ = 0.8 nM. Moreover, the compounds having better affinity and selectivity binding profiles towards 5-$HT_{2A}$ were selected and tested on rat ileum, to determine their effect on 5HT induced contractions. Those more selective towards 5-$HT_{1A}$ receptors were studied in vivo on several behavioral tests.

*Keywords:* Picolinamide derivatives; Synthesis; 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ ligands; Binding assays, *in vitro* assay, behavioural tests.

## 1. INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) [1-3] plays a crucial role in several pivotal physiological and pathophysiological processes such as circadian rhythms, sleep and wake regulation, mood and emotions, sexual behaviors, aggression and anxiety as well as memory and learning processes, sleep thermoregulation, thalamic blood pressure and nociception[4]. It is apparent that the serotonergic system offers therapeutic potential, and several research groups have focused their attention on this specific subject. The 5-HT receptors are widely expressed throughout the brain and in many key structures responsible for cognition and basic brain functions and it's possible to recognize a pathophysiological role in neurodegenerative disorders like Alzheimer's, Huntington's and Parkinson's diseases [5]. As a local mediator, 5-HT is also present in other tissues including the gut, the blood, as vasoactive agent, and immune system.

Among the six families of G-protein-coupled receptors - GPCR 5-HT receptors (5-$HT_{1-7}$ each of them further divided into different subtypes, amounting to fifteen receptors), 5-$HT_{1A}$ mediates effects on a wide range of psychiatric disorders, and its involvement in prostate, bladder and other cancer types was reported [6, 7]. The 5-$HT_2$ receptor family comprises of 3 subtypes namely 5-$HT_{2A}$, 5-$HT_{2B}$ and 5-$HT_{2C}$. Among these, 5-$HT_{2A}$ receptor is the most abundant 5-HT receptor in the human brain and it is involved in the pathophysiology of a variety of neuropsychiatric and neurodegenerative diseases and in the aging of brain [8]. Peripherically, it regulates also intestinal motility and secretions, by means of the conspicuous presence of 5-HT-containing enterochromaffin cells; 5-HT causes diarrhea if present in excess, and constipation if any at fault [3, 9]. 5-$HT_{2C}$ receptors are highly localized in the choroid plexus and are moderately observed in the hypothalamus, globus pallidus, and substantia nigra. Furthermore, the 5-$HT_{2C}$ receptor displays multiple actions on various neurotransmitters and receptors; abnormalities of 5-$HT_{2C}$ receptors are associated with psychiatric diseases such as depression, schizophrenia, drug abuse, anxiety and eating disorder [10]. Finally it's already known that 5-$HT_{2C}$ blockade can prevent the extrapyramidal side effects induced by atypical antipsychotics [11]. The long-chain N-1-substituted N-4-arylpiperazines (LCAPs) have been explored as privileged structures, already known for their high affinity toward many subtypes of 5-HTRs [12]. The significance of the respective parts of the LCAP structures on the 5-$HT_{1A}$ receptor affinity, intrinsic activity, and selectivity has been the subject of numerous published structure-activity relationship (SAR) studies. Extended studies, around this class of compounds, has been devoted to understand both the role of the heterocyclic nucleus and the substituent on the piperazine scaffold in the ligand-receptor interaction and, consequently, the role of different chain spacers between the tail of the molecule and a great number of different heterocyclic fragments used as head [13]. Our research

group has been developing for a long time more selective serotoninergic ligands [14-17] in order to have novel pharmacological tools that could improve our knowledge of the signal transduction mechanism leading to compounds with high affinity and selectivity.

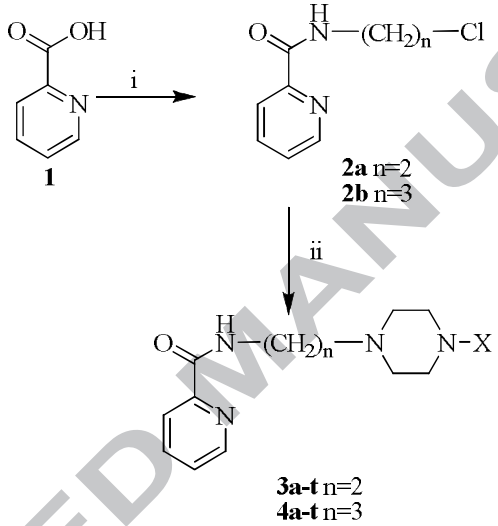
A previously described study focused on the synthesis and pharmacological evaluation of a set of arylpiperazine derivatives containing a N'-cyanopicolinamidine nucleus; the binding data reported in this study identified this original scaffold as an optimal structural element to enhance 5-$HT_{2A}$ receptor affinity [16]. In continuation of our research program, we designed a new set of derivatives where the piperazine-N-alkyl moiety has been linked to a picolinic fragment as terminal part of LCAPs (Scheme 1); this choice was made considering this scaffold as a molecular simplification of derivatives previously synthesized, embodying the N'-cyanopicolinamidine nucleus[16]. A similar investigation was previously made substituting the N'-cyanoisonicotinamidine scaffold with a simpler isonicotinic nucleus [17] and demonstrating that a simple amide bond instead of a cyanoamidine group, provided compounds characterized by 5-$HT_{1A}$, 5-$HT_{2A}$ and mixed 5-$HT_{1A}$/5-$HT_{2C}$ affinity and selectivity. Consequently, also in this case, we decided to investigate the influence of the substitution of a cyanoamidine group with a simple amide bond in terms of binding affinity/selectivity profile. The picolinic scaffold was linked via two and three methylene spacing units to the N-4-aryl-substituted piperazines and this choice was done in order to obtain a complete and comparative structure-affinity and structure-selectivity relationship study. Moreover, the obtained picolinamide derivatives are effectively close to some 2-quinolinamide analogues already reported by Graulich et al. [18] as 5-$HT_{1A}$, D4.2 and $\alpha_2$ receptors ligands. All the new compounds were tested for their affinity to 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors and the multireceptor profiles of promising derivatives were also evaluated in terms of binding affinities for dopaminergic ($D_1$, $D_2$) and adrenergic ($\alpha_1$, $\alpha_2$) receptors. Moreover, the compounds showing better affinity and selectivity binding profile towards 5-$HT_{2A}$ receptors have been tested by *in vitro* assay to evaluate their agonist or antagonist activity towards 5-HT-evoked contractions. Whereas, compounds with a better affinity/selectivity profile towards 5-$HT_{1A}$ have been evaluated by *in vivo* assay (i.e. behavioural tests), to determine their functional activity.

## 2. RESULTS AND DISCUSSION

### 2.1. Chemistry

The synthetic strategy used for the preparation of the picolinamide derivatives is summarized in Scheme 1. Picolinic acid (1) reacted with 2-chloroethanamine hydrochloride or 3-chloropropan-1-amine hydrochloride in acetonitrile, in presence of *N,N'*-dicyclohexylcarbodiimide (DCC),

hydroxybenzotriazole (HOBt) and triethylamine (TEA) to give the corresponding chloroalkylpicolinamides **2a** and **2b**. Subsequent condensation of intermediates **2a** and **2b** with the appropriate 4-X-substituted-piperazine, performed in acetonitrile ($CH_3CN$) with potassium carbonate ($K_2CO_3$) and sodium iodide (NaI), under reflux, provided the final compounds **3a-t** and **4a-t**. Each final product was purified by chromatography on silica gel column and further crystallized from the appropriate solvent. Each compound was characterized by $^1$H-NMR, $^{13}$C-NMR and triple quadrupole mass spectrometry (API 2000 Applied Biosystem). $^1$H-NMR, $^{13}$C-NMR and MS data for all final compounds were consistent with the proposed structures.




**Scheme 1.** Reagents and conditions: (i) $Cl(CH_2)_nNH_2 \cdot HCl$, DCC, HOBt, TEA, $CH_3CN$, r.t., 24h; (ii) 4-X-substituted-piperazine, $K_2CO_3$, NaI, $CH_3CN$, 70°C, 24 h.

### 2.2 *In Vitro* Receptor Binding

Several of the synthesized derivatives showed affinities in the nanomolar range towards 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors (Table 1 and 2). Besides the outstanding 5-$HT_{2A}$ receptor affinity and selectivity of compound **3b** ($K_i$ = 0.0224 nM), other interesting $K_i$ values were those of compounds **3c** (9.33 nM), **3f** (48.5 nM), **3a** (53.2 nM), **4q** (1.68 nM), **4p** (45.3 nM), **4s** (77.8 nM) and **4k** (90.4 nM). Moreover compounds **3j, 3k** and **3o**, showed the most interesting affinity/selectivity profile towards 5-$HT_{1A}$ receptors with $K_i$ values of 0.344, 0.183 and 0.046 nM respectively, whereas compound **3s** presented an attractive 5-$HT_{2C}$ activity with $K_i$ value of 0.8 nM. The two series, **3a-t** and **4a-t**, differ in the length of the connecting chain between the picolinic fragment and the piperazine ring. Unlike the previously reported series of arylpiperazines [14-17], where as general trend three units alkyl chain compounds showed the best affinity/selectivity profile towards 5-$HT_{1A}$ receptors, in this new series, the alkyl chain length, as

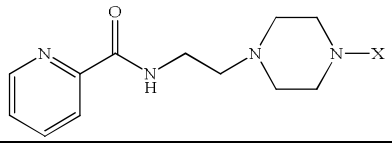
well as a simple amide bond instead of cyanoamidine group, don't seem to be decisive in determining a specific profile towards a specific 5-HT receptors subtype but the affinity/selectivity profile is more influenced by the particular substituent on the piperazine moiety. The pyrimidinyl group associated to a shorter chain spacer (n=2, **3o**), conferred the highest affinity and selectivity values for the 5-$HT_{1A}$ receptor. The presence of a 4-chlorophenyl (**3j**) or a 3,4-dichlorophenyl group (**3k**), associated always to a shorter chain spacer on the N-4 of the piperazine moiety, led also to compounds which exhibited high affinity for 5-$HT_{1A}$ receptor (**3j** $K_i$ = 0.344 nM and **3k** $K_i$ = 0.183). Instead, the furoyl and piperonyl moieties (**4q**, $K_i$ = 1.68 nM and **4p**, $K_i$ 45.3 nM, respectively) associated to a propyl chain spacer and the 2-methoxyphenyl and 4-methoxyphenyl moieties (**3b** $K_i$ = 0.0224 nM and **3c** $K_i$ = 9.33 nM, respectively), associated to an ethyl chain spacer, afforded a favorable affinity profile for 5-$HT_{2A}$ receptors. Finally, bis(4-fluorophenyl)methyl group, associated to a shorter chain spacer (n=2)**,** has led to a compound characterized by 5-$HT_{2C}$ activity with $K_i$ value of 0.8 nM (**3s**). Additionally, the affinity of the most active compounds (**3j**, **3k** and **3o**) on several other receptors ($\alpha_1$ and $\alpha_2$ adrenergic and $D_1$ and $D_2$ dopaminergic receptors) was examined in order to verify the selectivity of these compounds. Results are summarized in Table 3. All the compounds proved highly selective against dopaminergic receptors. Regarding $\alpha_1$ and $\alpha_2$ adrenergic receptors, only compounds **3j** and **3o** showed quite moderate affinity towards $\alpha_1$ (76 nM and 31 nM respectively); these data are very interesting considering the high degree of homology existing between the considered receptors and demonstrate that these compounds possess a very good binding profile.

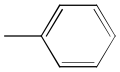
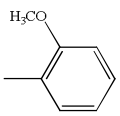
These results further support the choice of the picolinic nucleus not only for the preparation of serotoninergic ligands endowed with high 5-$HT_{1A}$ affinity, but also with a selective 5-$HT_{2A}$ and 5-$HT_{2C}$ activity. Anyway, a comparative study on the structure-affinity and structure-selectivity relationships between the picolinamide and the N'-cyanopicolinamidine derivatives emphasizes the cyanoamidine group as an important structural element able to produce a favorable 5-$HT_{2A}$ receptor affinity/selectivity profile [16]. Concerning the influence of the N-4 substituent of the piperazine moiety, the outstanding affinities of **3b**, **3c**, **4p** and **4q** towards 5-$HT_{2A}$ agree with data already reported in literature [19,20] where compounds embodying an ether group can act as hydrogen bond acceptors from key residues such as threonine and serine in the 5-$HT_{2A}$ receptor binding pocket [21]. The interesting binding result of compound **3b** is also in accordance with a recently reported [22] general pharmacophore model for ligands showing high experimental affinity, consisting of the two aromatic sites and one hydrogen bond donor (HBD) site. Moreover, the pyrimidinyl group associated to a shorter chain spacer (n=2, **3o**), conferred the highest affinity and selectivity for the 5-$HT_{1A}$ receptor whereas other moieties that led to compounds with high

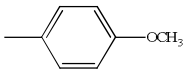
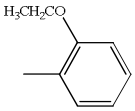
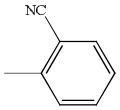
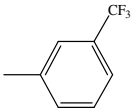
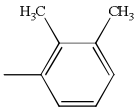
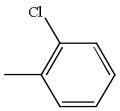
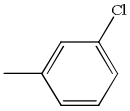
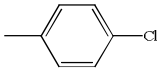
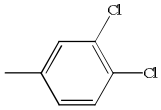
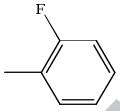
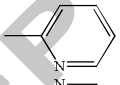
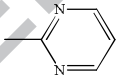
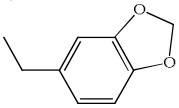
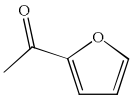
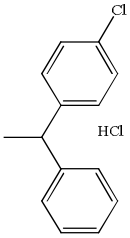
affinity for 5-$HT_{1A}$ receptor are 4-chlorophenyl (n=2, **3j**) and 3,4-dichlorophenyl (n=2, **3k**) groups. These results could be explained in terms of solvent accessibility and hydrophobic interaction with the receptor that are decisive compared to all other compounds [14]. In particular, it was already reported that the pyrimidinyl group, originally present in buspirone and later employed in many 2-pyrimidinylpiperazine analogues, produces a greater affinity for 5-$HT_{1A}$ receptor even if the plane of the 2-pyrimidinyl ring is parallel to piperazine one due to delocalization of the $sp^2/sp^3$ nitrogen into the aromatic system and bringing to only two low-energy conformations, which are essentially isoenergetic [23]. Noteworthy, there are many published SAR studies indicating that 5-$HT_{1A}$ receptor binds preferentially amide analogues containing a two carbon spacer chain [23] and in the present study all the high affinity ligands reported also belongs to derivatives supporting a shorter chain spacer (n=2). Instead, it should be observed that the data obtained with 4-chlorophenyl (n=2, **3j**) group, are in harmony with those already reported in literature [24]. In fact it was already demonstrated that inductive and mesomeric electronic effects of halogen atoms might be some times in competition, and in these molecules, the mesomeric effect, increasing the electronic density of the phenyl ring, could explain the enhancement of affinity in comparison with other analogues [25].

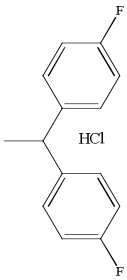
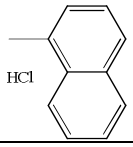
Finally, surprising results have been obtained when a bis(4-fluorophenyl)methyl group, already present in the selective 5-$HT_{2A}$ antagonist lomerizine, [26] was associated to an ethyl chain spacer. Compound **3s**, in fact, showed a favorable affinity profile for 5-$HT_{2C}$ receptors. The same substitution, when done on an isonicotinic scaffold conferred a favorable affinity profile for 5-$HT_{2A}$ receptors, when linked through an ethyl chain, whereas associated to a longer chain spacer, conferred the highest affinity and selectivity for the 5-$HT_{1A}$ receptor. Anyway, the obtained result is particularly interesting and outlines for compound **3s** a profile as chemical tool useful for the pharmacological study of the 5-$HT_{2C}$ receptors.

**Table 1.** Binding affinities values of compounds **3a–t** for 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors.



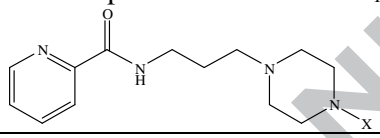

| Comp. | X | Receptor affinity [a]Ki ± SD (nM) | | |
|---|---|---|---|---|
| | | 5-$HT_{1A}$ [$^3$H]8OH-DPAT | 5-$HT_{2A}$ [$^3$H]Ketanserin | 5-$HT_{2C}$ [$^3$H]Mesulergine |
| **3a** |  | 161 ± 13 | 53.2 ± 3.6 | $>10^4$ |
| **3b** |   | 599 ± 25.9 | 0.0224± 0.0047 | $>10^4$ |

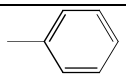
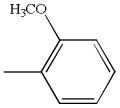
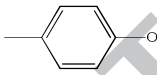
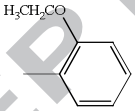
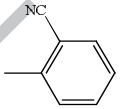
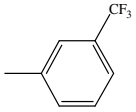
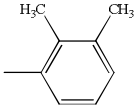
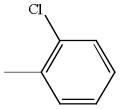
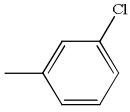
| | | | | |
|---|---|---|---|---|
| **3c** |  | 138 ± 28.5 | 9.33 ± 0.67 | >$10^4$ |
| **3d** |  | 55.4 ± 1.6 | >$10^4$ | >$10^4$ |
| **3e** |  | 74.8 ± 1.9 | 616 ± 43.7 | >$10^4$ |
| **3f**[*] |  | 1320 ± 12 | 48.5 ± 2.7 | 140 ± 2.5 |
| **3g** |  | 215 ± 7 | >$10^4$ | >$10^4$ |
| **3h** |  | 412 ± 40.5 | >$10^4$ | 268 ± 25 |
| **3i** |  | 267 ± 7.6 | 309 ± 41 | 90.9 ± 3.1 |
| **3j** |  | 0.344 ± 0.025 | 178 ± 22.9 | 564 ± 34.7 |
| **3k** |  | 0.183 ± 0.003 | 74.1 ± 0.5 | >$10^4$ |
| **3l** |  | 158 ± 42 | >$10^4$ | >$10^4$ |
| **3m** |  | 82 ±0.9 | 241 ± 21.5 | 79.4 ± 2.2 |
| **3n** |  | 32.7 ± 2.1 | >$10^4$ | >$10^4$ |
| **3o** |  | 0.046 ± 0.01 | >$10^4$ | >$10^4$ |
| **3p** |  | >$10^4$ | >$10^4$ | 61.2 ± 2.3 |
| **3q** |  | >$10^4$ | >$10^4$ | >$10^4$ |
| **3r** |  | 540 ± 8.4 | 504 ± 40.4 | 49.8± 8.2 |

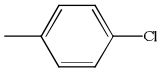
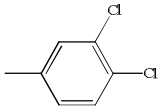
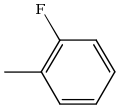
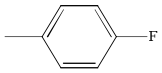
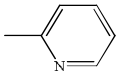
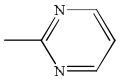
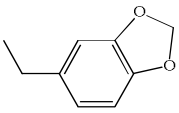
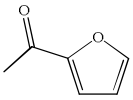
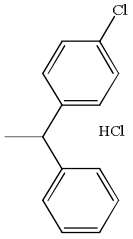
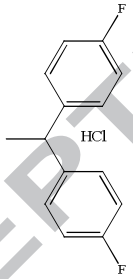
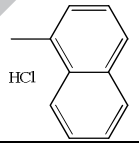
| | | | | |
|---|---|---|---|---|
| **3s** |  | $>10^4$ | 218 ± 9.3 | 0.8 ± 0.05 |
| **3t** |  | 20.8 ± 0.6 | 91.1 ±1.18 | 116 ± 15.8 |

[a]For purpose of comparison, 8-OH-DPAT, Ketanserin and Mesulergine bind 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors with values of 0.80, 0.85 and 1.90 nM, respectively, under these assay conditions. Each value is the mean ± SD of three determinations, as described in Experimental Section. *This derivative was already described in Preparation of heterocyclic D3 receptor ligands for treatment of central nervous disorders (DE 4425146 1996/01/18)

**Table 2.** Binding affinities values of compounds **4a–t** for 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors.



| Comp. | X | Receptor affinity [a]Ki ± SD (nM) 5-$HT_{1A}$ [$^3$H]8OH-DPAT | 5-$HT_{2A}$ [$^3$H]Ketanserin | 5-$HT_{2C}$ [$^3$H]Mesulergine |
|---|---|---|---|---|
| **4a** |  | 104 ± 7.2 | 277 ± 29 | 111 ± 6.7 |
| **4b*** |  | 40.1 ± 0.7 | $>10^4$ | 128±11.9 |
| **4c** |  | $>10^4$ | $>10^4$ | $>10^4$ |
| **4d** |  | 85.6 ± 2.3 | 119 ± 7 | 80 ± 2.07 |
| **4e** |  | 108 ± 12.6 | $>10^4$ | 128 ± 53.2 |
| **4f*** |  | 17.9 ± 0.9 | $>10^4$ | $>10^4$ |
| **4g** |  | 88.7 ± 0.5 | $>10^4$ | 105 ± 13.5 |
| **4h** |  | 71.6 ± 1.9 | 763 ± 38.4 | 1050 ± 101 |
| **4i** |  | 59.5 ± 2.9 | 80.7 ± 1.6 | 101 ± 26.9 |

| | | | | |
|---|---|---|---|---|
| **4j** |  | 68 ± 1.6 | 371 ± 23.7 | 39.7 ± 3.2 |
| **4k** |  | 181 ± 12.5 | 90.4 ± 2.05 | 9.57 ± 0.3 |
| **4l** |  | 230 ± 5.8 | 327 ± 17.2 | $>10^4$ |
| **4m** |  | $>10^4$ | 161 ± 20.1 | 11.7 ± 1.4 |
| **4n** |  | 495 ± 4.5 | $>10^4$ | 140 ± 41.1 |
| **4o** |  | 500 ± 27.2 | 16.8 ± 7.9 | $>10^4$ |
| **4p** |  | $>10^4$ | 45.3 ± 5.7 | $>10^4$ |
| **4q** |  | $>10^4$ | 1.68 ± 0.4 | $>10^4$ |
| **4r** |  | 443 ± 14.8 | 725 ± 42.7 | $>10^4$ |
| **4s** |  | 52.7 ± 2.7 | 77.8 ± 2.1 | 117 ± 6.1 |
| **4t** |  | 44.1 ± 0.2 | 213 ± 15.3 | $>10^4$ |

[a]For purpose of comparison, 8-OH-DPAT, Ketanserin and Mesulergine bind 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors with values of 0.80, 0.85 and 1.90 nM, respectively, under these assay conditions. Each value is the mean ± SD of three determinations, as described in Experimental Section.[*]These derivatives were already described in Amide-based phenyl piperazine derivatives and their salts, preparation method and application for treating benign prostatic hyperplasia (CN 103980195 2014/08/13) (**3b)** and Preparation of heterocyclic D3 receptor ligands for treatment of central nervous disorders (DE 4425146 1996/01/18) (**4f)**

**Table 3**. Affinities of compounds **3j**, **3k** and **3o** for $\alpha_1$, $\alpha_2$, $D_1$ and $D_2$ receptors.

| Comp. | Ki (nM) | | | |
|---|---|---|---|---|
| | **$\alpha_1$ [$^3$H]Prazosin** | **$\alpha_2$ [$^3$H]RX 821002** | **$D_1$ [$^3$H]SCH-23390** | **$D_2$ [$^3$H]Methyl-spiperone** |
| **3j** | 76 | N.C. | N.C. | 2300 |
| **3k** | 31 | 4200 | 610 | 250 |
| **3o** | N.C. | N.C. | N.C. | N.C. |

*N.C.:* Ki not calculable..

### 2.3 *In Vitro* evaluation of 5-HT-evoked contractions

Successively, the compounds with better affinity/selectivity binding profiles towards 5-$HT_{2A}$ receptors have been tested by *in vitro* assay to determine their activity towards 5-HT-evoked contractions. Results are summarized in Table 4. In the rat ileum, 5-$HT_{2A}$ receptors are located on smooth muscles and their activation by 5-HT is known to induce contraction. Consequently, 5-$HT_{2A}$ antagonists depress 5-HT-induced contractions in the rat ileum [27]. According to Briejer and colleagues, we have shown that 5-HT contracted the rat ileum longitudinal muscle. In preliminary experiments we found that the neuronal blocker tetrodotoxin (0.3 μM), the muscarinic receptor antagonist atropine (1 μM), the adrenergic receptor antagonists phentolamine ($10^{-6}$ M) plus propranolol ($10^{-6}$ M) did not affect the contractions to 5-HT. By contrast, ketanserin (0.1 μM), at concentration that blocks 5-$HT_{2A}$ receptors, depressed the contractions induced by 5-HT. Collectively, these results suggest that 5-HT contracts the ileum by acting on 5-$HT_{2A}$ receptors located on smooth muscle and that muscarinic or adrenergic receptors are not involved. Table 3 shows the potency (expressed by the $IC_{50}$ value) and the efficacy (expressed by the $E_{max}$ value) of the compounds under investigation in inhibiting 5-HT-induced contractions in the rat ileum (a pharmacological assay useful to detect an action at 5-$HT_{2A}$ receptors). All the compounds under investigation, with the exception of **3a** and **3c** ($E_{max}$ less than 25%), significantly inhibited the contractions induced by 5-HT. The rank order of efficacy was: **4p>4s>4q>3f>3b>4o>4k.** Concerning the potency, these compounds displayed potency approximately in the $10^{-7}$-$10^{-6}$ M range, being **4p** the most potent compound. Specifically, the rank order of potency was **3f>4k>4q>4o>4s>3b>4p**. Considering both potency and efficacy, **3f** and **4q** appear to be the most promising compounds in antagonizing 5-HT-induced contractions. Finally, none of the compounds under investigation, contracted, *per se*, the rat ileum.

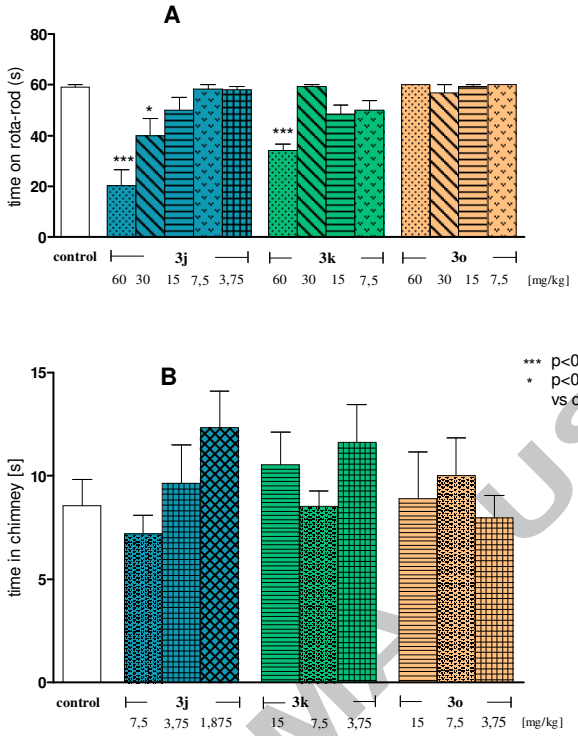
**Table 4.** Potency (indicated by the $EC_{50}$ values) and efficacy (indicated by the $E_{max}$ values) of compounds **3a**, **3b**, **3c**, **3f**, **4k**, **4o**, **4p, 4q** and **4s** in inhibiting 5-HT ($10^{-5}$ M) - induced contractions in the rat ileum.

| Compounds | n | $EC_{50}$ (95% Confidence Intervals) | $E_{max}$ (95% Confidence Intervals) |
|---|---|---|---|
| **3a** | 6 | Inactive | Inactive |
| **3b** | 6 | $4.25 \times 10^{-6}$ ($7.70 \times 10^{-8}$-$2.35 \times 10^{-5}$) M | 33.72 (3.946 to 63.49) |
| **3c** | 6 | Inactive | Inactive |
| **3f** | 6 | $1.61 \times 10^{-7}$ ($3.38 \times 10^{-8}$-$7.67 \times 10^{-5}$) M | 48.41 (24.84 to 71.98) |
| **4k** | 6 | $7.70 \times 10^{-7}$ ($5.35 \times 10^{-8}$-$2.12 \times 10^{-6}$) M | 26.70 (-11.83 to 65.22) |
| **4o** | 6 | $8.28 \times 10^{-7}$ ($3.35 \times 10^{-8}$-$2.04 \times 10^{-5}$) M | 32.13 (14.43 to 49.83) |
| **4p** | 6 | $6.48 \times 10^{-6}$ ($3.43 \times 10^{-6}$-$1.22 \times 10^{-5}$) M | 75.53 (61.16 to 89.89) |
| **4q** | 6 | $7.77 \times 10^{-7}$ ($1.85 \times 10^{-10}$-$3.26 \times 10^{-6}$) M | 53.15 (-91.61 to 197.9) |
| **4s** | 6 | $2.04 \times 10^{-6}$ ($8.42 \times 10^{-8}$-$4.95 \times 10^{-5}$) M | 68.44 (28.36 to 108.5) |

### 2.4 *In Vivo* Behavioral tests

Compounds **3j, 3k** and **3o,** with the highest 5-$HT_{1A}$ receptor affinity and selectivity were selected for further functional *in vivo* studies. First part of experiments included: locomotor activity and motor coordination tests, generally accepted as basic in central activity investigations of new agents [28]. The second one was focused on potential serotoninergic activity of investigated molecules. Firstly, it should be noted that the tested compounds **3j** and **3k** administered at higher doses (60 and 30 mg/kg for **3j**; 60 mg/kg for **3k**), impaired motor coordination assessed in the rotarod (Fig. 1A). Whereas, all compounds did not change the behaviour of mice in the chimney test (Fig. 1B). The rotarod and chimney tests were used to evaluate the ability of drug treatment to interfere with motor coordination. The rotarod test is used to assess not only a rodent's motor coordination but also its sense of balance [28,29]. The chimney test can be used only as complementary to other tests which determine muscle relaxant activity. Therefore, motor incoordination, observed only in the rotarod test, may reflect the depressive effect of drugs on the central nervous system (CNS) and can influence on the results of the other tests [28,30].

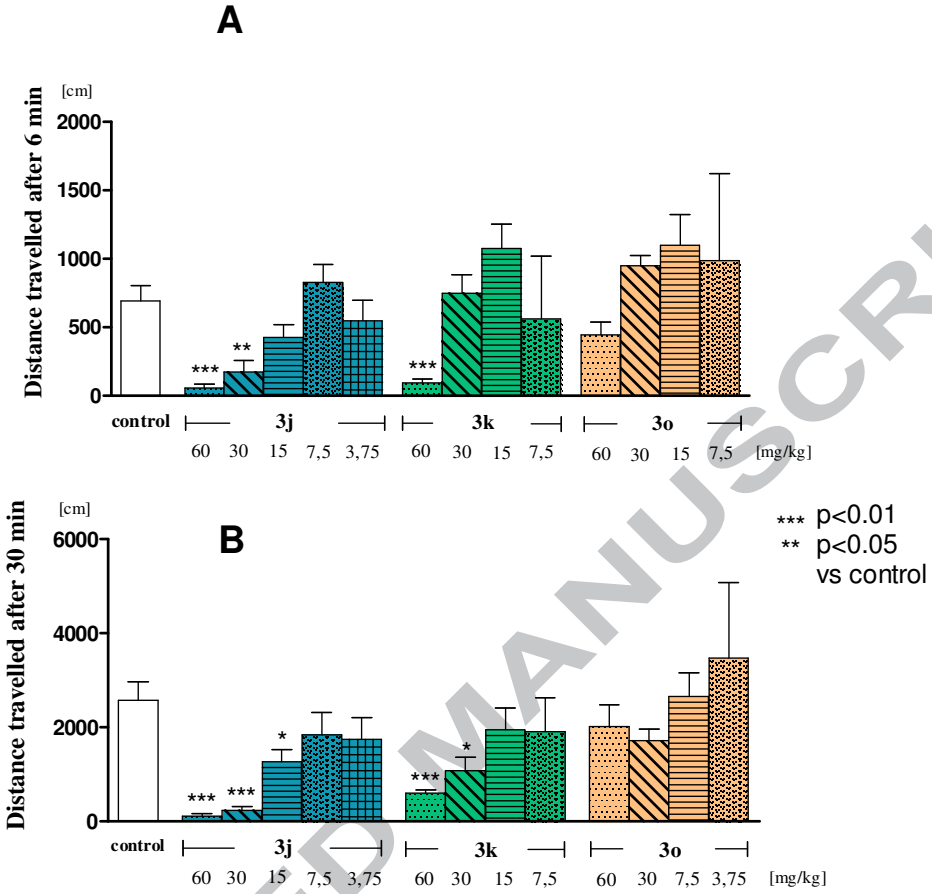
**Figure 1.** The influence of the tested compounds **3j, 3k** and **3o** (3.75 - 60 mg/kg) on motor coordination in mice evaluated in rotarod **[A]** and chimney **[B]** tests.




Investigated compounds were injected ip 60 min before the test. Data are expressed as mean ±SEM values of the 2 independent experiments. One-way ANOVA showed significant changes in the time spent on the rota-rod ($F_{(13,79)}$=11.13; $p<0.0001$). Dunnett's post hoc test confirmed a significant decrease in time of mice spent on rota-rod after the administration of compound **3j** (60 and 30 mg/kg) and **3k** (60 mg/kg), ($p<0.001$; $p<0.05$ and $p<0.001$, respectively).

Similarly, in the locomotor activity test, the same compounds i.e. **3j** and **3k** at the same doses (60 and 30 mg/kg for **3j**; 60 mg/kg for **3k**) decreased spontaneous motility after 6 (Fig.2A) and 30 min (Fig.2B). The effect observed after 30 min, was also expressed for lower doses of both compounds (15 mg/kg for **3j** and 30 mg/kg for **3k**).

**Figure 2**.The influence of the tested compounds **3j, 3k** and **3o** (3.75 - 60 mg/kg) on the spontaneous locomotor activity of mice.

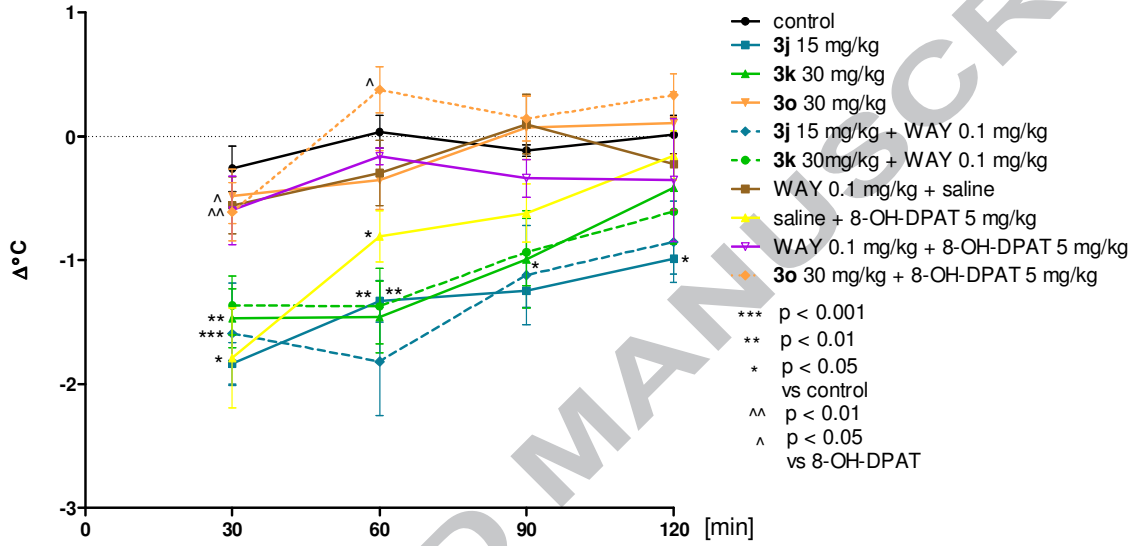



Investigated compounds were injected ip 60 min before the test. Locomotor activity was noted after 6 and 30 min. Data are expressed as mean ±SEM values of the 1 independent experiment. ***p<0.001; **p<0.01; * p<0.05 vs appropriate control. One-way ANOVA showed significant changes in the locomotor activity of mice in 6 min ($F_{(13,75)}$=4.206; p<0.001 and $F_{(13,69)}$=2.577; p<0.01]. Dunnett's post hoc test confirmed a significant decrease in locomotor activity of mice after the administration of compound **3j** (60 and 30 mg/kg) and **3k** (60 mg/kg) after 6 min of observation (p<0.001; p<0.01 and p<0.001, respectively) as well as after 30 min of observation: **3j** 60 and 30 mg/kg (p<0.001) and 15 mg/kg (p<0.05) and **3k** 60 mg/kg (p<0.001) and 30 mg/kg (p<0.05).

Based on above results, doses for further experiments were chosen. As already demonstrated the hypothermia induced by the 5-$HT_{1A}$ receptor agonist 8-OH-DPAT in mice was connected with the activation of presynaptic 5-$HT_{1A}$ receptors [31] and was abolished by 5-$HT_{1A}$ receptor antagonists such as WAY-100635 [32] or 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)piperazine (MP 3022) [33]. Thus, the hypothermia produced by the tested compounds in mice was regarded as a measure of presynaptic 5-$HT_{1A}$ receptor agonistic activity. Compound **3j** (15 mg/kg) produced a long-term decrease of the body temperature (up to 120 min after treatment). Compound **3k** (30 mg/kg) decreased the body temperature in 30 and 60 min after treatment. These effects of both compounds are comparable to the effect of 8-OH-DPAT (statistically significant effect in 30 and 60 min) (Fig. 3). Consequently these results suggest that **3j** and **3k** show features

of presynaptic 5-$HT_{1A}$ receptor agonists. Compound **3o**, did not change the body temperature of normothermic mice, but administered concomitantly with 8-OH-DPAT reversed its hypothermic effect. Compound **3o** mimicked the action of WAY-100635 in this experiment and it seems to behave as a presynaptic 5-$HT_{1A}$ receptor antagonist (Fig. 3).

**Figure 3.** Effects of the investigated compounds **3j** (15 mg/kg) and **3k, 3o** (30 mg/kg), 8-OH-DPAT (5 mg/kg) and WAY 100635 (0.1 mg/kg) on the body temperature in mice.

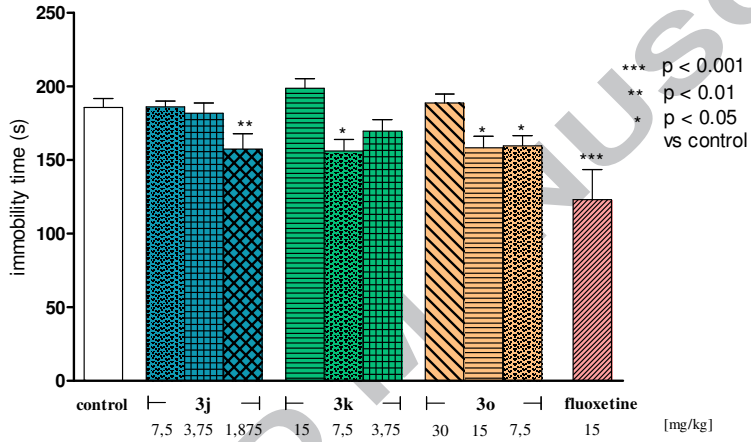



Body temperature was measured during the total period of 180 min (60 min before and 120 min after the tested compound injection). Data are expressed as mean ±SEM values of the 1 independent experiment. *** p<0.001; **p<0.01; *p<0.05 vs control group, ^^ p<0.01; ^ p<0.05 vs 8-OH-DPAT-treated group (Bonferroni's test). Two-way ANOVA revealed statistically significant effects of the compound ($F_{(9,242)}$=17.17; p<0.0001) and dose $F_{(3,242)}$=14.24; p<0.0001). Bonferroni's post hoc test revealed a significant decrease in mice body temperature after administration of compound **3j** (at the dose 15 mg/kg) in 30 (p<0.001), 60 (p<0.01), 90 and 120 min (p<0.05) and compound **3k** (at the dose 30 mg/kg) in 30 and 60 min (p<0.01) and 8-OH-DPAT (5 mg/kg) in 30 and 60 min (p<0.05).WAY 100635 (0.1 mg/kg) reversed the effect of 8-OH-DPAT in 30 min (p<0.05) and compound **3o** (30 mg/kg), similarly to WAY reversed the effect of 8-OH-DPAT in 30 and 60 min (p<0.01 and p<0.05).

The neurotransmitter 5-HT has been implicated in the pathophysiology and treatment of depression. Perhaps the strongest evidence for the role of serotonergic system in the depression is the efficacy of antidepressants that target the 5-HT transporter – SSRI (e.g. fluoxetine, paroxetine or escitalopram). The preclinical and clinical evidence support the involvement of different 5-HT receptors in the therapeutic action of antidepressant and anxiolytic drugs. Among 5-HT receptors, presynaptic and postsynaptic 5-$HT_{1A}$ subtypes appear to be very beneficial for the antidepressant action [34]. Two new antidepressants - vilazodone, approved in 2011; and vortioxetine approved in 2013 - inhibit 5-HT reuptake and show partial agonism at 5-$HT_{1A}$ receptors [35]. What is more, the azapirones e.g. buspirone, showing preferential activation of presynaptic 5-$HT_{1A}$ receptors, are used to treat long-term anxiety [34]. As well as improving the onset of action of SSRIs, 5-$HT_{1A}$ agents are known to exert their own, potentially therapeutic, actions. 5-$HT_{1A}$ ligands with partial

agonist activity seem to possess antianxiety [36], antidepressant [36], antiaggressive [37] effects. Thus, in the present study, the potential antidepressant and anxiolytic profile of the investigated compounds was in the forced swimming test (FST) and in the elevated plus-maze (EPM) test [38] in mice [39], respectively. The results obtained indicate that all tested compounds: **3j** (1.875 mg/kg), **3k** (7.5 mg/kg) and **3o** (15 and 7.5 mg/kg) revealed antidepressant-like properties, shortening the immobility time of mice to various extents in the FST (Fig. 4).

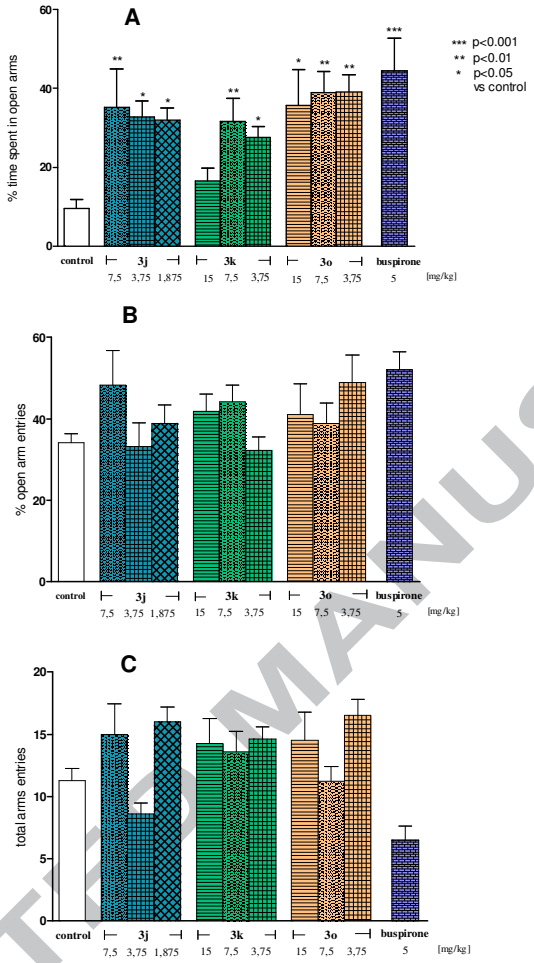
**Figure 4.**The influence of the investigated compounds **3j, 3k** and **3o** on the total duration in the forced swim test in mice (FST).




The investigated compounds were administered i p 1 h before the test. The values represent means ± SEM of the 1 independent experiment. ***p<0.001; **p<0.01; * p<0.05 vs control (Dunnett's test). One-way ANOVA showed significant changes in immobility time after administration of the compound **3j** ($F_{(3,27)}$ = 3,808; $p$<0.05), **3k** ($F_{(3,27)}$ = 6,725; $p$<0.01) and **3o** ($F_{(3,27)}$ = 5,670; $p$<0.01). Dunnett's post hoc test confirmed a significant reduction in immobility time after the administration of compounds **3j** applied at the dose of 1.875 mg/kg (p<0.01), **3k** at the dose of 7.5 mg/kg (p<0.05), and **3o** applied at the doses of 15 and 7.5 mg/kg (p<0.05). Also fluoxetine (20 mg/kg) induced a significant reduction in the immobility time (p<0.001).

In addition, fluoxetine at the dose 15mg/kg, used as a reference drug, caused statistically significant decrease in immobility time. The antidepressant-like effect of investigated compounds seems to be specific, since their antidepressant doses had no influence on the spontaneous activity of mice. Additionally, the same compounds active in the FST test (**3j, 3k** and **3o)**, exhibited characteristics of anxiolytic drugs. In fact, these compounds showed anxiolytic-like activity, increasing in a statistically significant manner, the time spent in the open arms of the EPM. Buspirone (5 mg/kg), used as a reference anxiolytic drug, also prolonged time spent in the open arms of the EPM (Fig. 5).

**Figure 5.** The influence of the investigated compounds **3j, 3k** and **3o** on elevated plus-maze performance in mice - percentage of time spent in open arms [**A**], the percentage of the open arm entries [**B**] and total arm entries **[C].**




The results are expressed as mean ± SEM of the 1 independent experiment; *** p <0.001; ** p<0.01; * p<0.05 vs control (Dunnett's test). One-way ANOVA showed significant changes in percentage of time spent in open arms of EPM ($F_{(10,68)}$=2.89; p<0.01 [**A**] and in the total arm entries ($F_{(10,72)}$=3.483; p<0.001) [**C**]. There were no significant changes in the percentage of open arm entries ($F_{(10,81)}$=1.564; p>0.05). **[B].** Dunnett's post hoc test confirmed a significant increase in time spent in open arms after the administration of compounds **3j, 3k** and **3o.** Compound **3j** significantly increased time spent in open arms at the dose of 7.5, 3.75 and 1.875 mg/kg (p<0.01 and p<0.05), whereas compound **3k** was active at doses 7.5 and 3.75 mg/kg (p<0.01 and p<0.05) and **3o** at doses 15, 7.5 and 3.75 mg/kg (p<0.05 and p<0.01). Also buspirone (5 mg/kg) induced a significant increase in time spent in open arms (p<0.001)[**A**].

Consequently, the functional profile of the investigated 5-$HT_{1A}$ receptor ligands suggests their potential anxiolytic- and/or antidepressant-like activity.

Moreover, to determine the postsynaptic 5-$HT_{1A}$ receptor agonistic effects of the tested compounds, their ability to induce lower lip retraction (LLR) in rats was tested. It is commonly accepted that the 8-OH-DPAT induced LLR in rats depends on stimulation of postsynaptic 5-$HT_{1A}$ receptors and this effect is inhibited by WAY-100635 [33, 40, 41]. Hence, the ability of the tested compounds to inhibit the 8-OH-DPAT-induced LLR was regarded as postsynaptic 5-$HT_{1A}$

receptor antagonistic activity. Compound **3o**, given alone, induced LLR in rats, and this effect was comparable to that after 8-OH-DPAT administration and was abolished by WAY 100635. Interestingly, **3o** administered jointly with 8-OH-DPAT, reversed its effect. The LLR induced by 8-OH-DPAT was significantly reduced by WAY 100635 (Table 5). Based on the above results, it could be speculated that compound **3o** acts as a partial agonist of postsynaptic 5-$HT_{1A}$ receptor. The remaining compounds, similarly to WAY 100635, did not mimic the effect of 8-OH-DPAT in that test (Table 5). It means that these compounds did not behave as postsynaptic 5-$HT_{1A}$ receptor neither antagonist nor agonist.

**Table 5.** Induction of lower lip retraction (LLR) by the investigated compounds **3j** (at the dose of 30 mg/kg), **3k**, and **3o** (at the dose of 30 mg/kg) and WAY 100635 (**A**) and their effect on the 8-OH-DPAT-induced LLR (**B**) in rats.

| treatment | dose mg/kg | mean ± SEM LLR score | |
|---|---|---|---|
| | | A | B |
| vehicle | - | 0.0 ± 0.0 | 2.875 ± 0.08183*** |
| **3j** | 15 | 0.4 ± 0.1 | 2.5 ± 0.3162 |
| **3k** | 30 | 0.1429± 0.09221 | 2.3 ± 0.2550 |
| **3o** | 30 | 2.714 ± 0.1844*** | 2.0 ± 0.2236$^{\#\#}$ |
| WAY 100635 | 0.3 | 0.0 ± 0.0 | 1.667± 0.1054$^{\#\#\#}$ |

Investigated compounds ip and WAY 100635 sc were administrated 15 min before the test (**A**), or 45 min before 8-OH-DPAT (1 mg/kg, sc) (**B**). The results are expressed as mean ± SEM of the 1 independent experiment; *** $p< 0.001$ vs vehicle (**A**), ## $p<0.01$ vs vehicle + 8-OH-DPAT (**B**) (Newman-Keuls test). One-way ANOVA showed significant changes in LLR of rats (ANOVA: $F_{(9,54)}$=36,48. Newman-Keuls test revealed that 8-OH-DPAT and compound **3o** (30 mg/kg) induced LLR in rats ($p<0.001$). LLR caused by 8-OH-DPAT were significantly reversed by WAY 100635 and compound **3o** ($p < 0.001$, $p < 0.01$, respectively

To sum up, the obtained results suggest that compound **3j** and **3k** showed features of presynaptic 5-$HT_{1A}$ receptor agonist, whereas compound **3o** showed features of presynaptic antagonist and postsynaptic partial agonist and further in vivo studies will be required to prove definite functional status of compound **3o**. All the compounds revealed significant anxiolytic properties in a wide range of doses, and weaker antidepressant-like activity.

## 3. Conclusion

We have described the synthesis of a new series of arylpiperazines as 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ ligands (**3a-t** and **4a-t**), containing a picolinic fragment as terminal part of LCAPs. Compounds **4p** ($K_i$ = 45.3 nM) and **4q** ($K_i$ = 1.68 nM), associated to a propyl chain spacer and **3b** ($K_i$ = 0.0224 nM), and **3c** ($K_i$ = 9.33 nM), associated to an ethyl chain spacer, afforded a favorable affinity profile for 5-$HT_{2A}$ receptors. Instead, considering both potency and efficacy, **3f** and **4q** appear to be the most promising compounds in antagonizing 5-HT induced contractions in the rat ileum.

Moreover compounds **3j** (*K*i = 0.344 nM), **3k** (Ki = 0.183) and **3o** (*K*i = 0.046 nM) associated to an ethyl chain spacer, with the highest 5-$HT_{1A}$ receptor affinity and selectivity were selected for further functional *in vivo* studies in order to investigate the functional activity. The obtained results suggest that compounds **3j** and **3k** show features of presynaptic 5-$HT_{1A}$ receptor agonists, whereas, compound **3o**, showed features of presynaptic antagonist and postsynaptic partial agonist. Additionally compounds **3j**, **3k**, and **3o** revealed significant anxiolytic properties in a wide range of doses, and weaker antidepressant-like activity. Furthermore data presented in this study demonstrate that the simple amide bond of the picolinic derivatives in place of a cyanoamidine group, characterizing the N'-cyanopicolinamidine analogues [16], doesn't determine a general trend towards 5-$HT_{2A}$ receptors but provide compounds characterized by 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ affinity and selectivity.

## 4. EXPERIMENTAL SECTION

### 4.1 Chemistry

#### 4.1.1.General procedures

All reagents and substituted piperazines were commercially available from Sigma-Aldrich. All reactions were monitored by TLC, carried out on Merck 60G $F_{254}$ plates with fluorescent indicator and the plates were visualized with UV light (254 nm). Each final compound and each intermediate were purified by silica gel column chromatography (Macherey-Nagel 60 0,063-0,2mm/70-230 mesh). Some final compounds were obtained in a pure form after conversion in the corresponding hydrochloride salts. $^1$H-NMR spectra were recorded on Varian Mercury Plus 400 MHz instrument. Unless otherwise stated, all spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm using $Me_4Si$ as internal standard. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), m (multiplet), q (quartet), qt (quintet), dd (double doublet), ddd (double dd), bs (broad singlet). Mass spectra of the final products were performed on API 2000 Applied Biosystem ESI-triple quadrupole mass spectrometer. Melting points were determined using a Buchi B-540 hot-stage instrument and are uncorrected. Where analyses are indicated only by the symbols of the elements, results obtained are within ± 0.4% of the theoretical values. Solutions were dried over $Na_2SO_4$ and concentrated with Buchi R-114 rotavapor at low pressure.

#### 4.1.2. Synthesis of the chloroalkylpicolinamides (Scheme 1: compounds 2a and 2b)

*N,N'*-Dicyclohexylcarbodiimide (DCC, 9.28 g, 0.045 mol) and hydroxybenzotriazole (HOBt, 6.08 g, 0.045 mol) were added to a mixture of picolinic acid (**1**) (5.00 g, 0.041 mol) in acetonitrile (50

ml) and the reaction was stirred for 1 hour at 0°C. Then, triethylamine (TEA, 4.55 g, 0.045 mol) and the appropriate 2-chloroethanamine hydrochloride (4.75g, 0.041 mol) or 3-chloropropan-1-amine hydrochloride (5.33 g, 0.041 mol) was added to the reaction mixture and the resulting solution was stirred for 24 hours at room temperature. The mixture was then cooled to 0°C in order to precipitate N,N′-Dicyclohexylurea (DCU) that was filtered by Gooch. The filtrate was evaporated and after dissolution in DCM, the residue was washed with $NaHCO_3$, water and brine. The combined organic layers were dried on anhydrous $Na_2SO_4$ and concentrated in *vacuo*. The crude chloroalkylpicolinamides were purified by column chromatography (dichloromethane/methanol 9:1 (v/v)), yielding N-(2-chloroethyl)picolinamide (**2a**, 6.17 g, 82%), Mp: 80-81°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 3.61 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.4*); 3.82 (t, 2H, -C<u>H</u>$_2$-Cl, *J=5.4*); 7.40 (t, 1H, *J=7.0*); 7.85 (t, 1H, *J=7.0*); 8.19 (d, 1H, *J=7.6*); 8.40 (bs, 1H, N<u>H</u>); 8.56 (d, 1H, *J=5.8*). and N-(3-chloropropyl)picolinamide (**2b**, 6.35 g, 78%), Mp: 134-135°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.86 (m, 2H, -$CH_2$-C<u>H</u>$_2$-$CH_2$, *J=5.4*); 3.60 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.4*); 3.86 (t, 2H, -C<u>H</u>$_2$-Cl, *J=5.4*); 7.35 (t, 1H, *J=7.0*); 7.83 (t, 1H, *J=7.0*); 8.18 (d, 1H, *J=7.6*); 8.42 (d, 1H, *J=5.8*); 9.02 (bs, 1H, N<u>H</u>).

**4.1.3. General procedure for the preparation of picolinamide derivatives (3a-t, 4-a-t)**

A mixture of N-(2-chloroethyl)picolinamide (**2a**, 0.500 g, 2.71 mmol) or N-(3-chloropropyl)picolinamide (**2b**, 0.500 g, 2.52 mmol) and NaI (1.1 equiv.) in acetonitrile (30 mL) was stirred under reflux for 30 min. Then the appropriate 4-substituted arylpiperazine (1.0 equiv.) and anhydrous $K_2CO_3$ (1.1 equiv.) were added. The reaction mixture was stirred under reflux for 24 h. After cooling to room temperature, the mixture was filtered, concentrated to dryness and the residue was dissolved in dichloromethane (20 mL) and washed with $NaHCO_3$, water and brine. The combined organic layers were dried on anhydrous $Na_2SO_4$ and the solvent removed under *vacuum*. The crude mixture was purified by silica gel column chromatography using dichloromethane/methanol 9.5:0.5 (v/v) or ethyl acetate/methanol 9:1 (v/v) as eluent, according to the used arylpiperazine. The crude products were crystallized from diethyl ether, affording final compounds **3a-t** and **4a-t** (yield 11-86%). Final compounds **3s**, **4k** and **4t** were obtained in a pure form after conversion in the corresponding hydrochloride salts adding HCl ethereal solution to an ethanolic solution of the free bases. All derivatives were recrystallized by ethanol/diethyl ether. $^1$H-NMR, and MS data for all final compounds were consistent with the proposed structures.

**4.1.4. N-(2-(4-phenylpiperazin-1-yl)ethyl)picolinamide (3a)**

From **2a** and 1-phenylpiperazine.

Yield:18%; mp 102-103°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 2.67 (t, 2H, -C<u>H</u>$_2$-N$^1$, *J=5.4*); 2.70 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.24 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.62 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.4*); 6.87 (t, 1H, *J=6.9*); 6.94 (d, 2H, *J=8.4*); 7.27 (t, 2H, *J=8.4*); 7.41 (t, 1H, *J=7.0*); 7.83 (t, 1H, *J=7.0*); 8.18 (d, 1H, *J=7.6*); 8.37 (bs, 1H, N<u>H</u>); 8.55 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.27; 49.12; 53.02; 56.84; 116.01; 119.68; 122.19; 126.04; 129.094; 137.25; 148.18; 150.05; 151.28; 164.42.

ESI-MS: 311.2 $[M+H]^+$, 333.0 $[M+Na]^+$, 349.2 $[M+K]^+$.

Anal. ($C_{18}H_{22}N_4O$), C, H, N.

**4.1.5. N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)picolinamide (3b)**

From **2a** and 1-(2-methoxyphenyl)piperazine.

Yield:35%; mp 95-96°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 2.69 (t, 2H, -C<u>H</u>$_2$-N$^1$, *J=5.4*); 2.73 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.12 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.62 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.4*); 3.86 (s, 3H, OC<u>H</u>$_3$); 6.87 (d, 1H, *J=7.0*); 6.95-6.99 (m, 3H); 7.41 (t, 1H, *J=7.0*); 7.83 (t, 1H, *J=7.0*); 8.19 (d, 1H, *J=7.6*); 8.36 (bs, 1H, N<u>H</u>); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.56; 50.91; 53.52; 55.59; 57.20; 111.44; 118.41; 121.18; 122.44; 123.11; 126.24; 137.48; 141.58; 148.40; 150.36; 152.50; 164.64.

ESI-MS: 341.2 $[M+H]^+$, 363.2 $[M+Na]^+$, 379.2 $[M+K]^+$.

Anal. ($C_{19}H_{24}N_4O_2$), C, H, N.

**4.1.6. N-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)picolinamide (3c)**

From **2a** and 1-(4-methoxyphenyl)piperazine.

Yield:11%; mp 105-106°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 2.66 (t, 2H, -C<u>H</u>$_2$-N$^1$, *J=5.4*); 2.69 (bs, 4H, 2C<u>H</u>$_2$ pip.); 3.13 (bs, 4H, 2C<u>H</u>$_2$ pip.); 3.63 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.4*); 3.76 (s, 3H, -OC<u>H</u>$_3$); 6.84 (d, 2H, *J=8.7*); 6.89 (d, 2H, *J=8.7*); 7.41 (t, 1H, *J=7.0*); 7.83 (t, 1H, *J=7.0*); 8.18 (d, 1H, *J=7.6*); 8.36 (bs, 1H, N<u>H</u>); 8.55 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.53; 50.83; 53.36; 55.80; 57.09; 114.67; 118.36; 122.44; 126.27; 137.50; 145.94; 148.40; 150.30; 154.01; 164.65.

ESI-MS: 341.3 $[M+H]^+$, 363.2 $[M+Na]^+$.

Anal. ($C_{19}H_{24}N_4O_2$), C, H, N.

**4.1.7. N-(2-(4-(2-ethoxyphenyl)piperazin-1-yl)ethyl)picolinamide (3d)**

From **2a** and 1-(2-ethoxyphenyl)piperazine.

Yield:16%; mp 120-122°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 1.43 (t, 3H, -C<u>H</u>$_3$, *J=6.7*); 3.23 (t, 2H, -C<u>H</u>$_2$-N$^1$, *J=5.4*); 3.63 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.54 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.59 (q, 2H, -NH-

C$\underline{H}_2$, *J=5.1*); 4.07 (m, 2H, -OC$\underline{H}_2$); 6.86 (d, 1H, *J=7.3*); 6.89 (t, 1H, *J=8.7*); 6.97 (d, 1H, *J=7.3*); 7.04 (t, 1H, *J=8.7*); 7.44 (t, 1H, *J=7.0*); 7.84 (t, 1H, *J=7.0*); 8.12 (d, 1H, *J=7.6*); 8.59 (d, 1H, *J=5.8*); 8.67 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 15.20; 34.73; 47.55; 53.36; 56.81; 63.94; 112.62; 119.35; 121.36; 122.43; 124.82; 126.83; 137.61; 138.62; 148.79; 149.29; 151.61; 165.75.

ESI-MS: 355.1 $[M+H]^+$, 377.4 $[M+Na]^+$.

Anal. ($C_{20}H_{26}N_4O_2$), C, H, N.

**4.1.8. N-(2-(4-(2-cyanophenyl)piperazin-1-yl)ethyl)picolinamide (3e)**

From **2a** and 1-(2-cyanophenyl)piperazine.

Yield:23%; mp 74-75°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.69 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=5.4*); 2.72 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.27 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.62 (q, 2H, -NH-C$\underline{H}_2$, *J=5.1*); 6.97 (t, 1H, *J=9.8*); 7.02 (d, 1H, *J=7.3*); 7.42 (t, 1H, *J=7.0*); 7.47 (t, 1H, *J=7.9*); 7.54 (d, 1H, *J=7.3*); 7.84 (t, 1H, *J=8.0*); 8.19 (d, 1H, *J=7.6*); 8.34 (bs, 1H, N$\underline{H}$); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.54; 51.76; 53.22; 57.05; 106.16; 118.89; 121.92; 122.47; 126.28; 133.97; 134.59; 137.52; 148.38; 150.66; 159.62; 165.17.

ESI-MS: 336.0 $[M+H]^+$, 358.0 $[M+H]^+$.

Anal. ($C_{19}H_{21}N_5O$), C, H, N.

**4.1.9. N-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)picolinamide (3f)**

From **2a** and 1-(3-(trifluoromethyl)phenyl)piperazine.

Yield:12%; mp 107-108°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.69 (bs, 2H, -C$\underline{H}_2$-N$^1$); 2.71 (bs, 4H, 2C$\underline{H}_2$ pip.); 2.98 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.59 (q, 2H, -NH-C$\underline{H}_2$, *J=5.1*); 6.97 (t, 1H, *J=7.6*); 7.01 (d, 1H, *J=7.3*); 7.03 (d, 1H, *J=7.6*); 7.16 (t, 1H, *J=7.3*); 7.54 (d, 1H, *J=7.3*); 7.84 (t, 1H, *J=8.0*); 8.18 (d, 1H, *J=7.6*); 8.35 (bs, 1H, N$\underline{H}$); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.50; 48.92; 50.03; 57.05; 112.31; 116.01; 118.94; 122.45; 126.30; 129.74; 137.52; 138.87 148.41; 150.06; 152.15; 164.05.

ESI-MS: 379.3 $[M+H]^+$.

Anal. ($C_{19}H_{21}F_3N_4O$), C, H, N.

**4.1.10. N-(2-(4-(2,3-dimethylphenyl)piperazin-1-yl)ethyl)picolinamide (3g)**

From **2a** and 1-(2,3-dimethylphenyl)piperazine.

Yield:11%; mp 70-71°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:2.21 (s, 3H, -C$\underline{H}_3$); 2.26 (s, 3H, -C$\underline{H}_3$); 2.74 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=5.4*); 2.76 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 2.97 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.67

(q, 2H, -NH-C<u>H</u>$_2$, *J=4.7*); 6.89 (t, 1H, *J=7.0*); 6.91 (d, 1H, *J=7.9*); 7.07 (t, 1H, *J=7.0*); 7.42 (t, 1H, *J=7.3*); 7.84 (t, 1H, *J=8.0*); 8.18 (d, 1H, *J=7.6*); 8.41 (bs, 1H, N<u>H</u>); 8.58 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 14.17; 20.85; 36.42; 52.04; 53.82; 57.21; 116.93; 122.43; 125.31; 126.07; 126.32; 131.46; 137.50; 138.22; 148.46; 150.24; 151.55; 164.78.

ESI-MS: 339.2 $[M+H]^+$.

Anal. ($C_{20}H_{26}N_4O$), C, H, N.

**4.1.11. N-(2-(4-(2-chlorophenyl)piperazin-1-yl)ethyl)picolinamide (3h)**

From **2a** and 1-(2-chlorophenyl)piperazine.

Yield:32%; mp 89-90°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.76 (t, 2H, -C<u>H</u>$_2$-N$^1$, *J=5.4*); 2.79 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.16 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.68 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.1*); 6.97 (t, 1H, *J=7.6*); 7.07 (d, 1H, *J=7.3*); 7.20 (t, 1H, *J=7.3*); 7.34 (d, 1H, *J=7.6*); 7.42 (t, 1H, *J=7.0*); 7.84 (t, 1H, *J=7.0*); 8.18 (d, 1H, *J=7.6*); 8.40 (bs, 1H, N<u>H</u>); 8.57 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.35; 51.06; 53.46; 57.14; 120.67; 122.46; 124.06; 126.34; 127.82; 129.00; 130.88; 137.51; 148.45; 150.04; 150.21; 164.78.

ESI-MS: 345.4 $[M+H]^+$.

Anal. ($C_{18}H_{21}ClN_4O$), C, H, N.

**4.1.12. N-(2-(4-(3-chlorophenyl)piperazin-1-yl)ethyl)picolinamide (3i)**

From **2a** and 1-(3-chlorophenyl)piperazine.

Yield:23%; mp 95-96°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.64 (t, 2H, -C<u>H</u>$_2$-N$^1$, *J=5.4*); 2.67 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.8*); 3.23 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.8*); 3.63 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.1*); 6.77 (d, 1H, *J=8.1*); 6.80 (d, 1H, *J=8.3*); 6.88 (s, 1H); 7.15 (t, 1H, *J=7.9*); 7.41 (t, 1H, *J=7.0*); 7.84 (t, 1H, *J=7.3*); 8.18 (d, 1H, *J=7.9*); 8.35 (bs, 1H, N<u>H</u>); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.49; 48.92; 53.04; 57.05; 114.09; 115.94; 119.47; 122.43; 126.30; 130.23; 135.17; 137.50 148.41; 150.27; 152.57; 164.65.

ESI-MS: 345.3 $[M+H]^+$, 367.2 $[M+Na]^+$.

Anal. ($C_{18}H_{21}ClN_4O$), C, H, N.

**4.1.13. N-(2-(4-(4-chlorophenyl)piperazin-1-yl)ethyl)isonicotinamide (3j)**

From **2a** and 1-(4-chlorophenyl)piperazine.

Yield:22%; mp 110-111°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.65 (t, 2H, -C<u>H</u>$_2$-N$^1$, *J=5.4*); 2.68 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.19 (t, 4H, 2C<u>H</u>$_2$ pip., *J=4.7*); 3.62 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.1*); 6.83 (d, 2H, *J=8.8*); 7.18 (d, 2H, *J=8.8*); 7.41 (t, 1H, *J=7.3*); 7.83 (t, 1H, *J=8.0*); 8.18 (d, 1H, *J=7.6*); 8.35 (bs,

1H, NH); 8.54 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.24; 49.11; 52.86; 56.82; 117.19; 122.20; 124.50; 126.07; 128.92; 137.27; 141.17; 149.87; 150.02; 164.43.

ESI-MS: 345.0 $[M+H]^+$, 367.1 $[M+Na]^+$, 383.1 $[M+K]^+$.

Anal. ($C_{18}H_{21}ClN_4O$), C, H, N.

**4.1.14. N-(2-(4-(3,4-dichlorophenyl)piperazin-1-yl)ethyl)picolinamide (3k)**

From **2a** and 1-(3,4-dichlorophenyl)piperazine.

Yield:20%; mp 115-116°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.67 (t, 2H, -CH$_2$-N$^1$, *J=5.4*); 2.67 (t, 4H, 2CH$_2$ pip., *J=4.7*); 3.20 (t, 4H, 2CH$_2$ pip., *J=4.7*); 3.61 (q, 2H, -NH-CH$_2$, *J=5.1*); 6.75 (d, 1H, *J=8.8*); 6.95 (d, 1H, *J=8.7*); 7.25 (s, 1H); 7.42 (t, 1H, *J=7.3*); 7.84 (t, 1H, *J=8.0*); 8.18 (d, 1H, *J=7.6*); 8.34 (bs, 1H, NH); 8.55 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.48; 48.92; 52.92; 57.04; 115.52; 117.41; 122.33; 122.44; 126.31; 130.63; 133.00; 137.52; 148.40; 150.25; 150.91; 164.65.

ESI-MS: 379.1 $[M+H]^+$.

Anal. ($C_{18}H_{20}Cl_2N_4O$), C, H, N.

**4.1.15. N-(2-(4-(2-fluorophenyl)piperazin-1-yl)ethyl)picolinamide (3l)**

From **2a** and 1-(2-fluorophenyl)piperazine.

Yield:16%; mp 56-57°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.75 (t, 2H, -CH$_2$-N$^1$, *J=5.4*); 2.71 (t, 4H, 2CH$_2$ pip., *J=4.7*); 3.15 (t, 4H, 2CH$_2$ pip., *J=4.7*); 3.62 (q, 2H, -NH-CH$_2$, *J=5.1*); 6.94 (d, 1H, *J=7.9*); 6.98 (t, 1H, *J=7.6*); 7.02-7.04 (m, 2H); 7.41 (t, 1H, *J=7.3*); 7.84 (t, 1H, *J=8.0*); 8.18 (d, 1H, *J=7.6*); 8.35 (bs, 1H, NH); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.53; 50.75; 53.34; 57.15; 116.23; 119.13; 122.46; 122.67; 124.62; 126.27; 137.51; 148.39; 150.32; 156.10; 158.32; 164.65.

ESI-MS: 329.7 $[M+H]^+$.

Anal. ($C_{18}H_{21}FN_4O$), C, H, N.

**4.1.16. N-(2-(4-(4-fluorophenyl)piperazin-1-yl)ethyl)picolinamide (3m)**

From **2a** and 1-(4-fluorophenyl)piperazine.

Yield:18%; mp 109-111°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.65 (t, 2H, -CH$_2$-N$^1$, *J=5.4*); 2.68 (t, 4H, 2CH$_2$ pip., *J=4.7*); 3.15 (t, 4H, 2CH$_2$ pip., *J=4.7*); 3.62 (q, 2H, -NH-CH$_2$, *J=5.4*); 6.88 (d, 2H, *J=8.2*); 6.96 (d, 2H, *J=8.2*); 7.41 (t, 1H, *J=7.3*); 7.84 (t, 1H, *J=8.0*); 8.18 (d, 1H, *J=7.6*); 8.35 (bs, 1H, NH); 8.55 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.52; 50.39; 53.25; 57.06; 115.61; 117.93; 122.44; 126.28; 137.50; 148.21; 150.29; 156.18; 158.55; 164.65.

ESI-MS: 329.7 $[M+H]^+$, 351.4 $[M+Na]^+$.

Anal. ($C_{18}H_{21}FN_4O$), C, H, N.

**4.1.17. N-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)picolinamide (3n)**

From **2a** and 1-(pyridin-2-yl)piperazine.

Yield:35%; mp 114-116°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.64 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 2.67 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=5.4*); 3.58 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.62 (q, 2H, -NH-C$\underline{H}_2$, *J=5.1*); 6.61 (d, 1H, *J=7.0*); 6.63 (d, 1H, *J=8.8*); 7.41 (t, 1H, *J=7.0*); 7.47 (t, 1H, *J=7.0*); 7.83 (t, 1H, *J=7.6*); 8.18 (d, 2H, *J=7.3*); 8.38 (bs, 1H, N$\underline{H}$); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.51; 45.49; 53.07; 57.17; 107.32; 113.54; 122.43; 126.27; 137.48; 137.66; 148.18; 148.40; 150.29; 159.80; 164.65.

ESI-MS: 312.1 $[M+H]^+$,334.2 $[M+Na]^+$, 350.0 $[M+K]^+$.

Anal. ($C_{17}H_{21}N_5O$), C, H, N.

**4.1.18. N-(2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethyl)picolinamide (3o)**

From **2a** and 1-(pyrimidin-2-yl)piperazine.

Yield:22%; mp 108-110°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.58 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 2.66 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=4.6*); 3.62 (q, 2H, -NH-C$\underline{H}_2$, *J=5.4*); 3.85 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 6.47 (t, 1H, *J=8.0*); 7.41 (t, 1H, *J=7.0*); 7.83 (t, 1H, *J=7.6*); 8.18 (d, 1H, *J=7.6*); 8.30 (d, 2H, *J=7.6*); 8.38 (bs, 1H, N$\underline{H}$); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.51; 43.95; 53.15; 57.24; 110.07; 122.44; 126.27; 137.49; 148.40; 150.86; 157.93; 162.08; 164.17.

ESI-MS: 313.1 $[M+H]^+$, 335.0 $[M+Na]^+$.

Anal. ($C_{16}H_{20}N_6O$), C, H, N.

**4.1.19. N-(2-(4-(piperonyl)piperazin-1-yl)ethyl)picolinamide (3p)**

From **2a** and 1-(piperonyl)piperazine.

Yield:19%; mp 92-94°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.49 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=5.4*); 2.54 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 2.61 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.42 (s, 2H, -C$\underline{H}_2$-); 3.58 (q, 2H, -NH-C$\underline{H}_2$, *J=5.4*); 5.93 (s, 2H, -OC$\underline{H}_2$); 6.73 (bs, 2H); 6.85 (s, 1H); 7.41 (t, 1H, *J=7.0*); 7.83 (t, 1H, *J=7.3*); 8.18 (d, 1H, *J=7.9*); 8.29 (bs, 1H, N$\underline{H}$); 8.55 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.60; 53.15; 53.24; 57.12; 62.96; 101.09; 108.07; 109.76; 122.44; 122.48; 126.24; 132.22; 137.48; 146.81; 147.83; 148.32; 150.33; 164.62.

ESI-MS: 369.4 $[M+H]^+$.

Anal. ($C_{20}H_{24}N_4O_3$), C, H, N.

**4.1.20. N-(2-(4-(furoyl)piperazin-1-yl)ethyl)picolinamide (3q)**

From **2a** and 1-(furoyl)piperazine.

Yield:36%; mp 95-96°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 2.57 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 2.65 (t, 2H, -C$\underline{H}_2$-N$^{1}$, *J=5.8*); 3.60 (q, 2H, -NH-C$\underline{H}_2$, *J=5.1*); 3.83 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 6.46 (d, 1H, *J=8.1*); 6.98 (d, 1H, *J=8.3*); 7.42 (t, 1H, *J=7.0*); 7.47 (t, 1H, *J=7.0*); 7.84 (t, 1H, *J=7.3*); 8.18 (d, 1H, *J=7.9*); 8.34 (bs, 1H, N$\underline{H}$); 8.56 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 36.44; 53.33; 57.07; 111.47; 116.55; 122.46; 126.35; 137.54; 143.86; 148.40; 150.77; 159.82; 164.96.

ESI-MS: 329.3 $[M+H]^+$, 351.0 $[M+Na]^+$, 367.0 $[M+K]^+$.

Anal. ($C_{17}H_{20}N_4O_3$), C, H, N.

**4.1.21. N-(2-(4-(4-chlorobenzhydryl)piperazin-1-yl)ethyl)picolinamide (3r)**

From **2a** and 1-(4-chlorobenzhydryl)piperazine.

Yield:10%; mp 41-42°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 2.43 (t, 2H, -C$\underline{H}_2$-N$^{1}$, *J=5.8*); 2.54 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 2.61 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.57 (q, 2H, -NH-C$\underline{H}_2$, *J=5.1*); 4.21 (s, 1H, -C$\underline{H}$-); 7.18 (d, 2H, *J=7.3*); 7.22 (t, 1H, *J=7.6*); 7.24 (d, 2H, *J=7.0*); 7.35 (bs, 4H); 7.40 (t, 1H, *J=7.0*); 7.82 (t, 1H, *J=7.0*); 8.16 (d, 1H, *J=7.6*); 8.28 (bs, 1H, N$\underline{H}$); 8.55 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, DMSO) δ: 36.34; 51.76; 53.25; 56.87; 74.48; 122.21; 126.03; 127.14; 127.80; 128.59; 128.65; 129.16; 132.53; 137.27; 141.38; 142.21; 148.13; 150.04; 164.40.

ESI-MS: 435.9 $[M+H]^+$.

Anal. ($C_{25}H_{27}ClN_4O$), C, H, N.

**4.1.22. N-(2-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)ethyl)picolinamide (3s)**

From **2a** and 1-bis(4-fluorophenyl)methyl)piperazine.

Yield:13%; mp 191-193°C. $^{1}$H-NMR (400 MHz, DMSO) δ: 2.31 (t, 2H, -C$\underline{H}_2$-N$^{1}$, *J=5.8*); 2.75 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.09 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.63 (q, 2H, -NH-C$\underline{H}_2$, *J=5.1*); 4.54 (s, 1H, -C$\underline{H}$-); 7.13 (t, 4H, *J=8.5*); 7.42 (t, 4H, *J=7.6*); 7.59 (t, 1H, *J=7.0*); 7.98 (t, 1H, *J=7.0*); 8.02 (d, 1H, *J=7.0*); 8.63 (d, 1H, *J=7.6*); 9.09 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, DMSO) δ: 34.39; 48.40; 52.13; 55.69; 72.25; 116.03; 116.23; 122.67; 127.38; 130.08; 138.52; 149.13; 150.32; 160.67; 163.09.

ESI-MS: 437.3 $[M+H]^+$.

Anal. ($C_{25}H_{26}F_2N_4O$), C, H, N.

**4.1.23. N-(2-(4-(naphthalen-1-yl)piperazin-1-yl)ethyl)picolinamide (3t)**

From **2a** and 1-(naphthalen-1-yl)piperazine.

Yield:15%; mp 66-68°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.79 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 2.88 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=5.4*); 3.21 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.70 (q, 2H, -NH-C$\underline{H}_2$, *J=6.1*); 7.10 (d, 1H, *J=7.3*); 7.40 (d, 1H, *J=7.6*); 7.42 (t, 1H, *J=7.3*); 7.47 (t, 2H, *J=7.0*); 7.54 (d, 1H, *J=7.6*); 7.65 (d, 1H, *J=5.8*); 7.82 (d, 1H, *J=7.3*); 8.18 (d, 1H, *J=7.3*); 8.21 (d, 1H, *J=7.3*); 8.42 (bs, 1H, N$\underline{H}$); 8.58 (d, 1H, *J=5.8*). $^{13}$C-NMR (400 MHz, DMSO) δ: 36.49; 52.96; 53.83; 57.26; 114.95; 122.47; 123.73; 123.82; 125.60; 126.06; 126.33; 128.64; 129.07; 134.97; 137.53; 148.45; 149.67; 150.26; 164.77.

ESI-MS: 361.4 $[M+H]^+$, 383.2 $[M+Na]^+$.

Anal. ($C_{22}H_{24}N_4O$), C, H, N.

**4.1.24. N-(3-(4-phenylpiperazin-1-yl)propyl)picolinamide (4a)**

From **2b** and 1-phenylpiperazine.

Yield: 28%; mp 47-48°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.84 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.4*); 2.59 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=5.4*); 2.66 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.29 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.58 (q, 2H, -NH-C$\underline{H}_2$, *J=5.4*); 6.86 (t, 1H, *J=7.6*); 6.93 (d, 2H, *J=7.3*); 7.27 (t, 2H, *J=8.2*); 7.35 (t, 1H, *J=8.0*); 7.81 (t, 1H, *J=7.1*); 8.16 (d, 1H, *J=7.4*); 8.41 (d, 1H, *J=5.8*); 9.05 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.65; 39.28; 49.02; 53.34; 57.47; 116.05; 119.66; 122.14; 125.96; 129.11; 137.18; 148.03; 150.20; 151.41; 164.45.

ESI-MS: 325.1 $[M+H]^+$, 347.2 $[M+Na]^+$.

Anal. ($C_{19}H_{24}N_4O$), C, H, N.

**4.1.25. N-(3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl)picolinamide (4b)**

From **2b** and 1-(2-methoxyphenyl)piperazine.

Yield: 45%; mp 105-106°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.84 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.4*); 2.60 (t, 2H, -C$\underline{H}_2$-N$^1$, *J=5.4*); 2.70 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.18 (t, 4H, 2C$\underline{H}_2$ pip., *J=4.7*); 3.60 (q, 2H, -NH-C$\underline{H}_2$, *J=5.4*); 3.86 (s, 3H, -OC$\underline{H}_3$); 6.86 (d, 1H, *J=7.6*); 6.94-6.96 (m, 3H, *J=7.6*); 7.37 (t, 1H, *J=8.0*); 7.81 (t, 1H, *J=7.3*); 8.17 (d, 1H, *J=7.6*); 8.50 (d, 1H, *J=5.8*); 9.10 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.80; 39.67; 50.74; 53.83; 55.57; 57.88; 111.35; 118.27; 121.21; 122.41; 123.09; 126.18; 137.41; 141.70; 148.29; 150.51; 152.52; 164.68.

ESI-MS: 355.3 $[M+H]^+$, 377.4 $[M+Na]^+$, 393.3 $[M+K]^+$.

Anal. ($C_{20}H_{26}N_4O_2$), C, H, N.

**4.1.26. N-(3-(4-(4-methoxyphenyl)piperazin-1-yl)propyl)picolinamide (4c)**

From **2b** and 1-(4-methoxyphenyl)piperazine.

Yield: 17%; mp 89-91°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:1.84 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.4*); 2.58 (t, 2H, -C$\underline{H}_2$-N[1], *J=5.4*); 2.66 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.18 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.59 (q, 2H, -NH-C$\underline{H}_2$, *J=5.4*); 3.77 (s, 3H, -OC$\underline{H}_3$); 6.86 (d, 2H, *J=8.5*); 6.90 (d, 2H, *J=8.5*); 7.36 (t, 1H, *J=8.0*); 7.81 (t, 1H, *J=7.3*); 8.17 (d, 1H, *J=7.6*); 8.43 (d, 1H, *J=5.8*); 9.05 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.90; 39.57; 50.73; 53.71; 55.79; 57.73; 114.68; 118.33; 122.39; 126.18; 137.41; 146.11; 148.26; 150.48; 153.96; 164.68.

ESI-MS: 355.4 $[M+H]^+$, 377.4 $[M+Na]^+$, 393.4 $[M+K]^+$.

Anal. ($C_{20}H_{26}N_4O_2$), C, H, N.

**4.1.27. N-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)propyl)picolinamide (4d)**

From **2b** and 1-(2-ethoxyphenyl)piperazine.

Yield: 17%; mp 65-66°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:1.44 (t, 3H, -C$\underline{H}_3$, *J=6.7*); 1.89 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.4*); 2.64 (t, 2H, -C$\underline{H}_2$-N[1], *J=5.4*); 2.75 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.23 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.59 (q, 2H, NH-C$\underline{H}_2$, *J=5.4*); 4.05 (q, 2H, -OC$\underline{H}_2$, *J=6.9*); 6.86 (d, 1H, *J=7.6*); 6.94 (bs, 3H); 7.38 (t, 1H, *J=8.0*); 7.82 (t, 1H, *J=7.3*); 8.17 (d, 1H, *J=7.6*); 8.50 (d, 1H, *J=5.8*); 9.04 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 15.16; 25.75; 39.37; 50.34; 53.77; 57.65; 63.78; 112.72; 118.30; 121.25; 122.39; 123.00; 126.19; 137.41; 141.54; 148.32; 150.46; 151.82; 164.74.

ESI-MS: 369.4 $[M+H]^+$, 391.3 $[M+Na]^+$.

Anal. ($C_{21}H_{28}N_4O_2$), C, H, N.

**4.1.28. N-(3-(4-(2-cyanophenyl)piperazin-1-yl)propyl)picolinamide (4e)**

From **2b** and 1-(2-cyanophenyl)piperazine.

Yield: 47%; mp 109-110°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 1.84 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.4*); 2.61 (t, 2H, -C$\underline{H}_2$-N[1], *J=5.4*); 2.71 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.33 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.60 (q, 2H, -NH-C$\underline{H}_2$, *J=5.1*); 7.01 (d, 1H, *J=8.0*); 7.03 (d, 1H, *J=7.3*); 7.39 (t, 1H, *J=7.6*); 7.48 (t, 1H, *J=7.3*); 7.56 (d, 1H, *J=7.3*); 7.83 (t, 1H, *J=7.6*); 8.19 (d, 1H, *J=7.6*); 8.49 (d, 1H, *J=7.6*); 8.97 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.88; 39.47; 51.63; 53.50; 57.55; 106.16; 118.67; 118.74; 121.87; 122.50; 126.24; 134.06; 134.69; 137.51; 148.16; 150.49; 156.05; 164.59.

ESI-MS: 350.3 $[M+H]^+$, 372.0 $[M+Na]^+$.

Anal. ($C_{20}H_{23}N_5O$), C, H, N.

**4.1.29. N-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)propyl)picolinamide (4f)**

From **2b** and 1-(3-(trifluoromethyl)phenyl)piperazine.

Yield: 23%; mp 52-54°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:1.90 (m, 2H, -$CH_2$-C<u>$H_2$</u>-$CH_2$, *J=5.4*); 2.73 (t, 2H, -C<u>$H_2$</u>-N[1], *J=5.4*); 2.76 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.01 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.62 (q, 2H, -NH-C<u>$H_2$</u>, *J=4.7*); 6.95 (t, 1H, *J=7.3*); 6.99 (t, 1H, *J=7.3*); 7.02 (d, 1H, *J=6.9*); 7.19 (d, 1H, *J=7.3*); 7.37 (t, 1H, *J=8.0*); 7.82 (t, 1H, *J=7.3*); 8.17 (d, 1H, *J=7.6*); 8.52 (d, 1H, *J=5.8*); 9.03 (bs, 1H, -N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.90; 39.56; 48.87; 53.36; 57.74; 112.37; 116.02; 119.00; 122.46; 126.27; 129.81; 137.50; 139.27; 148.21; 150.44; 151.78; 164.25.

ESI-MS: 393.3 $[M+H]^+$.

Anal. ($C_{20}H_{23}F_3N_4O$), C, H, N.

**4.1.30. N-(3-(4-(2,3-dimethylphenyl)piperazin-1-yl)propyl)picolinamide (4g)**

From **2b** and 1-(2,3-dimethylphenyl)piperazine.

Yield: 45%; mp 47-49°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:1.89 (m, 2H, -$CH_2$-C<u>$H_2$</u>-$CH_2$, *J=5.4*); 2.21 (s, 3H, -C<u>$H_3$</u>); 2.26 (s, 3H, -C<u>$H_3$</u>); 2.65 (bs, 4H, 2C<u>$H_2$</u> pip.); 2.71 (t, 2H, -C<u>$H_2$</u>-N[1], *J=5.4*); 3.02 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.59 (q, 2H, -NH-C<u>$H_2$</u>, *J=4.7*); 6.92 (d, 1H, *J=7.6*); 6.94 (d, 1H, *J=7.3*); 7.10 (t, 1H, *J=7.6*); 7.40 (t, 1H, *J=7.4*); 7.82 (t, 1H, *J=7.6*); 8.18 (d, 1H, *J=7.6*); 8.54 (d, 1H, *J=7.6*); 9.00 (bs, 1H, -N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 14.21; 20.86; 25.87; 39.44; 52.02; 54.09; 57.71; 116.71; 122.43; 125.16; 126.09; 126.21; 131.46; 137.44; 138.25; 148.32; 150.48; 151.80; 164.74.

ESI-MS: 353.0 $[M+H]^+$.

Anal. ($C_{21}H_{28}N_4O$), C, H, N.

**4.1.31. N-(3-(4-(2-chlorophenyl)piperazin-1-yl)propyl)picolinamide (4h)**

From **2b** and 1-(2-chlorophenyl)piperazine.

Yield: 27%; mp 185-187°C. $^1$H-NMR (400 MHz, DMSO) δ:2.03 (m, 2H, -$CH_2$-C<u>$H_2$</u>-$CH_2$, *J=5.4*); 3.17 (t, 4H, 2C<u>$H_2$</u> pip., *J=4.7*); 3.21 (t, 2H, -C<u>$H_2$</u>-N[1], *J=5.4*); 3.38 (t, 4H, 2C<u>$H_2$</u> pip., *J=4.7*); 3.53 (q, 2H, NH-C<u>$H_2$</u>, *J=5.4*); 7.08 (t, 1H, *J=7.0*); 7.18 (d, 1H, *J=7.4*); 7.22 (t, 1H, *J=7.0*); 7.35 (d, 1H, *J=7.4*); 7.62 (t, 1H, *J=8.0*); 8.06 (t, 1H, *J=7.3*); 8.32 (d, 1H, *J=7.6*); 8.65 (d, 1H, *J=5.8*); 9.05 (bs, 1H, N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 24.10; 36.79; 48.02; 51.54; 53.93; 121.42; 122.23; 125.53; 127.13; 127.97; 128.71; 130.91; 138.70; 147.87; 148.57; 149.97; 164.32.

ESI-MS: 359.2 $[M+H]^+$.

Anal. ($C_{19}H_{23}ClN_4O$), C, H, N.

**4.1.32. N-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)picolinamide (4i)**

From **2b** and 1-(3-chlorophenyl)piperazine.

Yield: 72%; mp 77-78°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:1.84 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.4*); 2.58 (t, 2H, -C$\underline{H}_2$-N[1], *J=5.4*); 2.63 (t, 4H, 2C$\underline{H}_2$ pip., *J=5.1*); 3.28 (t, 4H, 2C$\underline{H}_2$ pip., *J=5.1*); 3.59 (q, 2H, NH-C$\underline{H}_2$, *J=5.4*); 6.80 (m, 2H); 6.88 (s, 1H, *J=7.3*); 7.17 (t, 1H, *J=8.2*); 7.36 (t, 1H, *J=7.4*); 7.81 (t, 1H, *J=7.6*); 8.17 (d, 1H, *J=7.3*); 8.40 (d, 1H, *J=7.6*); 9.03 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.92; 39.51; 48.81; 53.36; 57.70; 114.02; 115.91; 119.41; 122.41; 126.22; 130.27; 135.19; 137.44; 148.22; 150.48; 152.70; 164.62.

ESI-MS: 359.2 $[M+H]^+$, 381.3 $[M+Na]^+$, 397.3 $[M+K]^+$.

Anal. ($C_{19}H_{23}ClN_4O$), C, H, N.

**4.1.33. N-(3-(4-(4-chlorophenyl)piperazin-1-yl)propyl)picolinamide (4j)**

From **2b** and 1-(4-chlorophenyl)piperazine.

Yield: 21%; mp 81-83°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:1.84 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.8*); 2.58 (t, 2H, -C$\underline{H}_2$-N[1], *J=5.4*); 2.64 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.25 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.58 (q, 2H, -NH-C$\underline{H}_2$, *J=5.4*); 6.86 (d, 2H, *J=8.2*); 7.20 (d, 2H, *J=8.2*); 7.36 (t, 1H, *J=7.4*); 7.81 (t, 1H, *J=7.6*); 8.17 (d, 1H, *J=7.3*); 8.39 (d, 1H, *J=7.6*); 9.05 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.64; 39.30; 49.02; 53.16; 57.47; 117.13; 122.17; 124.42; 125.97; 128.95; 137.19; 147.96; 150.04; 150.23; 164.39.

ESI-MS: 359.2 $[M+H]^+$.

Anal. ($C_{19}H_{23}ClN_4O$), C, H, N.

**4.1.34. N-(3-(4-(3,4-dichlorophenyl)piperazin-1-yl)propyl)picolinamide (4k)**

From **2b** and 1-(3,4-dichlorophenyl)piperazine.

Yield: 37%; mp 92-93°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ:1.84 (m, 2H, -$CH_2$-C$\underline{H}_2$-$CH_2$, *J=5.8*); 2.58 (t, 2H, -C$\underline{H}_2$-N[1], *J=5.4*); 2.63 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.26 (bs, 4H, 2C$\underline{H}_2$ pip.); 3.60 (q, 2H, -NH-C$\underline{H}_2$, *J=5.4*); 6.76 (d, 1H, *J=8.8*); 6.96 (s, 1H); 7.29 (d, 1H, *J=8.8*); 7.37 (t, 1H, *J=7.4*); 7.82 (t, 1H, *J=7.6*); 8.17 (d, 1H, *J=7.3*); 8.40 (d, 1H, *J=7.6*); 9.02 (bs, 1H, N$\underline{H}$). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.90; 39.49; 48.80; 53.21; 57.65; 115.42; 117.35; 122.33; 122.44; 126.26; 130.68; 133.03; 137.47; 148.17; 150.45; 151.03; 164.62.

ESI-MS: 393.1 $[M+H]^+$.

Anal. ($C_{19}H_{22}Cl_2N_4O$), C, H, N.

**4.1.35. N-(3-(4-(2-fluorophenyl)piperazin-1-yl)propyl)picolinamide (4l)**

From **2b** and 1-(2-fluorophenyl)piperazine.

Yield: 29%; mp 64-65°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 1.84 (m, 2H, -$CH_2$-C<u>$H_2$</u>-$CH_2$, *J=5.4*); 2.60 (t, 2H, -C<u>$H_2$</u>-N[1], *J=5.4*); 2.69 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.21 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.61 (q, 2H, -NH-C<u>$H_2$</u>, *J=5.1*); 6.95-7.06 (m, 4H); 7.38 (t, 1H, *J=7.4*); 7.83 (t, 1H, *J=7.6*); 8.18 (d, 1H, *J=7.6*); 8.49 (d, 1H, *J=7.6*); 9.05 (bs, 1H, N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.88; 39.56; 50.63; 53.67; 57.75; 116.26; 119.00; 122.44; 122.61; 124.68; 124.71; 126.18; 137.43; 148.22; 150.54; 154.79; 164.63.

ESI-MS: 343.4 $[M+H]^+$, 365.4 $[M+Na]^+$.

Anal. ($C_{19}H_{23}FN_4O$), C, H, N.

**4.1.36. N-(3-(4-(4-fluorophenyl)piperazin-1-yl)propyl)picolinamide (4m)**

From **2b** and 1-(4-fluorophenyl)piperazine.

Yield: 26%; mp 62-64°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 1.84 (m, 2H, -$CH_2$-C<u>$H_2$</u>-$CH_2$, *J=5.4*); 2.58 (t, 2H, -C<u>$H_2$</u>-N[1], *J=5.4*); 2.66 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.21 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.59 (q, 2H, -NH-C<u>$H_2$</u>, *J=5.1*); 6.90 (d, 2H, *J=8.0*); 6.97 (t, 2H, *J=8.0*); 7.36 (t, 1H, *J=7.4*); 7.81 (t, 1H, *J=7.6*); 8.16 (d, 1H, *J=7.3*); 8.41 (d, 1H, *J=7.6*); 9.03 (bs, 1H, N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.90; 39.51; 50.26; 53.55; 57.63; 115.64; 117.90; 122.42; 126.22; 137.45; 148.21; 150.42; 156.18; 158.55; 164.68.

ESI-MS: 343.4 $[M+H]^+$, 365.5 $[M+Na]^+$.

Anal. ($C_{19}H_{23}FN_4O$), C, H, N.

**4.1.37. N-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)picolinamide (4n)**

From **2b** and 1-(pyridin-2-yl)piperazine.

Yield: 26%; mp 102-103°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ:1.85 (m, 2H, -$CH_2$-C<u>$H_2$</u>-$CH_2$, *J=5.4*); 2.57 (t, 2H, -C<u>$H_2$</u>-N[1], *J=5.4*); 2.60 (bs, 4H, 2C<u>$H_2$</u> pip.); 3.59 (q, 2H, -NH-C<u>$H_2$</u>, *J=5.1*); 3.63 (bs, 4H, 2C<u>$H_2$</u> pip.); 6.61 (d, 1H, *J=8.4*); 6.63 (t, 1H, *J=7.3*); 6.66 (t, 1H, *J=7.3*); 7.36 (t, 1H, *J=7.3*); 7.48 (t, 1H, *J=7.6*); 7.81 (t, 1H, *J=7.3*); 8.19 (d, 1H, *J=7.6*); 8.43 (d, 1H, *J=7.6*); 9.02 (bs, 1H, N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.97; 39.45; 45.39; 53.40; 57.75; 107.24; 113.45; 122.39; 126.18; 137.42; 137.68; 148.23; 148.38; 150.47; 159.83; 164.64.

ESI-MS: 326.1 $[M+H]^+$, 348.2 $[M+Na]^+$.

Anal. ($C_{18}H_{23}N_5O$), C, H, N.

**4.1.38. N-(3-(4-(pyrimidin-2-yl)piperazin-1-yl)propyl)picolinamide (4o)**

From **2b** and 1-(pyrimidin-2-yl)piperazine.

Yield: 86%; mp 89-91°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 1.84 (m, 2H, -$CH_2$-C<u>H</u>$_2$-$CH_2$, *J=5.4*); 2.54 (bs, 4H, 2C<u>H</u>$_2$ pip.); 2.57 (t, 2H, -C<u>H</u>$_2$-N[1], *J=5.4*); 3.60 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.4*); 3.91 (bs, 4H, 2C<u>H</u>$_2$ pip.); 6.47 (t, 1H, *J=8.1*); 7.37 (t, 1H, *J=7.3*); 7.82 (t, 1H, *J=7.3*); 8.17 (d, 1H, *J=7.6*); 8.29 (d, 2H, *J=7.0*); 8.48 (d, 1H, *J=7.6*); 9.02 (bs, 1H, N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.94; 39.45; 43.83; 53.49; 57.81; 109.97; 122.40; 126.18; 137.44; 148.23; 150.93; 157.95; 162.17; 165.26.

ESI-MS: 327.2 $[M+H]^+$.

Anal. ($C_{17}H_{22}N_6O$), C, H, N.

**4.1.39. N-(3-(4-(piperonyl)piperazin-1-yl)propyl)picolinamide (4p)**

From **2b** and 1-(piperonyl)piperazine.

Yield: 17%; mp 82-83°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 1.80 (bs, 6H); 2.52 (bs, 6H); 3.45 (s, 2H, -C<u>H</u>$_2$-); 3.55 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.4*); 5.93 (s, 2H, -OC<u>H</u>$_2$); 6.72 (bs, 2H); 6.85 (s, 1H); 7.82 (t, 1H, *J=7.3*); 8.16 (d, 1H, *J=7.6*); 8.49 (d, 1H, *J=7.3*); 8.86 (bs, 1H, N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 26.02; 39.31; 52.93; 53.52; 57.49; 63.08; 101.10; 108.09; 109.82; 122.42; 122.53; 126.15; 132.11; 137.42; 146.84; 147.85; 148.21; 150.51; 164.64.

ESI-MS: 383.0 $[M+H]^+$.

Anal. ($C_{21}H_{26}N_4O_3$), C, H, N.

**4.1.40. N-(3-(4-(furoyl)piperazin-1-yl)propyl)picolinamide (4q)**

From **2b** and 1-(furoyl)piperazine.

Yield: 56%; mp 54-56°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 1.82 (qt, 2H, -$CH_2$-C<u>H</u>$_2$-$CH_2$, *J=5.4*); 2.59 (bs, 6H); 3.58 (q, 2H, NH-C<u>H</u>$_2$, *J=5.4*); 3.90 (bs, 4H, 2C<u>H</u>$_2$ pip.); 6.47 (d, 1H, *J=7.3*); 6.99 (d, 1H, *J=7.6*); 7.41 (t, 1H, *J=7.3*); 7.46 (t, 1H, *J=7.6*); 7.83 (t, 1H, *J=7.6*); 8.18 (d, 1H, *J=7.9*); 8.52 (d, 1H, *J=7.3*); 8.99 (bs, 1H, N<u>H</u>). $^{13}$C-NMR (400 MHz, $CDCl_3$) δ: 25.85; 39.41; 53.65; 57.62; 111.47; 116.51; 122.48; 126.27; 137.53; 143.83; 148.21; 150.45; 159.25; 164.58.

ESI-MS: 343.3 $[M+H]^+$, 365.2 $[M+Na]^+$, 381.2 $[M+K]^+$.

Anal. ($C_{18}H_{22}N_4O_3$), C, H, N.

**4.1.41 N-(3-(4-(4-chlorobenzhydryl)piperazin-1-yl)propyl)picolinamide (4r)**

From **2b** and 1-(4-chlorobenzhydryl)piperazine.

Yield: 11%; mp 37-39°C. $^{1}$H-NMR (400 MHz, $CDCl_3$) δ: 1.80 (m, 2H, -$CH_2$-C<u>H</u>$_2$-$CH_2$, *J=5.4*); 2.51 (bs, 4H, 2C<u>H</u>$_2$ pip.); 2.54 (bs, 6H); 3.53 (q, 2H, -NH-C<u>H</u>$_2$, *J=5.1*); 4.25 (s, 1H, -C<u>H</u>-); 7.19 (t, 1H, *J=7.3*); 7.21-7.27 (m, 4H); 7.34-7.37 (m, 4H); 7.42 (t, 1H, *J=7.6*); 7.82 (t, 1H, *J=7.6*); 8.15

(d, 1H, *J=7.9*); 8.43 (d, 1H, *J=7.3*); 8.79 (bs, 1H, NH). $^{13}$C-NMR (400 MHz, DMSO) δ: 24.36; 34.65; 48.38; 53.19; 56.35; 75.58; 122.17; 126.59; 127.62; 127.82; 129.04; 129.20; 129.27; 132.18; 137.33; 141.58; 142.32; 148.54; 149.02; 165.54.

ESI-MS: 449.2 $[M+H]^+$, 471.1 $[M+Na]^+$.

Anal. ($C_{26}H_{29}ClN_4O$), C, H, N.

**4.1.42 N-(3-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)propyl)picolinamide (4s)**

From **2b** and 1-bis(4-fluorophenyl)methyl)piperazine.

Yield:22%; mp 116-118°C. $^1$H-NMR (400 MHz, $CDCl_3$) δ: 2.25 (m, 2H, -$CH_2$-$CH_2$-$CH_2$, *J=5.4*); 3.00 (bs, 4H, 2$CH_2$ pip.); 3.13 (bs, 4H, 2$CH_2$ pip.); 3.50 (t, 2H, -$CH_2$-N$^1$, *J=5.8*); 3.61 (q, 2H, -NH-$CH_2$, *J=5.1*); 4.53 (s, 1H, -CH-); 7.16 (t, 4H, *J=8.5*); 7.43 (t, 4H, *J=7.6*); 7.46 (t, 1H, *J=7.6*); 7.83 (t, 1H, *J=7.6*); 8.09 (d, 1H, *J=7.9*); 8.39 (bs, 1H, NH); 8.54 (d, 1H, *J=7.3*). $^{13}$C-NMR (400 MHz, DMSO) δ: 24.43; 36.47; 48.25; 54.90; 58.74; 75.13; 116.14; 116.34; 122.17; 126.46; 129.43; 137.42; 148.28; 149.23; 161.63; 165.19.

ESI-MS: 451.3 $[M+H]^+$.

Anal. ($C_{26}H_{28}F_2N_4O$), C, H, N.

**4.1.43. N-(3-(4-(naphthalen-1-yl)piperazin-1-yl)propyl)picolinamide (4t)**

From **2b** and 1-(naphthalen-1-yl)piperazine.

Yield:22%; mp 201-203°C. $^1$H-NMR (400 MHz, DMSO) δ: 2.20 (m, 2H, -$CH_2$-$CH_2$-$CH_2$, *J=5.4*); 3.38 (t, 4H, 2$CH_2$ pip., *J=4.7*); 3.51 (t, 2H, -$CH_2$-N$^1$, *J=5.4*); 3.62 (t, 4H, 2$CH_2$ pip., *J=4.7*); 3.73 (q, 2H, -NH-$CH_2$, *J=5.4*); 7.21 (d, 1H, *J=7.3*); 7.43 (t, 1H, *J=8.0*); 7.50 (m, 2H, *J=7.3*); 7.63 (d, 1H, *J=8.3*); 7.72 (t, 1H, *J=5.8*); 7.85 (d, 1H, *J=7.3*); 8.16 (d, 1H, *J=8.3*); 8.18 (d, 1H, *J=8.3*); 8.25 (t, 1H, *J=7.6*); 8.28 (bs, 1H, NH); 8.71 (d, 1H, *J=7.0).* $^{13}$C-NMR (400 MHz, DMSO) δ: 24.36; 36.43; 50.96; 52.65; 54.60; 115.27; 122.64; 122.80; 124.83; 125.62; 125.85; 126.06; 127.33; 127.42; 128.40; 128.71; 135.14; 147.36; 147.64; 164.17.

ESI-MS: 375.2 $[M+H]^+$.

Anal. ($C_{23}H_{26}N_4O$), C, H, N.

**4.2. In Vitro Receptor Binding**

**4.2.1. General procedures**

The newly synthesized compounds were tested for *in vitro* affinity for serotonin 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors by radioligand binding assays. The following specific radioligands and tissue sources were used: (a) serotonin 5-$HT_{1A}$ receptor, [$^3$H]-8-OH-DPAT, rat brain cortex; (b)

serotonin 5-$HT_{2A}$ receptor, [$^3$H]ketanserin, rat brain cortex; (c) serotonin 5-$HT_{2C}$ receptor, [$^3$H]mesulergine, rat brain cortex.

Non-specific binding was determined as described in the experimental section, and specific binding as the difference between total and non-specific binding. Blank experiments were carried out to determine the effect of 5% DMSO on the binding and no effects were observed. Competition experiments were analyzed by PRISM 5 (GraphPadPrism®, 1992-2007, GraphPad Software, Inc., La Jolla, CA, USA) to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding ($IC_{50}$), with six concentrations of test compounds, each performed in triplicate. The $IC_{50}$ values obtained were used to calculate apparent inhibition constants ($K_i$) by the method of Cheng and Prussoff [42], from the following equation: $K_i = IC_{50}/(1+S/K_D)$ where S represents the concentration of the hot ligand used and $K_D$ its receptor dissociation constant ($K_D$ values, obtained by Scatchard analysis [43], were calculated for each labeled ligand).

The more active compounds on serotonin receptors have been selected and evaluated for their affinity for dopaminergic ($D_1$ and $D_2$) and adrenergic ($\alpha_1$ and $\alpha_2$) receptors. The following specific radioligands and tissue sources were used: (a) dopamine $D_1$ receptor [$^3$H]SCH-23390, human recombinant (CHO cells); (b) dopamine $D_2$ receptor [$^3$H]methyl-spiperone, human recombinant (HEK-293 cells); (c) $\alpha_1$adrenergic receptor [$^3$H]prazosin, rat cerebral cortex; (d) $\alpha_2$ adrenergic receptor [$^3$H]RX 821002, rat cerebral cortex.

The experiments on these receptors ($D_1$, $D_2$, $\alpha_1$ and $\alpha_2$) were performed at Eurofins-Cerep SA Le Bois l'Evêque - BP 30001 - 86600 CELLE L'EVESCAULT – France (Study Number: 100041183) according the Standardized Operational Protocols in place, following the experimental protocols already described for $D_1$[44], $D_2$ [45], $\alpha_1$[46] and $\alpha_2$ [47].

#### 4.2.2. 5-$HT_{1A}$ binding assay

Radioligand binding assays were performed following a published procedure [48]. Cerebral cortex from male Sprague-Dawley rats (180–220 g) was homogenized in 20 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.7 at 22°C) with a Polytron PT10, Brinkmann Instruments (setting 5 for 15 sec), and the homogenate was centrifuged at 50000 *g* for 10 min at 0°C. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37°C, and centrifuged at 50000 *g* for 10 min. The final pellet was resuspended in 80 volumes of the Tris-HCl buffer containing 10 µM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbate. To each assay tube was added the following: 0.1 mL of the drug dilution (0.1 mL of distilled water if no competing drug was added), 0.1 mL of [$^3$H]-8-hydroxy-2-(di-n-propylamino)tetralin ([$^3$H]-8-OH-DPAT) (170.0 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA) in the same buffer as above to achieve a final assay

concentration of 1 nM, and 0.8 mL of resuspended membranes. The tubes were incubated for 30 min at 37°C, and the incubations were terminated by *vacuum* filtration through Whatman GF/B filters (Brandel Biomedical Research and Laboratories Inc., Gaithersburg, MD, USA). The filters were washed twice with 5 mL of ice-cold Tris-HCl buffer, dried and immersed into vials containing 8 ml of Complete LSC-Cocktail (Perkin Elmer). The radioactivity bound to the filters was measured by liquid scintillation spectrometer (Packard TRI-CARB® 2000CA - Packard BioScience s.r.l., Pero, Milan, Italy). Specific [$^3$H]-8-OH-DPAT binding was defined as the difference between binding in the absence and presence of 5-HT (10 μM).

#### 4.2.3. 5-HT$_{2A}$ and 5-HT$_{2C}$ binding assays

Radioligand binding assays were performed as previously reported by Herndon et al. [49]. Briefly, frontal cortical regions of male Sprague-Dawley rats (180-220 g) were dissected on ice and homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris HCl, 0.5 mM EDTA, and 10 mM $MgCl_2$ at pH 7.4) with a Polytron PT10 (setting 5 for 15 sec) and centrifuged at 3000 *g* for 15 min. The pellet was resuspended in buffer (1:30 w/v), incubated at 37°C for 15 min and then centrifuged twice more at 3000 *g* for 10 min (with resuspension between centrifugations). The final pellet was resuspended in buffer that also contained 0.1% ascorbate and $10^{-5}$ M pargyline.

Assays were performed in triplicate in a 2.0 mL volume containing 5 mg wet weight of tissue and 0.4 nM [$^3$H] ketanserin hydrochloride (47.3 Ci/mmol; Perkin Elmer Life Sciences, Boston, MA, USA) for 5-HT$_{2A}$ receptor assays, and 10 mg wet weight of tissue and 1 nM [$^3$H]mesulergine (83.1 Ci/mmol; Amersham Biosciences Europe GmbH) for 5-HT$_{2C}$ receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT$_{2A}$ assay. In the 5-HT$_{2C}$ assays, mianserin (1.0 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block binding to 5-HT$_{2A}$ receptors. Tubes were incubated for 15 min at 37°C, filtered on Schliecher and Schuell (Keene, NH, USA) glass fibre filters presoaked in polyethylene imine, and washed with 10 mL of ice-cold buffer. The filters, dried and immersed into vials containing 8 ml of Complete LSC-Cocktail (Perkin Elmer), were counted at an efficiency of 50%.

### 4.3. In Vitro Assays

#### 4.3.1.General procedures

Male rats (Sprague-Dawley, 160-200 g; Harlan Laboratories, S. Pietro al Natisone, Italy) were manipulated and cared for in strict compliance with the Principles of laboratory animal care (NIH publication n° 86–23, revised 1985) and the Italian D.L. no.116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Animal housing complied with recent pharmacological guidance [50]. All animals weighing 160-200 g were used after a 1 week acclimation period (temperature 23±2°C; humidity 60%, free access to water and standard food).

#### 4.3.2. Ileum preparation and evaluation of 5-HT-evoked contractions

Rats were asphyxiated using $CO_2$ and segments (1-1.5 cm) of ileum were removed, flushed of luminal contents, and placed in Krebs solution (119 mM NaCl, 4.75 mM KCl, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.5 mM $MgSO_4$, and 11 mM Glucose). The segments were prepared as previously described [51]: the segments were set up in such a way as to record contractions mainly from the longitudinal axis, in an organ bath containing 20 mL of Krebs solution, bubbled with 95% $O_2$ and 5% $CO_2$ and maintained at 37 °C. The tissues were connected to an isotonic transducer (load: 0.5 g), connected to PowerLab system (Ugo Basile, Comerio, Italy). Ileal segments were equilibrated for 60 min [51] followed by three repeated additions of submaximal concentration of 5-HT ($10^{-5}$ M) in order to record stable control contractions. To evaluate the inhibitory activity, the responses were observed in the presence of increasing concentrations ($10^{-8}$-$10^{-5}$ M). In preliminary experiments, the effect of 5-HT was observed in the presence of the neuronal blocker tetrodotoxin (0.3 μM), the muscarinic receptor antagonist atropine (1 μM), the adrenergic receptor antagonists phentolamine ($10^{-6}$ M) plus propranolol ($10^{-6}$ M) and the $5\text{-}HT_{2A}$ antagonist ketanserin (0.1 μM). The contact time for each concentration was 10 min. The compounds were dissolved in DMSO. DMSO (<0.01%) did not modify 5-HT-induced contractions. Results are expressed as mean (SEM). The concentration of the compounds that produced 50% inhibition of 5-HT-induced contractions ($IC_{50}$) or maximal inhibitory effect ($E_{max}$) were used to characterize compounds potency and efficacy, respectively. The $IC_{50}$ and $E_{max}$ values were calculated with the aid of a computer program (Graphpad Prism 5).

### 4.4. In Vivo Behavioral tests

#### 4.4.1. General procedures.

The experiments were performed on male Swiss albino mice (18 - 24 g) obtained from the Farm of Laboratory Animals, Jacek Kołacz, Warsaw and male Wistar rats (250-300 g) purchased from a licensed breeder (Farm of Laboratory Animals, Z. Lipiec, Brwinow, Poland). Mice were kept in groups of eight – ten, rats were caged in groups of six in an environmentally controlled rooms (ambient temperature 22 ± 1 °C; relative humidity 50 - 60%; 12:12 light:dark cycle, lights on at 8:00). The animals were allowed to acclimatize with the environment for one week before commencement of the experiments. Standard laboratory food (LSM, Agropol-Motycz, Poland)

and filtered water were available *ad libitum*. All the experimental procedures were carried out, according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Directive for the Care and Use of Laboratory of 24 November 1986 (86/609/EEC), and approved by the Local Ethics Committee for Animal Experimentation (23/2016). The investigated compounds (**3j, 3k** and **4o**) in all tests were administered intraperitoneally (i.p.), dissolved in DMSO (its final concentration of 0.1 %) and then diluted by aqueous solution of 0.5 % methylcellulose and injected 60 min before the tests. 8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT, Sigma-Aldrich),and N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridynyl)cyclohexane carboxamide trihydrochloride (WAY-100635, Sigma-Aldrich) were used in the form of aqueous solutions and injected subcutaneously (s.c.), while reference drugs (buspirone and fluoxetine) were administered i.p., diluted in saline. All compounds were given in a volume of 2 ml/kg (rats) or 10 ml/kg (mice). The control animals received an equivalent volume of the solvent at the respective time before the tests. All the experiments were conducted in the light phase between 09.00 a.m. and 14.00 p.m. Animals were used only once in each test. The experiments were performed by an observer unaware of the treatment administered. The results were calculated by the two-way analysis of variance (ANOVA) (body temperature test) and one-way ANOVA (other tests), followed by the Dunnett's, Bonferroni's and Newman-Keuls' post hoc tests. The results are presented as mean ± standard errors (SEM). The level of $p<0.05$ was considered as statistically significant. All the figures were prepared by the GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego, California, USA), www.graphpad.com.

#### 4.4.2. Spontaneous locomotor activity

Spontaneous locomotor activity was measured using an animal activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, OH, USA), as was described in details previously [52]. The animals were placed individually in an actometer for 30 min. A distance travelled by a tested mouse was measured after 6 min (corresponded with the time duration of the FST and near (5 min) to the EPM test, respectively) and 30 min to characterize dynamics of changes. The cages were cleaned up with 70% ethanol after each mouse.

#### 4.4.3. Motor coordination.

The effects of investigated compounds on motor coordination was evaluated in the rota-rod [18, 53] and chimney tests [18,54]. In the first test, motor impairments were measured, defined as the inability to keep balance on a rotating rod (at constant speed of 18 rpm) for 1 min. In the second

test, motor impairments were assessed by mouse inability to climb up the tube backwards (3 cm in inner diameter, 25 cm long) within 60 s. Before the tests, the animals were trained once a day for 3 days. The animals, able to stay on the rotating rod or to leave the chimney for 60 s, were approved for experiments.

**4.4.4. Effects on body temperature.**

Body temperature in normothermic mice was measured in animal's rectum with a thermistor thermometer during a total period of 180 min (60 min before and 120 min after tested compound injection). The mean value from the first two measurements (60 and 30 min before drug administration) was assumed as initial temperature (ti). The final temperature (tf) was measured 30, 60, 90 and 120 min after the injection of tested compounds at a dose of 30 mg/kg. Body temperature changes ($\Delta t$) were calculated according to the formula: $\Delta t = tf - ti$ [55].

**4.4.5. Lower Lip Retraction (LLR) in Rats.**

LLR was assessed according to the method described by Berendsen et al. [40]. The rats were individually placed in cages (30 cm x 25 cm x 25 cm) and were scored three times at 15, 30, and 45 min after administration of the tested compounds, as follows: 0) lower incisors not visible, 0.5) partly visible, 1) completely visible. The total maximum scores amounted to 3 for each rat. In a separate experiment, the effect of the tested compounds or WAY 100635 on the LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The compounds and WAY 100635 (0.3 mg/kg) were administered 45 and 15 min, respectively, before 8-OH-DPAT and the animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

**4.4.6. Forced swimming test (FST, Porsolt's test) in mice.**

The experiment was carried out according to the method of Porsolt et al. [39]. Mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 10 cm of water maintained at 23 - 25°C, and were left there for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session.

**4.4.7. Elevated plus-maze test.**

The EPM studies were carried out on mice according to the method of Lister [38]. The EPM apparatus was made of Plexiglas and consisted of two open (30 ×5 cm) and two enclosed (30 × 5 × 15 cm) arms. The arms extended from a central platform of 5 × 5 cm. The apparatus was

mounted on a Plexiglas base, raising it 38.5 cm above the floor, and illuminated by a red light. The test consisted of placing a mouse in the center of the apparatus (facing an open arm) and allowing it to freely explore. The number of entries into the open arms and the time spent in these arms were scored for a 5 min test period. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: the total number of arm entries; the percentage of arm entries into the open arms; and the time spent in the open arms expressed as a percentage of the time spent in both the open and closed arms. Anxiolytic activity was indicated by increases in the time spent in open arms and in the number of open arm entries. The total number of entries into either type of arm was used additionally as a measure of overall motor activity.

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**New 5-HT$_{1A}$, 5HT$_{2A}$ and 5HT$_{2C}$ receptor ligands containing a picolinic nucleus: Synthesis, *in vitro* and *in vivo* pharmacological evaluation.**

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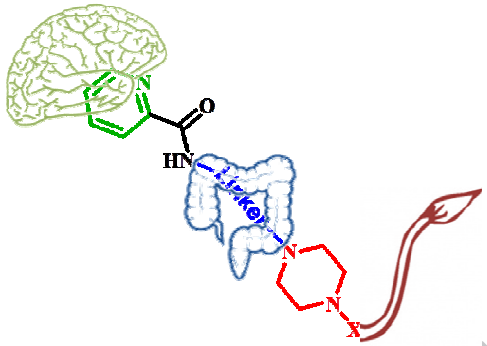

Highlights

>Serotonin is involved in physiological and pathophysiological processes> Picolinamide derivatives, linked to an arylpiperazine moiety>The combination of structural elements known to be critical for affinity to serotoninergic receptors>In binding studies, several molecules showed high affinity, selectivity and functional activity at 5-$HT_{1A}$, 5-$HT_{2A}$ and $5HT_{2C}$ receptors.