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Use of imidazo[1,5-*a*]quinoline scaffold as the pharmacophore in the design of bivalent ligands of central benzodiazepine receptors

Marco Paolino [a,*], Mario Saletti [a], Jacopo Venditti [a], Federica Castriconi [a], Germano Giuliani [a], Samuele Maramai [a], Alessandra Toti [b], Carla Ghelardini [b], Rosanna Matucci [b], Narcy Alcazar Villalobos [c], Maurizio Anzini [a,1], Andrea Cappelli [a]

[a] *Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Via A. Moro 2, 53100 Siena, Italy*
[b] *Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino (NEUROFARBA) – Sez. Farmacologia e Tossicologia, Università di Firenze, Viale G. Pieraccini 6, 50139 Firenze, Italy*
[c] *Medicines Discovery Institute, Cardiff University, Park Place, CF10 3AT Cardiff, Wales, United Kingdom*

A B S T R A C T

The imidazo[1,5-*a*]quinoline scaffold of central benzodiazepine receptor (CBR) ligands was used as the pharmacophore in the design of bivalent ligands bearing spacers showing variable length and different physicochemical features. The newly designed compounds were synthesized along with the corresponding reference monovalent compounds bearing the corresponding spacers terminated with a *tert*-butoxycarbonyl group. The novel compounds were tested in binding assays with different CBR preparations such as the cerebral cortex from male CD-1 albino mice or the human recombinant α1β3γ2 and α2β3γ2 γ-aminobutyric acid type A receptors ($GABA_A$Rs) stably expressed in mouse L(tk-) cells. The tested compounds showed IC_{50} values from the sub-micromolar up to the nanomolar range with very similar inhibition constants values for the two isoforms of $GABA_A$Rs. The similarity in the affinity between the bivalent ligands and the corresponding monovalent ones appeared to rule out any bivalent interactions of these ligands with the two isoforms of $GABA_A$Rs. Similarly, both series were able to inhibit the binding of radiolabeled flumazenil to $GABA_A$Rs in cortical membranes of albino CD-1 mice, but most of the tested compounds showed biphasic inhibition curves, suggesting the existence of two well-distinct populations of binding sites. Finally, some CBR ligands selected from the bivalent ligands (i.e. **6a**,**c**) and from the reference monovalent ligands (i.e. **7a**) were then tested *in vivo* for their potential pharmacological effects, evaluating four classical benzodiazepine actions such as anticonvulsant, anxiolytic, locomotor, and anti-amnesic activities. All the tested compounds showed anticonvulsant and anxiolytic properties with neither muscle relaxant effect nor learning and memory impairments.

1. Introduction

The central neurotransmitter γ-aminobutyric acid (GABA) exerts its action through both GABA type A ($GABA_A$Rs) and type B ($GABA_B$Rs) receptors, modulating the excitability of many central nervous system (CNS) pathways.[1] $GABA_A$Rs are chloride ions channel belonging to ligand-gated ion channels (LGICs) of the Cys-loop superfamily, to which nicotinic acetylcholine, glycine, zinc-activated, and 5-HT_3 receptors also belong. Cys-loop receptors are targets of many drugs and feature the five-subunit assembly forming pentameric arrangements around a central ion-conducting pore.[1] The $GABA_A$R function is regulated by allosteric sites interacting with a large diversity of agents in addition to the agonist binding site.[2] The most known positive modulators of the $GABA_A$Rs is the classical 1,4-benzodiazepine diazepam (**1**, Figure 1).

The positive allosteric modulators (PAMs) of $GABA_A$Rs are an important class of drugs used as sedatives, anticonvulsants, anxiolytics, and muscle relaxants. On the other hand, negative $GABA_A$Rs modulators (i.e. inverse agonists) show anxiogenic and convulsant effects.[3–6] Finally, neutral modulators, [i.e. the imidazo[1,5-*a*][1,4]benzodiazepines flumazenil (**2**, Figure 1)] interact with $GABA_A$Rs but do not show any functional efficacy. Therefore, flumazenil is considered to antagonize the activity of both positive and negative $GABA_A$Rs modulators acting *via* the central benzodiazepine receptor (CBR). Positive modulators are documented as molecules with amnesic effects in animal and man,[7–10] whereas negative modulators were supposed to possess procognitive properties.[11,12] Unfortunately, the employment of CBR inverse agonists in the treatment of neurological disorders connected with cognitive impairment was limited by their anxiogenic and convulsant effects.[13]

Even if a large variety of different subunits (i.e. α_{1-6}, β_{1-4}, γ_{1-4}, δ, ε, π,

* Corresponding author.
E-mail address: paolino3@unisi.it (M. Paolino).
[1] Present address, Via del bosco 19, 53019 Castelnuovo Berardenga, Italy.

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θ and ρ_{1-3}) was cloned and sequenced, most of $GABA_A$Rs are constituted by the combination of α, *β*, and *γ*-subunits organized in a 2:2:1 stoichiometry.[14] Among the possible combinations stemming from the co-assembly of the subunits, barely the receptors composed by a γ_2 or γ_3[15] subunit combined with α_1, α_2, α_3, or α_5 ones appear to bind CBR ligands with noteworthy affinity, and the binding domain is recognized to be located at the interface between α and *γ* subunits.[16] Molecular genetics and pharmacological investigations suggested for α_1 subunit an important role in the sedative and muscle relaxant effects of the non-selective CBR agonists, while α_2 or α_3 subunits are associated with anxiolytic and anticonvulsant effects.[17–19] The recognition of the physiological and pharmacological roles of α subunits in $GABA_AR$ functions has renewed the general interest with the assumption that new drugs could be obtained with fewer side-effects or different therapeutic uses with respect to the classical benzodiazepines.[20–27] Many CBR ligands have been developed showing different $GABA_AR$ subtype selectivity: selective binding (i.e. by establishing a receptor-ligand complex with a particular receptor subtype) or by selective efficacy (i.e. by obtaining a biological response after binding to the receptor).[20,28].

Interestingly, imidazo[1,5-*a*][1,4]benzodiazepine derivatives **3** have been reported to show the full range of intrinsic efficacy, which was modulated in a rather subtle manner by the substitution pattern,[29,30] and similar results were obtained when the seven-membered ring of the benzodiazepine system was contracted as in the series of imidazo[1,5-*a*]quinoxaline derivatives **4**.[31–36] Moreover, the work made by the Upjohn researchers suggested the existence of a low affinity-binding site on $GABA_A$Rs,[37] and the structure of imidazo[1,5-*a*]quinoxaline derivatives **4** was easily translated into the imidazo[1,5-*a*]quinoline one of **5** (see Figure 1). Compound **5a** was identified as a drug candidate for the treatment of anxiety, but its development was discontinued for safety reason.[38]

Intriguingly, the structure–activity relationship (SAR) analysis suggested that the presence of bulky substituents was tolerated by CBR binding site when they are located in the ligand region corresponding to positions 4 and 5 of the imidazo[1,5-*a*]quinoline nucleus consistently in agreement with that we observed with our 5-HT_3 receptor ligands based on quinoline structure.[39,40]

The structural analogies between $GABA_A$ and 5-HT_3 receptors stimulated the application of our design approach previously used in studying 5-HT_3 receptors and leading to the discovery of multivalent ligands.[41,42] Intriguingly, the docking studies performed on a 5-HT_3 receptor model allowed us to ascertain the existence of several potential accessory binding sites. Thus, we assumed that multivalency in 5-HT_3 receptor could involve receptor domains different from the main binding site.[41] A similar information was not available for $GABA_A$Rs. Thus, in the first part of this project, we performed a careful exploration of the SAR of imidazo[1,5-*a*]quinoline derivatives **5** in the interaction with CBR.[43] The results of such study allowed us to identify a suitable pharmacophore candidate, which was employed in the second part described in the present paper, for the design of bivalent CBR ligands **6a-e** (Figure 2).

In particular, our approach consisted in the design of bivalent ligands **6a-e** and the corresponding reference monovalent ligands **7a-e**, in which the second imidazo[1,5-*a*]quinoline pharmacophore is replaced by a *tert*-butoxy group. In our approach, reference monovalent and bivalent ligands were designed to directly compare binding affinity data and to

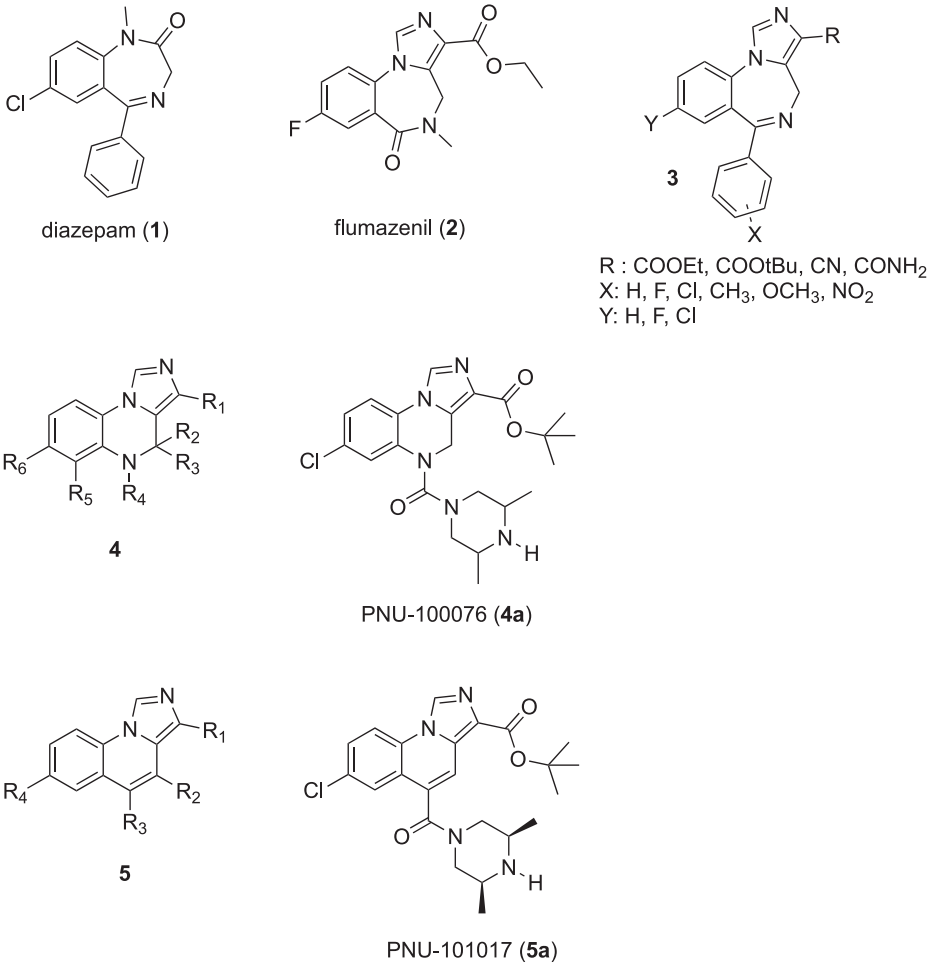


Fig. 1. Structure of reference CBR ligands showing different scaffolds.

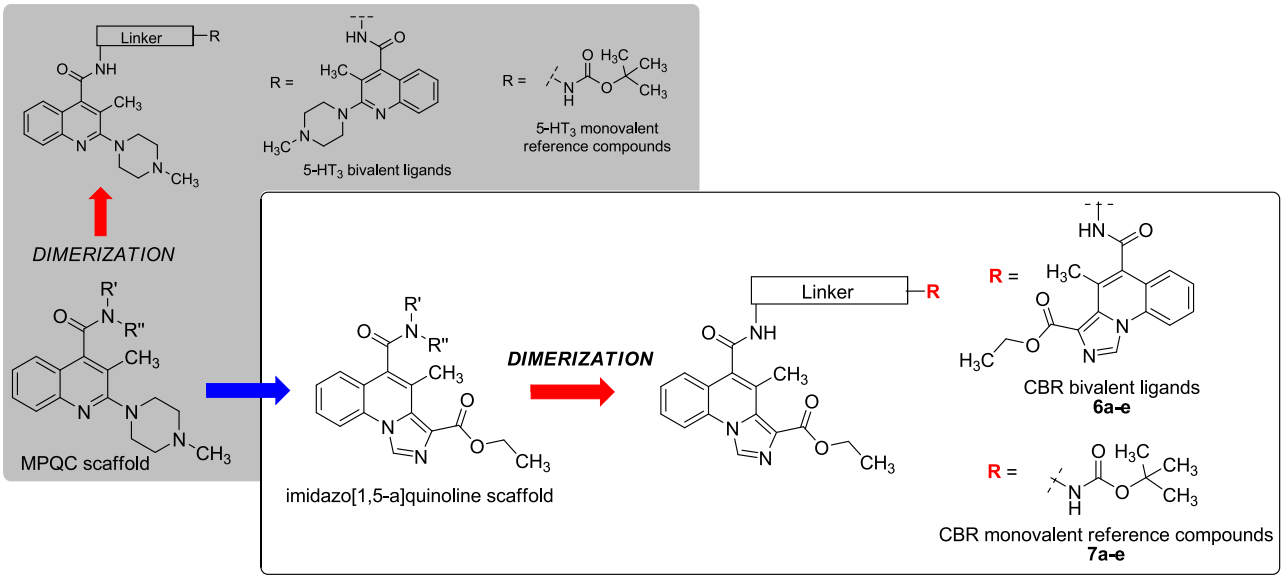


Fig. 2. Application of the imidazo[1,5-*a*]quinoline scaffold in the design of bivalent and monovalent CBR ligands **6a-e** and **7a-e**.

properly evaluate the multivalent approach.[41,42,44–46] In particular, the bivalent interaction of a bivalent ligand should be able to produce an increase in the binding affinity with respect to that shown by the corresponding monovalent one. These newly designed compounds were synthesized by means of the chemistry previously developed in our laboratories (see Supporting Information) and were evaluated in binding assays by using different CBR preparations (i. e. in cerebral cortex from male CD-1 albino mice or in mouse L(tk-) cells stably expressing human recombinant α1β3γ2 and α2β3γ2 $GABA_A$Rs) and by using different experimental set-up (see below). Moreover, a few selected CBR ligands (**6a**,**c** and **7a**) were then tested *in vivo* for their potential pharmacological effects, by taking into consideration four benzodiazepine actions such as anticonvulsant, anxiolytic, locomotor, and anti-amnesic activities.

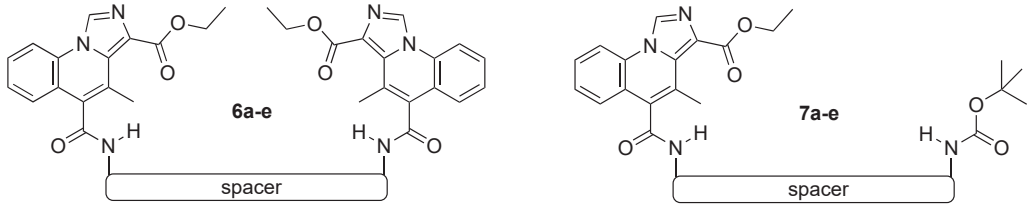
2. Results and discussion

2.1. In vitro binding

The affinity of bivalent ligands **6a-e** and the corresponding reference monovalent ligands **7a-e** for CBR in cortical membranes of albino CD-1 mice was measured by means of competition experiments against the radiolabeled antagonist [^{3}H]flumazenil and the results of the binding studies are summarized in Table 1.

The most surprising result was the rather peculiar behaviour of most of the tested compounds in the test system used in this preliminary

Table 1
Inhibition of [^{3}H]flumazenil specific binding to CBR in cerebral cortex from male CD-1 albino mice of bivalent ligands **6a-f** and reference monovalent ligands **7a-e**.[a]



Compd	Spacer	Methylene equivalents	*Ki* (nM)	*Ki* LAS (nM)	*Ki* HAS (nM)
			Monophasic curve	*Biphasic curve*	*Biphasic curve*
6a	$-(CH_2)_6-$	6		60	0.022
6b	$-(CH_2)_7-$	7		12	0.00068
6c	$-(CH_2)_8-$	8		52	0.050
6d	$-(CH_2-CH_2-O)_2-CH_2-CH_2-$	8	18		
6e	$-(CH_2-CH_2-O)_7-CH_2-CH_2-$	23	41		
7a	$-(CH_2)_6-$	6		35	0.000065
7b	$-(CH_2)_7-$	7		6.4	0.000061
7c	$-(CH_2)_8-$	8		51	0.0050
7d	$-(CH_2-CH_2-O)_2-CH_2-CH_2-$	8	12		
7e	$-(CH_2-CH_2-O)_7-CH_2-CH_2-$	23	0.66		
1				3.5	0.00000049
2				1.3	0.00000029

[a] Brain cortex membranes were incubated with 0.2 nM [^{3}H]flumazenil ([^{3}H]Ro 15-1788) in the absence and presence of increasing concentrations of the indicated compounds. All incubations were performed for 90 min at 0 °C (i. e. in ice) in the assay buffer. Non-specific binding was determined in the presence of 10 μM of diazepam. Concentration-response curves were analyzed using the curve-fitting program GraphPad Prism. Ten concentrations of displacers were examined each in duplicate. Each value was the average of 4–6 independent experiments. The K_i values were calculated from the IC_{50} by the method of Cheng and Prusoff using the K_d value (0.47 nM) obtained for [^{3}H]flumazenil.

biological evaluation. In fact, most of the tested compounds showed biphasic inhibition curves suggesting the existence of two well-distinct populations of binding sites. Even more surprising was the apparent affinity of some of the tested compound for the high affinity site, which was estimated to be in the sub-picomolar range. The most potent ligands in the interaction with the high affinity binding site were the reference monovalent ligands **7a-c** and the corresponding bivalent ligands **6a-c**, with reasonable affinity modulations in relation to the structural variations. For instance, the heptamethylene spacer was optimal for the binding potency in both the series **6a-c** and **7a-c** as previously observed in the bivalent ligands for $5HT_3$ receptors.[41] On the other hand, the affinity of these compounds for the low affinity site was markedly lower with *Ki* values spanning from the sub-micromolar up to the nanomolar range, with stringent analogies with the values displayed by the ligands showing monophasic inhibition curves. The structure-affinity relationship analysis failed in revealing evidence of bivalent interactions of these compounds at CBR binding site since the bivalent ligands **6a-e** appeared to be less potent than the corresponding reference monovalent ligands **7a-e**, in which the second imidazo[1,5-*a*]quinoline pharmacophore is replaced by a *tert*-butoxy group.

A large body of evidence reported in the literature supports the existence of a biphasic binding at CBRs and of very high affinity sites.[47–52] The intriguing work performed by Metha and Shank[52] took into consideration the possible role of several parameters of the experimental set-up (i.e. brain areas, incubation temperature and time, radioligand etc.) in the biphasic binding. Thus, the results obtained in albino CD-1 mice required to be challenged in a different test system. Therefore, CBRs affinity for both bivalent derivatives **6a-e** and monovalent ligands **7a-e** was measured by means of competition experiments by using the same radioligand (i.e. [^{3}H]flumazenil), but different receptors and experimental binding setup. In particular, the binding of the compounds to the benzodiazepine site was measured in human recombinant α1β3γ2 and α2β3γ2 $GABA_A$Rs. Thus, mouse L(tk) cells stably expressing human α1β3γ2 and α2β3γ2 $GABA_A$Rs were generated by transfection of the individual subunits in the dexamethasone-inducible expression vector pMSGneo in mouse L(tk-) cells[53,54] and were used in the binding assay by following a previously reported procedure (for the details see Supporting Information).

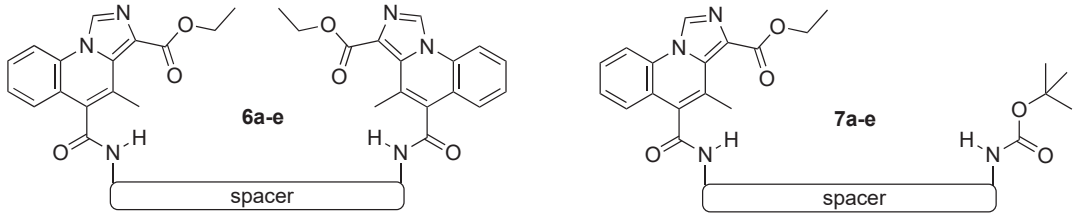
The results of the binding studies are summarized in Table 2.

In this test system, the binding profiles of the tested compounds were rather different from the ones obtained in the experimental set-up employing cerebral membrane preparations from albino CD-1 mice. In fact, the tested compounds were found to inhibit the specific binding of radiolabeled flumazenil at the human recombinant α1β3γ2 and α2β3γ2 $GABA_A$Rs showing roughly monophasic inhibition curves with *Ki* values from the sub-micromolar up to the nanomolar range very similar to that shown by diazepam (used as the reference compound in the binding assays). In particular, nanomolar *Ki* values were observed in both the series of bivalent **6a-c** and the corresponding monovalent ligands **7a-c**, with slightly better affinity values in the monovalent ligand series with respect to the bivalent series. Interestingly, the *Ki* values measured in this test system roughly correspond to those obtained for the low affinity site of CD-1 mice. Overall, these results appeared to rule out significant bivalent interactions and rather suggest monovalent interactions of these ligands with CBR. However, the role of each parameter of the experimental set-up (i.e. brain areas, incubation temperature and time, radioligand etc.) should be carefully investigated in a systematic pharmacological work to understand the molecular bases of the biphasic binding of these compounds in CD-1 mice.

2.2. *In vivo efficacy*

The selection of the compounds for the *in vivo* tests represented a very complex task because of the complexity of the binding profiles shown by the newly synthesized molecules. Overall, two divalent ligands (i.e. **6a** and **6c**) and a reference monovalent ligand (i.e. **7a**) were selected as the most representatives of the most interesting compounds to be examined in mice for their pharmacological effects. Four potential benzodiazepine actions were considered: the anticonvulsant action evaluated by means of the compounds against pentylenetetrazole-induced convulsions, the potential anxiolytic effects screened using the light/dark box test, the myorelaxant effect with the rota rod test, and finally the mouse learning and memory impairment evaluated by the passive avoidance test. The anticonvulsant activity of the compounds was studied by means of pentylenetetrazole [6,7,8,9-tetrahydro-5*H*-tetrazoloazepine (PTZ)] as a chemical convulsant agent (Table 3).

Table 2
Inhibition of [^{3}H]flumazenil specific binding to CBR in mouse L(tk-) cells stably expressing human recombinant α1β3γ2 and α2β3γ2 $GABA_A$Rs of bivalent ligands **6a–e** and reference monovalent ligands **7a–e**.[a]



Compd	Spacer	Methyleneequivalents	*Ki* (nM) α1β3γ2	*Ki* (nM) α2β3γ2
6a	$-(CH_2)_6-$	6	74	45
6b	$-(CH_2)_7-$	7	74	74
6c	$-(CH_2)_8-$	8	64	56
6d	$-(CH_2-CH_2-O)_2-CH_2-CH_2-$	8	129	100
6e	$-(CH_2-CH_2-O)_7-CH_2-CH_2-$	23	245	210
7a	$-(CH_2)_6-$	6	10	14
7b	$-(CH_2)_7-$	7	31	33
7c	$-(CH_2)_8-$	8	39	42
7d	$-(CH_2-CH_2-O)_2-CH_2-CH_2-$	8	615	400
7e	$-(CH_2-CH_2-O)_7-CH_2-CH_2-$	23	42	63
Diazepam			31	22

[a] Cell membranes were incubated with 4 nM [^{3}H]flumazenil ([^{3}H]Ro 15–1788]) in the absence and presence of increasing concentrations of the indicated compounds. All incubations were performed for 1 h at 4 °C in the assay buffer. Non-specific binding was determined in the presence of 1–3 μM of **TP003** (see the Supporting Information for details). The % inhibition of [^{3}H]Ro15-1788 binding was plotted as a function of compound concentration and the IC_{50} calculated. From the IC_{50} values, the Ki values were calculated using the method of Cheng and Prusoff using the K_d values obtained for [^{3}H]Ro15-1788.

Table 3
Effect of **6a**, **6c**, and **7a** on convulsions induced by pentylenetetrazole.[a]

Treatments	Dose mg/kg	Convulsion latency (min)	Death latency (min)
vehicle		19.3 ± 4.1	28.4 ± 4.8
6a	20	22.8 ± 5.5	40.5 ± 4.9*
6c	20	20.7 ± 4.8	41.8 ± 6.5*
7a	20	24.2 ± 3.9	43.3 ± 7.1*

[a] Pentylenetetrazole (90 mg/kg i.p.) was injected 30 min after the administration of compounds. Each value represents the mean ± s.e.m of at least 8 mice per group. *P<0.05 vs vehicle-treated animals.

As shown in Table 3, 20 mg/kg of **6a**, **6c**, and **7a** were able to significantly delay the mouse death latency in comparison to the control animals treated with vehicle. No effect was recorded concerning the time of the onset of convulsions (convulsion latency).

On the other hand, the effects of compounds on mouse anxiety in comparison to diazepam was evaluated using the light/dark box apparatus, the results are summarized in Table 4.

All compounds were challenged at the doses of 10 mg/kg and 20 mg/kg while diazepam was administered at 1 mg/kg. The time spent by vehicle-treated animals in the light box was 112.1 ± 10.9 s, this value was not changed by the treatment with **6a**, **6c**, and **7a**, at 10 mg/kg (114.3 ± 9.5 s, 96.7 ± 8.4 s, and 109.7 ± 8.7 s, respectively). All compounds at the higher dose potentiated this parameter up to 166.2 ± 8.2 s for **6a**, 173.4 ± 9.5 s for **6c**, and 144.7 ± 11.2 s for **7a**. Bivalent ligands **6a** and **6c**, and diazepam significantly increased the persistent time in light box up to 174.9 ± 10.9 s.

The effect of the acute administration of compounds (20 mg/kg, p.o.) on motor coordination was assessed using the rota rod test as a screening method to highlight any myorelaxant effect (Table 5).

Mice were challenged to perform the test before and after treatments (every 15 min) and the number of falls in 30 *sec* were counted. All compounds did not show a muscle relaxant effect since no increasing number of falls were detected after treatments in comparison to control animals injected with vehicle.

Finally, to investigate the effect of compounds on learning and memory, mice performance on passive avoidance test was analysed. This test allows to analyse if compounds show amnesic effects in a similar way to benzodiazepines. The difference between the retention latencies of compounds-treated mice and vehicles-treated mice was not statistically significant, meaning of no negative effect on learning and memory. On the contrary, the reference drug diazepam showed an amnesic effect after injection at the dose of 1 mg/kg (Table 6).

3. Conclusions

The imidazo[1,5-*a*]quinoline scaffold already reported in high affinity CBR ligands[43] was here employed as the pharmacophore in the design of bivalent CBR ligands **6a-e** bearing spacers showing variable length from six to twenty-three methylene equivalents and different physicochemical features. The newly designed compounds **6a-e** were synthesized exploiting previously developed procedures along with the corresponding reference monovalent compounds **7a-e** bearing the corresponding spacers terminated with a *tert*-butoxycarbonyl (Boc) group. The affinity to $GABA_A$Rs was evaluated in binding assays by using different CBR preparations (such as the cerebral cortex from male CD-1 albino mice or the human recombinant α1β3γ2 and α2β3γ2 receptors stably expressed in mouse L(tk-) cells) and different experimental set-up. The results obtained in the human recombinant α1β3γ2 and α2β3γ2 $GABA_A$Rs showed that the tested compounds were able to inhibit radiolabelled flumazenil, displaying monophasic inhibition curves, with *Ki* values from the sub-micromolar up to the nanomolar range. The similar inhibition constants values at α1- and α2-subunit containing $GABA_A$Rs showed an apparent lack of selectivity across the two isoforms. Nanomolar *Ki* values were obtained in both the series of homo-bivalent **6a-c** and the corresponding monovalent ligands **7a-c**, which showed slightly better affinity values. The similarity in the affinity between the bivalent ligands and the corresponding monovalent ones appeared to rule out any bivalent interactions of these ligands with the two isoforms of $GABA_A$Rs. Similarly, both the series of bivalent **6a-c** and the corresponding monovalent ligands **7a-c** were able to inhibit the radiolabeled flumazenil to $GABA_A$Rs in in cortical membranes of albino CD-1 mice, but most of the tested compounds showed biphasic inhibition curves, which suggested the existence of two well-distinct populations of binding sites. Interestingly, the inhibition constant values measured for the low affinity site roughly correspond to those obtained in the human recombinant α1β3γ2 and α2β3γ2 $GABA_A$Rs. Surprisingly, the apparent affinity of some of the tested compound for the high affinity site was estimated to be in the sub-picomolar range. Giving the lack of an unconfutable explanation for this surprising results, further studies are obviously necessary to evaluate the reproducibility of this observation. Finally, some CBR ligands selected from the bivalent ligands (i.e. **6a**,**c**) and from the reference monovalent ligands (i.e. **7a**) were then tested for their potential pharmacological *in vivo* effects in mice. Four potential benzodiazepine actions such as anticonvulsant, anxiolytic, locomotor, and anti-amnesic activities were taken into consideration. All the tested compounds showed anticonvulsant and anxiolytic properties without muscle relaxant and learning and memory negative effects.

Table 4
Effect of **6a**, **6c**, and **7a** in comparison to diazepam in the light/dark box test.[a]

Treatments	Dose mg/kg	Time in light (*sec*)
Vehicle		112.1 ± 10.9
6a	10	114.3 ± 9.5
6a	20	166.2 ± 8.2**
6c	10	96.7 ± 8.4
6c	20	173.4 ± 9.5**
7a	10	109.7 ± 8.7
7a	20	144.7 ± 11.2**
diazepam	1.0	174.9 ± 10.9**

[a] All compounds were administered 30 min before the test. Each value represents the mean ± s.e.m of at least 8 mice per group. **P<0.01 vs vehicle-treated animals.

Table 5
Lack of effect of **6a**, **6c**, and **7a** on mice rota rod test (falls in 30 *sec*).[a]

Treatments	Dose mg/kg	Time (min)				
		0	15	30	45	60
Vehicle		5.0 ± 0.4	3.1 ± 0.4	1.6 ± 0.0	1.0 ± 0.2	0.2 ± 0.3
6a	20	4.9 ± 0.3	3.2 ± 0.3	1.9 ± 0.4	0.7 ± 0.2	0.1 ± 0.2
6c	20	4.5 ± 0.4	3.0 ± 0.3	1.4 ± 0.2	0.7 ± 0.3	0.2 ± 0.3
7a	20	5.1 ± 0.3	3.3 ± 0.3	1.8 ± 0.3	0.8 ± 0.2	0.2 ± 0.2

[a] Each value represents the mean ± s.e.m. of at least 8 mice per group.

Table 6
Lack of effect of **6a**, **6c**, and **7a** in the mouse passive avoidance test.[a]

Treatments	Dose mg/kg	Training session (s)	Retention session (s)	Δ
Saline		15.6 ± 3.2	92.4 ± 8.7	76.8
CMC		16.6 ± 3.5	90.7 ± 9.2	74.1
6a	20	15.3 ± 4.1	86.1 ± 7.1	70.8
6c	20	14.8 ± 3.2	91.8 ± 9.1	77.0
7a	20	18.1 ± 2.9	95.5 ± 9.3	77.4
Diazepam	1.0	17.4 ± 3.8	61.0 ± 6.8**	43.6

[a] All compounds and diazepam were administered immediately after punishment. Each value represent the mean ± s.e.m of at least 8 mice per group. **P<0.01 in comparison to saline/CMC treated mice.

CRediT authorship contribution statement

Marco Paolino: Writing – original draft, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Mario Saletti:** Formal analysis. **Jacopo Venditti:** Formal analysis. **Federica Castriconi:** Investigation, Formal analysis. **Germano Giuliani:** Investigation, Formal analysis. **Samuele Maramai:** Writing – review & editing, Formal analysis, Data curation. **Alessandra Toti:** Investigation, Formal analysis, Data curation. **Carla Ghelardini:** Methodology, Investigation, Formal analysis, Data curation. **Rosanna Matucci:** Methodology, Investigation, Formal analysis, Data curation. **Narcy Alcazar Villalobos:** Methodology, Investigation, Formal analysis, Data curation. **Maurizio Anzini:** Project administration, Investigation. **Andrea Cappelli:** Writing – original draft, Supervision, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Chemical procedures and characterization data of compounds **6a-e** and **7a-e**. Experimental details for *in vitro* and *in vivo* studies. This material is available free of charge via the Internet at. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2024.118006.

Data availability

Data will be made available on request.

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