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European Journal of Medicinal Chemistry 44 (2009) 124–130
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Substituted benzo[*d*]oxazol-2(3*H*)-one derivatives with preference for the σ_1 binding site☆

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Received 18 November 2007; received in revised form 12 March 2008; accepted 18 March 2008
Available online 27 March 2008

Abstract

We describe here the synthesis and the binding interaction with σ_1 and σ_2 receptors of a series of new benzo[*d*]oxazol-2(3*H*)-one derivatives variously substituted on the *N*-benzyl moiety. The results of binding studies confirm the notion that the benzoxazolone moiety confers preference towards σ_1 sites and establish that the ability to bind to σ_1, but not to σ_2 receptors, is strongly affected by the kind and the position of the substituents introduced in the *N*-benzyl ring. In fact, compounds with substitutions in *para*-position with atoms of Cl, H or F or with a CH_3 group exhibit a higher affinity for σ_1 receptors than the corresponding *ortho*-substituted compounds. The highest affinity and selectivity, with K_i values of 0.1 and 427 nM for σ_1 and σ_2 receptors, respectively, and a corresponding $K_i\sigma_2/K_i\sigma_1$ selectivity ratio of 4270 were found for the Cl-substituted compound.

These results indicate that benzo[*d*]oxazol-2(3*H*)-one derivatives are among the most selective and σ_1 receptor-preferring ligands currently available.

Keywords: σ-Receptors; Benzo[*d*]oxazol-2(3*H*)-one derivatives; Radioligand binding assays

1. Introduction

The σ-binding sites were first defined and classified as being an opioid receptor subtype [1]. Later on, further investigations demonstrated that σ receptors were distinct from opioid and phencyclidine receptors and at least two distinct σ receptor subtypes have been pharmacologically characterized [2–4] and designated σ_1 and σ_2 [5].

The σ_1 receptor subtype has been purified and cloned from several animal species and humans [6,7] and its sequence shows significant homology with sterol C_8–C_7 isomerase from fungi. The molecular identity of σ_2 receptor has not been fully determined [6,7], although a number of studies have presented evidence linking σ_2 receptor to potassium channels and intracellular calcium release in NCB-20 cells [8,9]. Ligands displaying preferential affinity for the σ_1 receptor subtype are (+)-benzomorphans such as (+)-pentazocine and (+)-*N*-allylnormetazocine (NANM, SKF-10,047) whereas haloperidol and 1,3-di-(2-tolyl)guanidine (DTG) exhibit high affinity for both receptor subtypes [8]. (+)-Pentazocine shows a very low affinity for σ_2 receptors and represents a typical selective agonist used as tritiated ligand to label σ_1 receptors. Several compounds bind selectively the σ_1 receptors and σ_1 pharmacophoric models have been proposed by Glennon [10–12] and Gund [13]. The antipsychotic haloperidol and 1,3-di(2-tolyl)guanidine (DTG) possess high affinity for both σ subtypes [4]. DTG is the most used σ_2 radioligand but it needs a σ_1 masking agent.

☆ A preliminary account of this work was presented at 5th Joint Meeting on Medicinal Chemistry, June 17–21, 2007, Portoroz, Slovenia.

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doi:10.1016/j.ejmech.2008.03.011

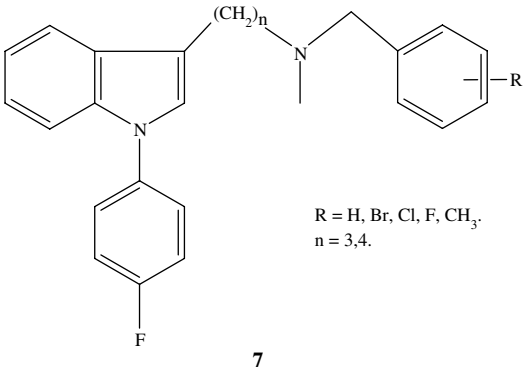
The σ_1 and σ_2 receptors have a widespread distribution in the central nervous system and in a variety of tissues and organs [8,9]. The σ_1 receptors have been shown to have modulatory roles among which are modulatory effects on neurotransmitter systems such as dopaminergic, serotoninergic, muscarinic systems [5,14,15] and on the NMDA-stimulated neurotransmitters release [16]. Moreover, σ_1 receptors are involved in neuroprotective and antiamnesic activity [17], modulation of opioid analgesia [18] and attenuation of cocaine-induced locomotor activity and toxicity [19]. In addition, σ_1 antagonists have shown to be effective against negative symptoms of schizophrenia without producing extrapyramidal side effects [20,21].

The σ_2 receptors may contribute to the acute side effects of typical neuroleptic drugs and σ_2 antagonists attenuate the extrapyramidal effects, dystonic reactions and tardive dyskinesia [2,8,14,22,23] suggesting their potential use in the treatment of psychosis [20,21]. Furthermore, σ_2 receptors are involved in regulation of cell proliferation and maintenance of cell viability. They are highly expressed in several tumoral cell lines [24,25], where σ_2 agonists produce morphological changes and apoptosis. The σ_2 receptor agonists promote Ca^{2+} release from endoplasmatic reticulum and mitochondrial stores [26] with subsequent cell death by caspase-independent apoptosis [25]. Apoptosis may also be induced in tumoral cells by regulation of the sphingolipid pathway [27]. Therefore, σ_2 agonists may be useful as novel anticancer agents and as imaging agents in cancer diagnosis by positron emission tomography (PET) [28–31] and single photon emission computed tomography (SPECT) [32,33].

In our previous work [34] we described the synthesis and the σ receptor affinities of a series of substituted indolylalkylamino derivatives **7** (Fig. 1) in which the length of the intermediate alkylene chain was varied ($n = 3$, 4) and the benzyl group was variously substituted at the phenyl ring. The indole derivatives [34] have been designed, according to the σ_1 receptor model proposed by Glennon [10–12], with the assumption that the indole moiety may interact with a primary hydrophobic site corresponding to Glennon's phenyl "B" region [10–12], the basic nitrogen atom linked by an alkylene chain to the indole moiety may interact with a receptor proton-donor site and the substituted *N*-benzyl moiety may bind the secondary hydrophobic phenyl "A" region of the σ_1 receptor model, modulating the binding affinity of the compounds for σ_1 or σ_2 receptors.

From the obtained results [34] it appeared that substitutions in the phenyl ring, as well as the length of the interspacing alkylene chain, modulated the σ_1 and σ_2 affinity of those compounds. The highest σ_2 binding affinity was reached by the butylene derivative **7** (R = 2,4-$(CH_3)_2$, $n = 4$), whose $K_i\sigma_2$ value was 5.9 nM, with a $K_1\sigma_1/K_1\sigma_2$ selectivity ratio of 22. Conversely, the compound with the greater affinity for σ_1 was the unsubstituted propylene derivative **7** (R = H, $n = 3$), whose $K_i\sigma_1$ value was 20.8 nM; the ratio of $K_1\sigma_1$ to $K_1\sigma_2$ was 0.05.

Considering that the benzoxazolone moiety is present in compounds characterized by high affinity and selectivity towards σ_1 receptors, among which compound **8** (Fig. 1) possesses a $K_1\sigma_1$ value of 8.5 nM, with a selectivity ratio $K_1\sigma_2/K_1\sigma_1$ of



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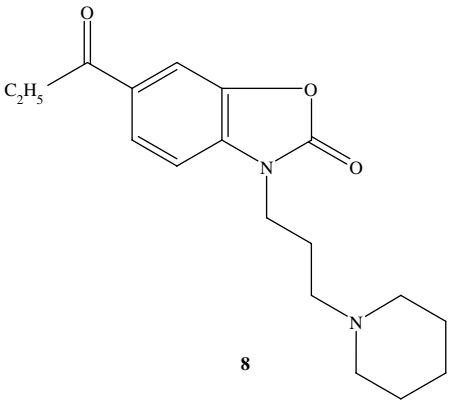

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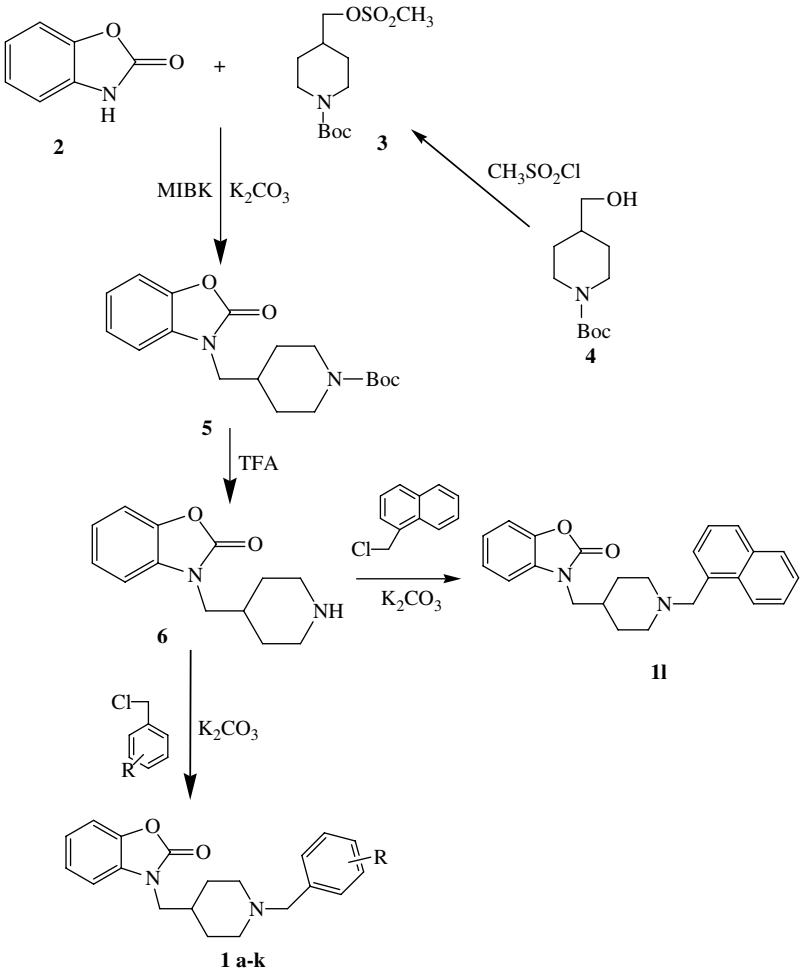
Fig. 1.

58 [35], we synthesized a series of new benzo[*d*]oxazol-2(3*H*)-one derivatives **1a–l** variously substituted on the *N*-benzyl moiety. In these compounds, the sequence formed by the benzoxazolone moiety linked through a 4-methylpiperidin-1-yl spacer to a benzyl group resembles that of compounds with pharmacophoric features predicted by the classical σ_1 receptor model proposed by Glennon [10–12]. Therefore, these compounds have been synthesized with the supposition that they will retain high affinity and selectivity towards σ_1 receptors and with the additional aim to investigate the influence of the different substitutions on the *N*-benzyl ring on σ_1 affinity and/or selectivity.

2. Chemistry

The substituted 3-[(1-benzylpiperidin-4-yl)methyl]benzo[*d*]oxazol-2(3*H*)-one derivatives **1a–l** have been synthesized (Scheme 1) by treating benzo[*d*]oxazol-2(3*H*)-one **2**, in the presence of K_2CO_3 and MIBK (methyl isobutyl ketone) as solvent, with *N*-Boc-piperidin-4-yl-methyl methanesulfonate **3**, which in turn has been obtained by treatment of the commercially available *N*-Boc-4-piperidinemethanol **4** with methanesulfonyl chloride. The obtained 3-[[*N*-Boc-piperidin-4-yl]methyl]benzo[*d*]oxazol-2(3*H*)-one **5** was deprotected with TFA to obtain the intermediate **6** which was alkylated with substituted benzylchlorides or with 1-naphtylmethyl chloride to obtain the expected compounds **1a–k** and **1l**, respectively.



Scheme 1. Synthesis route of flame retardant.

3. Results and discussion

In the newly synthesized derivatives **1a–l** the hydrophobic indole moiety present in the indole derivatives **7** [34] was replaced by the benzoxazolone group, the alkylene intermediate chain was substituted by the 4-methylpiperidin-1-yl spacer, with less conformational freedom with respect to the alkylene chain, and the piperidine basic nitrogen atom was linked to various substituted benzyl moieties, whose importance for the binding to σ_1 or σ_2 sites have been already described for the indole derivatives **7**.

The K_i values of compounds **1a–l** were determined, from the corresponding IC_{50} values, for both receptor types and the σ_2/σ_1 selectivity ratios were evaluated for each compound (Table 1).

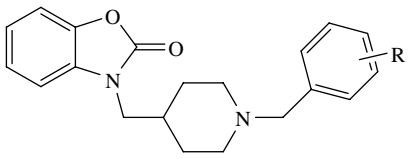
From the obtained results it appears that the substitutions on the phenyl ring can strongly affect the σ_1 and σ_2 binding affinity of these compounds.

With the exception of the derivatives **1j** and **1l**, characterized by a very weak affinity for both σ_1 and σ_2 receptors, all compounds exhibit, with various degree, preference for σ_1 receptor sites. The compounds with the highest affinity were the unsubstituted derivative **1a** and the corresponding *para*-substituted derivatives **1c**, **1f** and **1h**. The best result was reached by the 4-chloro substituted compound **1c**, whose $K_i\sigma_1$ value was 0.1 nM, with a $K_1\sigma_2/K_1\sigma_1$ selectivity ratio of 4270.

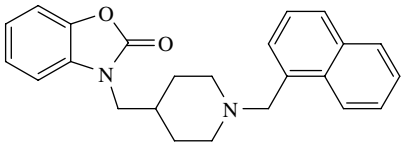
In this work we referred to the σ_1 receptor model proposed by Glennon [10–12], which includes an amine binding site flanked by two hydrophobic domains, the primary hydrophobic binding site that binds phenyl "B" and a secondary binding site that binds phenyl "A", placed at certain optimal distances to each other. A nitrogen atom is an essential pharmacophoric element for the binding of ligands to σ_1 receptors. Ablordeppey et al. [35] studied the nature of the nitrogen atom as it binds the σ_1 receptors in order to know if a lone pair of electrons on the nitrogen was needed for binding or if protonation of the nitrogen occurs on binding. The conclusions of this study implicate that the lone pair of the electrons on the ligands probably becomes protonated at σ_1 receptor and that a protonated N atom may be required for σ_1 receptor binding [35].

To the receptor affinity of our compounds may contribute the basic piperidine nitrogen atom interacting with a receptor proton-donor site. The aromatic portion of the benzoxazolone moiety may interact with the receptor hydrophobic "B" region proposed by Glennon. Moreover, the electronegative atoms of the benzoxazolone group may contribute to the binding

Table 1
Binding affinities of benzo[*d*]oxazol-2(3*H*)-one derivatives for [^{3}H]-(+)-pentazocine (σ_1) and [^{3}H]-DTG (σ_2) binding sites in rat liver homogenate



1 a-k



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Compound	R	$K_i\sigma_1$ (nM)	n_H	$K_i\sigma_2$ (nM)	n_H	σ_2/σ_1 ratio
1a	H	3.6 ± 0.3	1.22 ± 0.11	246 ± 127	0.67 ± 0.27	69
1b	2-Cl	61 ± 2	0.76 ± 0.11	299 ± 73	2.29 ± 1.19	5
1c	4-Cl	0.1 ± 0.03	0.74 ± 0.16	427 ± 102	0.79 ± 0.12	4270
1d	2,4-$(Cl)_2$	258 ± 58	0.99 ± 0.18	382 ± 121	2.36 ± 0.78	1.5
1e	2-F	23 ± 2	0.56 ± 0.07	213 ± 19	0.77 ± 0.20	9
1f	4-F	3.9 ± 0.04	0.85 ± 0.09	170 ± 45	1.87 ± 0.13	44
1g	2-CH_3	33 ± 4	0.55 ± 0.04	338 ± 59	0.87 ± 0.22	10
1h	4-CH_3	2.9 ± 0.2	1.09 ± 0.07	116 ± 0.6	0.79 ± 0.09	40
1i	2,4-$(CH_3)_2$	30 ± 11	0.80 ± 0.21	187 ± 24	0.61 ± 0.05	6
1j	2,4,6-$(CH_3)_3$	6230 ± 802	1.13 ± 0.16	2881 ± 807	1.75 ± 0.75	0.5
1k	4-Phenyl	394 ± 67	1.09 ± 0.16	>10 μM	–	>25
1l	–	1057 ± 17	0.84 ± 0.13	1292 ± 186	1.08 ± 0.17	1.2
(+)-Pentazocine		15 ± 3	0.88 ± 0.14	327 ± 166	0.78 ± 0.25	22
DTG		180 ± 22	1.3 ± 0.2	130 ± 46	1.5 ± 0.67	0.72
Haloperidol		5.7 ± 1	0.55 ± 0.07	235 ± 71	0.85 ± 0.19	41

Competition data of three separate determinations performed in duplicate were averaged by fitting in a four parameters curve by means of the SigmaPlot software and calculated IC_{50} values and Hill's coefficient (n_H) reported as means ± SE. The corresponding K_i values were obtained by means of the Cheng–Prusoff equation.

affinity. In effect an electronegative atom such as O or S is frequently present in very potent σ_1 ligands between aromatic component and the classical alkyl or cycloalkyl intermediate spacer linked to the basic nitrogen atom [13,36].

Gund et al. [13] concluded, on the basis of a molecular modeling study of σ_1 receptors ligands, that, besides the primary hydrophobic binding region "B", there could be a secondary binding region that may surround the oxygen or sulphur atom of the molecules. On the other hand, the obtained results suggest that the substituted benzyl group, linked to the nitrogen atom of the 4-methylpiperidin-1-yl intermediate sequence may interact favourably with another hydrophobic binding site resembling the receptor binding site of the "phenyl A region" proposed by Glennon's σ_1 receptor model [10–12]. The highest affinity of the nonsubstituted compound **1a** and the series **1c**, **1f** and **1h** may be due to a possible preferential orientation of the phenyl ring in the hydrophobic receptor region of the phenyl "A" and to a steric hindrance to the free rotation which may be determined by the presence of substitution in the *ortho*-position. The very high affinity of the *para*-chloro derivative **1c** may depend on a strong interaction of the chlorine atom into a small hydrophobic receptor pocket which can accept other small *para*-substituents like in compounds **1f** and **1h**, but is inaccessible to substituents of greater dimension as the phenyl group (**1k**) or the 1-naphtylmethyl moiety (**1l**).

In conclusion the results in this study indicate that substitutions in the benzene ring may be very important for the σ_1 affinity and selectivity. Compound **1c**, which showed the highest affinity and selectivity towards σ_1 receptors (about 4000-fold), can be considered a new selective σ_1 over σ_2 ligand belonging to a new class of potent receptor ligands which bind preferentially to σ_1 receptor subtype.

4. Experimental section

4.1. Chemistry

Melting points were determined with a Buchi 510 capillary apparatus, and are uncorrected. Infrared spectra in nujol mulls were recorded on a Jasko FT 200 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian Gemini 200 spectrometer, chemical shifts are reported as δ (ppm) in $CDCl_3$ solution. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. ESI-MS spectra were obtained on a PE-API I spectrometer by infusion of a solution of the sample in MeOH. Elemental analyses (C, H, N) were performed on a Carlo Erba analyzer and were within ±0.3 of the theoretical value.

*4.1.1. [1-(tert-Butoxycarbonyl)piperidin-4-yl] methyl methanesulfonate **3** [37]*

N-Boc-4-piperidinemethanol **4** (1.5 g, 6.98 mmol) was dissolved in 5 ml of CH_2Cl_2 and the solution was stirred at 0 °C in an ice-bath. Triethylamine (0.85 g, 8.37 mmol) was added to the solution and then an equimolar amount of methanesulfonyl chloride (0.96 g, 8.37 mmol) was added dropwise. The

temperature was allowed to reach room temperature and the mixture was stirred for additional 1 h. The organic phase was washed with water, dried over sodium sulphate, filtered and evaporated under reduced pressure. The oily residue solidificated upon cooling to afford a yellow-light solid: yield 1.72 g (84 %); melting point 76–78 °C.

IR cm^{-1} (nujol): 1690 cm^{-1}; 1168 cm^{-1}; 1360 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.23 (m, 2H, H_3–H_5, pip.); 1.45 (s, 9H, CH_3, Boc); 1.74 (m, 2H, $H_{3'}$–$H_{5'}$, pip.); 1.91 (m, 1H, H_4, pip.); 2.70 (m, 2H, H_2–H_6, pip.): 3.01 (s, 3H, OSO_2CH_3); 4.06 (d, 2H, pip.–CH_2–OSO_2CH_3); 4.14 (m, 2H, $H_{2'}$–$H_{6'}$ pip.). Anal. calcd. for $C_{12}H_{23}NO_5S$ (MW 293.38): C, 49.13; H, 7.90; N, 4.77%; found: C, 48.90; H, 7.77: N, 4.65%.

4.1.2. 3-[[1-(tert-Butoxycarbonyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***5***

A mixture of **2** (0.8 g, 5.86 mmol), K_2CO_3 (0.81 g, 5.86 mmol) and **3** (1.72 g, 5.86 mmol) in 50 ml of methyl isobutyl ketone (MIBK) was heated under reflux at 150 °C for 16 h. After cooling the inorganic salts were filtered off and the solvent was evaporated under reduced pressure. The residue was extracted with ethyl acetate (3 × 50 ml) and the organic phase was washed with distilled water. The collected organic phases were dried over sodium sulphate, filtered and concentrated under reduced pressure. The solid residue was crystallized from ethanol: yield 1.95 g (70%); melting point 117–119 °C.

IR cm^{-1} (nujol): 1681 cm^{-1}; 1781 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.31 (m, 2H, H_3–H_5, pip.); 1.46 (s, 9H, CH_3, Boc); 1.70 (m, 2H, $H_{3'}$–$H_{5'}$, pip.); 2.07 (m, 1H, H_4, pip.); 2.70 (m, 2H, H_2–H_6, pip.); 3.72 (d, 2H, N–CH_2–pip.); 4.15 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 6.94–7.27 (m, 4H, arom.). Anal. calcd. for $C_{18}H_{24}N_2O_4$ (MW 332.39): C, 65.04; H, 7.28; N, 8.43%; found: C, 64.90; H, 7.15; N, 8.30%.

4.1.3. 3-[(Piperidin-4-yl)methyl]benzo[d]oxazol-2(3H)-one ***6***

Compound **5** (1.0 g, 3.00 mmol) was deprotected with 2 ml of trifluoroacetic acid at room temperature, overnight. The solution was concentrated at reduced pressure and the residue was poured into water and basified with NaOH 10% solution (pH 10). The white solid precipitate was filtered, washed with water and used without further purification: 0.65 g (93%); melting point 124–126 °C.

IR cm^{-1} (nujol): 1765 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.20–188 (m, 4H, $H_{3,3'}$–$H_{5,5'}$, pip.); 2.06 (m, 1H, H_4, pip.); 2.50–3.30 (m, 4H, $H_{2,2'}$–$H_{6,6'}$, pip.); 3.72 (d, 2H, N–CH_2–pip.); 3.86 (br s, 1H, NH, disappearing on deuteration); 6.96–7.30 (m, 4H, arom.). Anal. calcd. for $C_{13}H_{16}N_2O_2$ (MW 232.29): C, 67.22; H, 6.94; N, 12.06%; found: C, 67.00; H, 6.85; N, 12.00%.

4.1.4. 3-[(1-Benzylpiperidin-4-yl)methyl]benzo[d] oxazol-2(3H)-one ***1a***

A mixture of **7** (0.15 g, 0.64 mmol), K_2CO_3 (0.09 g, 0.64 mmol) and benzylchloride (0.08 g, 0.64 mmol) in 50 ml of acetone was heated under reflux for 5 h. After cooling the inorganic salts were filtered off and the solvent was evaporated under reduced pressure. The residue was washed with water, then with ethyl ether to afford **1a** as a chromatographically pure solid: yield 0.16 g (77%); melting point 126–128 °C.

IR cm^{-1} (nujol): 1772 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.37–2.03 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.91 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.51 (s, 2H, N–CH_2–Ar); 3.72 (d, 2H, N–CH_2–pip.); 6.94–7.40 (m, 9H, arom.). MS: *m/z* 323 [MH^+]. Anal. calcd. for $C_{20}H_{22}N_2O_2$ (MW 322.4): C, 74.51; H, 6.88; N, 8.69%; found: C, 74.40; H, 6.85; N, 8.60%.

In an analogous way the following compounds **1b–l** were similarly obtained.

4.1.5. 3-[[1-(2-Chlorobenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1b***

Yield (%): 74; melting point (°C): 163–167; IR cm^{-1} (nujol): 1768 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.33–2.17 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.94 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.61 (s, 2H, N–CH_2–Ar); 3.73 (d, 2H, N–CH_2–pip.); 6.93–7.54 (m, 8H, arom.). MS: *m/z* 357 [MH^+], 359 [$MH^+ + 2$]. Anal. calcd. for $C_{20}H_{21}ClN_2O_2$ (MW 356.85): C, 67.32; H, 5.93; N, 7.85%; found: C, 67.50; H, 6.15; N, 7.90%.

4.1.6. 3-[[1-(4-Chlorobenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1c***

Yield (%): 65; melting point (°C): 122–127; IR cm^{-1} (nujol): 1772 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.30–2.04 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.88 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.46 (s, 2H, N–CH_2–Ar); 3.72 (d, 2H, N–CH_2–pip.); 6.94–7.34 (m, 8H, arom.). MS: *m/z* 357 [MH^+], 359 [$MH^+ + 2$]. Anal. calcd. for $C_{20}H_{21}ClN_2O_2$ (MW 356.85): C, 67.32; H, 5.93; N, 7.85%; found: C, 67.45; H, 5.95; N, 7.60%.

4.1.7. 3-[[1-(2,4-Dichlorobenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1d***

Yield (%): 84; melting point (°C): 169–174; IR cm^{-1} (nujol): 1769 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.32–2.17 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.90 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.56 (s, 2H, N–CH_2–Ar); 3.73 (d, 2H, N–CH_2–pip.); 6.93–7.49 (m, 7H, arom.). MS: *m/z* 391 [MH^+], 393 [$MH^+ + 2$]. Anal. calcd. for $C_{20}H_{20}Cl_2N_2O_2$ (MW 391.29): C, 61.39; H, 5.15; N, 7.16%; found: C, 61.55; H, 5.00; N, 7.30%.

4.1.8. 3-[[1-(2-Fluorobenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1e***

Yield (%): 64; melting point (°C): 137–140; IR cm^{-1} (nujol): 1770 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.31–2.10 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.93 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.58 (s, 2H, N–CH_2–Ar); 3.72 (d, 2H, N–CH_2–pip.); 6.93–7.45 (m, 8H, arom.). MS: *m/z* 341 [MH^+]. Anal. calcd. for $C_{20}H_{21}FN_2O_2$ (MW 340.39): C, 70.57; H, 6.22; N, 8.23%; found: C, 70.50; H, 6.15; N, 8.10%.

4.1.9. 3-[[1-(4-Fluorobenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1f***

Yield (%): 96; melting point (°C): 144–148; IR cm^{-1} (nujol): 1768 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) ppm (δ): 1.37–2.03 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.90 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.45 (s, 2H, N–CH_2–Ar); 3.71 (d, 2H, N–CH_2–pip.); 6.93–7.34 (m, 8H, arom.). MS: *m/z* 341 [MH^+]. Anal. calcd. for $C_{20}H_{21}FN_2O_2$ (MW 340.39): C, 70.57; H, 6.22; N, 8.23%; found: C, 70.30; H, 6.05; N, 8.05%.

4.1.10. 3-[[1-(2-Methylbenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1g***

Yield (%): 79; melting point (°C): 129–132; IR cm^{-1} (nujol): 1768 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) 1.30–2.06 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.36 (s, 3H, CH_3); 2.88 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.44 (s, 2H, N–CH_2–Ar); 3.71 (d, 2H, N–CH_2–pip.); 6.94–7.32 (m, 8H, arom.). MS: *m/z* 337 [MH^+]. Anal. calcd. for $C_{21}H_{24}N_2O_2$ (MW 336.43): C, 74.97; H, 7.19; N, 8.33%; found: C, 74.70; H, 7.10; N, 8.40%.

4.1.11. 3-[[1-(4-Methylbenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1h***

Yield (%): 79; melting point (°C): 118–121; IR cm^{-1} (nujol): 1769 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) 1.35–2.05 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.34 (s, 3H, CH_3); 2.90 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.46 (s, 2H, N–CH_2–Ar); 3.71 (d, 2H, N–CH_2–pip.); 6.95–7.40 (m, 8H, arom.). MS: *m/z* 337 [MH^+]. Anal. calcd. for $C_{21}H_{24}N_2O_2$ (MW 336.43): C, 74.97; H, 7.19; N, 8.33%; found: C, 74.78; H, 7.25; N, 8.45%.

4.1.12. 3-[[1-(2,4-Dimethylbenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1i***

Yield (%): 66; melting point (°C): 119–122; IR cm^{-1} (nujol): 1770 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) 1.25–2.14 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.32 (s, 6H, $2 \times CH_3$); 2.90 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.40 (s, 2H, N–CH_2–Ar); 3.71 (d, 2H, N–CH_2–pip.); 6.90–7.37 (m, 7H, arom.). MS: *m/z* 351 [MH^+]. Anal. calcd. for $C_{22}H_{26}N_2O_2$ (MW 350.45): C, 75.40; H, 7.48; N, 7.99%; found: C, 75.35; H, 7.35; N, 8.05%.

4.1.13. 3-[[1-(2,4,6-Trimethylbenzyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1j***

Yield (%): 64; melting point (°C): 114–120; IR cm^{-1} (nujol): 1753 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) 1.20–2.08 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.30 (m, 9H, $3 \times CH_3$); 2.82 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.40 (s, 2H, N–CH_2–Ar); 3.68 (d, 2H, N–CH_2–pip.); 6.80–7.30 (m, 6H, arom.). MS: *m/z* 365 [MH^+]. Anal. calcd. for $C_{23}H_{28}N_2O_2$ (MW 364.48): C, 75.79; H, 7.74; N, 7.69%; found: C, 75.65; H, 7.62; N, 7.69%.

4.1.14. 3-[[1-(4-Biphenyl)piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1k***

Yield (%): 70; melting point (°C): 159–163; IR cm^{-1} (nujol): 1770 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) 1.35–2.07 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.96 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.55 (s, 2H, N–CH_2–Ar); 3.73 (d, 2H, N–CH_2–pip.); 6.94–7.66 (m, 13H, arom.). MS: *m/z* 399 [MH^+]. Anal. calcd. for $C_{26}H_{26}N_2O_2$ (MW 398.50): C, 78.36; H, 6.58; N, 7.03%; found: C, 78.20; H, 6.55; N, 7.15%.

4.1.15. 3-[[1-[(Naphtalen-1-yl)methyl]piperidin-4-yl]methyl]benzo[d]oxazol-2(3H)-one ***1l***

Yield (%): 71; melting point (°C): 150–153; IR cm^{-1} (nujol): 1770 cm^{-1}. ^1H NMR ($CDCl_3$–TMS) 1.30–2.10 (m, 7H, H_2–$H_{3,3'}$–$H_{5,5'}$–H_6–H_4, pip.); 2.97 (m, 2H, $H_{2'}$–$H_{6'}$, pip.); 3.70 (s, 2H, N–CH_2–Ar); 3.88 (d, 2H, N–CH_2–pip.); 6.92–8.33 (m, 11H, arom.). MS: *m/z* 373 [MH^+]. Anal. calcd. for $C_{24}H_{24}N_2O_2$ (MW 372.46): C, 77.39; H, 6.49; N, 7.52%; found: C, 77.22; H, 6.45; N, 7.45%.

4.2. Pharmacology

4.2.1. Radioligand binding assays

Binding assays were performed on rat liver membranes according to the methods of Hellewell [9], slightly modified as previously described [34]. Briefly, for σ_1 receptor assay 250 μg of rat liver homogenate were incubated for 120 min at 37 °C with 1 nM [^3H]-(+)-pentazocine (Perkin-Elmer, specific activity 34.9 Ci/mmol) in 50 mM Tris–HCl, pH 8.0, 0.5 mL final volume. Nonspecific binding was defined in the presence of 10 μM haloperidol. The reaction was stopped by vacuum filtration through GF/B glass-fiber filters presoaked with 0.5% polyethylenimine, followed by rapid washing with 2 ml ice-cold buffer. Filters were placed in 3 ml scintillation cocktail and the radioactivity determined by liquid scintillation counting.

For σ_2 receptor assay, 150 μg of rat liver homogenate were incubated for 120 min at room temperature with 3 nM [^3H]-DTG (PerkinElmer, specific activity 58.1 Ci/mmol) in 50 mM Tris–HCl, pH 8.0, 0.5 mL final volume. (+)-Pentazocine (100 nM) and haloperidol (10 μM) were used to mask σ_1 receptors and to define nonspecific binding, respectively.

Competition studies were done using at least 11 different concentrations of the ligand under investigation. As internal controls, three increasing concentrations of unlabelled (+)-pentazocine (σ_1 receptors) or DTG (σ_2 receptors) were always included. The compounds were prepared as 10 mM stock solutions in 100% DMSO and diluted with Tris–HCl buffer on the day of the experiment. The final DMSO concentration in the incubation tubes was maintained at 0.1%.

IC_{50} values and Hill's coefficients n_H were calculated by nonlinear regression using a four parameters curve-fitting algorithm of the SigmaPlot software, and are the means ± SE of three separate determinations performed in duplicate. The corresponding K_i values were obtained by means of the Cheng–Prusoff equation, using the K_d values obtained in saturation experiments performed on rat liver homogenate, and previously reported [34].

Acknowledgment

This research was carried out with the financial support from Italian Ministry for University and Research (MIUR) Rome, Italy (PRIN 2003).

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