Dipartimento di Chimica Farmaceutica e Tossicologica[1], Università degli Studi di Napoli "Federico II", Napoli, Dipartimento di Farmacologia "G. Segre" [2], Università di Siena, Italy

Synthesis and *in vitro* pharmacological evaluation of a new series of 5-HT_{1A} 5-HT_{2A} and 5-HT_{2C} receptor ligands containing a norbornene nucleus

F. Fiorino[1], B. Severino[1], F. De Angelis[1], E. Perissutti[1], E. Magli[1], F. Frecentese[1], A. Esposito[1], P. Massarelli[2], C. Nencini[2], B. Viti[2], V. Santagada[1], G. Caliendo[1]

Received April 4, 2009, accepted May 7, 2009

Prof. Giuseppe Caliendo, Dipartimento di Chimica Farmaceutica e Tossicologica Università degli Studi di Napoli "Federico II", Via D. Montesano, 49, 80131 Napoli, Italy
caliendo@unina.it

Pharmazie 64: 555–564 (2009) *doi: 10.1691/ph.2009.9593*

A series of 4-substituted piperazine derivatives bearing a norbornene nucleus have been prepared and their affinity for serotonin 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors has been evaluated. Compounds showing the highest affinity have been selected and evaluated on dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors. The combination of structural elements (heterocyclic nucleus, oxyalkyl chain and 4-substituted piperazine) known to be critical in order to have affinity on serotonin receptors and the proper selection of substituents led to compounds with higher receptor specificity and affinity. In binding studies, several molecules showed affinity in nanomolar range towards 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors and moderate to no affinity for other relevant receptors (D_1, D_2, α_1 and α_2). Compound **2q** 4-[2-[4-(3,4-dichlorophenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione ($K_i = 1.13$ nM), was the most active and selective derivative for the 5-HT_{2C} receptor with respect to other serotonin, dopaminergic and adrenergic receptors. Moreover, compound **3p** showed mixed 5-HT_{2A}/5-HT_{2C} activity with affinity values in nanomolar range.

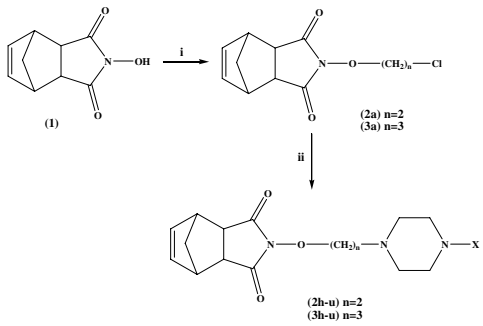
1. Introduction

Evidence suggests that serotonin (5-hydroxytryptamine, 5-HT), is an important neurotransmitter in the central and peripheral nervous systems (CNS and PNS, respectively), implicated in numerous physiological and pathophysiological processes (Baumgarten et al. 1987; Martin et al. 1998; Barnes et al. 1999; Hoyer et al. 2002; Shimada et al. 2008). In fact, serotonin receptors (5-HTR_s) may be involved in impulsivity and alcoholism (De Vry et al. 1995; File et al. 1996), in the different phases of sleep (Neckelmann et al. 1996), sexual behavior, appetite control, thermoregulation, cardiovascular function (Sleight et al. 1991; Saxena et al. 1995) and recently has been found to show growth-promoting activity and to be functionally related to oncogenes (Dizeyi et al. 2004). In particular, the 5-HT_{1A} receptor belongs to the superfamily of G-protein-coupled receptors and negatively coupled to adenylyl cyclase found in high concentration in the limbic system, where it is thought to play a role in emotional processes and represents a major target for research and drug development due to its implication in the pathophysiology and treatment of major neuropsychiatric disorders, including depression, schizophrenia and anxiety. Moreover, the 5-HT_{2A} receptor is known to play a key role in the action of psychedelics as well as being a therapeutic target for the treatment of schizophrenia (Parker et al. 2008), whereas the 5-HT_{2C} receptor is considered to be an attractive target for the design of novel drugs for treatment of CNS-related diseases such as obesity, obsessive compulsive disorder, and sexual dysfunction, even if few 5-HT_{2C} selective agonists are known so far (Shimada et al. 2008).

5-HT_{1A}R, 5-HT_{2A}R and 5-HT_{2C}R are G protein coupled receptors (GPCRs) (Sleight et al. 1991; Bikker et al. 1998) that show an amino acid composition similar to adrenergic and dopaminergic receptors. In particular, the 5-HT_{1A}R transmembrane amino acid sequence presents 45% homology with the respective part of the α_1-adrenergic receptor (Trump-Kallmeyer et al. 1992).

Several classes of agents are already known for their high affinity toward these receptors and, from a chemical point of view, they can be subdivided into different classes (aminotetralines, ergolines, arylpiperazines, indolylalkylamines, aporphines, arylalkylpiperidines, indoles and aryloxyalkylamines). One of the most studied group is that of long-chain arylpiperazine (LCAPs), (Lopez-Rodriguez et al. 2002; Pessoa-Mahana et al. 2003) that have provided interesting drugs acting on the CNS (buspirone, ziprasidone, aripiprazol) and compounds with a potential therapeutical profile (adatanserin, mazapertine, flesinoxan, lecozotan, bifeprunox, tandospirone) which, among others, exert their action via 5-HT_{1A} and 5-HT_{2A} receptors. Their diversified receptor binding profiles and intrinsic activities,

Scheme



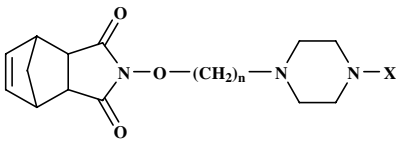
Reagents and conditions: (i) $Br(CH_2)_nCl$, NaOH, absolute EtOH, 70 °C, 24 h; (ii) 4-X-substituted-piperazine, K_2CO_3, NaI, CH_3CN, reflux, 24 h

depending on either the kind of substituent attached to the N-4 atom of the piperazine moiety or the nature of an amide or imide terminal fragment, open the possibility for discovery of new potent therapeutic agents (Zajdel et al. 2007). The influence of each part of the LCAP structures on the 5-HT_{1A} and 5-HT_{2A} receptor affinity, intrinsic activity, and selectivity has been the subject of many SAR studies, whereas there are few evidences regarding the interaction of the LCAP structures on the 5-HT_{2C} receptor. In our laboratories, there has been an ongoing effort to develop new 5-HT_{1A} agents (Caliendo et al. 1993, 1995, 1996, 1999, 2000, 2001, 2002; Fiorino et al. 2005, 2008) with high affinity and selectivity over other serotoninergic, dopaminergic and adrenergic receptors. In continuation of our research program, the aim of this work was also to better clarify the structural features needed in order to make the LCAPs able for the interaction with 5-HT_{2A} and 5-HT_{2C} receptors. Based on this consideration we have analyzed a new set of derivatives where the piperazine-N-alkyl moiety has been linked to a norbornene fragment (Scheme); introducing this heterocyclic nucleus in already reported compounds afforded arylpiperazine derivatives with high affinity and selectivity towards 5-HT_{1A} receptor (Fiorino et al. 2005). The relevance of the norbornene nucleus as terminal fragment was already supported by unconstrained molecular dynamics simulations that showed a very stable orientation and position of the norbornene part, emphasizing its favourable properties as anchoring group (Fiorino et al. 2005). In this paper, we describe a new series of derivatives in which, this bicyclic nucleus has been linked via two or three methylene spacing units to piperazines substituted in position 4 with aliphatic and/or aromatic moieties, aiming to further explore the interaction with 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors. In particular, the choice of aliphatic substituents on the N-4 of the piperazine moiety, that could appear in contrast with the statement that protonable nitrogen and aromatic system are necessary for binding of aminergic ligand, was done in order to obtain a complete structure-affinity and structure-selectivity relationship study. Instead, the role of oxygen atom in the chain linker was already clarified by molecular modelling studies showing that the oxygen leads to more favourable changes in steric as well as electrostatic interaction between the norbornene derivatives and the receptor (Fiorino et al. 2005). Moreover, the multireceptor profiles of promising derivatives were also evaluated in terms of binding affinities for dopaminergic (D_1, D_2) and adrenergic (α_1, α_2) receptors.

2. Investigations, results and discussion

The general strategy for the synthesis of the target compounds (Table 1) is summarized in Scheme 1. The general

Table 1: Affinities of compounds 2h–u and 3h–u for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors



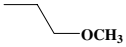
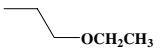
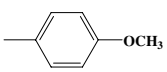
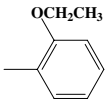
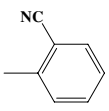
Compd.	Receptor affinity $K_i \pm$ SD (nM)				
	X	n	5-HT_{1A} [^{3}H]8OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine
2h		2	no affinity	$>10^4$	$>10^4$
2i		2	no affinity	$>10^4$	1590 ± 524
2l		2	no affinity	$>10^4$	$>10^4$
2m		2	no affinity	25.3 ± 2.6	6.79 ± 0.08
2n		2	5.67 ± 0.3	381 ± 16	10.9 ± 4.9
2o		2	4.82 ± 0.05	83.7 ± 1.9	6.61 ± 0.26

Table 1: Continued

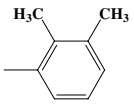
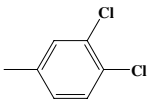
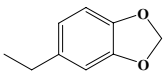
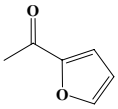
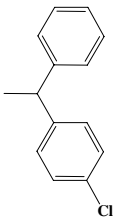
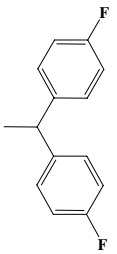
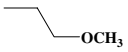
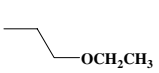
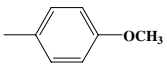
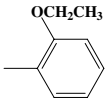
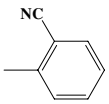
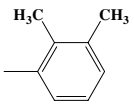
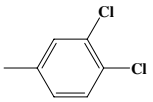
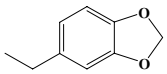
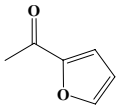
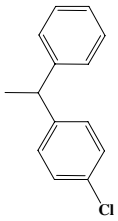
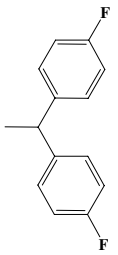
Compd.	Receptor affinity $K_i \pm SD$ (nM)				
	X	n	5-HT_{1A} [^{3}H]8OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine
2p		2	15.1 ± 0.8	14.8 ± 1.2	17.4 ± 1.0
2q		2	no affinity	153 ± 70	1.13± 0.16
2r		2	no affinity	no affinity	no affinity
2s		2	no affinity	3880 ± 172	145 ± 48
2t		2	no affinity	$>10^4$	no affinity
2u		2	10.3 ± 0.6	344 ± 57	10.2 ± 1.2
3h		3	no affinity	$>10^4$	19.4 ± 0.8
3i		3	no affinity	no affinity	no affinity
3l		3	no affinity	$>10^4$	11.0 ± 5.7
3m		3	no affinity	71.4 ± 1.0	4.56 ± 0.37
3n		3	$>10^4$	397 ± 24	5.02 ± 0.22
3o		3	$>10^4$	18.8 ± 1.0	no affinity
3p		3	no affinity	7.48 ± 0.18	5.16 ± 0.12
3q		3	$>10^4$	13.4 ± 1.4	no affinity

Table 1: Continued

Compd.	Receptor affinity $K_i \pm SD$ (nM)				
	X	n	5-HT_{1A} [^{3}H]8OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine
3r		3	no affinity	28.8 ± 1.6	1720 ± 62
3s		3	3530 ± 231	2690 ± 204	794 ± 6
3t		3	433 ± 38	25.6 ± 1.3	no affinity
3u		3	1070 ± 88	25.3 ± 3.6	2820 ± 154

For purpose of comparison, 8-OH-DPAT, Ketanserine and Mesulergine binds 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

procedure is as follows: alkylation of the starting heterocycle endo-N-hydroxy-5-norbornene-2,3-dicarboximide with 1-bromo-2-chloroethane or 1-bromo-3-chloropropane, in presence of NaOH in absolute ethanol, gave the corresponding chloro-alkyl norbornene derivatives **2a** and **3a**. Subsequent condensation of compounds **2a** and **3a** with the desired 4-substituted-piperazine, performed in CH_3CN in the presence of K_2CO_3 and NaI, under reflux, provided the final compounds **2h–u** (n = 2) and **3h–u** (n = 3), respectively. Purification of each final product was obtained by chromatography on silica gel column and further by crystallization from the appropriate solvent. All new compounds gave satisfactory elemental analyses and were characterized by ^{1}H NMR and mass spectrometry (LCQ-MS Thermoquest-Ion trap). ^{1}H NMR and MS data for all final compounds were consistent with the proposed structures.

Twenty-four piperazine derivatives (**2h–u**; **3h–u**) were synthesized and evaluated for their activity and selectivity. Introduction of a norbornene nucleus as terminal part, slight modifications concerning the alkyl spacer chain length (three to four atoms) and the introduction of new aliphatic and aromatic substituents on the N-4 piperazine moiety are depicted in the Fig. Several of the newly synthesized molecules showed affinity in the nanomolar range towards 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors and moderate to no affinity for other relevant receptors (D_1, D_2, α_1 and α_2) (Table 1 and 2). The outstanding 5-HT_{2C} receptor affinity of compound **2q** (K_i = 1.13 nM), is of particular interest, while **3q** (K_i = 13.4 nM), showed the most interesting selectivity profile with a good affinity towards 5-HT_{2A} activity, whereas compound **3p** presented a mixed 5-HT_{2A}/5-HT_{2C} activity with K_i values of 7.48/5.16 nM respectively.

The two series, **2h–u** and **3h–u**, differ in the length of the connecting chain between the exocyclic oxygen atom and the piperazine ring. Unlike the first series of norbornene derivatives (Fiorino et al. 2005), where three units alkyl chain compounds showed the best affinity/selectivity profile towards 5-HT_{1A} receptors, in this second series, the alkyl chain length does not seem to be decisive in determining a general trend but the affinity/selectivity profile is more influenced by the particular substituent on the piperazine moiety.

A 3,4-dichlorophenyl group as N-4 piperazine substituent, associated to a shorter chain spacer (n = 2, **2q**), conferred the highest affinity and selectivity for the 5-HT_{2C} receptor. Instead, 2,3-dimethylphenylpiperazine moiety, associated to a longer chain spacer (n = 3, **3p**), afforded a favorable mixed affinity profile for 5-HT_{2A}/5-HT_{2C} receptors. Additionally, 5-HT_{1A} receptor affinity and selectivity of the tested compounds were always lower than those observed for the first series of norbornene derivatives (Fiorino et al. 2005).

Successively, the affinity of the most active compounds (**2m**, **2n**, **2o**, **2p**, **2q**, **3m**, **3n** and **3p**) on several other receptors (α_1 and α_2 adrenergic and D_1 and D_2 dopaminergic receptors) was examined in order to verify their selectivity. Results are summarized in Table 2. All the compounds proved highly selective against dopaminergic receptors with K_i values of above 10^4 nM except for compound **2n**, which exhibited a K_i value of 7.92 nM on the D_2 receptor, whereas compound **3p** showed quite moderate affinity (84.7 nM). Regarding α_1 and α_2 receptors,

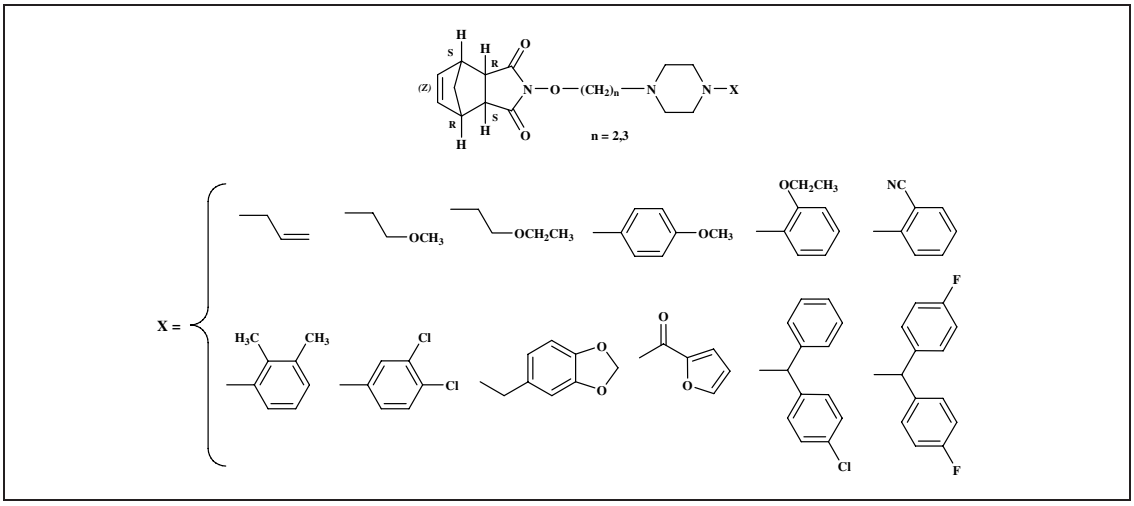


Fig.:
General structures

only compound **3n** showed quite a moderate affinity (162 nM) towards α_2 receptors.

These results further support the choice of the norbornene 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 mixed 5-HT_{2A}/5-HT_{2C} activity, as well as compounds with high 5-HT_{2C} affinity and selectivity.

The high difference in affinity observed between this new series of norbornene derivatives and the previously described series (Fiorino et al. 2005) demonstrates again that, besides the alkyl chain length, also the substituents on the N-4 of the piperazine moiety represents a critical structural feature in determining 5-HT_{1A} receptor affinity and selectivity. In fact, in contrast to all other substituents generally reported in the literature and the substituents utilized in our previous series with the same norbornene nucleus (Fiorino et al. 2005), the new aliphatic and aromatic derivatives exhibit a poor or lower affinity and selectivity for this receptor. The only exceptions could be represented by compounds **2n**, **2o** and **2u** where a good nanomolar affinity on the 5-HT_{1A} receptor is associated to a concomitant nanomolar affinity on the 5-HT_{2C} receptor and a quite poor affinity on the 5-HT_{2A} receptor. These results can help to clarify the role of N-4 position of the piperazine moiety on the 5-HT_{1A} receptor affinity and selectivity. In fact, the newly selected 4-substituted piperazines were included in this study in order to investigate the influence of size of the substituent in position 4 on the ligand affinity and selectivity. Zlatovic et al. (2006) have recently reported that some arylpiperazines can interact directly with the hydrophobic part of the 5-HT_{1A} receptor binding site and ligands embodying larger substituents in position 4 on the aromatic ring of the arylpiperazine moiety form weaker complexes with the receptor, because of steric interactions between the substituent in position 4 and Trp 358, and/or the backbone of TM6. In fact, when the position 4 on the aromatic ring is not substituted and associated to a shorter alkyl chain (n = 2, **2n**, **2o** and **2p**) we still have a nanomolar affinity on the 5-HT_{1A} receptor but not selectivity over other serotoninergic receptors. Instead, when the position 4 is not substituted but associated to a longer alkyl chain (n = 3, **3n**, **3o** and **3p**) that, however, leads to a longer structure, we do not find any affinity. Therefore, these aspects can be useful to explain the smaller affinity and selectivity that these new piperazine derivatives have shown; in these compounds, in fact, a non aromatic moiety or a larger substituent are less favorable to the interaction with the receptor. Based on this consideration we can suppose that, also the lack of affinity of the 4-methoxyphenylpiperazine derivatives (**2m** and **3m**) on 5-HT_{1A} receptor, apparently clashing with the high affinity and selectivity of the previously reported 2-methoxyphenylpiperazine derivative (Fiorino et al. 2005), could be due, as recently reported (Zlatovic et al. 2006), to the unfavorable steric interactions of the methoxy group in position 4 of the aromatic ring with Trp 358, and in part with the backbone of TM6. Moreover, results obtained with compound **2n** (K_i value of 5.67 nM) and **3n** (K_i value > 104), supporting an *o*-ethoxyphenylpiperazine moiety, compared with *o*-methoxyphenylpiperazine derivatives (**2b** and **3b**), previously re-

Table 2: Affinities of compounds 2m, 2n, 2o, 2p, 2q, 3m, 3n and 3p for D_1, D_2, α_1 and α_2 receptors

Compd.	Receptor affinity $K_i \pm$ SD (nM)			
	D_1	D_2	α_1	α_2
	[^{3}H]SCH-23390	[^{3}H]spiperone	[^{3}H]prazosin	[^{3}H]yohimbine
2m	$>10^4$	$>10^4$	no affinity	$>10^4$
2n	$>10^4$	7.92 ± 0.197	1290 ± 108	3100 ± 227
2o	350 ± 15.6	$>10^4$	$>10^4$	1110 ± 425
2p	$>10^4$	646 ± 12.6	550 ± 32.3	1150 ± 106
2q	$>10^4$	$>10^4$	$>10^4$	2730 ± 206
3m	no affinity	no affinity	no affinity	$>10^4$
3n	180 ± 4.8	$>10^4$	723 ± 56.8	162 ± 11.3
3p	$>10^4$	84.7 ± 0.62	249 ± 11.0	497 ± 17.6

ported (Fiorino et al. 2005), showing high 5-HT_{1A} receptor affinity and selectivity, could be discussed in order to better explain the influence of the different substituents in *ortho* position on the phenylpiperazine moiety. In fact, the simple change from $-OCH_3$ to $-OCH_2CH_3$ produces a reduction of affinity by five orders of magnitude. This results could be, probably, addressed to unfavorable steric interaction of the ethoxy group supported by longer oxyimido moiety (**3n**; n = 3) with the surrounding receptor residues and loss of the hydrogen bond to Asn-386, that molecular modeling studies (Fiorino et al. 2005) suggested as important for *o*-methoxy analogues. In fact, increasing the length of the substituent in ortho position by one methylene group, the substituent is pulled out of interaction with Asn-386. On the contrary the shorter oxyimido chain (**2n**; n = 2) allows the phenyl ring to stay into the receptor, leading a quite favorable interaction of the ethoxy group and the side chain of Asn-386 by hydrogen bond.

Finally, also the bad affinity/selectivity profile of the derivatives characterized with the presence of a biaryl group (**2t**, **2u**, **3t** and **3u**) on the N-4 position can be explained, as reported in the literature (Abou-Gharbia et al. 1999), because of asteric interaction that determines the formation of weaker complex with the receptor. The only exception could be represented by compound **2u** (K_i = 10.3 nM) that showed high 5-HT_{1A} receptor affinity, but unfortunately associated to a concomitant nanomolar affinity on the 5-HT_{2C} receptor (K_i = 10.2 nM) and a consequently quite poor selectivity.

4-[2-[4-(3,4-Dichlorophenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2q**) with K_i = 1.13 nM was the most active and selective derivative for the 5-HT_{2C} receptor with respect to other serotoninergic, dopaminergic and adrenergic receptors. This result is particularly interesting also because there are few evidences regarding the interaction of the LCAPs structures on the 5-HT_{2C} receptor. In fact, only some piperazine analogs were disclosed as potent and selective 5-HT_{2C} agonist, but unfortunately no supporting *in vivo* data was reported (Isaac et al. 2005). Interestingly nanomolar affinity on the 5-HT_{2C} receptor was shown also by compounds **3h**, **3l**, **3m**, and **3n**, but with lower affinity/selectivity profile. Furthermore, compounds **3h** and **3l** could appear particularly interesting because they showed nanomolar affinity on the 5-HT_{2C} receptor, no affinity for other serotonin receptor and they are devoid of aromatic part on piperazine moiety.

Additionally, the mixed 5-HT_{2A}/5-HT_{2C} affinity (K_i values of 7.48/5.16 nM respectively), showed by compound **3p** with a concomitant fairly good affinity on D_2 receptor, is of particular interest and outlines a potential atypical antipsychotic profile for this derivative.

In summary, we have synthesized a new series of 4-substituted piperazines linked to a norbornene nucleus via two or three methylene spacing units. The binding data presented in this study have shed additional light on the influence of the LCAPs on the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors affinity and selectivity. In accordance with already reported data, larger or aliphatic substituents on the N-4 of the piperazine moiety are not tolerated even when norbornene nucleus has been used as terminal fragment, in order to obtain derivatives with a better profile of affinity/selectivity on the 5-HT_{1A} receptor. Simultaneously, we have disclosed two interesting compounds as 5-HT_{2C} and mixed 5-HT_{2A}/5-HT_{2C} ligands (**2q** and **3p** respectively), with a potential therapeutical profile as antiepileptic, anxiolytic or atypical antipsychotic agents.

3. Experimental

3.1. Synthesis

All reagents and substituted piperazines were commercial products purchased from Aldrich. Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. ^{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). Mass spectra of the final products were determined using LCQ Thermoquest-Ion trap mass spectrometry. Where analyses are indicated only by the symbols of the elements, results obtained are within ± 0.4% of the theoretical values. All reactions were followed by TLC, carried out on Merck silica gel 60 F_{254} plates with fluorescent indicator and the plates were visualized with UV light (254 nm). Preparative chromatographic purifications were performed using silica gel column (Kieselgel 60). Solutions were dried over Na_2SO_4 and concentrated with Büchi rotary evaporator at low pressure.

3.1.1. Synthesis of 4-(2-chloroethoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2a**)

A solution of absolute ethanol (50 mL) and sodium hydroxide 0.72 g (0.018 mol) was reacted with 3.22 g (0.018 mol) of commercially available endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide and 2.58 g (0.018 mol) of 1-bromo-2-chloroethane at 70 °C for 24 h. Afterwards the mixture was cooled to room temperature, concentrated to dryness and the residue diluted in water (40 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried on anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (diethyl ether/ethanol 9 : 1 (v/v)). The combined and evaporated product fractions were crystallized from diethyl ether/hexane, yielding 3.5 g (80%) of white solid: mp 56–58 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.50 (d, 1 H J = 9.1); 1.76 (dt, 1 H J = 9.1); 3.18 (dd, 2H, J = 1.4, 2.8); 3.42 (s, 2 H); 3.58 (t, 2 H, J = 6.4); 4.09 (t, 2 H, J = 5.9); 6.16 (t, 2 H, J = 1.8).

3.1.2. Synthesis of 4-(3-Chloropropoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3a**)

A solution of absolute ethanol (50 mL) and sodium hydroxide 0.72 g (0.018 mol) was reacted with 3.22 g (0.018 mol) of commercially available endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide and 2.83 g (0.018 mol) of 1-bromo-3-chloropropane at 70 °C for 24 h. Afterwards the mixture was cooled to room temperature, concentrated to dryness and the residue diluted in water (40 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried on anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (diethyl ether/ethanol 9 : 1 (v/v)). The combined and evaporated product fractions were crystallized from diethyl ether/hexane, yielding 4.30 g (94%) of the desired product as a white solid: m.p.: 59–61 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.50 (d, 1 H J = 9.1); 1.76 (dt, 1 H J = 9.1); 2.16 (q, 2 H, J = 6.2); 3.18 (dd, 2 H, J = 1.4, 2.8); 3.42 (s, 2 H); 3.58 (t, 2 H, J = 6.4); 4.09 (t, 2 H, J = 5.9); 6.16 (t, 2 H, J = 1.8).

*3.1.3. General procedure for the condensation of 4-substituted piperazines with derivatives **2a** and **3a***

A mixture of 4-(2-chloroethoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione **2a** or 4-(3-chloro-propoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione **3a** (0.006 mol), and NaI (0.009 mol) was stirred under reflux for 30 min. Then, the appropriate 4-substituted alkyl or arylpiperazine (0.03 mol) and anhydrous K_2CO_3 (0.009 mol) were added. The reaction mixture was stirred under reflux for 24 h. After cooling, the mixture was filtered, concentrated to dryness and the residue was dissolved in water (50 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried on anhydrous Na_2SO_4 and the solvent removed under vacuum. The crude mixtures were purified by silica gel column chromatography using diethyl ether/methanol 8 : 2 (v/v) as eluent. The crude products were recrystallized from diethyl ether.

3.1.4. Synthesis of 4-[2-[4-allylpiperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2h**)

From **2a** and 1-allylpiperazine. Yield: 54%; m.p. 75–76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 2.50 (bs, 4 H, $2CH_2$ pip.); 2.55 (bs, 4 H, $2CH_2$ pip.); 2.68 (t, 2 H, N^1-CH_2, J = 7.3); 2.98 (d, 2 H, J = 6.6); 3.17 (s, 2 H); 3.41 (s, 2 H); 4.05 (t, 2 H, O–CH_2, J = 6.5); 5.12 (dd, 2 H); 5.80 (m, 1 H); 6.14 (s, 2 H). ESI–MS: 332.1 $[M + H]^+$.

($C_{18}H_{25}N_3O_3$), C, H, N

3.1.5. Synthesis of 4-[2-[4-(2-methoxyethyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2i**)

From **2a** and 1-(2-methoxyethyl)piperazine. Yield: 55%; m.p. 70–72 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 1.82 (bs, 4 H, 2 CH_2 pip.); 2.56 (bs, 4 H, 2 CH_2 pip.); 2.58 (t, 2 H, N^4-CH2 pip., J = 5.1); 2.67 (t, 2 H, N^1-CH_2, J = 7.3); 3.16 (s, 2 H); 3.33 (s, 3 H, OCH_3); 3.41 (s, 2 H); 3.48 (t, 2 H CH_2O, J = 5.1); 4.05 (t, 2 H, O–CH_2, J = 6.5); 6.14 (s, 2 H). ESI–MS: 350.3 $[M + H]^+$; 372.3 $[M + Na]^+$; 388.3 $[M + K]^+$.
($C_{18}H_{27}N_3O_4$), C, H, N

3.1.6. Synthesis of 4-[2-[4-(2-ethoxyethyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2l**)

From **2a** and 1-(2-ethoxyethyl)piperazine. Yield: 68%; m.p. 215–216 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.19 (t, 3 H); d, 1 H, J = 8.9); 1.68 (bs, 4 H, 2CH_2 pip.); 1.77 (d, 1 H, J = 8.9); 2.56 (bs, 4 H, 2 CH_2 pip.); 2.67 (t, 2 H, N^1-CH_2, J = 7.3); 3.18 (s, 2 H); 3.29 (t, 2 H, N^4-CH_2 pip., J = 5.1); 3.41 (s, 2H); 3.45 (t, 2 H CH_2O, J = 5.1); 3.52 (q, 2 H, OCH_2, J = 6.2); 4.05 (t, 2 H, O–CH_2, J = 6.5); 6.20 (s, 2 H). ESI–MS: 364.2 $[M + H]^+$.
($C_{19}H_{29}N_3O_4$), C, H, N.

3.1.7. Synthesis of 4-[2-[4-(p-methoxyphenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2m**)

From **2a** and *p*-methoxyphenylpiperazine. Yield: 54%; m.p. 96–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 2.69 (bs, 4 H, 2 CH_2 pip.); 2.74 (t, 2 H, N^1-CH_2, J = 7.3); 3.10 (bs, 4 H, 2CH_2 pip.); 3.17 (s, 2 H); 3.42 (s, 2 H); 3.76 (s, 3 H, OCH_3); 4.10 (t, 2 H, O–CH_2, J = 6.5); 6.16 (s, 2 H); 6.81 (d, 2 H, J = 8.7); 6.88 (d, 2 H, J = 8.7). ESI–MS: 398.1 $[M + H]^+$.
($C_{22}H_{27}N_3O_4$), C, H, N.

3.1.8. Synthesis of 4-[2-[4-(o-ethoxyphenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2n**)

From **2a** and *o*-ethoxyphenylpiperazine. Yield: 61%; m.p. 81–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (t, 3 H, -CH_3, J = 6.9); 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 2.72 (bs, 4 H, 2 CH_2 pip.); 2.74 (t, 2 H, N^1-CH_2, J = 7.3); 3.13 (bs, 4 H, 2 CH_2 pip.); 3.18 (s, 2 H); 3.42 (s, 2 H); 4.04 (q, 2 H, OCH_2, J = 6.9); 4.11 (t, 2 H, O–CH_2, J = 6.5); 6.16 (s, 2 H); 6.82–6.95 (m, 4 H). ESI–MS: 412.3 $[M + H]^+$; 434.1 $[M + Na]^+$; 450.1 $[M + K]^+$.
($C_{23}H_{29}N_3O_4$), C, H, N

3.1.9. Synthesis of 4-[2-[4-(2-cyanophenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2o**)

From **2a** and 2-(piperazin-1-yl)benzonitrile. Yield: 50%; m.p. 80–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 2.75 (bs, 4 H, 2CH_2 pip.); 2.77 (t, 2 H, N^1-CH_2, J = 7.3); 3.18 (s, 2 H); 3.25 (bs, 4 H, 2CH_2 pip.); 3.42 (s, 2 H); 4.10 (t, 2 H, O–CH_2, J = 6.5); 6.16 (s, 2 H); 6.97–7.00 (m, 2 H); 7.45 (t, 1 H, J = 8.0); 7.53 (d, 1 H, J = 8.0). ESI–MS: 394.0 $[M + H]^+$; 415.1 $[M + Na]^+$.
($C_{22}H_{24}N_4O_3$), C, H, N

3.1.10. Synthesis of 4-[2-[4-(2,3-dimethylphenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2p**)

From **2a** and 1-(2,3-dimethylphenyl)piperazine. Yield: 67%; m.p. 146–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 2.20 (s, 3 H); 2.25 (s, 3 H); 2.69 (bs, 4 H, 2 CH_2 pip.); 2.76 (t, 2 H, N^1-CH_2, J = 7.3); 2.91 (bs, 4 H, 2 CH_2 pip.); 3.19 (s, 2 H); 3.43 (s, 2 H); 4.11 (t, 2 H, O–CH_2, J = 6.5); 6.16 (s, 2 H); 6.88 (m, 2H); 7.04 (t, 1H, J = 7.7). ESI–MS: 396.4 $[M + H]^+$; 418.1 $[M + Na]^+$; 434.1 $[M + K]^+$.
($C_{23}H_{29}N_3O_3$), C, H, N

3.1.11. Synthesis of 4-[2-[4-(3,4-dichlorophenyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2q**)

From **2a** and 1-(3,4-dichlorophenyl)piperazine. Yield: 56%; m.p. 104–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 2.66 (bs, 4 H, 2 CH_2 pip.); 2.73 (t, 2 H, N^1-CH_2, J = 7.3); 3.16 (bs, 4 H, 2 CH_2 pip.); 3.18 (s, 2 H); 3.42 (s, 2 H); 4.09 (t, 2 H, O–CH_2, J = 6.5); 6.15 (s, 2 H); 6.70 (d, 1 H, J = 2.5); 6.93 (s, 1 H); 7.25 (d, 1 H, J = 2.5). ESI–MS: 437.7 $[M + H]^+$.
($C_{21}H_{23}Cl_2N_3O_3$), C, H, N

3.1.12. Synthesis of 4-[2-[4-((benzo[d][1,3]dioxol-5-yl)methyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2r**)

From **2a** and 1-((benzo[*d*][1,3]dioxol-6-yl)methyl)piperazine. Yield: 58%; m.p. 93–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 2.46 (bs, 4 H, 2 CH_2 pip.); 2.53 (bs, 4 H, 2 CH_2 pip.); 2.67 (t, 2 H, N^1-CH_2, J = 7.3); 3.15 (s, 2 H); 3.40 (s, 2 H); 3.42 (s, 2 H); 4.05 (t, 2 H, O–CH_2, J = 6.5); 5.92 (s, 2 H, OCH_2O); 6.13 (s, 2 H); 6.72 (m, 2 H); 6.83 (s, 1 H). ESI–MS: 426.2 $[M + H]^+$.
($C_{23}H_{27}N_3O_5$), C, H, N

3.1.13. Synthesis of 4-[2-[4-(2-furoyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2s**)

From **2a** and 1-(2-furoyl)piperazine. Yield: 47%; m.p. 115–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 2.58 (bs, 4 H, 2 CH_2 pip.); 2.71 (t, 2 H, N^1-CH_2, J = 7.3); 3.18 (s, 2 H); 3.42 (s, 2 H); 3.82 (bs, 4 H, 2 CH_2 pip.); 4.07 (t, 2 H, O–CH_2, J = 6.5); 6.15 (s, 2 H); 6.46 (d, 1 H, J = 2.9); 6.97 (d, 1 H, J = 2.9); 7.46 (m, 1 H). ESI–MS: 386.3 $[M + H]^+$; 408.2 $[M + Na]^+$; 424.2 $[M + K]^+$.
($C_{20}H_{23}N_3O_5$), C, H, N

3.1.14. Synthesis of 4-[2-[4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2t**)

From **2a** and 1-(4-chlorobenzhydryl)piperazine. Yield: 57%; m.p. 62–65 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47 d, 1 H, J = 8.9); 1.73 (d, 1 H, J = 8.9); 2.40 (bs, 4 H, 2 CH_2 pip.); 2.54 (bs, 4 H, 2 CH_2 pip.); 2.67 (t, 2 H, N^1-CH_2, J = 7.3); 3.13 (s, 2 H); 3.39 (s, 2 H); 4.04 (t, 2 H, O–CH_2, J = 6.5); 4.20 (s, 1 H); 6.12 (s, 2 H); 7.17–7.35 (m, 9 H). ESI–MS: 493.1 $[M + H]^+$.
($C_{28}H_{30}ClN_3O_3$), C, H, N

3.1.15. Synthesis of 4-[2-[4-(bis(4-fluorophenyl)methyl)piperazin-1-yl]ethoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**2u**)

From **2a** and 1-(bis(4-fluorophenyl)methyl)piperazine. Yield: 67%; m.p. 54–56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.73 (d, 1 H, J = 8.9); 2.39 (bs, 4 H, 2 CH_2 pip.); 2.55 (bs, 4 H, 2 CH_2 pip.); 2.68 (t, 2 H, N^1-CH_2, J = 7.3); 3.13 (s, 2 H); 3.39 (s, 2 H); 4.04 (t, 2 H, O–CH_2, J = 6.5); 4.20 (s, 1 H); 6.12 (s, 2 H); 6.92–6.97 (m, 4 H); 7.30–7.33 (m, 4 H). ESI–MS: 494.9 $[M + H]^+$.
($C_{28}H_{29}F_2N_3O_3$), C, H, N

3.1.1.6. Synthesis of 4-[3-[4-allylpiperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3h**)

From **3a** and 1-allylpiperazine. Yield: 58%; m.p. 250–251 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 1.81 (q, 2 H, –CH_2–, J = 6.6, 7.3); 2.05 (bs, 4 H, 2 CH_2 pip.); 2.50 (t, 2 H, N^1–CH_2, J = 7.3); 2.53 (bs, 4 H, 2 CH_2 pip.); 2.99 (d, 2 H, J = 6.6); 3.17 (s, 2 H); 3.42 (s, 2 H); 3.98 (t, 2 H, O–CH_2, J = 6.5); 5.13 (m, 2 H); 5.82 (m, 1 H); 6.15 (s, 2 H). ESI–MS: 346.0 $[M + H]^+$.
($C_{19}H_{27}N_3O_3$), C, H, N

3.1.17. Synthesis of 4-[3-[4-(2-methoxyethyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3i**)

From **3a** and 1-(2-methoxyethyl)piperazine. Yield: 70%; m.p. 207–208 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 1.79 (q, 2 H, –CH_2–, J = 6.6, 7.3); 2.47 (t, 2 H, N^4–CH_2 pip., J = 5.1); 2.49 (bs, 4 H, 2 CH_2 pip.); 2.51 (bs, 4 H, 2 CH_2 pip.); 2.54 (t, 2 H, N^1–CH_2, J = 7.3); 3.16 (s, 2 H); 3.33 (s, 3 H, OCH_3); 3.41 (s, 2 H); 3.48 (t, 2 H CH_2O, J = 5.8); 3.97 (t, 2 H, O–CH_2, J = 6.5); 6.14 (s, 2 H). ESI–MS: 364.3 $[M + H]^+$.
($C_{19}H_{29}N_3O_4$), C, H, N

3.1.18. Synthesis of 4-[3-[4-(2-ethoxyethyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3l**)

From **3a** and 1-(2-ethoxyethyl)piperazine. Yield: 58%; m.p. 208–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.16 (t, 2 H, J = 6.9) 1.48 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 1.82 (q, 2 H, –CH_2–, J = 6.6, 7.3); 1.98 (bs, 4 H, 2 CH_2 pip.); 2.48 (bs, 4 H, 2 CH_2 pip.); 2.50 (t, 2 H, N^4–CH_2 pip., J = 5.1); 2.57 (t, 2 H, N^1–CH_2, J = 7.3); 3.17 (s, 2 H); 3.41 (s, 2 H); 3.45 (q, 2 H, OCH_2, J = 6.2); 3.53 (t, 2 H CH_2O, J = 5.8); 3.97 (t, 2 H, O–CH_2, J = 6.5); 6.15 (s, 2 H). ESI–MS: 377.9 $[M + H]^+$.
($C_{20}H_{31}N_3O_4$), C, H, N

3.1.19. Synthesis of 4-[3-[4-(p-methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3m**)

From **3a** and *p*-methoxyphenylpiperazine. Yield: 25%; m.p. 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 1.86 (q, 2 H, –CH_2–, J = 6.6, 7.3); 2.55 (t, 2 H, N^1–CH_2, J = 7.3); 2.61 (bs, 4 H, 2 CH_2 pip.); 3.08 (bs, 4 H, 2 CH_2 pip.); 3.18 (s, 2 H); 3.42 (s, 2 H); 3.76 (s, 3 H, OCH_3); 4.02 (t, 2 H, O–CH_2, J = 6.5); 6.16 (s, 2 H); 6.81 (d, 2 H, J = 7.3); 6.88 (d, 2 H, J = 7.3). ESI–MS: 412.4 $[M + H]^+$; 434.3 $[M + Na]^+$; 450.3 $[M + K]^+$.
($C_{23}H_{29}N_3O_4$), C, H, N

3.1.20. Synthesis of 4-[3-[4-(o-Ethoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3n**)

From **3a** and *o*-ethoxyphenylpiperazine. Yield: 48%; m.p. 121–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (t, 3 H, CH_3 J = 6.9); 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 1.87 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.55 (t, 2 H, N^1-CH_2, J = 7.3); 2.64 (bs, 4 H, 2 CH_2 pip.); 3.10 (bs, 4 H, 2 CH_2 pip.); 3.18 (s, 2 H); 3.42 (s, 2 H); 4.02 (q, 2 H, OCH_2, J = 6.9); 4.08 (t, 2 H, $O-CH_2$, J = 6.5); 6.16 (s, 2 H); 6.82–6.94 (m, 4 H). ESI−MS: 426.5 $[M + H]^+$; 448.3 $[M + Na]^+$; 464.0 $[M + K]^+$.
($C_{24}H_{31}N_3O_4$), C, H, N

3.1.21. Synthesis of 4-[3-[4-(2-cyanophenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3o**)

From **3a** and 2-(piperazin-1-yl)benzonitrile. Yield: 47%; m.p. 104–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 1.85 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.57 (t, 2 H, N^1-CH_2, J = 7.3); 2.65 (bs, 4 H, 2 CH_2 pip.); 3.18 (s, 2 H); 3.20 (bs, 4 H, 2 CH_2 pip.); 3.42 (s, 2 H); 4.01 (t, 2 H, $O-CH_2$, J = 6.5); 6.16 (s, 2 H); 6.97–7.00 (m, 2 H); 7.44 (t, 1 H, J = 6.5); 7.53 (dd, 1 H, J = 6.5). ESI−MS: 407.3 $[M + H]^+$; 429.2 $[M + Na]^+$.
($C_{23}H_{26}N_4O_3$), C, H, N

3.1.22. Synthesis of 4-[2-[4-(2,3-dimethylphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3p**)

From **3a** and 1-(2,3-dimethylphenyl)piperazine. Yield: 60%; m.p. 94–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 1.87 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.20 (s, 3 H, CH_3); 2.25 (s, 3 H, CH_3); 2.55 (t, 2 H, N^1-CH_2, J = 7.3); 2.59 (bs, 4 H, 2 CH_2 pip.); 2.88 (bs, 4 H, 2 CH_2 pip.); 3.18 (s, 2 H); 3.42 (s, 2 H); 4.02 (t, 2 H, $O-CH_2$, J = 6.5); 6.17 (s, 2 H); 6.88 (m, 2 H); 7.04 (t, 1 H, J = 7.6). ESI−MS: 410.5 $[M + H]^+$; 432.3 $[M + Na]^+$; 448.3 $[M + K]^+$.
($C_{24}H_{31}N_3O_3$), C, H, N

3.1.23. Synthesis of 4-[2-[4-(3,4-dichlorophenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3q**)

From **3a** and 1-(3,4-dichlorophenyl)piperazine. Yield: 72%; m.p. 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 1.84 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.54 (t, 2 H, N^1-CH_2, J = 7.3); 2.58 (bs, 4 H, 2 CH_2 pip.); 3.14 (bs, 4 H, 2 CH_2 pip.); 3.18 (s, 2 H); 3.42 (s, 2 H); 4.01 (t, 2 H, $O-CH_2$, J = 6.5); 6.16 (s, 2 H); 6.71 (d, 1 H, J = 6.9); 6.93 (s, 1 H); 7.24 (d, 1 H, J = 6.9). ESI−MS: 450.2 $[M + H]^+$; 472.1 $[M + Na]^+$.
($C_{22}H_{25}Cl_2N_3O_3$), C, H, N

3.1.24. Synthesis of 4-[2-[4-((benzo[d][1,3]dioxol-5-yl)methyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3r**)

From **3a** and 1-((benzo[d][1,3]dioxol-6-yl)methyl)piperazine. Yield: 62%; m.p. 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 1.80 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.45 (bs, 4 H, 2 CH_2 pip.); 2.46 (t, 2 H, N^1-CH_2, J = 7.3); 2.53 (bs, 4 H, 2 CH_2 pip.); 3.16 (s, 2 H); 3.39 (s, 2 H); 3.41 (s, 2 H); 3.96 (t, 2 H, $O-CH_2$, J = 6.5); 5.92 (s, 2 H, OCH_2O); 6.14 (s, 2 H); 6.72 (m, 2 H); 6.83 (s, 1 H). ESI−MS: 441.2 $[M + H]^+$.
($C_{24}H_{29}N_3O_5$), C, H, N

3.1.25. Synthesis of 4-[2-[4-(2-furoyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3s**)

From **3a** and 1-(2-furoyl)piperazine. Yield: 50%; m.p. 94–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (d, 1 H, J = 8.9); 1.75 (d, 1 H, J = 8.9); 1.82 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.48 (bs, 4 H, 2 CH_2 pip.); 2.52 (t, 2 H, N^1-CH_2, J = 7.3); 3.18 (s, 2 H); 3.42 (s, 2 H); 3.78 (bs, 4 H, 2 CH_2 pip.); 4.00 (t, 2 H, $O-CH_2$, J = 6.5); 6.15 (s, 2 H); 6.45 (d, 1 H, J = 3.2); 6.96 (d, 1 H, J = 3.2); 7.46 (m, 1 H). ESI−MS: 400.2 $[M + H]^+$; 422.0 $[M + Na]^+$; 438.0 $[M + K]^+$.
($C_{21}H_{25}N_3O_5$), C, H, N

3.1.26. Synthesis of 4-[2-[4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3t**)

From **3a** and 1-(4-chlorobenzhydryl)piperazine. Yield: 74%; m.p. 52–54 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47 (d, 1 H, J = 8.9); 1.73 (d, 1 H, J = 8.9); 1.78 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.38 (bs, 4 H, 2 CH_2 pip.); 2.48 (bs, 4 H, 2 CH_2 pip.); 2.50 (t, 2 H, N^1-CH_2, J = 7.3); 3.16 (s, 2 H); 3.40 (s, 2 H); 3.96 (t, 2 H, $O-CH_2$, J = 6.5); 4.18 (s, 1 H); 6.14 (s, 2 H); 7.15–7.35 (m, 9 H). ESI−MS: 506.3 $[M + H]^+$.
($C_{29}H_{32}ClN_3O_3$), C, H, N

3.1.27. Synthesis of 4-[2-[4-(bis(4-fluorophenyl)methyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**3u**)

From **3a** and 1-(bis(4-fluorophenyl)methyl)piperazine. Yield: 60%; m.p. 124–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (d, 1 H, J = 8.9); 1.74 (d, 1 H, J = 8.9); 1.79 (q, 2 H, $-CH_2-$, J = 6.6, 7.3); 2.36 (bs, 4 H, 2 CH_2 pip.); 2.47 (bs, 4 H, 2 CH_2 pip.); 2.49 (t, 2 H, N^1-CH_2, J = 7.3); 3.16 (s, 2 H); 3.41 (s, 2 H); 3.96 (t, 2 H, $O-CH_2$, J = 6.5); 4.18 (s, 1 H); 6.14 (s, 2 H); 6.93–6.97 (m, 4 H); 7.30–7.34 (m, 4 H). ESI−MS: 508.0 $[M + H]^+$.
($C_{29}H_{31}F_2N_3O_3$), C, H, N

3.2. Pharmacology

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 more active compounds on serotonin receptors have been selected and evaluated for their affinity for dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors. All the compounds were dissolved in ethanol or in 5% DMSO. 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; (d) dopamine D_1 receptor [^{3}H]SCH-23390, rat striatum; (e) dopamine D_2 receptor [^{3}H]spiperone, rat striatum; (f) $\alpha_1$adrenergic receptor [^{3}H]prazosin, rat brain cortex; (g) α_2 adrenergic receptor [^{3}H]yohimbine, 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 the "Easy Fit" program (Easy Fit 1.4, 1989–1991) 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 (1973) 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, (Scatchard et al. 1949) were calculated for each labeled ligand).

3.2.1. 5-HT_{1A} binding assay

Radioligand binding assays were performed as previously reported by Schlegel et al. (1986) 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 s), 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 0.1 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, and 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).

3.2.2. 5-HT_{2A} and 5-HT_{2C} binding assays

Radioligand binding assays were performed as reported by Herndon et al. (1992) 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 s) 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 (88.0 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 (87.0 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 Schleicher and Schuell (Keene, NH, USA) glass fibre filters presoaked in polyethylene imine, and washed with 10 mL of ice-cold buffer. Filters were counted at an efficiency of 50%.

3.2.3. D_1 Dopaminergic binding assay

The binding assay for D_1 dopaminergic receptors was that described by Billard et al. (1985) Corpora striata were homogenized in 30 vol. (w/v) ice cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50000 g with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 μL [^{3}H]SCH-23390 (85.0 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA) to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]SCH-23390 binding was defined as the difference between binding in the absence or in the presence of 0.1 μM piflutixol.

3.2.4. D_2 Dopaminergic binding assay

The procedure used in the radioligand binding assay was reported in detail by Creese et al. (1977) Corpora striata were homogenized in 30 vol. (w/v) ice cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50000 g with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice cold Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline (pH 7.1 at 37 °C). Each assay tube contained 50 μL [^{3}H]spiperone (15.7 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA) to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (3 mg fresh tissue). The tubes were incubated for 15 min at 37 °C and the incubation was terminated by rapid filtration under *vacuum* through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]spiperone binding was defined as the difference between binding in the absence or in the presence of 1 μM (+)-butaclamol.

3.2.5. α_1 Adrenergic binding assay

The procedure used in the radioligand binding assay has been reported in detail by Greengrass and Bremner (1979). Brain cortex was homogenized in 30 vol. (w/v) ice-cold 50 mM Tris-HCl buffer, (pH 7.2 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 50000 g with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50mM ice-cold Tris-HCl, (pH 7.4 at 25 °C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]prazosin (80.5 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA) to achieve a final concentration of 0.4 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl, buffer (pH 7.2 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]prazosin binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

3.2.6. α_2 Adrenergic binding assay

The procedure used in the radioligand binding assay was reported in detail by Perry and U'Prichard (1981). Brain cortex was homogenized in 30 vol. (w/v) ice-cold 5 mM tris-HCl, 5 mM EDTA buffer (pH 7.3 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged three times for 10 min at 50000 *g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris-HCl, 0.5 mM EDTA (pH 7.5 at 25 °C). Each assay tube contained 50 μL drug solution, 50 μL [^{3}H]yohimbine (80.5 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA) to achieve a final concentration of 1 nM, and 900 μL resuspended membranes (10 mg fresh tissue). The tubes were incubated for 30 min at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl, 0.5 mM EDTA buffer (pH 7.5 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [^{3}H]yohimbine binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

Acknowledgements: The NMR spectral data were provided by Centro di Ricerca Interdipartimentale di Analisi Strumentale, Università degli Studi di Napoli "Federico II". The assistance of the staff has been gratefully appreciated.

References

Abou-Gharbia MA, Childers WE, Fletcher Jr H, McGaughey G, Patel U, Webb MB, Yardley J, Andree T, Boast C, Kucharik RJ, Marquis Jr K, Morris H, Scerni R, Moyer JA (1999) Synthesis and SAR of adatanserin: novel adamantyl aryl- and heteroarylpiperazines with dual serotonin 5-HT(1A) and 5-HT(2) activity as potential anxiolytic and antidepressant agents. J Med Chem 42: 5077–5094.

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38: 1083–1152.

Baumgarten HG, Gother M (1997) Serotoninergic Neurons and 5-HT Receptors in the CNS. Handb Exp. Pharm. Springer-Verlag, Berlin, Vol. 129.

Billard W, Ruperto V, Crosby G, Iorio Barnett LC (1984) Characterization of the binding of 3H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. Life Sci 35: 1885–1893.

Bikker JA, Trump-Kallmeyer S, Humblet S (1998) G-Protein coupled receptors: models, mutagenesis, and drug design. J Med Chem 41: 2911–2927.

Caliendo G, Di Carlo R, Meli R, Perissutti, E, Santagada V, Silipo C, Vittoria A (1993) Synthesis and trazodone-like pharmacological profile of 1- and 2-[3-[4-(X)-1-piperazinyl]propyl]benzotriazoles. Eur J Med Chem 28: 969–974.

Caliendo G, Di Carlo R, Greco G, Meli R, Novellino E, Perissutti E, Santagada V (1995) Synthesis and biological activity of benzotriazole derivatives structurally related to trazodone. Eur J Med Chem 30: 77–84.

Caliendo G, Greco G, Grieco P, Novellino E, Perissutti E, Santagada V, Barbarulo D, Esposito E, De Blasi A (1996) Structure-affinity relationship studies on benzotriazole derivatives binding to 5-HT receptor subtypes. Eur J Med Chem 31: 207–213.

Caliendo G, Fiorino F, Greco P, Perissutti E, Santagada V, Albrizio S, Spadola L, Bruni G, Romeo MR (1999) Synthesis and binding affinities for 5-HT1A, 5-HT2A and 5-HT2C receptors of a series of 1- and 2-(4-arylpiperazinylalkyl)-4-(benzoyl)-1,2,3-triazole derivatives. Eur J Med Chem 34: 719–727.

Caliendo G, Fiorino F, Grieco P, Perissutti E, Santagada V, Severino B, Bruni G, Romeo MR (2000) Synthesis of new 1,2,3-benzotriazin-4-one-arylpiperazine derivatives as 5-HT_{1A} serotonin receptor ligands. Bioorg Med Chem 8: 533–538.

Caliendo G, Fiorino F, Perissutti E, Severino B, Gessi S, Cattabriga E, Borea PA, Santagada V (2001) Synthesis by microwave irradiation and binding properties of novel 5-HT1A receptor ligands. Eur J Med Chem 36: 873–886.

Caliendo G, Fiorino F, Perissutti E, Severino B, Scolaro D, Gessi S, Cattabriga E, Borea PA, Santagada V (2002) A convenient synthesis by microwave heating and pharmacological evaluation of novel benzoyltriazole and saccharine derivatives as 5-HT1A receptor ligands. Eur J Pharm Sci 16: 15–28.

Cheng YC, Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22: 3099–3108.

Creese I, Schneider R, Snyder SH (1977) 3H-Spiroperidol labels dopamine receptors in pituitary and brain. Eur J Pharmacol 46, 377–381.

De Vry J (1995) 5-HT_{1A} receptor agonists: recent developments and controversial issues.
Psychopharmacology 121: 1–26.

Dizeyi N, Bjartell A, Nilsson E, Hansson J, Gadaleanu V, Cross N and Abrahamsson PA (2004) Expression of serotonin receptors and role of serotonin in human prostate cancer tissue and cell lines. Prostate 59: 328–336.

EasyFit 1.4, 1989–1991, Matteo Vaccari and Mario Negri Institute, Milan, Italy.

File SE (1996) Recent developments in anxiety, stress, and depression. Pharmacol Biochem Behav 54: 3–12.

Fiorino F, Perissutti E, Severino B, Santagada V, Cirillo D, Terracciano S, Massarelli P, Bruni G, Collavoli E, Renner C, Caliendo G (2005) New 5-hydroxytryptamine1A receptor ligands containing a norbornene nucleus: synthesis and in vitro pharmacological evaluation. J Med Chem 48: 5495–5503.

Fiorino F, Severino B, De Angelis F, Perissutti E, Frecentese F, Massarelli P, Bruni G, Collavoli E, Santagada V, Caliendo G (2008) Synthesis and in-vitro pharmacological evaluation of new 5-HT1A receptor ligands containing a benzotriazinone nucleus. Arch Pharm Chem 341: 20–27.

Greengrass P, Bremner R (1979) Binding characteristics of 3H-prazosin to rat brain alpha-adrenergic receptors. Eur J Pharmacol 55: 323–326.

Herndon JL, Ismaiel A, Ingher SP, Teitler M, Glennon RA (1992) Ketanserin analogues: structure-affinity relationships for 5-HT_2 and 5-HT_{1C} serotonin receptor binding. J Med Chem 35: 4903–4910.

Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71: 533–554.

Isaac M (2005) Serotonergic 5-HT2C receptors as a potential therapeutic target for the design antiepileptic drugs. Curr Top Med Chem 5: 59–67.

Lopez-Rodriguez ML, Ayala D, Benhamu B, Morcillo MJ, Viso A(2002) Arylpiperazine derivatives acting at 5-HT1A receptors. Curr Med Chem 9: 443–469.

Martin GR, Eglen RM, Hoyer D, Hamblin MW, Yocca F (1998) Advances in Serotonin Receptor Research; Molecular Biology, Signal Transmission, and Therapeutics. Ann. N.Y. Acad. Sci, New York.

Neckelmann D, Bjorkum AA, Bjorvatn B, Ursin R (1996) Sleep and EEG power spectrum effects of the 5-HT1A antagonist NAN-190 alone and in combination with citalopram. Behav Brain Res 75: 159–168.

Parker MA, Kurrasch DM, Nichols DE (2008) The role of lipophilicity in determining binding affinity and functional activity for 5-HT_{2A} receptor ligands. Bioorg Med Chem 16: 4661–4669.

Perry BD, U' Prichard DC (1981) [3H]Rauwolscine (alpha-yohimbine): a specific antagonist radioligand for brain alpha 2-adrenergic receptors. Eur J Pharmacol 76: 461–464.

Pessoa-Mahana H, Araya-Maturana R, Saitz CB, Pessoa-Mahana CD (2003) A synthetic overview of new molecules with 5-HT1A binding affinities. MiniReviews in Med Chem 3: 77–93.

Saxena PR (1995) Serotonin receptors: subtypes, functional responses and therapeutic relevance. Pharmacol Ther 66: 339–368.

Scatchard G (1949) The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51: 660–672.

Schlegel JR, Peroutka SJ (1986) Nucleotide interactions with 5-HT1A binding sites directly labeled by [3H]-8-hydroxy-2-(di-n-propylamino)tetralin ([3H]-8-OH-DPAT). Biochem Pharmacol 35: 1943–1949.

Shimada I, Maeno K, Kazuta K, Kubota H, Kimizuka T, Kimira Y, Hatanaka K, Naitou Y, Wanibuchi F, Sakamoto S, Tsukamoto S (2008) Synthesis and structure-activity relationships of a series of substituted 2-(1H-furo[2,3-g]indazol-1-yl)ethylamine derivatives as 5-HT2C receptor agonists. Bioorg Med Chem 16: 1966–1982.

Sleight AJ, Pierce PA, Schmidt AW, Hekmatpanah CR, Peroutka SJ (1991) In: Peroutka SJ (ed.) Serotonin Receptor Subtypes; Wiley-Liss., New York, p. 211.

Trump-Kallmeyer S, Hoflack J, Bruinvels A, Hibert M (1992) Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. J Med Chem 35: 3448–3462.

Zajdel P, Subra G, Bojarski AJ, Duszynska B, Tatarczynska E, Nikiforuk A, Chojnacka-Wójcik E, Pawłowski M, Martinez J (2007) Novel class of arylpiperazines containing N-acylated amino acids: their synthesis, 5-HT1A, 5-HT2A receptor affinity, and in vivo pharmacological evaluation. Bioorg Med Chem 15: 2907–2919.

Zlatović MV, Šukalović VV, Schneider C, Roglić GM (2006) Interaction of arylpiperazine ligands with the hydrophobic part of the 5-HT1A receptor binding site. Bioorg Med Chem 14: 2994–3001.