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Full Paper

Synthesis and Docking of Novel 3-Indolylpropyl Derivatives as New Polypharmacological Agents Displaying Affinity for 5-HT_{1A}R/SERT

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A series of novel 3-indolylpropyl derivatives was synthesized and evaluated for their binding affinities at the serotonin-1A receptor subtype (5-HT_{1A}R) and the 5-HT transporter (SERT). Compounds **11b** and **14b** exhibited the highest affinities at the 5-HT_{1A}R ($K_i = 43$ and 56 nM), whereas compounds **11c** and **14a** were the most potent analogs at the SERT ($K_i = 34$ and 17 nM). On the other hand, compounds **14b** and **11d** showed potent activity at both targets, displaying a profile that makes them promising leads for the search for novel potent ligands with a dual mechanism of action. Molecular docking studies in all the compounds unveiled relevant drug–target interactions, which allowed rationalizing the observed affinities.

Keywords: Docking / 5-Hydroxytryptamine 1A receptor / Indole / Piperazinylpropylindole derivatives / Serotonin transporter

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Introduction

Polypharmacology is a novel paradigm in drug discovery, which is based on the concept that "promiscuous" drugs, that is, targeting simultaneously multiple specific receptors, would be more efficacious and/or safer than selective compounds [1]. However, the rational design of polypharmacological agents is a difficult task, particularly if the addressed targets exhibit high structural or functional diversity.

The serotonin-1A receptor subtype (5-HT_{1A}R) and the 5-HT transporter (SERT) are the main molecular targets of therapeutic agents currently used for the treatment of anxiety and/or depression [2–4]. In addition, they represent

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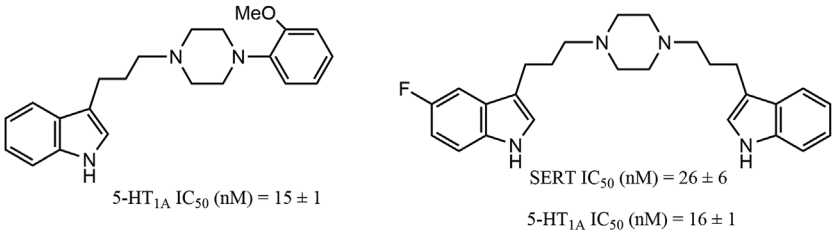


Figure 1. Chemical structure of 3-indolylpropyl derivatives and their affinity values upon SERT and/or 5-HT_{1A} receptor.

an attractive combination for the search of novel multitarget drugs [5]. However, both types of proteins are highly different. Thus, the 5-HT_{1A}R is a G-protein-coupled receptor (GPCR), containing seven transmembrane domains, whose stimulation inhibits adenylyl cyclase-mediated responses [6]. On the other hand, SERT is a plasma membrane protein containing 12 putative transmembrane domains, which belongs to the neurotransmitter/sodium symporter (NSS) family, and is in charge of terminating actions of 5-HT by reuptake of the monoamine into the nerve terminals [7]. Despite these differences, both proteins recognize 5-HT as the main ligand, suggesting that the indole moiety might act as a common pharmacophore in both targets.

For several years, we have been interested in developing new indolic compounds containing a C-5-substituted 3-indolylpropyl fragment as the pharmacophore, for drugs acting at both the SERT and the 5-HT_{1A}R. In the search of multitarget bioactivity, this structural portion has been either connected to different moieties such as arylpiperazine or utilized to develop dimeric *bis*-piperazinyl indole derivatives [8–10]. Thus, some of these compounds have shown affinity for the two aforementioned targets, a condition which has been proposed as a mechanism to overcome the poor efficacy and slow clinical action of current antidepressants [11]. An example of these structures and their corresponding affinities is shown in Fig. 1.

Keeping in mind the concept of developing dual-acting agents, we conducted a new exploratory study focused to the synthesis, docking, and biological evaluation of a new set of indolic derivatives. In order to evaluate the impact of novel scaffolds on the binding properties, in this work, the 3-indolylpropyl moiety was connected to the following frameworks: 5-methoxy-1-benzazepinone, 1-benzyl-3-indolylmethylpiperazine, and 1-benzyl-3-indolylmethyl-4-aminophenylpiperazine. The selection of these scaffolds was made considering the following general criteria: Compounds **4a–d** (benzazepinone series) were synthesized in order to evaluate whether this moiety could be beneficial for activity considering that some 3-benzazepine derivatives have exhibited good affinity for 5-HT_{1A}R [12–14].

Although the benzazepinone series lacks a basic amine, a requirement apparently essential for the binding of compounds to the 5-HT_{1A}R, we decided to explore binding studies on this family mainly based on a recent report showing that cannabinoid Δ^9-THCV (a compound which also lacks a protonable nitrogen, Fig. 2), exhibited affinity (2060 nM) for the human 5-HT_{1A}R expressed in CHO cells.

Compounds **11a–d** were based on homodimers previously reported by us [9], since preliminary docking studies indicated that reducing the length of the aliphatic chain linker (connecting indole moieties) to n = 1 might increase both SERT and 5-HT_{1A} affinity. Finally, compounds **14a–d** were conceived taking into account the good 5-HT_{1A} bioactivity displayed by indolylpropylarylpiperazine derivatives [9], and the notion that *bis*-indole ligands show also good SERT affinity.

Results and discussion

Chemistry

The synthesis of compounds **4a–d** was carried out by the Fischer reaction between commercially available C-4 substituted phenylhydrazines and 3,4-dihydro-2*H*-pyran under reflux conditions in a three-step sequence as is outlined in Scheme 1. The 3-(3-indolyl)-1-propanol analogs **2a–d** were obtained in good yield (65–80%) and subsequently reacted with tosyl chloride to provide tosylates **3a–d** (56–63% yield), which finally reacted with 6-methoxy-1-benzazepin-2-one, obtained by Schmidt reaction of 5-methoxy-1-tetralone with sodium azide [15], to afford compounds **4a–d** (36–61% yield).

On the other hand, compounds **11a–d** were obtained in moderate yield (35–46%) by a nucleophilic substitution reaction between 3-(3-indolylpropyl)piperazines **5a–d** (61–72% yield) and 3-indolylmethyl methanesulfonate **10a**, as shown in Scheme 2.

The synthesis of the 3-indolylmethyl methanesulfonate **10a** was achieved by a four-step sequence as follows: Vilsmeier–Häack reaction of 1*H*-indole **6a** in a $POCl_3$/DMF mixture to provide **7a**, *N*-benzylation to provide **8a** followed by carbonyl reduction to afford alcohol **9a**, which was finally reacted with

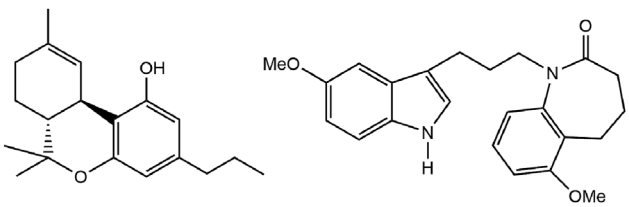
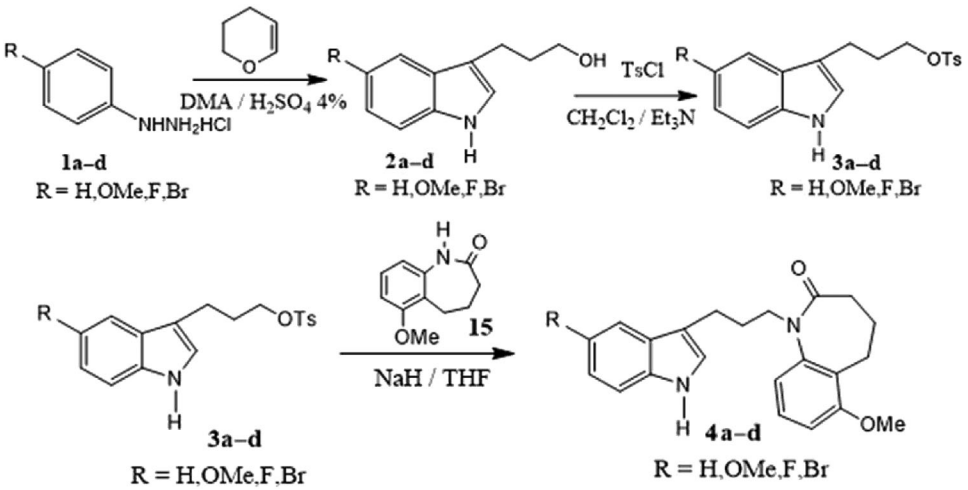


Figure 2. Cannabinoid Δ^9-THCV and **4b** derivative.



Scheme 1. Synthesis of compounds **4a**–**d**.

mesylchloride to give the expected *O*-mesylate derivative **10a** (Scheme 3), which was immediately utilized due its rapid decomposition.

Finally, compounds **14a**–**d** were obtained according to the retrosynthetic strategy shown in Scheme 4.

Thus, tosylates **3a–b** were reacted with 4-nitrophenylpiperazine to achieve nitroindolylpiperazine derivatives **12a**–**b** (51–64% yield), which were further reduced with iron in powder, to give 4-aminoarylpiperazinyl indol derivatives **13a–b** (42–53% yield; Scheme 5).

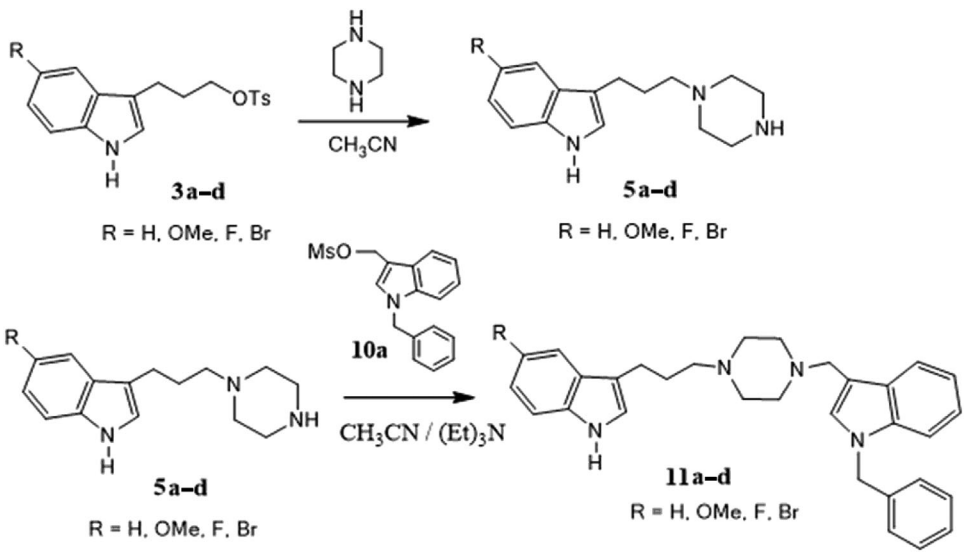
The indolylmethyl-*O*-mesylates **10b–c** (R = F, Br) were synthesized in a similar procedure as described for **10a**. Finally, the mesylates **10a–c** were immediately reacted with 4-amino-phenylpiperazinylpropylindole derivatives **13a–b,** to provide the *bis*-indoles **14a–d** (38–44% yield; Scheme 6).

Pharmacology and molecular docking

Table 1 summarizes the affinity measurements for all compounds at the 5-HT_{1A} receptor and the SERT.

*1-[3-(1H-3-Indolyl)-propyl]-1,3,4,5-tetrahydrobenzo[b]-azepin-2-one derivatives (**4a**–**d**)*

Regarding the effects of compounds **4a**–**d** upon the 5-HT_{1A} receptor, the most striking observation was that only the



Scheme 2. Synthesis of compounds **11a**–**d**.

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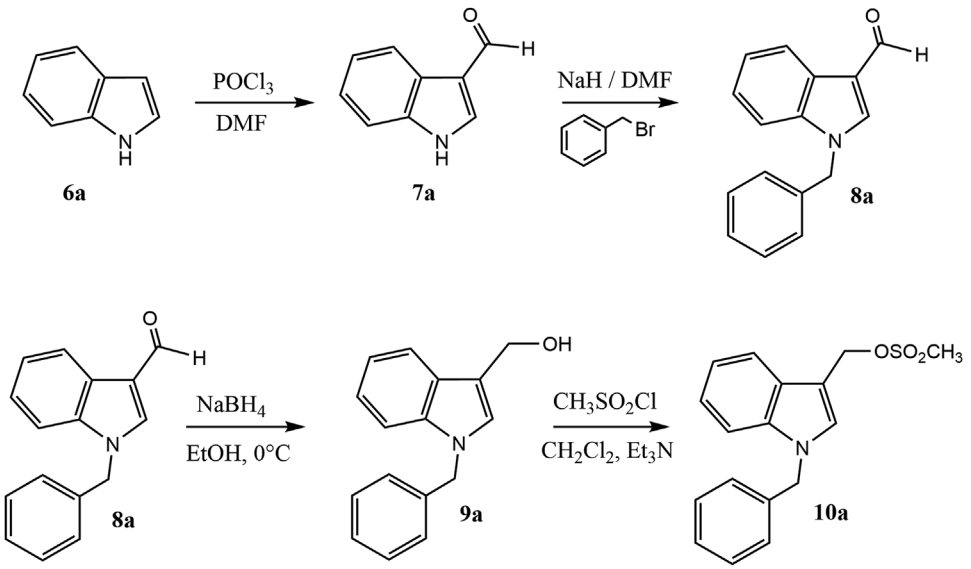
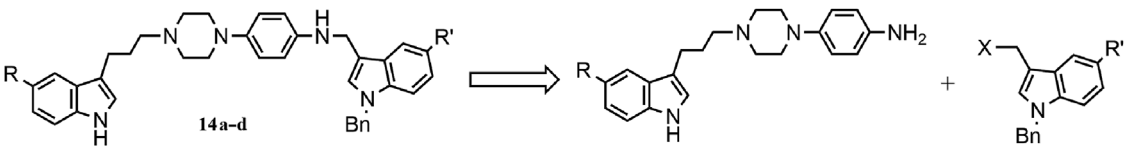
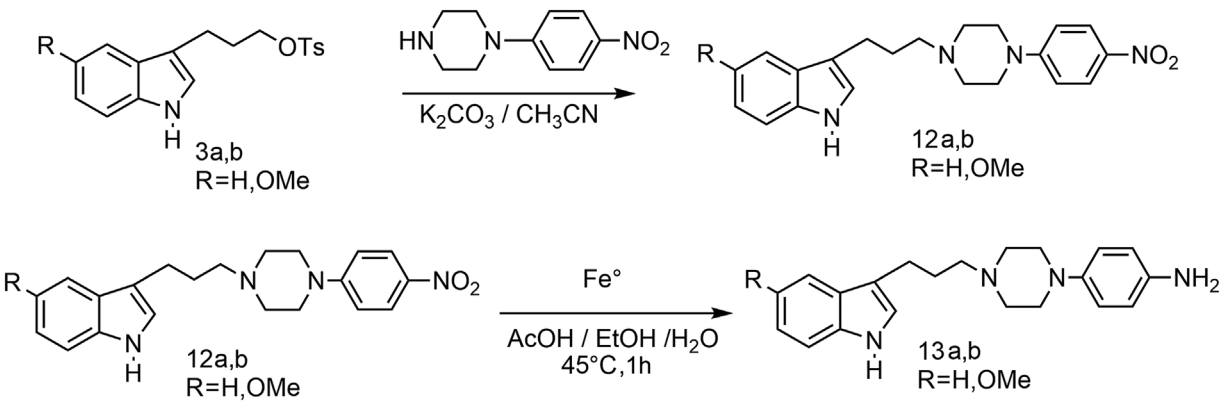


Scheme 3. Synthetic sequence for the preparation of **10a**.



Scheme 4. Retrosynthetic analysis of compounds **14a–d**.



Scheme 5. Synthetic sequence for the preparation of compounds **13a–b**.

5-methoxy derivative **4b** displayed affinity in the nanomolar range. Moreover, no affinity was detected for any of these compounds at the SERT. These results indicate that the presence of a 1-benzazepinone nucleus connected to an indolepropyl framework is detrimental for the affinity at both targets, as compared with previously reported indolealkylarylpiperazine derivatives [8, 9, 16, 17]. This is likely due to the benzazepinone nucleus, which lacks a protonable nitrogen

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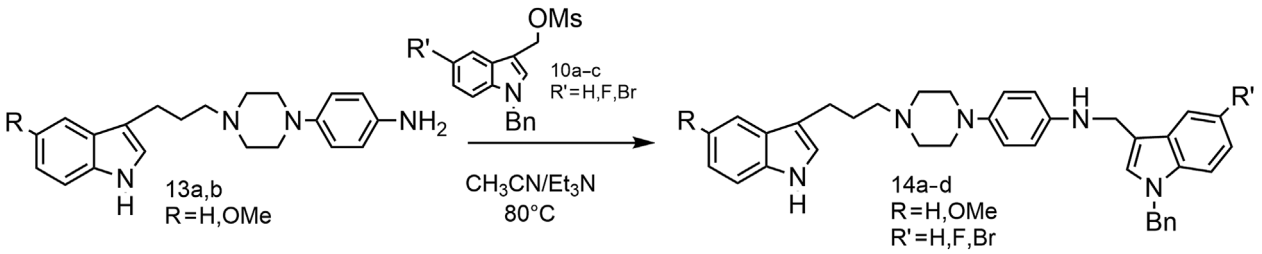
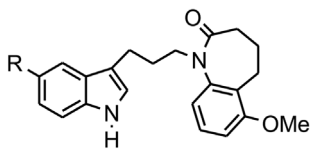


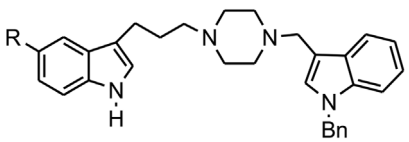
Scheme 6. Synthesis of compounds **14a–d**.

and is not capable to establish critical coulombic interactions with negatively charged residues at both receptors [18]. This was confirmed by docking experiments of compounds **4a–d** at the 5-HT_{1A} receptor, which revealed that although these ligands locate at the same binding site described for indolylalkylpiperazines, they do not establish an ionic interaction with Asp116. Nevertheless, it is interesting to see that interactions with residues such as Phe361, Phe362, Ser199, and Ile113 were observed for compounds **4b** and **4c**, which might explain the affinity exhibited by these compounds. Notably, compound **4b** showed an additional interaction with Cys187, which has been shown to be critical

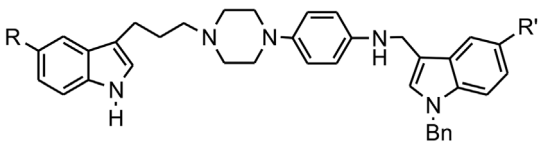
Table 1. Binding affinities of compounds 4a–d, 11a–d, and 14a–d at SERT and 5-HT1A receptor.



4a–d



11a–d



14a–d

Compound	R	R′	5-HT_{1A} K_i (nM) ± SD	SERT K_i (nM) ± SD
8-OH-DPAT[a)]	–	–	4.7 ± 0.6	ND
Fluoxetine[a)]	–	–	ND	13.7 ± 0.9
4a	H	–	>10000	>10000
4b	OMe	–	169 ± 15	>10000
4c	F	–	3226 ± 37	>10000
4d	Br	–	>10000	>10000
11a	H	–	994 ± 46	91 ± 14
11b	OMe	–	43 ± 3.9	>10000
11c	F	–	908 ± 15	34 ± 7
11d	Br	–	292 ± 10	99 ± 8.6
14a	H	H	168 ± 28	17 ± 0.8
14b	OMe	F	56 ± 5.3	365 ± 24
14c	H	F	>10000	57 ± 5.1
14d	H	Br	>10000	>10000

ND: not determined.
[a)]The affinity values of fluoxetine and 8-OH-DPAT are included as reference compounds for SERT and 5-HT1A receptor, respectively.

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for stabilizing other potent indolic 5-HT_{1A} ligands [9, 19]. These results are illustrated in Fig. 3, which shows the most stable docking poses for the most and least potent compounds of this series (**4b** and **4d**, respectively). As can be seen, both compounds adopt clearly different orientations, and in the case of **4b**, the indole moiety (NH) establishes a direct interaction with Cys187, in addition to an exclusive hydrogen bond interaction between the 5-methoxy group of the indolic ring with Ser199 at 3.7 Å. On the other hand, the benzazepinone ring appears located in a position favorable to interact with Phe361 and Phe362.

With regard to compound **4c**, which showed an intermediate affinity value, it docked into the 5-HT_{1A} receptor adopting a position between the most and least favorable binding modes (not shown). Thus, our results revealed that although the lack of a basic nitrogen reduces the affinity of indolepropyl derivatives at the 5-HT_{1A} receptor, its presence is not essential for having compounds with nanomolar affinity for this target.

*1-Benzyl-3-{4-[3-(1H-3-indolyl)-propyl]-1-piperazinylmethyl}-1H-indole derivatives (**11a–d**)*

The analysis of the affinities showed again that the most potent compound at the 5-HT_{1A} receptor was the 5-methoxy derivative **11b** (Table 1). This is in agreement with previous reports of indole-alkylpiperazine derivatives, which showed that the introduction of a methoxyl substituent at C5 position elicits an increase of affinity at this target [17]. Interestingly, compound **11b** displayed a marked selectivity for the 5-HT_{1A} receptor in comparison with its effect on the SERT, a condition also observed for other methoxyindole derivatives in earlier studies [16, 17].

Docking experiments showed that the two most potent compounds of this series at the 5-HT_{1A} receptor (**11b** and **11d**) exhibited a binding mode in which the main contact was the well-described coulombic interaction between a protonated nitrogen atom of the piperazine ring and Asp116 [8, 9, 20, 21]. Interestingly, in the case of compounds **11a** and **11c**, this interaction was absent, which agrees with the lower affinity exhibited by these derivatives (Fig. 4). In addition, drugs docked into the 5-HT_{1A} receptor model in such a way that their aromatic rings appeared located in positions favorable to interact with an "aromatic cage" formed by Trp358, Phe361, Phe362, and Tyr195. An additional interaction with Ile189 was observed in the case of the most potent compound. As shown in Fig. 4, the receptor–ligand complexes with the highest affinity (as inferred from docking energies; see Supporting Information Figs. S1 and S2) showed that interactions between the aromatic rings of compounds and the aromatic cage at the receptor involved both the non-benzylated and benzylated indole rings, requiring the bending of the molecule around the piperazine ring. These aromatic interactions were favored by the presence of a methoxy group (**11b**), whereas in the case of the less potent members of this series, the bended conformation weakened the interaction with Asp116. This is illustrated in Fig. 4, which shows the most favorable poses of **11a** and **11b**.

In the case of the SERT, most of the compounds of this series displayed nanomolar affinity, being the C5 fluorinated derivative **11c** the most active (Table 1). These results agree with previous reports [9, 22] showing that the introduction of a fluorine atom at position C5 of the indole moiety generates

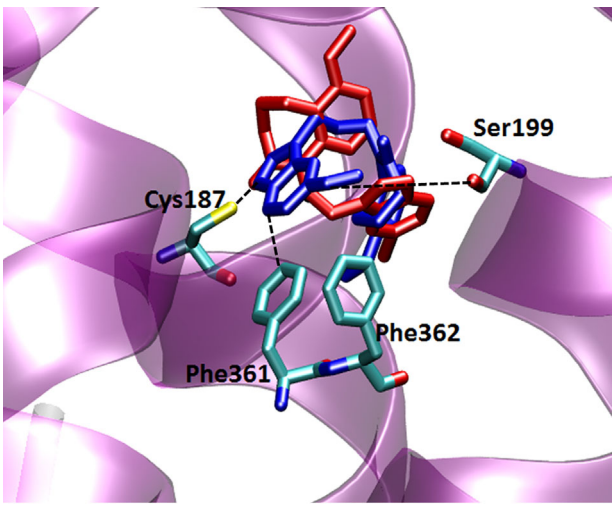

Figure 3. Superimposed structures of compounds **4b** (blue) and **4d** (red) docked into the binding site of the 5-HT_{1A} receptor. Main binding site amino acid residues (cyan) are rendered as stick models.

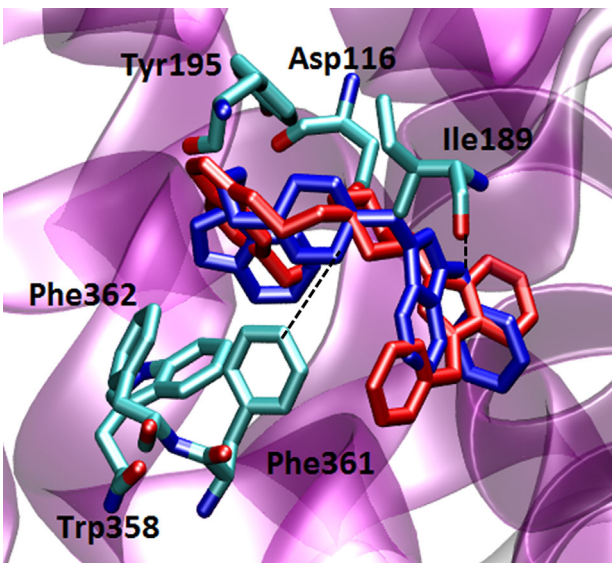

Figure 4. Superimposed structures of compounds **11a** (blue) and **11b** (red) docked into the binding site of the 5-HT_{1A} receptor. Main binding site amino acid residues (cyan) are rendered as stick models.

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an increase in affinity of indolylpropyl derivatives as compared with the unsubstituted derivative. Docking experiments revealed that the unsubstituted and halogenated compounds (i.e., **11a**, **11c**, and **11d**) exhibited similar binding modes (Fig. 5). Here, an electrostatic interaction between one of the protonated nitrogen atoms of the piperazine ring and Glu493 (although in some cases this interaction might also involve the adjacent Glu494) appears as the main interaction.

These results are somewhat different from those previously observed by us when evaluating similar piperazinylpropylindole derivatives [9]. Thus, in that earlier study, we reported that the main interaction underlying the affinity of these compounds at the SERT was a coulombic interaction established by a piperazine nitrogen with Asp400, a residue located a few angstroms closer to the extracellular domain of the SERT as compared with Glu493. Noteworthy, diverse studies (see for example [23, 24]) have shown that both Asp400 and/or Glu493 are critical for the binding of different arylpiperazine derivatives. In our view, an alternating interaction involving both residues might be the main molecular determinant explaining the affinity of these and other SERT ligands. Further experiments are necessary to evaluate the role of these residues (either individually or collectively) in the binding of piperazinylpropylindole derivatives and other drugs containing substituted or unsubstituted amino groups.

Additional interactions of molecules of this series with residues at the SERT included a π–cation interaction between one of the indole moieties and Arg104 and van der Waals interactions with Pro403 (compounds **11a**, **11c**, and **11d**), and a hydrogen bond between the other indole N-H with Asp400

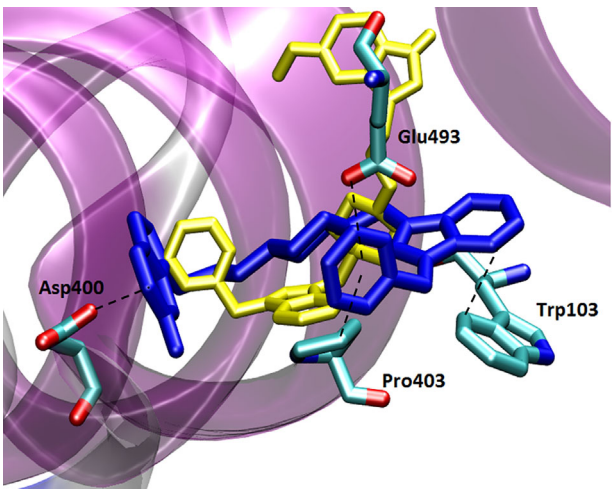

Figure 5. Superimposed structures of compounds **11b** (yellow) and **11c** (blue) docked into the binding site of the SERT. Main binding site amino acid residues (cyan) are rendered as stick models.

(**11c** and **11d**). A distinctive π–π interaction between the *N*-benzyl-indole moiety and Trp103 was also present only in the case of the most potent compound **11c**. Interestingly, the presence of a methoxyl group at position C5 of the non-benzylated indole moiety (compound **11b**) forced this derivative to adopt a binding mode that clearly differs from those obtained for all the other members of this series (Fig. 5). Therefore, many of the aforementioned interactions were not preserved in this derivative, which could explain its lack of affinity at the SERT.

*(1-Benzyl-1H-3-indolylmethyl)-(4-{4-[3-(1H-3-indolyl)-propyl]-1-piperazinyl}-aryl)amine derivatives (**14a–d**)*

The introduction of an aniline moiety between the *N*-benzyl-indole framework and the piperazine ring induced a 5–6-fold increase in the affinity of the compound for both the 5-HT_{1A} receptor and the SERT (compare **14a** with **11a**). Thus, considering the three series evaluated, **14a** was one of the most potent compounds at both targets, although it retained the relative selectivity of the unsubstituted derivative (one order of magnitude approximately) for the SERT over the 5-HT_{1A} receptor (Table 1). As could be predicted from the results obtained with the two other series, the introduction of a methoxyl group at the C5 position elicited an increase in the affinity for the 5-HT_{1A} receptor (**14b**). Interestingly, this was not accompanied by a total lack of effect at the SERT (as observed in the two other methoxylated derivatives **4b** and **11b**). Accordingly, **14b** and **11d** are the most promising compounds of the series evaluated, in terms of serving as leads for the search of novel potent ligands with a dual action. On the other hand, the introduction of a fluorine atom at the C5′ position of the *N*-benzyl-indole moiety induced a significant decrease of affinity at the 5-HT_{1A} receptor without affecting much the affinity for the SERT. Thus, compound **14c** was the most selective SERT ligand of the three series studied. Notably, the introduction of a bromine atom at the same position (**14d**) abolished the affinity of the compound at both protein targets. Docking experiments revealed that compounds of this series displayed interactions at both targets that were similar to those described in the previous analyses. Thus, the most potent compounds at the 5-HT_{1A} receptor (**14a** and **14b**) adopted a bent conformation that allowed the coulombic interaction with Asp116 and the interaction of the ligand aromatic rings with aromatic residues such as Trp358, Phe361, and Tyr195 (Fig. 6). Conversely, **14c** and **14d** (which showed no activity at this receptor) docked at the binding site in a more extended conformation, thus preventing the establishment of the most critical interactions.

In the case of the SERT, all compounds but **14d** exhibited a similar binding mode, in which the main interaction was the electrostatic contact established between one of the protonated nitrogen atoms of the piperazine ring and either Glu493 (**14a**) or Asp400 (**14b** and **14c**), Fig. 7. Likewise, the least potent compound (**14d**) showed a clearly different binding mode, which would explain its lack of affinity for the SERT.

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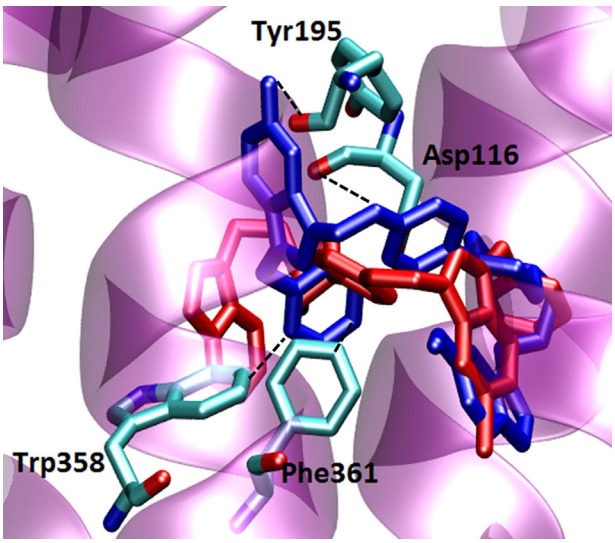

Figure 6. Superimposed structures of compounds **14b** (blue) and **14d** (red) docked into the binding site of the 5-HT_{1A} receptor. Main binding site amino acid residues (cyan) are rendered as stick models.

Conclusions

The purpose of this study was to examine the effect of incorporating bioactive heterocyclic rings to the indolylpropyl moiety in the search of novel ligands with a potential dual mechanism. Our results showed that although the presence of a basic nitrogen is important for the affinity of these drugs, it is still possible to find potent 5-HT_{1A} ligands in molecules lacking this function. This might enhance the structural variability that can be explored in the search of novel drugs acting at the 5-HT_{1A} receptor. In addition, our results were coherent with previous data regarding how substituents such as methoxyl groups and/or halogen atoms modulate the affinity of indolylpropyl derivatives at both the 5-HT_{1A} receptor and the SERT. Interestingly, some of the compounds showed a high selectivity for one target, whereas others could serve as leads for the development of ligands with dual activity. Since these effects were obtained with a few and relatively homogeneous series, it would be necessary to carry out further studies for a better understanding of the molecular basis underlying the selectivity and non-selectivity in this type of drugs.

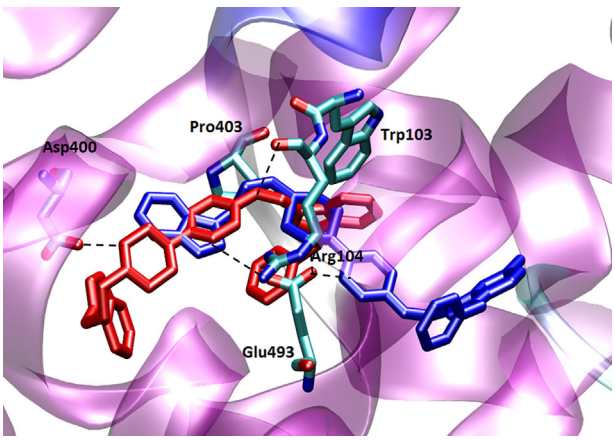

Figure 7. Superimposed structures of compounds **14a** (red) and **14d** (blue) docked into the binding site of the SERT. Main binding site amino acid residues (cyan) are rendered as stick models.

Experimental

Chemistry

General

Melting points were determined on a hot-stage apparatus and are uncorrected. The IR spectra were recorded in KBr discs on an FT-IR Bruker IFS 55 spectrophotometer and wavenumbers are reported in cm^{-1}. The ^{1}H and ^{13}C-NMR spectra were obtained on a Bruker DRX-300 spectrometer (300 and 75 MHz, respectively) in $CDCl_3$ or DMSO-d_6. Chemical shifts were recorded in ppm (δ) relative to TMS as an internal standard. *J* values are given in Hz. Micro-analyses were carried out on a Fisons EA 1108 analyzer. High-resolution mass spectra were recorded on a ThermoFinnigan MAT 95XP mass spectrometer and DSA–TOF AxION 2 TOF MS (Perkin Elmer, Shelton, CT, USA), positive mode. Silica gel Merck 60 (70–230 mesh) and aluminum sheets coated with silica gel 60 F_{254} were used for column and TLC chromatography, respectively.

The InChI codes of the investigated compounds are provided as Supporting Information.

*General procedure for the synthesis of 1-[3-(1H-indol-3-yl)-propyl]-4,5-dihydro-1H-benzo[b]azepin-2(3H)-one derivatives **4a–d**, with 1-[3-(1H-indol-3-yl)propyl]-6-methoxy-4,5-dihydro-1H-benzo[b]azepin-2(3H)-one (**4a**) as a model*

To a stirred solution of (0.1 g, 0.52 mmol) of 6-methoxy-1-benzazepin-2-one **15** [15] in dry THF (15 mL), sodium hydride NaH (0.03 g, 1.25 mmol) was added. The mixture was stirred at 0°C for 10 min and then slowly added to 3-(3-indolyl)propyl-4-methylbenzenesulfonate (0.17 g, 0.52 mmol) **3a** in dry THF (10 mL). After this time, the mixture was heated to 70°C during 40 h and quenched with water (50 mL). The reaction mixture was extracted with AcOEt 50 mL (2 × 25 mL) and the organic layers dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The obtained crude as an oil was purified by column chromatography on silica gel AcOEt/*n*-hexane (3:1) to afford (0.11 g, 61%) of a white solid **4a**. m.p. 104−106°C (recrystallized from AcOEt/*n*-hexane 1:0.5). IR_{vmax} (cm^{-1}): 3230 (N-H), 3030 (C-H Arom.), 2921 (C-H Aliph.), 1640 (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.79–1.83 (br s, 3H, indole-CH_2-**CH_2**-CH_2, and $COCH_2$-**CH**-CH_2 benzazepine ring),

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2.12–2.17 (br s, 3H, indole-**CH_2**-CH_2-CH_2, and CH_2-**CH**-CH_2 benzazepine ring), 2.4 (t, 2H, CO-CH_2-CH_2-**CH_2** benzazepine ring), 2.62 (t, 2H, CO-**CH_2**-CH_2 benzazepine ring, $J = 8$ Hz), 3.12 (br s, 1H, indole-CH_2-CH_2-**CH**-N), 3.4 (br s, 1H, indole-CH_2-CH_2-**CH**-N), 3.79 (s, 3H, OMe), 6.89–7.35 (m, 8H, Arom.), 10.78 (s, 1H, N-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.9, 22.1, 27.5, 28.4, 32.9, 46.4, 55.6, 108.4, 111.2, 113.5, 115.1, 117.9, 118.1, 120.8, 121.9, 123.2, 126.8, 127.5, 135.9, 142.8, 156.3, 171.8. HRMS: (EI) Calcd. for $C_{22}H_{24}N_2O_2$ (M^+): 348.1838. Found: 348.1836.

*6-Methoxy-1-[3-(5-methoxy-1H-indol-3-yl)propyl]-4,5-dihydro-1H-benzo[b]azepin-2(3H)-one (**4b**):* Prepared from 6-methoxy-1-benzazepin-2-one **15** (0.05 g, 0.26 mmol), sodium hydride (0.01 g, 0.42 mmol) and (0.1 g, 0.28 mmol) of tosylate **3b**, to afford pure **4b** (0.05 g, 51%) as an oil. $IR_{\nu max}$ (cm^{-1}): 3250 (N-H), 3033 (C-H Arom.), 2925 (C-H Aliph.), 1638 (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.75–1.81 (br s, 3H, indole-CH_2-**CH_2**-CH_2, and $COCH_2$-**CH**-CH_2 benzazepine ring), 2.14–2.18 (br s, 3H, indole-**CH_2**-CH_2-CH_2 and CH_2-**CH**-CH_2 benzazepine ring), 2.58 (t, 2H, CO-CH_2-CH_2-**CH_2** benzazepine ring), 2.62 (t, 2H, CO-**CH_2**-CH_2 benzazepine ring, $J = 8.1$ Hz), 3.33 (s, 2H, indole-CH_2-CH_2-**CH_2**-N), 3.71 (s, 3H, OMe), 3.79 (s, 3H, OMe), 6.81–7.25 (m, 7H, Arom.), 10.57 (s, 1H, N-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.6, 21.8, 27.0, 27.9, 32.7, 45.9, 54.7, 55.2, 99.3, 107.9, 110.4, 111.4, 112.9, 114.7, 122.3, 122.8, 126.7, 126.9, 130.9, 142.6, 152.3, 155.9, 170.9. HRMS: (EI) Calcd. for $C_{23}H_{26}N_2O_3$ (M^+): 378.1901. Found: 378.1910.

*1-[3-(5-Fluoro-1H-indol-3-yl)propyl]-6-methoxy-4,5-dihydro-1H-benzo[b]azepine-2(3H)-one (**4c**):* Prepared from 6-methoxy-1-benzazepine-2-one **15** (0.05 g, 0.26 mmol), sodium hydride (0.011 g, 0.46 mmol) and tosylate **3c** (0.091 g, 0.26 mmol) to afford pure **4c** (0.053 g, 55.2%) as a white solid. m.p. 107−108°C (recrystallized from AcOEt/*n*-hexane 1:0.5). $IR_{\nu max}$ (cm^{-1}): 3233 (N-H), 3010 (C-H Arom.), 2920 (C-H Aliph.), 1642 (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.80–1.85 (br s, 3H, indole-CH_2-**CH_2**-CH_2, and $COCH_2$-**CH**-CH_2 benzazepine ring), 2.02–2.07 (br s, 3H, indole-**CH_2**-CH_2-CH_2, and CH_2-**CH**-CH_2 benzazepine ring), 2.57 (t, 2H, CO-CH_2-CH_2-**CH_2** benzazepine ring), 2.62 (t, 2H, CO-**CH_2**-CH_2 benzazepine ring, $J = 8$ Hz), 3.35 (s, 2H, indole-CH_2-CH_2-**CH_2**-N), 3.79 (s, 3H, OMe), 6.88–7.25 (m, 7H, Arom.), 10.88 (s, 1H, N-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 21.1, 22.1, 27.5, 28.3, 33.1, 46.4, 55.8, 102.7 (d, $^2J_{C-F} = 17$ Hz), 108.8 (d, $^{2'}J_{C-F} = 19$ Hz), 112.2 (d, $^2J_{C-F} = 4.1$ Hz), 114.0 (d, $^3J_{C-F} = 9.8$ Hz) 122.8, (2x)123.9, 126.0 (d, $^{3'}J_{C-F} = 9.8$ Hz), (2x)127.0, 132.8, 143.1, 154.9 (d, $^1J_{C-F} = 211$ Hz), 156.5, 171.5. HRMS: (EI) Calcd. for $C_{22}H_{23}FN_2O_2$ (M^+): 366.1641. Found: 366.1645.

*1-[3-(5-Bromo-1H-indol-3-yl)propyl]-6-methoxy-4,5-dihydro-1H-benzo[b]azepin-2(3H)-one (**4d**):* Prepared from 6-methoxy-1-benzazepine-2-one **15** (0.06 g, 0.26 mmol), sodium hydride (0.011 g, 0.46 mmol) and tosylate **3d** (0.13 g, 0.32 mmol) to afford pure **4d** (0.049 g, 36.5%) as a white solid. m.p. 110−112°C (recrystallized from AcOEt/*n*-hexane 1:0.5). $IR_{\nu max}$ (cm^{-1}): 3231 (N-H), 3020 (C-H Arom.), 2925 (C-H Aliph.), 1636 (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.85–1.89 (br s, 3H, indole-CH_2-**CH_2**-CH_2, and $COCH_2$-**CH**-CH_2 benzazepine ring), 2.03–2.07 (br s, 3H, indole-**CH_2**-CH_2-CH_2, and CH_2-**CH**-CH_2 benzazepine ring), 2.5 (t, 2H, CO-CH_2-CH_2-**CH_2** benzazepine ring), 2.81 (br s, 2H, CO-**CH_2**-CH_2 benzazepine ring), 3.33 (s, 2H, indole-CH_2-**CH_2**-**CH_2**-N), 3.80 (s, 3H, OMe), 6.75–7.21 (m, 7H, Arom.), 10.80 (s, 1H, N-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.8, 21.7, 27.0, 27.6, 31.5, 45.8, 55.5, 107.0, 107.6, 110.0, (2x)111.6, 114.9, 122.0, 122.7, (2x)126.8, 132.7, 142.5, 152.1, 155.6, 170.5. HRMS: (EI) Calcd. for $C_{22}H_{23}BrN_2O_2$ (M^+): 426.0835. Found: 426.0830.

*General procedure for the synthesis of 1H-indol-3-carbaldehyde derivatives **7a–c**, with 1H-indole-3-carbaldehyde (**7a**) as a model*

To a solution of indole **6a** at 0°C, (500 mg, 4.27 mmol) in DMF (2.5 mL), a solution of recently prepared (1 h) $POCl_3$ (0.4 mL, 4.36 mmol) in DMF (2.5 mL) was added. The mixture was stirred for 30 min, then poured onto a water-ice mixture and turned basic by adding NaOH (0.5 M) to pH 12. The obtained white-yellow precipitate was filtered and dried to yield pure indole **7a** (526 mg, 85%). m.p. 181−182°C (recrystallized from AcOEt). $IR_{\nu max}$ (cm^{-1}): 3168 (N-H), 1634 (C=O), 1576 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.21 (m, 2H, 5-H, and 6-H), 7.49 (dd, 1H, 7-H, $J_o = 7.1$ Hz, $J_m = 1.5$ Hz), 8.1 (dd, 1H, 4-H, $J_o = 6.7$ Hz, $J_m = 1.9$ Hz), 8.26 (s, 1H, 2-H), 9.9 (s, 1H, CHO), 12.1 (s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 112.4, 118.2, 121.1, 122.1, 123.4, 124.1, 137.0, 138.4, 184.9. HRMS (EI) Calcd. for C_9H_7NO: 145.05276. Found (M^+): 145.05253.

*5-Fluoro-1H-indole-3-carbaldehyde (**7b**):* To a solution of indole **6b** (500 mg, 3.6 mmol) in DMF (2.5 mL) was added $POCl_3$ (0.37 mL, 4.00 mmol) in DMF (2.5 mL) to give pure **7b** (600 mg) in quantitative yield. m.p. 156−157°C (recrystallized from AcOEt). $IR_{\nu max}$ (cm^{-1}): 3224 (N-H), 1655 (C=O), 1590 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.15 (td, 1H, 6-H $J_o = 9.0$ Hz, $J_m = 3.0$ Hz), 7.49 (m, 1H, 7-H), 7.79 (dd, 1H, 4-H, $J_o = 9.0$ Hz, $J_m = 3.0$ Hz), 8.37 (s, 1H, 2-H), 9.95 (s, 1H, CHO), 12.0 (br s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 106.1 (d, $^{2'}J_{C-F} = 22.5$ Hz), 112.1 (d, $^2J_{C-F} = 23.9$ Hz), 114.2 (d, $^{3'}J_{C-F} = 9.1$ Hz), 115.0, 118.6 (d, $^4J_{C-F} = 4.2$ Hz), 125.1 (d, $^3J_{C-F} = 9.9$ Hz), 140.1, 159.2 (d, $^1J_{C-F} = 220.0$ Hz), 185.5. HRMS (EI) Calcd. for C_9H_6FNO: 163.04334. Found: 163.04328.

*5-Bromo-1H-indole-3-carbaldehyde (**7c**):* To a solution of indole **6c** (500 mg, 2.55 mmol) in DMF (5 mL) was added $POCl_3$ (0.26 mL, 2.85 mmol) in DMF (5 mL) to give pure **7c** (515 mg) in 90.1% yield. mp. 171−172°C (recrystallized from AcOEt). $IR_{\nu max}$ (cm^{-1}): 3210 (N-H), 1645 (CO), 1525 (CC). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.41 (dd, 1H, 6-H, $J_o = 9.0$ Hz, $J_m = 3.0$ Hz), 7.52 (d, 1H, 7-H, $J_o = 9.0$ Hz), 8.24 (d, 1H, 4-H, $J_m = 3.0$ Hz), 8.37 (s, 1H, 2-H), 9.95 (s, 1H, CHO), 11.9 (br s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 114.1, 115.9, 117.1, 123.7, 126.7, 128.4, 136.2, 136.9, 185.4. HRMS (EI) Calcd. for C_9H_6BrNO: 222.96328. Found: 222.96337.

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*General procedure for the synthesis of 1-benzyl-1H-indole-3-carbaldehyde derivatives **8a–c**, with 1-benzyl-1H-indole-3-carbaldehyde (**8a**) as a model*

To a solution of indole **7a** (500 mg, 3.44 mmol) in dry DMF (10 mL), NaH (123 mg, 5.16 mmol, 60% suspension in mineral oil) was slowly added. The reaction mixture was stirred and cooled to 5°C and benzyl bromide (816 mg, 4.8 mmol) was added dropwise. After stirring for 30 min, the mixture was poured onto a water-ice mixture affording a white-pink precipitate, which was filtered and dried to yield pure indole **8a** (607 mg, 75%). m.p. 94−95°C (recrystallized from AcOEt/*n*-hexane 1:1). IR_{vmax} (cm^{-1}): 3108 (C-H Arom.), 2815 (C-H Aliph.), 1661 (C=O), 1536 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.3 (s, 2H, Ar-**CH_2**-), 7.14–7.35 (m, 8H, 5-H, 6-H, 7-H, and **Ar**-CH_2), 7.7 (s, 1H, 2-H), 8.3 (m, 1H, 4-H), 9.97 (s, 1H, CHO). ^{13}C-NMR (75 MHz, DMSO-d_6): 50.4, 109.9, 118.0, 121.7, 122.6, 123.7, 125.0, (2x)126.8, 127.8, (2x)128.7, 134.9, 137.0, 138.1, 184.2. HRMS (EI) Calcd. for $C_{16}H_{13}NO$ (M^+): 235.09971. Found: 235.09946.

*1-Benzyl-5-fluoro-1H-indole-3-carbaldehyde (**8b**):* To a solution of indole **7b** (500 mg, 3.07 mmol) in dry DMF (10 mL), NaH (184 mg, 7.67 mmol) and benzyl bromide (734 mg, 4.29 mmol) were added to provide pure indole **8b** in quantitative yield. m.p. 137.2–138.9°C (recrystallized from AcOEt/*n*-hexane 1:1). IR_{vmax} (cm^{-1}): 3047 (C-H Arom.), 2920 (C-H Aliph.), 1663 (C=O), 1623 and 1582 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.57 (s, 2H, Ar-**CH_2**-), 7.2–7.8 (m, 7H, 6-H, 7-H, and **Ar**-CH_2), 7.82 (d, 1H, 4-H, $J_o = 9$ Hz), 8.56 (s, 1H, 2-H), 9.95 (s, 1H, CHO). ^{13}C-NMR (75 MHz, DMSO-d_6): 50.5, 106.5 (d, $^{2'}J_{C-F} = 25$ Hz), 112.2 (d, $^{2}J_{C-F} = 25.7$ Hz), 113.4 (d, $^{3'}J_{C-F} = 9.8$ Hz), 114.1, 117.8 (d, $^{4}J_{C-F} = 4.5$ Hz), 125.8 (d, $^{3}J_{C-F} = 11.3$ Hz), (2x)127.8, 128.4, (2x)129.3, 136.2, 137.0, 159.5 (d, $^{1}J_{C-F} = 235.5$ Hz), 185.2. HRMS (EI) Calcd. for $C_{16}H_{12}FNO$ (M^+): 253.09029. Found: 253.09022.

*1-Benzyl-5-bromo-1H-indole-3-carbaldehyde (**8c**):* To a solution of indole **7c** (500 mg, 2.23 mmol) in dry DMF (10 mL), NaH (134 mg, 5.58 mmol) and benzyl bromide (535 mg, 3.12 mmol) were added to give indole **8c** (560 mg, 80% yield). m.p. 113.1–115.2°C (recrystallized from AcOEt/*n*-hexane 1:1). IR_{vmax} (cm^{-1}): 3026 (C-H Arom.), 2921 (C-H Aliph.), 1663 (C=O), 1604 and 1565 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.56 (s, 2H, Ar-**CH_2**-), 7.2–7.5 (m, 6H, 7-H, $J_o = 6$ Hz, and **Ar**-CH_2), 7.60 (d, 1H, 6-H, $J_o = 9$ Hz), 8.26 (s, 1H, 2-H), 8.54 (s, 1H, 4-H), 9.95 (s, 1H, CHO). ^{13}C-NMR (75 MHz, DMSO-d_6): 50.4, 114.1, 115.9, 117.1, 123.7, 126.7, 127.0, (2x)127.8, 128.4, (2x)129.3, 136.2, 137.0, 142.2, 185.4. HRMS (EI) Calcd. for $C_{16}H_{12}BrNO$ (M^+): 313.01023. Found: 313.01022.

*General procedure for the synthesis of (1-benzyl-1H-indol-3-yl)methanol derivatives **9a–c**, with (1-benzyl-1H-3-indolyl)methanol (**9a**) as a model*

A solution of *N*-benzyl-3-formyl-1*H*-indole **8a** (500 mg, 2.12 mmol) in ethanol (30 mL), $NaBH_4$ (500 mg, 13.22 mmol) was added at 0°C and the mixture stirred for 45 min. The mixture was diluted with water (30 mL) and extracted with Et_2O (3 × 25 mL). The combined organic layers were dried on anhydrous Na_2SO_4 and concentrated *in vacuo*. The organic residue was purified by column chromatography on silica gel (AcOEt/*n*-hexane (1:1) to give pure **9a** (330 mg. 65%). m.p. 68−70°C (recrystallized from AcOEt/*n*-hexane 1:1). IR_{vmax} (cm^{-1}): 3386 (O-H), 3030 (C-H Arom.), 2924–2860 (C-H Aliph.), 1612 (C=C), 1555 (C=C), 1494 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.65 (d, 2H, -**CH_2**-OH, $J = 5.1$ Hz), 4.83 (m, 1H, OH), 5.35 (s, 2H, Ar-**CH_2**), 7.00 (t, 1H, $J = 7.4$ Hz, 5-H or 6-H), 7.09 (t, 1H, $J = 7.4$ Hz, 6-H or 5-H), 7.18–7.32 (m, 5H,-**C_6H_5**), 7.38 (s, 1H, 2-H), 7.41 (d, 1H, 7-H, $J = 8.1$ Hz), 7.62 (d, 1H, 4-H, $J = 7.9$ Hz). ^{13}C-NMR (75 MHz, $CDCl_3$): 49.8, 54.0, 109.8, 118.7, 119.0, 120.8, 121.2, (2x)126.9, 127.2, 127.9, (2x)128.2, 130.4, 136.4, 139.0. Anal. Calcd. for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Experimental, C, 80.88; H, 6.38; N, 5.89.

*(1-Benzyl-5-fluoro-1H-3-indolyl)methanol (**9b**):* To a solution of *N*-benzyl-3-formyl-1*H*-indole **8b** (300 mg, 1.18 mmol) in ethanol (25 mL), $NaBH_4$ (300 mg, 7.92 mmol) was added to give **9b** (50 mg. 17%). m.p. 60 − 62°C (recrystallized from AcOEt/*n*-hexane 1:1). IR_{vmax} (cm^{-1}): 3406 (O-H), 3062 (C-H Arom.), 2920 (C-H Aliph.), 1660 (C=C), 1191 (C-O). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.05 (t, 1H, OH, $J = 6$ Hz), 4.70 (d, 2H, –**CH_2**-OH, $J = 6$ Hz), 5.20 (s, 2H, Ar-**CH_2**), 7.0–7.3 (m, 8H, 2-H, 4-H, 6-H, and **Ar**-CH_2), 7.35 (dd, 1H, 7-H, $J_o = 6$ Hz and $J_m = 3$ Hz). ^{13}C-NMR (75 MHz, $CDCl_3$): 51.3, 53.8, 104.4 (d, $^{2'}J_{C-F} = 23.4$ Hz), 110.1 (d, $^{2}J_{C-F} = 26.4$ Hz), 112.4 (d, $^{3'}J_{C-F} = 8.3$ Hz), 114.1, 114.3 (d, $^{4}J_{C-F} = 4.5$ Hz), 122.3 (d, $^{3}J_{C-F} = 9.1$ Hz), (2x)126.7, 126.9, 128.2, (2x)128.9, 135.6, 157.6 (d, $^{1}J_{C-F} = 234$ Hz). HRMS: (EI) Calcd. for $C_{16}H_{14}FNO$ $(M+1)^+$: 256.1137. Found: 256.1135.

*(1-Benzyl-5-bromo-1H-indol-3-yl)methanol (**9c**):* A solution of *N*-benzyl-3-formyl-1*H*-indole **8c** (300 mg, 0.95 mmol) in ethanol (25 mL), $NaBH_4$ (300 mg, 7.92 mmol) was added to give **9c** (42 mg. 14%). m.p. 66 − 68°C (recrystallized from AcOEt/*n*-hexane 1:1). IR_{vmax} (cm^{-1}): 3422 (O-H), 3029 (C-H Arom.), 2921 (C-H Aliph.), 1663 (C=C), 1078 (C-O). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.06 (t, 1H, OH, $J = 6$ Hz), 4.84 (d, 2H, -**CH_2**-OH), 5.34 (s, 2H, Ar-**CH_2**), 7.10–7.40 (m, 6H,-C_6H_5, and 2-H), 7.43 (dd, 1H, 6-H, $J_o = 6$ Hz, $J_m = 3.0$ Hz), 7.61 (d, 1H, 7-H, $J = 9.0$ Hz), 8.55 (s, 1H, 4-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 51.2, 53.5, 111.9, 116.8, 117.9, 124.9, 127.0, (2x)127.1, 127.2, 128.6, (2x)129.3, 134.9, 136.1, 138.9. HRMS: (EI) Calcd. for $C_{16}H_{14}BrNO$ $(M+1)^+$: 316.0337. Found: 316.0336.

*General procedure for the synthesis of (1-benzyl-1H-indol-3-yl)methyl methanesulfonate derivatives **10a–c**, with (1-benzyl-1H-indol-3-yl)methyl methanesulfonate (**10a**) as a model*

To a solution of (1-benzyl-1*H*-indol-3-yl)methanol **9a** (500 mg, 2.1 mmol) in dichloromethane (20 mL), Et_3N (0.32 mL, 2.31 mmol) and mesylchloride (0.18 mL, 2.31 mmol) were added. The mixture was stirred for 1 h at 0°C. The mixture was subsequently concentrated under vaccum to give a white solid very unstable, being immediately utilized. In such sense, a rapid high-resolution mass spectrum was run to confirm the

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identity. HRMS: (EI) Calcd. for $C_{17}H_{17}NO_3S$ (M^+): 315.0929. Found: 315.0922.

*(1-Benzyl-5-fluoro-1H-indol-3-yl)methyl methanesulfonate (**10b**):* Prepared from (1-benzyl-5-fluoro-1*H*-indol-3-yl)methanol **9b** (500 mg, 1.96 mmol) in dichloromethane (20 mL), Et_3N (0.3 mL, 2.16 mmol) and mesylchloride (0.17 mL, 2.16 mmol). HRMS: (EI) Calcd. for $C_{17}H_{16}FNO_3S$ (M^+): 333.08349. Found: 333.08348.

*(1-Benzyl-5-bromo-1H-indol-3-yl)methyl methanesulfonate (**10c**):* Prepared from (1-benzyl-5-bromo-1*H*-indol-3-yl)methanol **9c** (500 mg, 1.58 mmol) in dichloromethane (20 mL), Et_3N (0.24 mL, 1.74 mmol) and mesylchloride (0.14 mL, 1.74 mmol). HRMS: (EI) Calcd. for $C_{17}H_{16}BrNO_3S$ (M^+): 393.0034. Found: 393.0035.

*General procedure for the synthesis of 3-{(4-[3-(1H-indol-3-yl)propyl]piperazin-1-yl)methyl}-1-benzyl-1H-indole derivatives **11a–d**, with 1-benzyl-3-{(4-[3-(1H-indol-3-yl)-propyl]piperazin-1-yl)methyl}-1H-indole (**11a**) as a model*
To a solution of (1-benzyl-1*H*-indol-3-yl)methyl methansulfonate **10a** (0.65 g, 2.17 mmol) in dry CH_3CN (20 mL) was added 3-(3-(piperazin-1-yl)propyl)-1*H*-indole **5a** (0.51 g, 2.1 mmol) and triethylamine (0.32 mL, 2.40 mmol). The mixture was heated under reflux conditions overnight. After this time, water (50 mL) was added and the reaction mixture was extracted with AcOEt (2 × 30 mL), dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure to give a crude, which was purified by column chromatography (AcOEt/*n*-hexane/MeOH 2:0.5:0.3) to provide pure **11a** (0.45 g, 46%, recrystallized from AcOEt/*n*-hexane 2:1). m.p. 108–110°C. $IR_{\nu max}$ (cm^{-1}): 3240 (N-H), 3030 (C-H Arom.), 2923 (C-H Aliph.). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.91 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.46 (t, 2H, $J = 8.2$ Hz, CH_2-CH_2-**CH_2**-Pip), 2.55 (m, 4H, Pip), 2.62 (m, 4H, Pip), 2.75 (t, 2H, Indole-**CH_2**-CH_2-CH_2, $J = 7.50$ Hz), 3.76 (s, 2H, Indole-**CH_2**-Ph), 5.25 (s, 2H, Pip–**CH_2**-Indole-Bn), 6.93 (s, 1H, **2-H**, Indole-Bn), 7.05–7.18 (m, 7H, Indole 2-H, 4-H, 5-H, 6-H, 7-H, and 2-H, 6-H Indole-**Bn**), 7.23–7.32 (m, 5H,5-H, 6-H **Indole**-Bn and 3-H, 4-H and 5-H Indole-**Bn**), 7.57 (d, 1H, $J = 7.8$ Hz, **7-H Indole**-Bn), 7.71 (d, 1H, $J = 7.7$ Hz, 4-H **Indole**-Bn), 8.22 (br s, 1H, N-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.0, 26.1, 49.1, (2x)51.7, (2x)52.0, 52.2, 57.3, 108.8, 109.8, 110.1, 115.1, 117.9, 118.1, 118.4, 118.6, 120.1, 120.9, (2x)125.9, 126.5, 126.6, 126.7, 127.3, (3x)127.8, 135.3, 135.7, 136.5. Anal. Calcd. for $C_{31}H_{34}N_4$: C, 80.48; H, 7.41; N, 12.11. Experimental: C, 80.46; H, 7.40; N, 12.09.

*1-Benzyl-3-{(4-[3-(5-methoxy-1H-indol-3-yl)-propyl]piperazin-1-yl)methyl}-1H-indole (**11b**):* Prepared from (1-benzyl-1*H*-indol-3-yl)methyl methanesulfonate **10a** (0.80 g, 2.67 mmol), triethylamine (0.36 mL,2.45 mmol) and 5-methoxy-3-(3-(piperazin-1-yl)propyl)-1*H*-indole **5b** (0.66 g, 2.42 mmol) to provide pure **11b** (0.513 g, 39%, recrystallized from AcOEt/*n*-hexane 2:1). mp. 107–109°C. $IR_{\nu max}$ (cm^{-1}): 3230 (N-H), 3022 (C-H Arom.), 2929 (C-H Aliph). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.92 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.54 (t, 2H, $J = 7.9$ Hz, CH_2-CH_2-**CH_2**-Pip), 2.58 (m, 4H, Pip), 2.7 (m, 6H, 4H-Pip, and Indole-**CH_2**-CH_2-CH_2), 3.80 (s, 3H, $-OCH_3$), 3.83 (s, 2H, Indole-**CH_2**-Ph), 5.23 (s, 2H, Pip–**CH_2**-Indole), 6.79 (dd, 1H, Indole **6-H**, $J_o = 8.7$ Hz, $J_m = 2.4$ Hz), 6.88 (s, 1H, **Indole**-Bn **2-H**), 7.01–7.22 (m, 11H, **Indole** 2-H, 4-H, 7-H, **Indole**-Bn **5-H, 6-H**, **7-H**, and Indole-**Bn** 2-H, 3-H, 4-H, 5-H, 6-H), 7.65 (d, 1H, $J = 7.2$ Hz, **Indole**-Bn **4-H**), 8.45 (br s, 1H, N-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.6, 26.9, 49.9, 51.1, 51.6, (2x)52.0, 52.2, 55.8, 57.4, 108.5, 109.8, 111.9, 114.9, 119.2, 119.4, 119.7, 121.9, 122.2, 122.4, (2x)127.7, 127.6, 127.8, 128.5, (3x)128.7, 129.0, 136.5, 137.1, 153.6. Anal. Calcd. for $C_{32}H_{36}N_4O$: C, 78.01; H, 7.37; N, 11.37. Experimental: C, 77.98; H, 7.36; N, 11.35.

*1-Benzyl-3-{(4-[3-(5-fluoro-1H-indol-3-yl)-propyl]piperazin-1-yl)methyl}-1H-indole (**11c**):* Prepared from (1-benzyl-1*H*-indol-3-yl)methyl methanesulfonate **10a** (0.71 g, 2.37 mmol), triethylamine (0.35 mL, 2.37 mmol) and 5-fluoro-3-(3-(piperazin-1-yl)propyl)-1*H*-indole **5c** (0.61 g, 2.34 mmol) to provide pure **11b** (0.46 g, 40%, recrystallized from AcOEt/*n*-hexane 2:1). m.p. 106–108°C. $IR_{\nu max}$ (cm^{-1}): 3414 (N-H), 3006 (C-H Arom.), 2933 (C-H Aliph.), 1579 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.87 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.46 (t, 2H, $J = 7.9$ Hz,CH_2-CH_2-**CH_2**-Pip), 2.58 (m, 4H, Pip), 2.67 (m, 6H, 4H-Pip, and Indole-**CH_2**-CH_2-CH_2), 3.80 (s, 2H, Indole-**CH_2**-Ph), 5.24 (s, 2H, Pip–**CH_2**-Indole), 6.87 (dd, 1H, Indole **6-H**, $J_o = 9.0$ Hz, $J_m = 2.4$ Hz), 6.93 (s, 1H, **Indole**-Bn **2-H**), 7.08–7.29 (m, 11H, **Indole** 2-H, 4-H, 7-H, **Indole**-Bn **5-H, 6-H, 7-H**, and Indole-**Bn** 2-H, 3-H, 4-H, 5-H, 6-H), 7.68 (d, 1H, $J = 7.1$ Hz, **Indole**-Bn **4-H**), 8.55 (br s, 1H, N-H), ^{13}C-NMR (75 MHz, $CDCl_3$): 22.3, 25.7, 49.1, (2x) 51.1, (2x)51.5, 51.8, 56.9, 102.7 (d, $^2J_{C-F} = 23.2$ Hz), 108.9, 109.1 (d,$^{2'}J_{C-F} = 26.3$ Hz), 109.2, 110.8 (d,$^{3'}J_{C-F} = 9.7$ Hz), 115.0 (d, $^4J_{C-F} = 4.8$ Hz), 118.6, 121.0, 122.3, (2x)125.9, 126.7, 126.8, 126.9 (d,$^{3'}J_{C-F} = 9.6$ Hz), 127.6, 127.7, (2x)127.8, 132.0, 135.7, 136.4, 156.6 (d, $^1J_{C-F} = 234$ Hz). Anal. Calcd. for $C_{31}H_{33}FN_4$: C, 77.47; H, 6.92; N, 11.66. Experimental: C, 77.51; H, 6.90; N, 11.63.

*1-Benzyl-3-{(4-[3-(5-bromo-1H-indol-3-yl)-propyl]piperazin-1-yl)methyl}-1H-indole (**11d**):* Prepared from (1-benzyl-1*H*-indol-3-yl)methyl methanesulfonate **10a** (0.62 g, 2.07 mmol), triethylamine (0.30 mL, 2.15 mmol) and 5-bromo-3-(3-(piperazin-1-yl)propyl)-1*H*-indole **5d** (0.64 g, 1.99 mmol) to provide pure **11d** (0.393 g, 35%) as an oil. $IR_{\nu max}$ (cm^{-1}): 3310 (N-H), 3011 (C-H Arom.), 2930 (C-H Aliph.), 1578 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.84 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.39 (t, 2H, $J = 7.9$ Hz, CH_2-CH_2-**CH_2**-Pip), 2.50 (m, 4H, Pip), 2.66 (m, 6H, **4H**-Pip, and Indole-**CH_2**-CH_2-CH_2), 3.74 (s, 2H, Indole-**CH_2**-Ph), 5.23 (s, 2H, Pip–**CH_2**-Indole), 6.86 (s, 1H, **Indole**-Bn **2-H**), 7.04–7.25 (m, 11H, **Indole** 2-H, 6-H, 7-H, **Indole**-Bn 2-H- 6-H and Indole-**Bn** 2-H, 3-H, 4-H, 5-H, 6-H, 7-H), 7.69 (d, 1H, $J = 7.2$ Hz, **Indole**-Bn 4-H), 7.73 (d, 1H, $J_m = 2.4$ Hz, **Indole** 4-H), 8.54 (br s, 1H, N-H). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.7, 26.9, 49.9, 52.8, (2x) 53.0, 58.0, 109.7, 111.1, 112.2, 112.4, 115.8, 119.3, 119.6, 121.5, 121.7, 122.5, 124.4, (2x)126.7, (2x)127.5, 127.9, (3x)128.7, (2x) 129.3, 134.8, 136.5, 137.4. Anal. Calcd. for $C_{31}H_{33}BrN_4$: C, 68.76; H, 6.14; N, 10.35. Experimental: C, 68.75; H, 6.13; N, 10.37.

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*General procedure for the synthesis of 3-{3-[4-(4-nitrophenyl)piperazin-1-yl]-propyl}-1H-indole derivatives **12a,b**, with 3-{3-[4-(4-nitrophenyl)piperazin-1-yl]-propyl}-1H-indole (**12a**) as a model*

To a solution of 3-(indol-3-yl)propyl-4-methylbenzenesulfonate **3a** (0.73 g, 2.2 mmol) in dry CH_3CN (50 mL), anhydrous K_2CO_3 (0.33 g, 2.42 mmol) and 4-nitrophenylpiperazine (0.46 g, 2.2 mmol) were added. The mixture was stirred under reflux condition for 6 h. After this time, the mixture was poured onto water (50 mL) and extracted with AcOEt (2 × 30 mL), dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure to give a crude, which was purified by column chromatography (AcOEt) to provide pure **12a** (0.42 g, 52%) as a yellow solid (recrystallized from AcOEt/*n*-hexane 1:1). mp. 158–160°C. IR_{vmax} (cm^{-1}): 3415 (N-H), 3049 (C-H arom), 2936–2843 (C-H aliph.), 1600 (NO_2, asym.), 1331 (NO_2, sym.). ^{1}H-NMR (DMSO-d_6): 1.86 (m, 2H, 2′-H), 2.41 (t, 2H, 3′-H, J = 6.8 Hz), 2.50 (s, 4H, 2″-H, 6″-H), 2.75 (t, 2H, 1′-H, J = 7.2 Hz), 3.44 (s, 4H, 3″-H, 5″-H), 6.71–6.76 (m, 3H, 6-H, 2‴-H, 6‴-H), 7.09 (t, 1H, 5-H, J = 7.3 Hz), 7.17 (s, 1H, 2-H), 7.38 (d, 1H, 4-H, J = 8.0 Hz), 7.56 (d, 1H, 7-H, J = 7.8 Hz), 8.07 (d, 2H, 3‴-H, 5‴-H, J = 9.3 Hz), 10.84 (bs., 1H, NH). ^{13}C-NMR (DMSO-d_6): 22.5, 27.0, (2x)46.2, (2x)52.3, 57.4, 111.4, (2x)112.5, 114.3, 118.1, 118.3, 120.8, 122.2, (2x)125.7, 127.2, 136.3, 136.8, 154.7. Anal. Calcd. for $C_{21}H_{24}N_4O_2$: C, 69.21; H, 6.64; N, 15.37. Experimental: C, 69.23; H, 6.65; N, 15.35.

*5-Methoxy-3-{3-[4-(4-nitrophenyl)piperazin-1-yl]propyl}-1H-indole (**12b**):* Prepared from 3-(5-methoxy-1*H*-indol-3-yl)propyl-4-methylbenzenesulfonate **3b** (1.4 g, 3.9 mmol), anhydrous K_2CO_3 (0.65 g, 4.7 mmol) and 4-nitrophenylpiperazine (0.81 g, 3.9 mmol) to provide pure **12b** (0.97 g, 63%, recrystallized from AcOEt/*n*-hexane 1:1). m.p. 166–167.1°C. IR_{vmax} (cm^{-1}): 3390 (N-H), 3030 (C-H arom), 2925–2850 (C-H aliph.), 1590 (NO_2 asym.), 1320 (NO_2 sym.). ^{1}H-NMR (DMSO-d_6): 2.05 (m, 2H, 2′-H), 2.63 (t, 2H, 3′-H, J = 6.9 Hz), 2.70 (s, 4H, 2″-H, 5″-H, piperazine), 2.80 (t, 2H, 1′-H, J = 7.0 Hz), 3.48 (s, 4H, 3″-H, 4″-H, piperazine), 3.89 (s, 3H, OCH_3), 6.82 (d, 2H, 2‴-H, 6‴-H, J = 9.42 Hz), 6.88 (dd, 1H, 6-H, J_o = 8.78 Hz and J_m = 2.4 Hz), 7.0 (s, 1H, 2-H), 7.05 (d, 1H, 4-H, J = 2.26 Hz), 7.27 (d, 1H, 7-H, J = 8.72 Hz), 8.02 (bs., 1H, NH), 8.13 (d, 2H, 3‴-H, 5‴-H, J = 9.37 Hz). ^{13}C-NMR (DMSO-d_6): 22.8, 26.6, (2x)46.6, (2x)52.4, 55.9, 57.9, 100.8, 111.7, 111.9, (2x)112.6, 115.5, 121.9, (2x)125.9, 127.7, 131.3, 138.4, 153.7, 154.7 ppm. Anal. calcd. for $C_{22}H_{26}N_4O_3$: C, 69.91; H, 6.64; N, 14.20. Experimental: C, 69.90; H, 6.66; N, 14.19.

*General procedure for the synthesis of 4-{4-[3-(1H-indol-3-yl)propyl]piperazin-1-yl}aniline derivatives **13a,b**, with 4-{4-[3-(1H-indol-3-yl)propyl]piperazin-1-yl}aniline (**13a**) as a model*

To a solution of 3-{3-[4-(4-nitrophenyl)piperazin-1-yl]propyl}-1*H*-indole **12a** (0.23 g, 0.63 mmol) in a mixture of H_2O/EtOH/AcOH (1:1:1), powder iron (0.21 g, 3.79 mmol) was added and stirred at 55−60°C for 40 min. Then water (100 mL) was added neutralized with $NaHCO_3$ and extracted with AcOEt (4 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness *in vacuo* to give a crude mixture which was finally purified by column chromatography (AcOEt/*n*-hexane/MeOH) (2:0.5:0.3) to provide pure **13a** (0.15 g, 71.1%) as a white solid (recrystallized from EtOH/*n*-hexane 2:1). mp. 128–130°C. IR_{vmax} (cm^{-1}): 3426 and 3328 (Ar-NH_2), 3414 (N-H indole), 3047 (C-H arom.), 2935–2840 (C-H aliph.). ^{1}H-NMR (DMSO-d_6): 2.26 (m, 2H, 2′-H), 2.61 (t, 2H, 3′-H, J = 6.5 Hz), 2.80 (s, 4H, 2″-H, 6″-H), 3.21 (t, 2H, 1′-H, J = 6.8 Hz), 3.46 (s, 4H, 3″-H, 5″-H), 4.53 (s, 2H, NH_2), 6.56 (m, 2H, 2‴-H, 6‴-H, J = 8.2 Hz), 6.77 (d, 2H, 3‴-H, 5‴-H, J = 8.3 Hz), 7.05 (t, 1H, 5-H, J = 7.1 Hz), 7.15 (m, 2H, 4-H and 6-H), 7.27 (s, 1H, 2-H), 8.01 (d, 1H, 7-H, J = 7.5 Hz), 10.41 (bs., 1H, NH indole) ppm. ^{13}C-NMR (DMSO-d_6): 22.4, 27.0, (2x)46.1, (2x)52.2, 57.4, 111.3, (2x)112.5, 114.2, 118.2, 118.4, 120.7, 122.1, (2x)125.5, 127.1, 136.4, 137.0, 142.7 ppm. Anal. Calcd. for$C_{21}H_{26}N_4$: C, 75.41; H, 7.84; N, 16.75. Experimental: C, 75.40; H, 7.85; N, 16.72.

*4-{4-[3-(5-Methoxy-1H-indol-3-yl)propyl]piperazin-1-yl}aniline (**13b**):* Prepared from 5-methoxy-3-{3-[4-(4-nitrophenyl)-piperazin-1-yl]-propyl}-1*H*-indole **12b** (0.8 g, 2.0 mmol), a mixture of H_2O/EtOH/AcOH (1:1:1) and powder iron (1.13 g, 20.3 mmol) to provide pure **13b** (0.68 g, 92%, recrystallized from EtOH/*n*-hexane 2:1). m.p. 133–134°C. IR_{vmax} (cm^{-1}): 3424 and 3330 (Ar-NH_2), 3395 (N-H indole), 3037 (C-H arom.), 2925–2842 (C-H aliph.). ^{1}H-NMR (DMSO-d_6): 2.03 (m, 2H, 2′-H), 2.65 (t, 2H, 3′-H, J = 7.01 Hz), 2.75 (s, 4H, 2″-H, 5″-H, piperazine), 2.86 (t, 2H, 1′-H, J = 7.0 Hz), 3.50 (s, 4H, 3″-H, 4″-H, piperazine), 3.73 (s, 2H, NH_2), 3.88 (s, 3H, OCH_3), 6.58 (d, 2H, 2‴-H, 6‴-H, J = 9.0 Hz), 6.73 (d, 2H, 3‴-H, 5‴-H, J = 8.9 Hz), 6.90 (dd, 1H, 6-H, J_o = 8.76 Hz and J_m = 2.42 Hz), 7.08 (d, 1H, 4-H, J = 2.31 Hz), 7.12 (s, 1H, 2-H), 7.29 (d, 1H, 7-H, J = 8.72 Hz), 9.08 (bs., 1H, NH indole) ppm. ^{13}C-NMR (DMSO-d_6): 22.7, 26.6, (2x)46.7, (2x) 52.3, 55.9, 58.0, 100.9, 111.6, 111.9, (2x)112.7, 115.4, 121.9, (2x)125.9, 127.7, 131.2, 138.2, 143.2, 154.7 ppm. Anal. Calcd. for $C_{22}H_{28}N_4O$: C, 72.50; H, 7.74; N, 15.37. Experimental: C, 72.52; H, 7.75; N, 15.40.

*General procedure for the synthesis of 4-{4-[3-(1H-indol-3-yl)propyl]piperazin-1-yl}-N-((1-benzyl-1H-indol-3-yl)-methyl)aniline derivatives **14a–d**, with 4-{4-[3-(1H-indol-3-yl)propyl]piperazin-1-yl}-N-((1-benzyl-1H-indol-3-yl)-methyl)aniline (**14a**) as a model*

A mixture of (1-benzyl-1*H*-indol-3-yl)methyl methanesulfonate **10a** (0.41 g, 1.37 mmol) in CH_3CN (20 mL), triethylamine (0.2 mL, 1.35 mmol), and 4-{4-[3-(1*H*-indol-3-yl)propyl]piperazin-1-yl}aniline **13a** (0.45 g, 1.35 mmol) was stirred under reflux for 24 h. After this time, water was added (20 mL) and the mixture extracted with AcOEt (3 × 30 mL). The combined organic layers were washed and dried over anhydrous Na_2SO_4. The organic portions were filtered and concentrated under vaccum to obtain a crude residue. The crude was purified by column chromatography on silica gel (AcOEt/*n*-hexane/MeOH) (2:0.5:0.3) to afford pure **14a** as an oil (0.33 g, 44.3%). IR_{vmax} (cm^{-1}): 3239 (N-H), 3030 (C-H Arom.), 2921 (C-H

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Aliph.), 1570 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.83 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.38 (t, 2H, $J = 7.9$ Hz, CH_2-CH_2-**CH_2**-Pip), 2.72 (t, 2H, $J = 7.9$ Hz, Indole-**CH_2**-CH_2-CH_2), 2.92 (m, 4H, Pip), 3.44 (br s, 5H, **N-H**, and **4H** Pip), 4.33 (s, 2H, Indole-**CH_2**-Phe), 5.36 (s, 2H, NH–**CH_2**-Indole), 6.62 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.73 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.95–7.43 (m, 13H, **Indole** 2-H, 5-H, 6-H, 7-H, Indole-CH_2-**Ph** 2-H,3-H, 4-H, 5-H, and 6-H, **Indole**-CH_2-Ph 2-H, 5-H, 6-H, and 7-H), 7.53 (d, 1H, $J = 7.1$ Hz, **Indole** 4-H), 7.65 (d, 1H, $J = 7.1$ Hz, **Indole**-CH_2-Ph 4-H), 10.77 (br s, 1H, N-H indole). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.0, 26.7, 48.4, 49.8, (2x)52.6, (2x)57.2, 59.2, 109.6, 109.8, 110.8, 112.5, 112.7, 113.9, 117.2, 117.6, 117.8, 118.2, 118.7, 120.3, 120.8, 121.7, (2x)126.5, 126.7, (2x)126.8, (2x)126.9, (2x)127.9, 135.7, 135.8, 137.8, 141.9, 142.6. Anal. Calcd. for $C_{37}H_{39}N_5$: C, 80.25; H, 7.10; N, 12.65. Experimental: C, 80.21; H, 7.11; N, 12.68.

*N-((1-Benzyl-5-fluoro-1H-indol-3-yl)methyl)-4-{4-[3-(5-methoxy-1H-indol-3-yl)propyl]piperazin-1-yl}aniline (**14b**):* Prepared from (1-benzyl-5-fluoro-1*H*-indol-3-yl)methyl methanesulfonate **10b** (0.52 g, 1.64 mmol), 4-{4-[3-(5-methoxy-1*H*-indol-3-yl)propyl]piperazin-1-yl}aniline **13b** (0.6 g, 1.65 mmol), and triethylamine (0.24 mL, 1.65 mmol) to afford pure **14b** (0.379 g, 38.4%) as an oil. IR_{vmax} (cm^{-1}): 3240 (N-H), 3035 (C-H Arom.), 2930 (C-H Aliph.), 1565 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.81 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.38 (t, 2H, $J = 7.9$ Hz, CH_2-CH_2-**CH_2**-Pip), 2.67 (t, 2H, $J = 7.9$ Hz, Indole-**CH_2**-CH_2-CH_2), 2.92 (m, 4H, Pip), 3.42 (br s, 5H, N-H, and Pip), 3.75 (s, 3H, OCH_3), 4.29 (s, 2H, Indole-**CH_2**-Ph), 5.36 (s, 2H, NH–**CH_2**-Indole), 6.60 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.72 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.89–7.44 (m, 12H, **Indole** 2-H, 6-H, 7-H, Indole-CH_2-**Ph,** 2-H, 3-H, 4-H, 5-H, 6-H, **Indole**-CH_2-Ph 2-H, 4-H, 6-H, 7-H), 7.52 (s, 1H, **Indole** 4-H), 10.60 (br s, 1H, N-H indole). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.5, 26.7, 49.2, (2x) 50.1, (2x)52.9, 55.2, 55.9, 57.7, 100.1, 103.9, 109.4 (d, $^{2'}J_{C-F} = 26.4$ Hz), 111.2 (d, $^2J_{C-F} = 20.4$ Hz), 112.6 (d, $^3J_{C-F} = 7.5$ Hz), 113.1 (d, $^4J_{C-F} = 4.7$ Hz), 114.2, 117.6, 117.8, 117.9, (2x)122.8, (2x)126.9, 127.2 (d, $^{3'}J_{C-F} = 7.8$ Hz), (2x)127.5, 127.6, (2x)128.6, 129.5, 131.3, 132.9, 138.0, 142.3, 142.8, 152.8, 156.6 (d, $^1J_{C-F} = 231$ Hz). Anal. Calcd. for $C_{38}H_{40}FN_5O$: C, 75.85; H, 6.70; N, 11.64. Experimental: C, 75.88; H, 6.72; N, 11.67.

*4-{4-[3-(1H-Indol-3-yl)propyl]piperazin-1-yl}-N-((1-benzyl-5-fluoro-1H-indol-3-yl)methyl)aniline (**14c**):* Prepared from (1-benzyl-5-fluoro-1*H*-indol-3-yl)methyl methanesulfonate **10b** (0.60 g, 1.89 mmol), 4-{4-[3-(1*H*-indol-3-yl)-propyl]piperazin-1-yl}aniline **13a** (0.60 g, 1.80 mmol) and triethylamine (0.26 mL, 1.80 mmol) to give pure **14c** (0.40, 39.2%) as an oil. IR_{vmax} (cm^{-1}): 3240 (N-H), 3028 (C-H Arom.), 2925 (C-H Aliph.), 1570 (C=C). ^{1}H-NMR ($CDCl_3$): 1.80 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.35 (t, 2H, $J = 7.9$ Hz, CH_2-CH_2-**CH_2**-Pip), 2.7 (t, 2H, $J = 7.9$ Hz, Indole-**CH_2**-CH_2-CH_2), 2.9 (m, 4H, Pip), 3.37 (br s, 5H, N-H, and Pip), 4.27 (s, 2H, Indole-**CH_2**-Ph), 5.34 (s, 2H, NH–**CH_2**–Indole), 6.58 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.69 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.87–7.42 (m, 13H, **Indole** 2-H, 5-H, 6-H, 7-H, Indole–CH_2–**Ph,** 2-H, 3-H, 4-H, 5-H, 6-H, **Indole**-CH_2-Ph 2-H, 4-H, 6-H, 7-H), 7.48 (s, 1H, **Indole** 4-H), 10.74 (br s, 1H, N-H indole). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.0, 26.7, 48.4, (2x)49.8, (2x)52.1, 52.6, 57.2, 102.9, 103.7, 108.9 (d, $^2J_{C-F} = 31.4$ Hz), 110.7 (d, $^{2'}J_{C-F} = 25.6$ Hz), 112.8 (d, $^3J_{C-F} = 7.54$ Hz), 113.9, 115.2 (d, $^4J_{C-F} = 4.7$ Hz), 117.2, 117.6, 117.8, 118.2, (2x)120.3, (2x) 121.7, (2x)126.5, 126.8 (d, $^{3'}J_{C-F} = 7.24$ Hz), (2x)128.0, 128.9, 132.4, 135.8, 137.5, 137.7, 141.9, 142.4, 156.7 (d, $^1J_{C-F} = 233$ Hz). Anal. Calcd. for $C_{37}H_{38}FN_5$: C, 77.73; H, 6.70; N, 12.25. Experimental: C, 77.75; H, 6.72; N, 12.24.

*4-{4-[3-(1H-Indol-3-yl)propyl]piperazin-1-yl}-N-((1-benzyl-5-bromo-1H-indol-3-yl)methyl)aniline (**14d**):* Prepared from (1-benzyl-5-bromo-1*H*-indol-3-yl)methyl methanesulfonate **10c** (0.40 g, 1.06 mmol), 4-{4-[3-(1*H*-indol-3-yl)propyl]piperazin-1-yl}aniline **13a** (0.37 g, 1.11 mmol) and triethylamine (0.16 mL, 1.14 mmol), to give pure **14d** (0.241 g, 36%) as an oil residue. IR_{vmax} (cm^{-1}): 3239 (N-H), 3032 (C-H Arom.), 2920 (C-H Aliph.), 1572 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.79 (q, 2H, $J = 7.6$ Hz, CH_2-**CH_2**-CH_2), 2.36 (t, 2H, $J = 7.8$ Hz, CH_2-CH_2-**CH_2**-Pip), 2.69 (t, 2H, $J = 7.9$ Hz, Indole-**CH_2**-CH_2-CH_2), 2.93 (m, 4H, Pip), 3.40 (br s, 5H, N-H, and Pip), 4.31 (s, 2H, Indole-**CH_2**-Ph), 5.36 (s, 2H, NH-**CH_2**-Indole), 6.62 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.71 (d, 2H, $J = 7.1$ Hz, Pip-**Ar**), 6.89–7.45 (m, 12H, **Indole** 2-H, 5-H, 6-H, 7-H, Indole-CH_2-**Ph,** 2-H, 3-H, 4-H, 5-H 6-H, **Indole**-CH_2-Ph 2-H, 6-H, 7-H), 7.49 (s, 1H, **Indole** 4-H), 7.52 (s, 1H, **Indole**-CH_2-Ph 4-H), 10.70 (br s, 1H, N-H indole). ^{13}C-NMR (75 MHz, $CDCl_3$): 22.1, 25.6, 48.1, 49.8, (2x)52.6, (2x)57.3, 59.1, 103.2, 108.5, 111.3, 112.4, 112.7, 113.8, 117.1, 117.4, 117.7, 118.1, 119.3, 120.1, 120.9, 121.5, 122.6, (2x)125.9, 126.4, (2x)126.8, 127.1, (2x)127.8, 135.7, 135.8, 138.1, 140.6, 143.2. Anal. Calcd. for $C_{37}H_{38}BrN_5$: C, 70.25; H, 6.05; N, 11.07. Experimental: C, 70.23; H, 6.06; N, 11.07.

Pharmacological evaluation

Binding assays

The affinity of compounds for the SERT was determined via a competitive binding assay, using [^{3}H]paroxetine as radioligand ($K_d = 0.07$ nM [25]) and membranes from human embryonic kidney (HEK-293) cells expressing the human SERT (RBHSTM400UA; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). The SERT membranes were thawed and diluted in assay buffer (50 mM Tris-HCl pH 7.4, 120 mM NaCl, 5 mM KCl) to a concentration of 10 μg/100 μL. Membranes (100 μL) were incubated for 30 min at 27°C with 50 μL 2 nM (final concentration) [^{3}H]paroxetine and 50 μL of the competing drugs at different concentrations (10^{-9} to 10^{-4} M) in a final volume of 500 μL. Incubations were terminated by filtration through Whatman GF/C filters that were previously soaked in 0.5% polyethyleneimine, using a cell harvester (Brandel Instruments, Gaithersburg, MD, USA). Radioactivity was counted in a Packard 1300 liquid scintillation counter with an efficiency of approximately 50%. Nonspecific binding was determined in the presence of 10 μM fluoxetine.

The affinity of the compounds toward the 5-HT_{1A} receptor was determined using [^{3}H]8-OH-DPAT as radioligand ($K_d = 0.87$ nM [26]) and membranes from HEK-293 cells

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expressing the human 5-HT_{1A} receptor (RBHS1AM400UA; PerkinElmer Life and Analytical Sciences), as previously described [8].

Inhibition curves were fitted using the sigmoidal dose–response curve (variable slope) equation built into GraphPad PRISM 5.01 (GraphPad Software, Inc., San Diego, CA, USA). The IC_{50} values were determined from four to five independent experiments, each in triplicate. K_i were determined from the IC_{50} values using the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + [S]/K_m)$ [27].

Molecular simulation

The crystal structure of the human 5-HT_{2B} receptor (Protein Data Bank, PDB, code 4IB4, 2.7 Å resolution; [28]) was used as template to build the 5-HT_{1A} receptor model. Models were prepared using MODELLER v9.9 [29] and the best model was stereochemically and energetically evaluated by the ANOLEA web service [30] and with PROCHECK [31]. In addition, the recently reported crystal structure of the human SERT (PDB, code 5I6X, 3.1 Å resolution; [32]) was used to perform docking calculations. Both protein structures were then embedded in a hydrated palmitoyl-oleyl-phosphatidyl-choline (POPC) bilayer membrane, solvated in a water box (SCP water model), and ions were added creating an overall neutral system in approximately 0.02 M NaCl. The final systems were subjected to a molecular dynamics simulation for 5 ns using Desmond software from Schrodinger Maestro [33]. The isobaric-isothermal ensemble (NPT, temperature of 310 K and 1 atm) was used to perform MD calculations. The equations of motion were integrated using a time step of 2 fs. The simulation time was sufficient to obtain an equilibrated system (root mean square deviation-RMSD- values <2 Å). All other conditions were as previously described [9, 34]. Docking of compounds, both in the SERT structure and the 5-HT_{1A} model, were performed with the AutoDock 4.2 suite [35], following protocols previously described elsewhere [34, 36]. The models used in this study were built and validated according to our previous publications [8, 9].

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