Bioorganic & Medicinal Chemistry 21 (2013) 7604–7611

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, docking and pharmacological evaluation of novel homo- and hetero-bis 3-piperazinylpropylindole derivatives at SERT and 5-HT$_{1A}$ receptor

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ARTICLE INFO

Article history:
Received 12 August 2013
Revised 15 October 2013
Accepted 24 October 2013
Available online 6 November 2013

Keywords:
Piperazinylpropylindole derivatives
Serotonin transporter
5-Hydroxytryptamine 1A receptor
Bivalent ligands
Docking

ABSTRACT

A series of 3-(3-(4-(3-(1*H*-indol-3-yl)propyl)piperazin-1-yl)propyl)-1*H*-indole derivatives (**3a–d** and **5a–f**) as homo- and hetero-*bis*-ligands, were synthesized and evaluated for in vitro affinity at the serotonin transporter (SERT) and the 5-HT$_{1A}$ receptor. Compounds **5b** and **5f** showed nanomolar affinities for both targets. The experimental data were rationalized according to results obtained from docking experiments. These findings are in agreement with our proposal that bis-indole derivatives can bind both targets, and might serve as leads in the quest of ligands endowed with a dual mechanism of action.

1. Introduction

Selective serotonin (5-HT) reuptake inhibitors (SSRIs, e.g., citalopram, fluoxetine or paroxetine) are drugs widely prescribed for patients suffering from depression and several other psychiatric disorders.[1] Despite the therapeutic usefulness and safety of these drugs, they still display significant side effects. In addition, as with other antidepressants, they exhibit a delayed onset of action, requiring at least 2–4 weeks of treatment before the appearance of clinical effects. More than a decade ago, it was shown that this delayed onset of action can be overcome in humans by the combined use of a SSRI plus pindolol, a 5-HT$_{1A}$ receptor antagonist.[2] This evidence has prompted the search of novel compounds exhibiting affinity for both the 5-HT transporter (SERT) and the 5-HT$_{1A}$ receptor.[3]

The design of bivalent ligands, that is, compounds containing two receptor-interacting moieties linked by a flexible chain, has been proposed as a strategy for the development of novel compounds that can act simultaneously on two different targets.[4]

As part of our ongoing efforts searching for leads to the development of novel centrally acting agents, we have recently reported a 3-arylpiperazinylpropylindole series displaying affinity in the low nanomolar range for the 5-HT$_{1A}$ receptor.[5] Considering that indole derivatives bearing electron-withdrawing groups at C5 are well-known SERT ligands,[3] we have designed a series of 5-substituted bis-indole derivatives connected by a propylpiperazine chain linker, with the idea that these compounds should exhibit dual activity, showing similarly high affinity at both the SERT and the 5-HT$_{1A}$ receptor.

2. Results and discussion

2.1. Chemistry

The synthesis of the C5-substituted homo-bis 3-(3-(4-(3-(1*H*-indol-3-yl)propyl)piperazin-1-l)propyl)-1*H*-indoles **3(a–d)** is outlined in Scheme 1. Fischer indole synthesis from commercially

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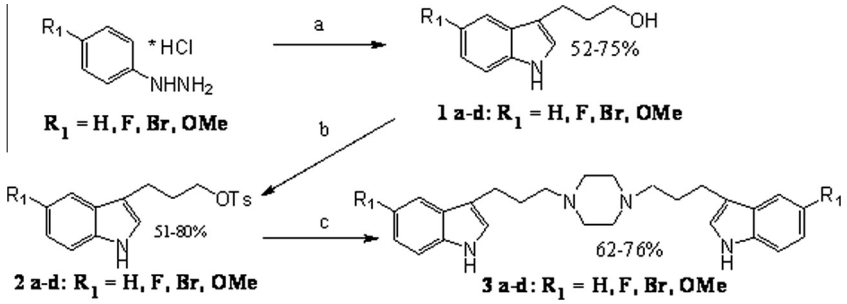
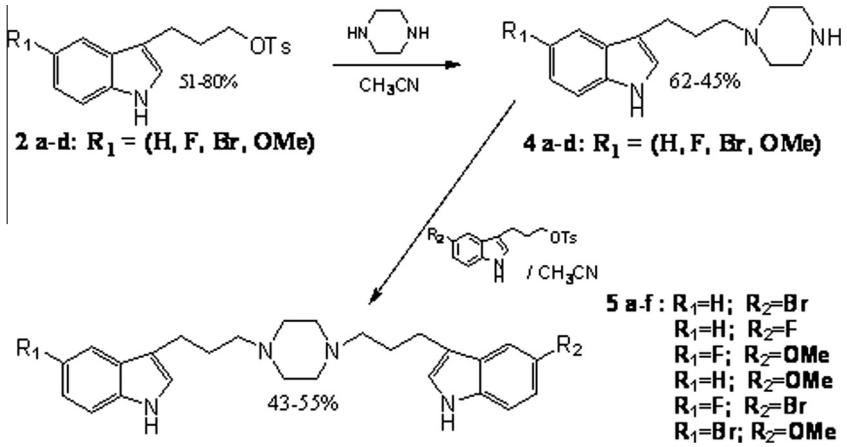
http://dx.doi.org/10.1016/j.bmc.2013.10.036



Scheme 1. Synthesis of 1,4-bis(3-(1*H*-3-indolyl)propyl)piperazine derivatives **(3a–d)**. Reagents and conditions: (a) 3,4-dihydropyran, 4% H_2SO_4, aq, DMA, 100 °C.; (b) TsCl, CH_2Cl_2, DMAP. (c) 1 equiv of piperazine, CH_3CN, K_2CO_3, reflux.



Scheme 2. Synthesis of 3-(3-(4-(3-(1*H*-indol-3-yl)propyl)piperazin-1-l)propyl)-1*H*-indole derivatives (**5a–f**).

Table 1
Binding affinities of series **3**(**a–d**) and **5**(**a–f**) at SERT and 5-HT_{1A} receptor

Compound	R_1	R_2	SERT IC_{50} (nM) ± SD	5-HT_{1A} IC_{50} (nM) ± SD
3a	H	—	37 ± 5	2700 ± 137
3b	F	—	13 ± 2	5700 ± 1200
3c	MeO	—	98 ± 21	599 ± 41
3d	Br	—	87 ± 18	1200 ± 214
5a	H	Br	137 ± 25	15 ± 3
5b	H	F	26 ± 6	16 ± 1
5c	F	MeO	14 ± 3	7200 ± 1300
5d	H	MeO	43 ± 14	4500 ± 1140
5e	F	Br	61 ± 18	4900 ± 1500
5f	Br	MeO	39 ± 2	128 ± 19
Citalopram[a]	—	—	3 ± 0.2	ND
8-OH-DPAT[a]	—	—	ND	6 ± 0.4

[a] The affinity values of citalopram and 8-OH-DPAT are included as reference compounds for SERT and 5-HT_{1A} receptor, respectively. ND: Not determined.

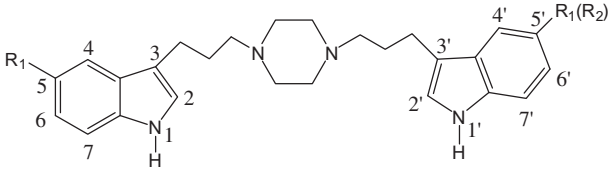
available 4-phenylhydrazines using 3,4-dihydropyran under reflux conditions in acidic medium provided the corresponding indolylpropanols **(1a–d)** in good yield.[6] This reaction proceeds by ring opening of the dihydropyrane ring under acid medium to provide in equilibrium the 4-hydroxy-butanal as intermediate, which reacts with the arylhydrazine derivatives affording the respective arylhidrazones, finally a [3,3] sigmatropic rearrangement followed by ammonia-loss with aromatization afforded series 1. The indolyl alcohols (**1a–d**) were subsequently converted into their corresponding tosylates (**2a–d**) by reaction with tosyl chloride in anhydrous CH_2Cl_2. Finally, the homo-bis-ligand series (**3a–d**) was obtained by coupling two equivalents of 3-(2-indolyl)propyl tosylate with 1 equivalent of piperazine in the presence of sodium carbonate in CH_3CN.

On the other hand, the bis-hetero piperazinyl propylindole derivatives **5a–f** were obtained in good yield in a two-step sequence from the respective tosylates **2a–d**, by nucleophilic displacement with the corresponding C-5 piperazinylpropylindole derivatives **4a–d** obtained by reaction of tosylates **2a–d** with piperazine, as is outlined in Scheme 2.

It should be noted that the substituents incorporated in the indole(s) moiety(ies) were selected on the basis of previous studies showing their ability to increase the binding affinity for either SERT or 5-HT_{1A} receptor.[3] In addition, we choice substituents allowing to evaluate in a small series of compounds, a range of properties such as different size, diverse electron-attracting or -donor properties, and other physicochemical features such halogen bonding properties.

2.2. Pharmacology and molecular docking

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



2.2.1. SERT

Regarding the activities of drugs upon the SERT, all compounds showed affinities in the nanomolar range, **3b** and **5c** being the most potent of each series (homo and hetero bis-ligands). The

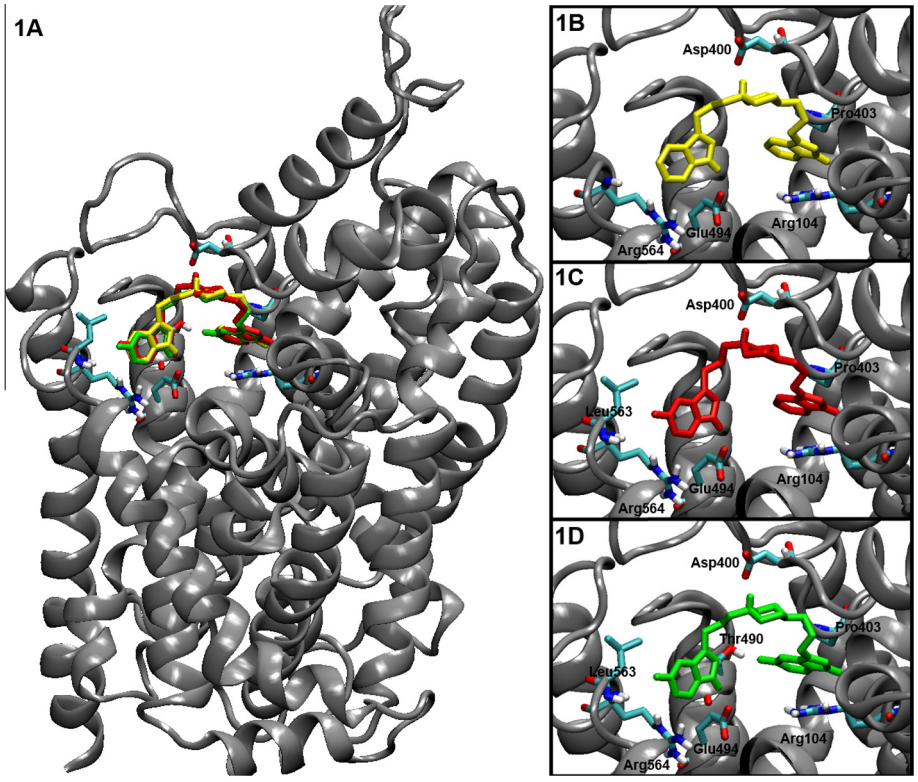


Figure 1. Ribbon diagram of the SERT model generated showing the putative binding site of compounds **3a** (yellow), **5b** (red) and **3b** (green) (1A). (1B–D) show closeups of docking poses of **3a** (yellow), **5b** (red) and **3b** (green), respectively. Main binding site amino acid residues (cyan) are rendered as stick models.

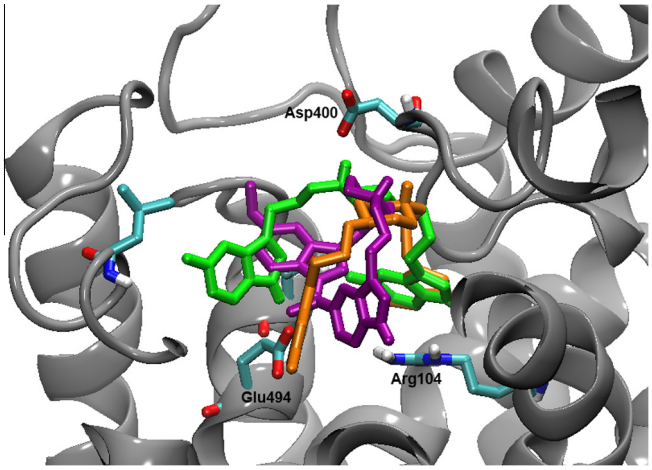


Figure 2. Superimposed structures of compounds **3b** (green), **3c** (purple) and **5a** (orange) docked into the binding site of the SERT. Main binding site amino acid residues (cyan) are rendered as stick models.

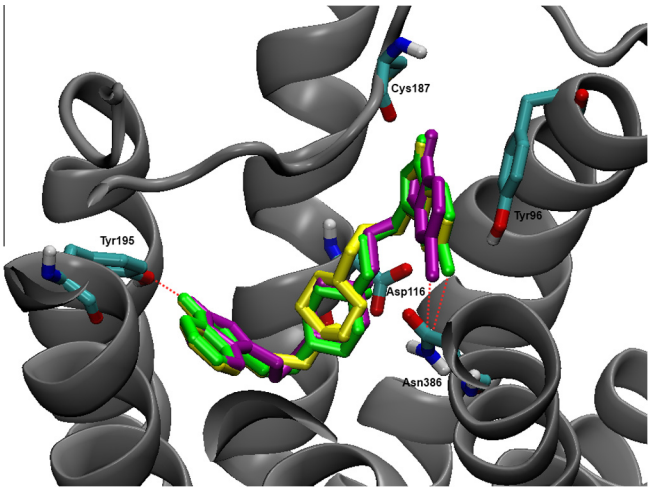


Figure 3. Superimposed structures of compounds **3a** (yellow), **5a** (purple) and **5b** (green) docked into the binding site of the 5-HT_{1A} receptor. Main binding site amino acid residues (cyan) are rendered as stick models.

introduction of two fluorine atoms at positions C5 and C5′ of the indole moieties (**3b**), generates an increase in affinity as compared with the unsubstituted derivative (**3a**). This result agrees with the observation that a fluorine atom at position 5 increases the SERT affinity of indolylpropyl derivatives.[3] This could be attributed to either an increase in the polarizability of neighboring hydrogens and the N–H bond (on the indole moiety) due to the electron-withdrawing nature of fluorine, or to a direct interaction of the halogen with a protein residue, or both.[7] This assumption is strongly supported by the results of molecular simulation. Figure 1A shows a general view of the binding site detected for all compounds tested. This site roughly coincides with the binding pocket described for tricyclic antidepressants in LeuTAa,[8,9] the bacterial homolog of the SERT, whose crystal structure was used to build the monoamine transporter model (see Methods). As shown in Figure 1B–D, docking experiments in the SERT for **3a**, **5b** and **3b**, revealed that these compounds exhibit similar binding modes, where a coulombic interaction between the protonated nitrogen atom of the piperazine ring (N-4) and Asp400 appears as a critical interaction (a similar binding mode was also observed for compound **5c**, although for the sake of clarity it is not shown in the figure). In addition, drugs docked into the SERT with one of the indole moieties establishing a π–cation interaction with Arg104 and van der

Waals interactions with Pro403, and the other forming a hydrogen bond between the indole N–H with Glu494 and a π–cation interaction with Arg564 (**3a**; Fig. 1B). Interestingly, an additional interaction with Leu563 arises due to the presence of a fluorine atom at C5 on the indole ring (**5b**; Fig. 1C), whereas an extra fluorine atom at C5′ (**3b**) leads to a new interaction (hydrogen bond) with Thr490 (**3b**; Fig. 1D). Thus, computational results allow the obtained experimental data to be rationalized.

Nevertheless, no other obvious structure-activity relationships can be established following the qualitative analysis (electronic and/or steric effects) of the different substituents located at positions C5 and C5′. For instance, in compounds **5d** and **3c** the presence of either one or two methoxyl groups leads to a gradual decrease in affinity as compared with the unsubstituted analog (**3a**). Considering only steric factors, one might expect a similar behavior when one or two bromine atoms are introduced at these positions. However, the dibrominated derivative (**3d**) showed higher affinity for SERT than the monosubstituted analog (**5a**). According to docking simulations, these results might be explained by the fact that compounds with the lowest affinities adopt different poses at the binding site, which prevent the analysis of all drugs as a sole family with similar binding modes. Figure 2 illustrates this finding by showing the binding modes of compounds **3b**, **3c** and **5a**, which displayed the highest and lowest affinities for the SERT, respectively. As can be seen, the least potent compounds (**3c** and **5a**) exhibit binding modes that clearly differ from that obtained for the difluorinated derivative **3b**.

2.2.2. 5-HT$_{1A}$ receptor

In contrast to SERT measured affinities, most of the assayed compounds displayed affinities in the micromolar range for the human 5-HT$_{1A}$ receptor, except the monohalogenated derivatives **5a** and **5b** which showed marked affinities for this target (IC_{50} = 15 and 16 nM, respectively). According to docking experiments, all our compounds exhibit a binding mode in which the main interaction was the well-described hydrogen bond between the protonated nitrogen atom of the piperazine ring and Asp116.[5,10–12] In the case of the unsubstituted compound **3a**, as well as the monohalogenated derivatives **5a** and **5b**, one of the indole moieties appeared located in a position favorable to establish a direct interaction with Cys187 and a π–π interaction with Tyr96, whereas the other indole establishes a hydrogen bond with the hydroxyl group of Tyr195 (Fig. 3). Thus, as illustrated in Figure 3, the increase in the affinity of the monohalogenated derivatives (**5a** and **5b**) as compared with their unsubstituted analog (**3a**) might be due to the appearance of an additional interaction between the halogen and Asn386.

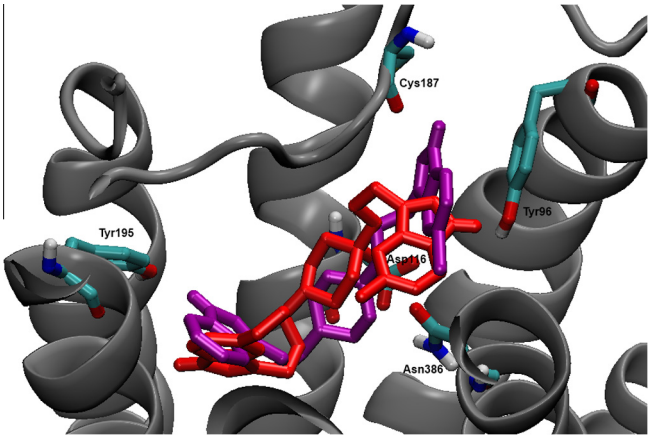


Figure 4. Superimposed structures of compounds **5a** (purple) and **5c** (red) docked into the binding site of the 5-HT$_{1A}$ receptor. Main binding site amino acid residues (cyan) are rendered as stick models.

The compounds with the lowest affinities, that is, **3b**, **5c**–**e**, adopted an orientation in which their indole moieties did not show the previously described interactions. This is illustrated in Figure 4, where the most and least potent compounds (**5a** and **5c**, respectively) are superimposed. As can be seen, the halogenated indole moiety of **5c** (red in Fig. 4) appears in an unfavorable position to establish interactions with Tyr96, Cys187 and Asn386, whereas the other indole ring does not display the interaction with Tyr195. Interestingly, compounds **3c**, **3d** and **5f**, which showed intermediate affinity values, docked into the 5-HT$_{1A}$ receptor with both indole moieties located between the most and the least favorable positions.

3. Conclusions

It is worth pointing out that at the least two compounds (**5b** and **5f**) showed high and similar affinities for the SERT and the 5-HT$_{1A}$ receptor. This result is in agreement with our proposal that bis-indole derivatives can bind both targets, and could serve as leads in the quest of ligands with a dual mechanism of action. Conversely, compounds **3a**, **3b**, **5c**–**e** exhibited a clear selectivity for the SERT. It is worth mentioning that this diversity of effects was obtained with a small and relatively homogeneous series of bivalent compounds, which highlights the importance of doing further studies in order to understand the basis underlying the structure-activity relationships of this type of drugs, since they might behave differently from their monovalent counterparts. In addition, further studies are necessary to evaluate the selectivity of these compounds over other monoaminergic receptors such as 5-HT$_{2A/2C}$, D_1–D_2 and/or α_1–α_2, as well as to assess whether they are agonists or antagonists at the addressed targets.

4. Experimental

4.1. Chemistry

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 ^1H 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. Microanalyses were carried out on a Fisons EA 1108 analyzer. High resolution mass spectra were recorded on a Thermo Finnigan MAT 95XP mass spectrometer. 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.

4.2. General procedure for the synthesis of 3-(1*H*-3-Indolyl)-1-propanol derivatives (1a–d). 3-(1*H*-3-Indolyl)-1-propanol (1a) as a model

To a stirred solution of commercial phenylhydrazine HCl (1.01 g, 6.9 mmol) in NN-DMA (10 mLl), H_2SO_4 4% w/w (10 mL, 0.04 mmol), and 3,4-dihydro-2*H*-pyran (0.63 mL, 6.9 mmol) were added, and the mixture was heated to 100 °C and then kept at reflux temperature for 2 h. After this time the reaction was quenched with water (50 mL) and extracted with AcOEt (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography on silica gel (AcOEt) to furnish 889 mg (74%) of a brown oil. IR cm^{-1}: 3417 (N–H), 3329 (O–H), 3055 (C–H arom), 2926 (C–H

aliph.). ^{1}H NMR ($CDCl_3$) δ: 2.02 (m, 2H, –CH_2–), 2.87 (t, 2H, Ar-CH_2–, J = 7.5 Hz), 3.72 (t, 2H, –CH_2–OH, J = 6.5 Hz), 6.93 (m, 1H, 2-H), 7.14 (t, 1H, 6-H, J = 7.0 Hz), 7.22 (td, 1H, 5-H, J_0 = 8.0 Hz, J_m = 1.1 Hz), 7.34 (d, 1H, 7-H, J = 7.4 Hz), 7.64 (d, 1H, 5-H, J = 7.8 Hz), 8.17 (s, 1H, N–H). ^{13}C–NMR (DMSO-d_6): 21.8, 33.3, 63.0, 111.6, 116.2, 119.3, 119.5, 121.9, 122.3, 127.9, 136.8. Anal. Calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.35; H, 7.42; N, 7.89.

4.2.1. 3-(5-Bromo-1*H*-3-indolyl)-1-propanol (1b)

Prepared from 4-bromo-phenylhydrazine HCl (0.49 g, 2.23 mmol), H_2SO_4 4% w/w (5.0 mL, 1.12 mmol), 3,4-dihydro-2*H*-pyran (0.20 mL, 2.14 mmol) in NN-DMA (3.0 mL), to afford pure **1b**, (0.31 g, 54%) as an oily product. IR (cm^{-1}): 3424–3250 (O–H and N–H), 2938–2879 (C–H aliph), 1459 (C=C arom.). ^{1}H NMR (DMSO-d_6): 1.78 (m, 2H, –CH_2–), 2.70 (t, 2H, Ar-CH_2–, J = 7.6 Hz), 3.47 (c, 2H, –CH_2–OH, J = 5.9 Hz), 4.46 (t, 1H, OH), 7.15–7.18 (m, 2H, 2-H and 6-H), 7.31 (d, 1H, 7-H, J = 8.6 Hz), 7.68 (d, 1H, 4-H, J = 1.4 Hz), 10.97 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6): 20.2, 32.8, 59.8, 110.3, 112.8, 113.9, 120.1, 122.7, 123.4, 128.6, 134.4. Anal. Calcd for $C_{11}H_{12}BrNO$: C, 51.99; H, 4.76; N, 5.51. Found: C, 51.75; H, 4.83, N, 5.53.

4.2.2. 3-(5-Fluoro-1*H*-3-indolyl)-1-propanol (1c)

Prepared from 4-fluoro-phenylhydrazine HCl (0.25 g, 1.54 mmol), H_2SO_4 4% w/w (2.5 mL, 0.01 mmol), 3,4-dihydro-2*H*-pyran (0.63 mL, 6.9 mmol) in NN-DMA (3.0 mL), to afford pure **1c** (0.155 g, 52%) as an oily product. IR (cm^{-1}): 3629–3250 (O–H y N–H), 3060 (C–H arom.), 2939 (C–H aliph), 1581 (C=C). ^{1}H NMR ($CDCl_3$): δ: 1.92 (2H, q, –CH_2–, J = 7.4 Hz), 2.21 (1H, br s, O*H*), 2.76 (2H, t, Ar-CH_2– J = 7.5 Hz), 3.68 (2H, t, –CH_2–OH, J = 6.5 Hz), 6.90 (1H, dt, J = 9.1 and 2.5 Hz), 6.96 (1H, d, 2-H, J = 2.0 Hz), 8.28 (1H, br s, –NH–), 7.20 (1H, d, J = 8.6 Hz, H-4 or H-7), 7.22 (1H, d, J = 8.4 Hz, H-7 or H-4). ^{13}C NMR: 21.3, 32.7, 62.4, 103.7 (d, $^2J_{C-F}$ = 23.2 Hz), 110.1(d, $^{2'}J_{C-F}$ = 26.3 Hz), 111.8 (d, $^{3'}J_{C-F}$ = 9.6 Hz), 115.8 (d, $^4J_{C-F}$ = 4.8 Hz), 123.4, 127.8 (d, $^3J_{C-F}$ = 9.6 Hz), 132.9, 157.6 (d, $^1J_{C-F}$ = 234 Hz). Anal. Calcd for $C_{11}H_{12}FNO$: C, 68.38; H, 6.26; N, 7.25. Found: C, 68.33; H, 6.31, N, 7.29.

4.2.3. 3-(5-Methoxy-3-indolyl)-1-propanol (1d)

Prepared from 4-methoxyphenylhydrazine HCl (0.5 g, 2.86 mmol), H_2SO_4 4% w/w (5.0 mL, 1.12 mmol), 3,4-dihydro-2*H*-pyran (0.26 mL, 2.86 mmol) in NN-DMA (5.0 mL), to afford pure **1d**, (0.44 g, 75%) as an oily product. IR (cm^{-1}): 3607–3200 (O–H and N–H), 2937–2850 (C–H), 1485–1624 C=C), 1213 (C–O), 1033 (C–O). ^{1}H NMR ($CDCl_3$) δ: 1.92 (q, 2H, –CH_2– J = 6.9 Hz), 2.40 (s, 1H, OH), 2.75 (t, 2H, Ar-CH_2– J = 7.3 Hz), 3.65 (t, 2H, –CH_2–OH, J = 6.50 Hz), 3.81 (s, 3H, OMe), 6.80–6.84 (m, 2H, 4-H and 6-H), 7.02 (d, 1H, 2-H J = 2.0 Hz), 7.13 (d, 1H, 7-H, J = 8.8 Hz), 8.14 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6) δ: 21.2, 32.5, 55.9, 62.3, 100.7, 111.7, 111.8, 115.2, 122.3, 127.6, 131.5, 153.5. HRMS: (EI) Calcd for $C_{12}H_{15}NO_2$ (M+): 205.11028. Found: 205.11115.

4.3. General procedure for the synthesis of 3-(1*H*-3-Indolyl)-1-propyl-4-methylbenzenesulfonate derivatives (2a–d). 3-(1*H*-3-Indolyl)-propyl-4-methylbenzenesulfonate (2a) as a model

To a solution of 3-(1*H*-3-indolyl)-1-propanol (0.89 g, 5.10 mmol) in dry CH_2Cl_2 (30 mL), triethylamine (0.3 mL, 2.17 mmol) tosyl chloride (1.17 g., 6.1 mmol) and catalytic concentrations of dimethylaminopyridine (DMAP) were added. The mixture was stirred at room temperature for 25 h, after this time the mixture was concentrated under vacuum and purified by column chromatography on silica gel AcOEt/*n*-Hex (1:2) to afford **2a** (1.08 g, 64%) as a pure solid. Mp 84–86 °C. IR cm^{-1}: 3386 (N–H), 3038 (C–H arom.), 2929–2903 (C–H aliph.). ^{1}H NMR ($CDCl_3$) δ: 2.05 (m, 2H, –CH_2–), 2.44 (s, 3H, CH_3–Ts), 2.82 (t, 2H, Ar-CH_2–, J = 7.3 Hz), 4.09 (t, 2H, –CH_2– OTs, J = 6.2 Hz), 6.92 (d, 1H, 2-H, J = 2.1 Hz), 7.10 (t, 1H, 6-H, J = 7.0 Hz), 7.19 (t, 1H, 5-H, J = 7.50 Hz), 7.32 (d, 2H, 3-H and 5-H Ts, J = 8.5 Hz), 7.35 (d, 1H, 4-H, J = 9.5 Hz), 7.50 (d, 1H, 7-H, J = 7.8 Hz), 7.77 (d, 2H, 2-H and 6-H, Ts, J = 8.5 Hz), 7.87 (br s 1H, N–H). ^{13}C NMR (DMSO-d_6): 21.3, 22.0, 29.5, 70.5, 111.6, 114.8, 119.0, 119.7, 122.2, 122.4, 127.6, 128.3 (2×), 130.2 (2×), 133.6, 136.8, 145.1. Anal. Calcd for: $C_{18}H_{19}N0_3S$ C (65.63), H (5.81), N (4.25), S (9.73); Found: C (65.68), H (5.68), N (4.33), S (9.75).

4.3.1. 3-(5-Bromo-3-indolyl)-propyl-4-methylbencensulfonate (2b)

Prepared from 3-(5-bromo-1*H*-3-indolyl)-1-propanol (0.24 g, 0.95 mmol), tosyl chloride (0.20 g, 1.04 mmol), triethylamine (0.15 mL, 1.08 mmol) and DMAP, to afford pure **2b** as a brown oil (0.20 g, 52%). IR (cm^{-1}): 3419 (N–H), 1596 (C–H arom), 1360 (SO_2). ^{1}H NMR (DMSO-d_6): 1.90 (m, 2H, –CH_2–), 2.41 (s, 3H, CH_3–Ts), 2.63 (t, 2H, Ar-CH_2–, J = 7.3 Hz), 4.04 (t, 2H, –CH_2– OTs, J = 6.3 Hz), 7.08 (d, 1H, 2-H, J = 2.2 Hz), 7.16 (dd, 1H, 6-H, J_0 = 8.6 Hz, J_m = 1.9 Hz), 7.29 (d, 1H, 7-H, J = 8.6 Hz), 7.45 (d, 2H, 3-H and 5-H, Ts, J = 8.2 Hz), 7.61 (d, 1H, 4-H, J = 1.7 Hz), 7.77 (d, 2H, 2-H and 6-H Ts, J = 8.2 Hz), 11.1 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6): 19.7, 20.6, 28.4, 70.0, 110.4, 112.2, 112.8, 120.0, 122.8, 123.6, 127.0(2×), 128.3, 129.6(2×), 132.0, 134.4, 144.3. Anal. Calcd for: $C_{18}H_{18}BrNO_3S$. C (52.95), H (4.44), N (3.43); Found: C (53.04), H (4.49), N (3.56).

4.3.2. 3-(5-Fluoro-1*H*-3-indolyl)-propyl-4-methylbencensulfonate (2c)

Prepared from 3-(5-Fluoro-1*H*-3-indolyl)-1-propanol. (0.35 g, 2.17 mmol), tosyl chloride (0.41 g, 2.17 mmol), triethylamine (0.30 mL, 2.17 mmol) and DMAP (as a catalyst), to afford pure **2c** as a brown solid (0.32 g, 51%). Mp: 75–77 °C. IR (cm^{-1}): 3391 (N–H), 1396 (SO_2), 1356 (SO_2). ^{1}H NMR (DMSO-d_6): 2.02 (q, 2H, –CH_2–, J = 7.0 Hz), 2.47 (s, 3H, CH_3–Ts), 2.77 (t, 2H, Ar-CH_2– J = 7.3 Hz), 4.09 (t, 2H, –CH_2–OTs, J = 6.2 Hz), 6.94 (m, 2H, 2-H y 6-H), 7.13 (dd, 1H, 4-H, J = 9.6 and 2.4 Hz), 7.28 (dd, 1H, J = 8.7 and 4.4 Hz, 7-H), 7.35 (d, 2H, 3-H and 5-H, Ts, J = 8.3 Hz), 7.81 (d, 2H, 2-H and 6-H Ts, J = 8.3 Hz), 8.05 (br s, 1H, –NH–). ^{13}C NMR (DMSO-d_6) δ: 21.2, 22.1, 29.3, 70.3, 103.9 (d, $^2J_{C-F}$ = 23.2 Hz), 110,8 (d, $^{2'}J_{C-F}$ = 26.3 Hz), 112,2 (d, $^{3'}J_{C-F}$ = 9.7 Hz), 114,9 (d, $^4J_{C-F}$ = 4,8 Hz), 124.0, 127.9 (d, $^3J_{C-F}$ = 9.5 Hz), (2×)128.3, (2×) 130.3, 133.2,133.4, 145.2, 158.0 (d, $^1J_{C-F}$ = 234 - Hz). HRMS: (EI) Calcd for $C_{18}H_{18}FNO_3S$ (M+): 347.09914. Found: 347.09929.

4.3.3. 3-(5-Methoxy-1*H*-3-indolyl)propyl-4-methylbencensulfonate (2d)

Prepared from 3-(5-methoxy-1*H*-3-indolyl)-1-propanol (0.51 g, 2.49 mmol), tosyl chloride (0.49 g, 2.49 mmol), triethylamine (0.34 mL, 2.49 mmol) and DMAP, to afford pure **2d** as a brown liquid (0.72 g, 80%). IR (cm^{-1}): 3416 (N–H), 3055 (C–H arom), 2982–2826 (C–H aliph), 1208 (C–O), 1171 (S=O). ^{1}H NMR ($CDCl_3$) δ: 2.02 (q, 2H, –CH_2–, J = 7.0 Hz), 2.43 (s, 3H, CH_3–Ts), 2.77 (t, 2H, Ar-CH_2–, J = 7.3 Hz), 3.85 (s, 3H, OMe), 4.08 (t, 2H, –CH_2–OTs, J = 6.3 Hz), 6.84 (dd, 1H, 6-H, J_0 = 8.8 Hz and J_m = 2.3 Hz), 6.88 (d, 1H, 2-H, J = 1.2 Hz), 6.94 (d, 1H, 4-H, J = 2.0 Hz), 7.23 (d, 1H, 7-H, J = 8.8 Hz), 7.30 (d, 2H, 3-H and 5-H, Ts, J = 8.1 Hz), 7.77 (d, 2H, 2-H and 6-H, Ts, J = 8.2 Hz), 7.89 (br s 1H, N–H). ^{13}C NMR (DMSO-d_6) δ: 20.8, 21.6, 28.9, 55.9, 70.0, 100.5, 111.8, 112.1, 114.1, 122.5, 127.5, 127.8, 129.8, 131.4, 133.1, 144.7, 153.8. HRMS: (EI) Calcd for $C_{19}H_{21}NO_4S$ (M+) = 359.11913 Found: 359.11981.

4.4. General procedure for the synthesis of 1,4-bis(3-(1*H*-3-indolyl)propyl)piperazine derivatives 3(a–d). 1,4-Bis(3-(1*H*-3-indolyl)propyl)piperazine (3a) as a model

To a solution of 3-(3-indolyl)propyl-4-methylbencensulfonate (**2a**) (0.420 g, 1.28 mmol) in CH_3CN (25 mL), K_2CO_3 (0.117 g, 1.28 mmol) and piperazine (0.055 g, 0.64 mmol) were added. The mixture was refluxed for 6 h and then poured onto water (200 mL), extracted with AcOEt (3 × 50 mL) and dried (Na_2SO_4). The crude was purified by column chromatography, eluting with MeOH/CH_2Cl_2 (1:8) to give pure **3a** (180 mg, 70% yield). Mp: 180–182 °C. IR (cm^{-1}): 3413 (N–H), 3053 (C–H arom), 1457 (C=C). ^1H NMR ($CDCl_3$) δ: 1.81 (q, 4H, CH_2–C*H*$_2$–CH_2, J = 7.1 Hz), 2.43 (t, 4H, –CH_2– pip, J = 7.4 Hz), 2.49 (br s 8H, pip.), 2.68 (t, 4H, Ar-CH_2–, J = 7.4 Hz), 6.95 (t, 1H, 5-H or 6- H, J = 7 Hz), 7.05 (t, 1H, 6-H or 5-H, J = 7.0 Hz), 7.10 (d, 1H, 2-H, J = 1.4 Hz), 7.32 (d, 1H, 7-H, J = 7.9 Hz), 7.49 (d, 1H, 4-H, J = 7.8 Hz), 10.80 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: (2×)22.8, (2×)27.2, (4×)52.6, (2×)57.7, (2×)111.8, (2×)114.6, (2×)118.5, (2×)118.7, (2×)121.2, (2×)122.6, (2×)127.6, (2×)136.8. HRMS: (EI) Calcd for $C_{26}H_{32}N_4$ (M+) = 400.2627, Found: 400.2625.

4.4.1. 1,4-Bis(3-(5-bromo-1*H*-3-indolyl)propyl)piperazine (3b)

Prepared from 3-(5-bromo-3-indolyl)propyl-4-methylbencensulfonate (0.370 g, 0.91 mmol), anhydrous potassium carbonate (0.125 g, 0.91 mmol) and piperazine (0.040 g, 0.46 mmol), to afford pure **3b**, (0.191 g, 76%). Mp: 163–165 °C. IR (cm^{-1}): 3421 (N–H), 3022 (C–H arom), 2867–2830 (C–H aliph), 1451 (C=C arom). ^1H NMR (DMSO-d_6): 1.75 (q, 4H, CH_2–C*H*$_2$–CH_2, J = 7.9 Hz), 2.30 (t, 4H, –CH_2–pip, J = 7.3 Hz), 2.39 (br s 8H, pip.), 2.66 (t, 4H, Ar-CH_2–, J = 7.1 Hz), 7.13–7.17 (m, 4H, 2-H and 6-H), 7.30 (d, 2H, 7-H, J = 8.6 Hz), 7.69 (d, 2H, 4-H, J = 1.6 Hz), 11,0 (s,1H, N–H). ^{13}C NMR (DMSO-d_6): (2×)21.4, (2×)26.7, (4x)52.3, (2×)56.8, (2×)110.2, (2×)112.7, (2×)113.8, (2×)120.1, (2×)122.6, (2×)123.4, (2×)128.7, (2×)134.3. HRMS: (EI) Calcd for $C_{26}H_{30}Br_2N_4$ (M+): = 556.08372, Found: 556.08437.

4.4.2. 1,4-Bis(3-(5-fluoro-1*H*-3-indolyl)propyl)piperazine (3c)

Prepared from 3-(5-fluoro-3-indolyl)propyl-4-methylbencensulfonate (0.350 g, 1.0 mmol), anhydrous potassium carbonate (0.138 g, 1.0 mmol) and piperazine (0.043 g, 0.50 mmol) to afford pure **3c**, (0.160 g, 73%). Mp: 189–191 °C. IR (cm^{-1}): 3422 (N–H), 3059 (C–H, arom), 2954–2820 (C–H aliph), 1626 (C=C), 1585 (C=C). ^1H NMR(DMSO-d_6): 1.62 (q, 4H, CH_2–C*H*$_2$–CH_2, J = 7.1 Hz), 2.19 (t, 4H, –CH_2–pip, J = 7.4 Hz), 2.29 (br s 8H, pip.), 2.50 (t, 4H, Ar-CH_2, J = 7.4 Hz), 6.74 (dt, 2H, 6-H, J_0 = 9.1 Hz, J_m = 2.4 Hz), 7.18 (s, 2H, 2-H), 7.24 (dd, 2H, 4-H, J_0 = 10.1 Hz, J_m = 2.4 Hz), 7.30 (dd,2H, 7-H, J_0 = 9.1 Hz, J_m = 4.6 Hz), 10.80 (s, 2H, N-H). ^{13}C-NMR (DMSO-d_6) δ: (2×)22.6, (2×)27.4, (4x)53.1, (2×)57.8, (2×)103.4 (d, $^2J_{C-F}$ = 22.8 Hz), (2×)109.3 (d, $^{2'}J_{C-F}$ = 26 Hz), (2×)112.6 (d, $^3J_{C-F}$ = 9.7 Hz), (2×)115.1 (d, $^4J_{C-F}$ = 4.7 Hz), (2×)124.8, 127.9 (d, $^{3'}J_{C-F}$ = 9.6 Hz), (2×)133.0, (2×)157.0 (d, $^1J_{C-F}$ = 231 Hz). HRMS: (EI) Calcd for $C_{26}H_{30}F_2N_4$ (M+): 436.24385. Found: 436.24425.

4.4.3. 1,4-Bis(3-(5-methoxy-1*H*-3-indolyl)propyl)piperazine (3d)

Prepared from 3-(5-methoxy-3-indolyl)propyl-4-methylbenzenesulfonate (0.3 g, 0.84 mmol), anhydrous potassium carbonate (0.116 g, 0.84 mmol) and piperazine (0.036 g, 0.42 mmol) to afford pure **3d**, (0.120 g, 62%). Mp: 178.0–180.0 °C. IR (cm^{-1}): 3221 (N–H), 3038 (C–H arom), 2994–2947 (C–H aliph), 1213 (C–O). ^1H NMR ($CDCl_3$) δ: 1.78 (m, 4H, CH_2–C*H*$_2$–CH_2), 2.37 (m, 4H, –CH_2–pip), 2.44 (br s, 8H, pip), 2.65 (t, 4H, Ar-CH_2, J = 7.2 Hz), 3.75 (s, 6H, OMe), 6.70 (br d 2H, 6-H, J = 8.7 Hz), 6.95 (s, 2H, 4-H), 7.05 (s, 2H, 2-H), 7.21 (d, 2H, 7-H, J = 8.7 Hz), 10.59 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) δ: (2×)21.8, (2×)27.8, (4×)54.8, (2×)57.2, (2×)99.6, (2×)110.4, (2×)111.4, (2×)113.4, (2×)122.4, (2×)126.9, (2×)127.5, (2×)130.9, (2×)152.3. HRMS: (EI) Calcd for $C_{28}H_{36}N_4O_2$ (M+): = 460.28383, Found: 460.28376.

4.5. General procedure for the synthesis of C-5-substituted 3-(3-Piperazinyl-1-propyl)-1*H*-indole derivatives 4(a–d). 3-(3-Piperazinyl-1-propyl)-1*H*-indol 4a as a model

A mixture of 3-(3-indolyl)propyl-4-methylbenzenesulfonate (0.5 g, 2.84 mmol) in acetonitrile (50 mL), anhydrous potassium carbonate (0.41 g, 3.0 mmol) and piperazine (0.49 g, 5.68 mmol) was stirred under reflux for 6 h. After this time water was added (30 mL) and the solution 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 the organic solvent evaporated to obtain a crude that was purified by column chromatography on silica gel (CH_2Cl_2, CH_3OH, $N(Et)_3$) (18:3:1) to give pure indolylpiperazine **4a** (0.50 g, 72%). Mp: 80–82 °C. IR (cm^{-1}): 3409–3295 (N–H indol and N–H pip.), 3054 (C–H arom), 2937–2840 (C–H aliph). ^1H NMR ($CDCl_3$) δ: 1.91 (q, 2H, CH_2–C*H*$_2$–CH_2-Pip, J = 7.6 Hz), 2.40–2.53 (m, 7H, N–H, *CH*$_2$-Pip, 2-H and 6-H Pip.), 2.76 (t, 2H, Ar-CH_2, J = 7.6 Hz), 2.91 (t, 4H, 3-H and 5-H Pip, J = 4.9 Hz), 6.93 (s, 1H, 2-H), 7.09 (t, 1H, 6-H, J = 7.0 Hz), 7.16 (t, 1H, 5-H, J = 7.0 Hz), 7.31 (d, 1H, 4-H J = 7.9 Hz), 7.59 (d, 1H, 7-H, J = 7.8 Hz), 8.49 (br s 1H, N–H). ^{13}C NMR (DMSO-d_6): 22.9, 27.0, (2×)45.8, (2×)54.3, 58.9, 111, 116.1, 118.8, 118.9, 121.1, 121.7, 127.5, 136.3. Anal. Calcd for $C_{15}H_{21}N_3$: C,74.03; H,8.70; N,17.27. Found: C, 74.61; H, 8.58; N, 17.22.

4.5.1. 5-Bromo-3-(3-piperazinyl-1-propyl)-1*H*-indol (4b)

Prepared from 3-(5-bromo-3-indolyl)-propyl-4-methylbenzenesulfonate (0.52 g, 1.50 mmol), anhydrous potassium carbonate (0.248 g, 1.80 mmol) and piperazine (0.25 g, 3.0 mmol), to afford **4b** as a pale-brown oil. Pure yield (0.23 g, 62%). IR (cm^{-1}): 3420 (N–H), 3020 (C–H arom), 2940–2880 (C–H aliph), 1463 (C=C arom). ^1H NMR: 1.72 (q, 2H, CH_2–C*H*$_2$–CH_2-Pip, J = 7.9 Hz), 2.30 (t, 2H, *CH*$_2$-Pip, J = 7.4 Hz), 2.57 (m, 4H, 2-H and 6-H,Pip), 2.80 (t, 2H, Ar-CH_2-, J = 7.8 Hz), 3.02 (m, 4H, 3-H and 5-H, Pip), 5.43 (1H, NH pip), 7.10–7.16 (m,2H, 6-H and 2-H), 7.33 (d, 1H, 7-H, J = 8.5 Hz), 7.62 (d, 1H, 4-H, J = 1.6 Hz), 11.58 (s,1H, NH). ^{13}C-NMR ($CDCl_3$): 22.2, 26.9, 2×(45.8), 2×(52.8), 57.2, 110.3, 112.6, 113.3, 120.8, 122.7, 123.3, 129.0, 135.0. Anal. Calcd for $C_{15}H_{20}BrN_3$: C, 55.91; H, 6.26; N, 13.04. Found: C, 55.76; H, 6.22; N, 12.92.

4.5.2. 5-Fluoro-3-(3-piperazinyl-1-propyl)-1*H*-indol (4c)

Prepared from 3-(5-fluor-3-Indolyl)-propyl-4-methylbenzenesulfonate (0.52 g, 1.50 mmol), anhydrous potassium carbonate (0.248 g, 1.80 mmol) and piperazine (0.26 g, 3.0 mmol) to afford pure **4c** (0.294, 75%). Mp: 168–170 °C. IR (cm^{-1}): 3387–3240 (N–H pip and N–H indol), 3030 (C–H arom), 2976–2938 (C–H aliph), 1475 (C=C arom). ^1H NMR (DMSO-d_6): 1.86 (m, 2H, CH_2–C*H*$_2$–CH_2–Pip), 2.62–2.83 (m, 6H, CH_2-Pip, 2-H and 6-H Pip.), 3.02–3.08 (m, 6H, Ar-CH_2, 3-H and 5-H Pip.), 3.60 (s, 1H, N–H), 6.87 (m, 1H, 6-H), 7.10–7.40 (m, 3H, 2-H, 4-H, 7-H), 11.2 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6) δ: 21.4, 25.6, (2×)48.3, (2×)51.6, 56.3, 102.3 (d, $^2J_{C-F}$ = 22.9 Hz), 108.4 (d, $^{2'}J_{C-F}$ = 26.2 Hz), 111.8 (d, $^{3'}J_{C-F}$ = 9.8 Hz), 113.7 (d, $^4J_{C-F}$ = 4.6 Hz), 123.7, 126.7 (d, $^{3'}J_{C-F}$ = 9.4 Hz), 132.2, 156 (d, $^1J_{C-F}$ = 231 Hz). HRMS (EI) Calcd for $C_{15}H_{21}FN_3^+$ (M+1) = 262.1719, Found: 262.1718.

4.5.3. 5-Methoxy-3-(3-piperazinyl-1-propyl)-1*H*-indol (4d)

Prepared from 3-(5-methoxy-3-indolyl)-propyl-4-methylbenzenesulfonate (0.35 g, 0.98 mmol), anhydrous potassium carbonate (0.275 g, 2.00 mmol) and piperazine (0.172 g, 2.0 mmol), to afford **4d** as a pale-brown oil. Pure yield (0.190 g, 70%). IR (cm^{-1}): 3406 (N–H), 2942 (C–H aliph), 1623 (C=C arom), 1215 (C–O). ^1H NMR ($CDCl_3$) δ: 1.87 (m, 4H, CH_2–C*H*$_2$–CH_2-Pip), 2.48 (t, 2H, –CH_2-Pip,

J = 7.0 Hz), 2.68 (m, 4H, 2-H and 6-H Pip), 2.72 (t, 2H, Ar-CH_2–J = 7.4 Hz), 3.10 (t, 4H, 3-H and 5-H Pip, J = 4.4 Hz), 3.84 (s, 3H, OMe), 5.47 (bs, 1H, N-H), 6.78 (dd, 1H, 6-H, J_0 = 8.8 Hz, J_m = 2.3 Hz), 6.9 (s, 1H, 2-H), 7.0 (d, 1H, 4-H, J_m = 2.3 Hz), 7.28 (d, 1H, 7-H, J_0 = 8.8 Hz), 9.71 (bs, 1H, N–H). ^{13}C NMR $CDCl_3$): 23.3, 27.3, 46.9, 54.0, 56.2, 59.2, 110.9, 111.9, 112.3, 122.5, 126.1, 128.0, 131.9, 153.7. HRMS (EI) Calcd for $C_{16}H_{23}N_3O$ (M+1) = 274.1919, Found: 274.1921.

4.6. General procedure for the synthesis of 3-(3-{4-[3-(3-indolyl)propyl]-1-piperazinyl}propyl)indol derivatives 5(a–f). 3-(3-{4-[3-(1*H*-3-indolyl)propyl]-1-piperazinyl}propyl)-5-methoxy-1*H*-indol (5d) as a model

A mixture of 3-(5-methoxy-3-indolil)propyl-4-methylbenzenesulfonate (0.155 g, 0.43 mmol) in acetonitrile (10 mL), anhydrous potassium carbonate (0.072 g, 0.52 mmol) and 3-(3-piperazinyl-1-propyl)-indol **4a** (0.1 g, 0.43 mmol) was stirred under reflux for 6 h. After this time water was added (35 mL) and extracted with AcOEt (3 × 50 mL). The combined organic layers were washed and dried over anhydrous Na_2SO_4. The organic portions were filtered and the organic solvent evaporated to obtain a crude that was purified by column chromatography (MeOH/CH_2Cl_2, 1:4) to afford pure **5d** (0.080 g, 43%). Mp: 139–140 °C, IR (cm^{-1}): 3223 (N–H), 3051 (C–H arom), 2944–2822 (C–H aliph), 1215 (C–O). ^{1}H NMR ($CDCl_3$) δ: 1.79 (m, 4H, (2×)CH_2–CH_2–CH_2-Pip), 2.37 (m, 4H, (2×)CH_2-Pip), 2.45 (br s, 8H, Pip), 2.65 (m, 4H, (2×)Ar-CH_2), 3.75 (s, 3H, OMe), 6.70 (dd, 1H, indole C-5 methoxylated, 6-H, J_0 = 8.7 and J_m = 2.3 Hz), 6.95 (m, 2H, 2-H and 6-H,unsubstituted indole ring), 7.49 (d, 1H, indole C-5 methoxylated, 7-H, J = 7.7 Hz), 7.06 (m, 2H, indole C-5 methoxylated, 2-H and unsubstituted indole ring 5-H), 7.15 (d, 1H, indole C-5 methoxylated, 4-H, J = 1.9 Hz), 7.32 (d, 1H, unsubstituted indole ring 7-H, J = 8.0 Hz), 7.21 (d, 1H, unsubstituted indole ring 4-H, J = 8.7 Hz), 10.67 (s, 1H, N–H), 10.8 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6): 2×(21.9), 4×(52.1), 3×(54.8), 2×(57.0), 99.5, 110.4, 110.8, 111.5, 113.6, 113.8, 117.6, 117.8, 120.3, 121.7, 122.4, 126.7, 127.0, 130.9, 135.8, 152.3. HRMS (EI) Calcd for $C_{27}H_{34}N_4O$ (M^+): 430.2732, Found: 430.27301.

4.6.1. 3-(3-{4-[3-(1*H*-3-Indolyl)propyl]-1-piperazinyl}propyl)-5-fluoro-1*H*-indol (5b)

Prepared from 3-(5-fluoro-3-indolyl)propyl-4-methylbenzenesulfonate (**2c**) (0.160 g, 0.46 mmol), anhydrous potassium carbonate (0.072 g, 0.52 mmol) and 3-(3-piperazinyl-1-propyl)-indol (**4a**) (0.108 g, 0.46 mmol), to afford pure **5b** (0.120 g, 62%). Mp: 193–194.5 °C. IR (cm^{-1}): 3263 (N–H), 3054 (C–H arom), 2963–2929 (C–H aliph), 1580 (C=C arom). ^{1}H NMR (DMSO-d_6) δ: 1.78 (m, 4H, (2×)CH_2–CH_2–CH_2-Pip), 2.34-2.43 (m, 12H, (2×)CH_2-Pip and 8H Pip), 2.66 (m, 4H, (2×)Ar-CH_2), 6.88 (td, 1H, indole C-5 fluorinated, 6-H, J_0 = 9.2 Hz, J_m = 2.4 Hz), 6.95 (t, 1H, unsubstituted indole ring 6-H, J = 7.4 Hz), 7.05 (t, 1H, unsubstituted indole ring 5-H, J = 7.1 Hz), 7.1 (d, 1H, unsubstituted indole ring 2-H, J = 1.7 Hz), 7.18 (d, 1H, indole C-5 fluorinated, 2-H, J = 1.8 Hz), 7.24 (dd, 1H, indole C-5 fluorinated, 7-H, J_0 = 10.1 and J_m = 2.4 Hz), 7.30 (d, 1H, indole C-5 fluorinated, 4-H, J = 6.6 Hz), 7.34 (d, 1H, unsubstituted indole ring 4-H, J = 7.3 Hz), 7.5 (d, 1H, unsubstituted indole ring 7-H, J = 7.7 Hz), 10.75 (s, 1H, N–H), 10.87 (s, 1H, N-H). ^{13}C NMR (DMSO-d_6): 21.7, 21.9, 26.4, 26.5, 4×(52.1), 56.8, 57.0, 102.4 (d, $^2J_{C-F}$ = 22.8 Hz), 108.3 (d, $^{2'}J_{C-F}$ = 26.2 Hz), 110.7, 111.6 (d, $^3J_{C-F}$ = 9.8 Hz), 113.8, 114.1(d, $^4J_{C-F}$ = 4.7 Hz), 117.5, 117.7, 120.3, 121.6, 123.9, 126.7, 126.9 (d, $^{3'}J_{C-F}$ = 9.6 Hz), 132.4, 135.8, 156.1(d, $^1J_{C-F}$ = 230.6 Hz). HRMS (EI) Calcd for $C_{26}H_{31}FN_4$ (M^+): 418.25327, Found: 418.25254.

4.6.2. 5-Fluoro-3-(3-{4-[3-(5-methoxy-1*H*-3-indolyl)propyl]piperazinyl}propyl)-1*H*-indol (5c)

Prepared from 3-(5-fluoro-3-indolyl)propyl-4-methylbenzenesulfonate (**2c**) (0.192 g, 0.54 mmol), anhydrous potassium carbonate (0.090 g, 0.65 mmol) and 3-(5-methoxy-3-piperazinyl-1-propyl)indol (**4d**) (0.150 g, 0.55 mmol) to afford pure **5c** (0.110 g, 45%). Mp: 156–158 °C. IR (cm^{-1}): 3226 (N–H), 3052 (C–H arom), 2947 (C–H aliph), 1582 (C=C arom), 1213 C–O). ^{1}H NMR ($CDCl_3$) δ: 1.87 (m,4H, (2×)CH_2–CH_2–CH_2-Pip), 2.52 (m, 4H, (2×)CH_2-Pip), 2.63 (br s, 8H, Pip.), 2.72 (m, 4H, Ar-CH_2–), 3.86 (s, 3H, OMe), 6.85 (dd, 1H, indole C-5 methoxylated, 4-H, J_0 = 8.8 Hz, J_m = 2.4 Hz), 6.92 (td, 1H, indole C-5 methoxylated, 6-H, J_0 = 9.1 Hz, J_m = 2.4 Hz), 6.97–7.03 (m, 4H, indole C-5 fluorinated, 2-H, 4-H, 6-H, and indole C-5 methoxylated, 2-H), 7.26 (d, 1H, indole C-5 fluorinated, 7-H, J = 8.7 Hz), 7.21 (dd, 1H, indole C-5 methoxylated, 7-H, J_0 = 9.5 J_m = 2.3 Hz), 7.98 (s, 1H, N–H), 8.12 (s, 1H, N–H). ^{13}C NMR ($CDCl_3$): 22.2, 22.3, 25.9, 26.1, 4×(52.0), 55.4, 57.3, 57.5, 100.1, 103.2 (d, $^2J_{C-F}$ = 23 Hz), 109.0 (d, $^{2'}J_{C-F}$ = 26.2 Hz), 111.2 (d, $^3J_{C-F}$ = 9.7 Hz), 111.3, 111.5, 115.0 (d, $^4J_{C-F}$ = 4.7 Hz), 115.5, 121.6, 122.6, 127.2 (d, $^{3'}J_{C-F}$ = 9.5 Hz), 128.5, 130.9, 132.2, 153.3, 156.4 (d, $^1J_{C-F}$ = 232.0 Hz). HRMS (EI) Calcd for $C_{27}H_{33}FN_4O$ (M^+): 448.26384, Found: 448.26293.

4.6.3. 5-Bromo-3-(3-{4-[3-(5-fluoro-1*H*-3-indolyl)propyl)piperazinyl)propyl)-1*H*-indol (5e)

Prepared from 3-(5-bromo-3-indolyl)propyl-4-methylbenzenesulfonate (**2b**) (0.240 g, 0.59 mmol), anhydrous potassium carbonate (0.098 g, 0.70 mmol) and 3-(5-fluoro-3-piperazinyl-1-propyl)-indol (**4c**) (0.155 g, 0.59 mmol) to afford **5e** (0.150 g, 52%). Mp: 181–182.5 °C. IR (cm^{-1}): 3228 (N–H), 3053 (C–H arom), 2952 C–H aliph.), 1580 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.76 (br s, 4H, (2×)CH_2–CH_2–CH_2-Pip), 2.30 (m, 4H, (2×)CH_2-Pip), 2.38 (br s, 8H, Pip.), 2.65 (m, 4H, (2×)Ar-CH_2), 6.88 (t, 1H, indole C-5 fluorinated, 6-H, J = 9 Hz), 7.13–7.33 (m,6H, indole C-5 brominated, 2-H, 6-H, 7-H, and indole C-5 fluorinated, 2-H, 4-H and 7-H), 7.68 (s, 1H, indole C-5 brominated, 4-H), 10.9 (s, 1H, N–H), 11.0 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6): 21.4, 21.7, 26.6, 26.7, 4×(52.4), 56.8, 57.0, 102.4 (d, $^2J_{C-F}$ = 23 Hz), 108.3 (d,$^{2'}J_{C-F}$ = 26.1 Hz), 110.3, 111.6 (d, $^3J_{C-F}$ = 9.6 Hz), 112.8, 113.9, 114.2 (d, $^4J_{C-F}$ = 4.8 Hz), 120.1, 122.7, 123.4, 123.8, 125.0, 127.0 (d, $^{3'}J_{C-F}$ = 9.6 Hz), 132.4, 134.4, 158.9 (d, $^1J_{C-F}$ = 242 Hz). HRMS (EI) Calcd for $C_{26}H_{30}BrFN_4$ (M^+): 496.16379, Found: 496.16311.

4.6.4. 5-Bromo-3-(3-(4-(3-(5-methoxy-1*H*-3-indolyl)propyl)piperazinyl)propyl)-1*H*-indol (5f)

Prepared from 3-(5-bromo-3-indolyl)propyl-4-methylbenzenesulfonate (**2b**) (0.20 g, 0.49 mmol) anhydrous potassium carbonate (0.096 g, 0.60 mmol) and 3-(5-methoxy-3-piperazinyl-1-propyl)indol (**4d**) (0.135 g, 0.49 mmol) to afford **5f** (0.137 g, 55%). Mp: 187.5–188.5 °C. IR (cm^{-1}): 3421 (N–H), 3046 (C–H arom), 2995 (C–H aliph). ^{1}H NMR (DMSO-d_6) δ: 1.77 (m, 4H, (2×)CH_2–CH_2–CH_2-Pip), 2.30 (m, 4H, (2×)CH_2-Pip), 2.35 (br s, 8H, Pip.), 2.65 (m, 4H, (2×)Ar-CH_2), 3.75 (s, 3H, OMe), 6.70 (dd, 1H, indole C-5 methoxylated, 6-H, J_0 = 6.60 Hz, J_m = 2.1 Hz), 6,96 (s, 1H, 2-H, indole C-5 methoxylated, or indole C-5 brominated), 7.05 (s,1H, 2-H, indole C-5 brominated or indole C-5 methoxylated), 7.17 (m, 3H, 4-H and 7-H, indole C-5 methoxylated), and 6-H indole C-5 brominated), 7.30 (d, 1H, indole C-5 brominated, 7-H, J = 6.6 Hz),7.69 (s, 1H, indole C-5 brominated, 4-H), 10.6 (s, 1H, N–H), 11.0 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6): 21.4, 21.9, 26.5, 26.7, 4×(52.4), 54.8, 56.8, 57.2, 99.6, 110.2, 110.3, 111.4, 112.8, 113.7, 113.8, 120.1, 122.3,122.6, 123.4, 126.9,128.7, 130.9,134.4, 152.3. HRMS (EI) Calcd for $C_{27}H_{33}BrN_4O$ (M^+): 508.18377, Found: 508.1829.

4.6.5. 3-(3-{4-[3-(1*H*-3-indolyl)propyl]-1-piperazinyl}propyl)-5-bromo-1*H*-indol (5a)

Prepared from 3-(5-bromo-3-indolyl)propyl-4-methylbenzenesulfonate (**2b**) (0.186 g, 0.46 mmol), anhydrous potassium carbonate (0.076 g, 0.55 mmol) and 3-(3-piperazinyl-1-propyl)indol (**4a**) (0.112 g, 0.46 mmol), to afford **5a** (0.115 g, 52%). Mp: 190.5–191.8 °C. IR (cm^{-1}): 3422 (N–H), 3027 (C–H arom), 2924 (C–H aliph). ^{1}H NMR (DMSO-d_6) δ: 1.78 (m, 4H, (2×)CH_2–CH_2–CH_2-Pip), 2.32 (m, 4H, (2×)CH_2-Pip), 2.38 (br s, 8H, pip.), 2.67 (m, 4H, (2×)Ar-CH_2), 6.96 (t, 1H, 6-H, unsubstituted indole ring, J = 7.8 Hz), 7.05 (t, 1H, 5-H, unsubstituted indole ring, J = 7.0 Hz), 7.10 (s, 1H, 2-H unsubstituted indole ring), 7.16 (m, 2H, 2-H indole C-5 brominated and 4-H unsubstituted indole ring), 7.32 (m, 2H, 6-H indole C-5 brominated and 7-H unsubstituted indole ring), 7.50 (d, 1H, 7-H indole C-5 brominated, J = 7.7 Hz), 7.69 (d, 1H, 4-H, indole C-5 brominated, J = 1.5 Hz), 10.74 (s, 1H, N–H), 10.99 (s, 1H, N–H). ^{13}C NMR (DMSO-d_6): 21.4, 21.9, 26.6, 26.7, 4×(52.4), 56.8, 57.2, 110.2, 110.8, 112.8, 113.8, 113.9, 117.5, 117.7, 120.1, 120.2, 121.6, 122.6, 123.4, 126.7, 128.7, 134.4, 135.8. HRMS (EI) Calcd for $C_{26}H_{31}BrN_4$: 478.17321, Found: 478.17282.

4.7. Pharmacology

The affinity of compounds for SERT was determined *via* a competitive binding assay, using [^{3}H]paroxetine as radioligand and a rat cortical membrane preparation, according to previously reported procedures.[13–15] Briefly, assays were carried out in a total volume of 1.0 ml, containing 50 mM Tris–HCL buffer (pH 7.7) containing NaCl 150 mM and KCl 5 mM, 0.35 mg rat cortical tissue (original wet weight), 0.2 nM radioligand and the compound to be tested at different concentrations (10^{-11}–10^{-4} M). After 6 min at 25 °C, incubations were terminated by rapid filtration, with two 5-mL washes of ice-cold buffer, through Whatman GF/B filters that were previously soaked in 0.05% polyethyleneimine, using a cell harvester (Brandel Instruments, Gaithersburg, MD). Radioactivity was counted in a Packard 1300 liquid scintillation counter with an efficiency of approximately 50%. Nonspecific binding of [^{3}H]paroxetine was defined in the presence of 3 μM citalopram.

The affinity of the compounds towards 5-HT_{1A} receptor was determined using [^{3}H]8-OH-DPAT as radioligand and membranes from human embryonic kidney (HEK-293) cells expressing the human 5-HT_{1A} receptor (RBHS1AM400UA; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA), as previously described.[5]

4.8. Molecular simulation

4.8.1. Target modeling

The crystal structure of the leucine transporter from *Aquifex aeolicus* (LeuTAa) at 1.9 Å resolution (Protein Data Bank, PDB, code 2A65)[16] was used as a template to build a rat SERT model. Models were prepared using MODELLER9v3[17] and the best models were evaluated stereochemically and energetically using PROSAII[18] and ANOLEA[19] servers and Procheck.[20] The transporter was then inserted into a POPC membrane, TIP3 solvated and ions were added creating an overall neutral system in approximately 0.02 M NaCl. The ions were equally distributed in a water box. The final system, which contained approximately 320,000 atoms, was subjected to a molecular dynamics (MD) simulation for 5 ns using NAMD 2.6.[21] All other conditions were as previously described.[22]

Models of human 5-HT_{1A} receptor were prepared as previously described[5] using MODELLER9v3 and the three-dimensional crystal structure of the β2-adrenergic receptor as template.[23]

4.8.2. Molecular docking

Docking of compounds, both in the SERT and 5-HT_{1A} models, were performed with the AutoDock 4.0 suite,[24] following protocols previously described elsewhere.[5,22]

Acknowledgments

We thank Dr. B. K. Cassels for critical reading of the manuscript. This work was partially funded by FONDECYT Grants 1090169 and 1130347 to HP-M, 1090037 and 1130185 to MR-P, 1100542 to PI-V and 11085002 to AF.

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