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Docking study, synthesis, and in vitro evaluation of fluoro-MADAM derivatives as SERT ligands for PET imaging

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ABSTRACT

In order to predict affinity of new diphenylsulfides for the serotonin transporter (SERT), a molecular modeling model was used to compare potential binding affinity of new compounds with known potent ligands. The aim of this study is to identify a suitable PET radioligand for imaging the SERT, new derivatives, and their precursors for a C-11 or F-18 radiolabeling, were synthesized. Two fluorinated derivatives displayed good in vitro affinity for the SERT (K_i = 14.3 ± 1 and 10.1 ± 2.7 nM) and good selectivity toward the other monoamine transporters as predicted by the docking study.

1. Introduction

The serotonin transporter (SERT) modulates serotonin (5-HT) levels in brain and thus plays a major role in the regulation of the serotoninergic neurotransmission.[1] As reported in literature, dysfunction of the SERT is involved in various neuropsychiatric disorders such as depression, schizophrenia, mental illness, obsessive compulsive disorders,[2–5] and degenerative pathologies such as Parkinson and Alzheimer diseases.[6,7] Correlations between SERT density and these diseases have been established encouraging the measurement of the SERT concentration. It should be of great interest to develop neuroimaging methods for the SERT quantification in living human brain. In this purpose, some radioligands from different structure family were synthesized, such as [^{123}I]β-CIT,[8] [^{123}I]5-iodo-6-nitroquipazine,[9] and [^{11}C]McN5652,[10] to image SERT by single photon emission computed tomography (SPECT) or positron emission tomography (PET). Unfortunately, their use to assess the SERT quantification has been limited due to their kinetics and/or important non-specific binding.[11,12]

Recently, a novel class of compounds, called diphenylsulfide, has been studied in order to obtain potent SPECT and PET tracers to image in vivo human SERT. The first diphenylsulfide ligand described as a potent SPECT tracer to image SERT was [^{123}I]IDAM which demonstrated good brain penetration, a moderate signal to noise ratio but in general, a superior pharmacological profile over the previous synthesized ligands.[13]

However to get higher resolution and sensitivity by PET imaging, and by the way to improve the quantification, a lot of analogs of diphenylsulfides radiolabeled with carbon-11 have been developed notably [^{11}C]DASB[14] which is widely used as tracer for SERT imaging in human[15] (for depressive disorders see review written by Meyer).[16] Several diphenylsulfides, especially modulated on the 4′-position where Y is an halogen or a fluoroalkyl chain (see Fig. 1), have been evaluated as potential SERT imaging agents for PET (see several examples listed by Jarkas et al.[17]). If SERT quantification by PET may be assessed by the use of carbon-11 tracers, no satisfying fluor-18 ligands (more widely used because especially of its half-life, $T_{1/2}$ = 109 min) are yet available. In fact, since few years, numerous fluor-18 radioligands based on diphenylsulfide has emerged such as [^{18}F]F-ADAM (called also [^{18}F]AFA),[18] [^{18}F]ACF,[19] [^{18}F]AFM,[20] [^{18}F]AFE,[21] or [^{18}F]EADAM.[22] Some of them have a limited use due to in vivo defluorination which may interfere with the quantification or kinetics not compatible with the half-life of F-18. In the aim to optimize the development of a suitable F-18 PET tracer to image in vivo human SERT, we have decided to design new structures through a docking study and we have prepared original fluorinated diphenylsulfides based on the MADAM structure (Fig. 1), compound developed in our laboratory.[23,24] MADAM presents in vitro high affinity and selectivity for the SERT ($K_{i\ SERT}$ = 1.65 nM, $K_{i\ DAT}$ > 1000 nM, $K_{i\ NET}$ = 325 nM). The methyl group in the 4′-position (Fig. 1) seems to be responsible of its good

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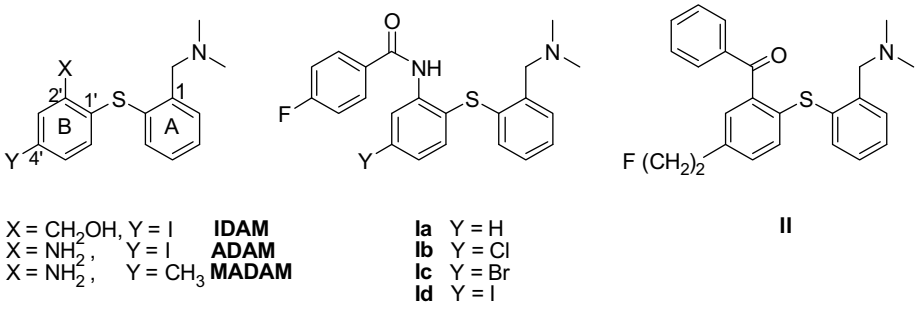


Figure 1. Literature SERT ligands: IDAM,[13] ADAM,[25] MADAM,[44] derivatives **Ia–d**,[26] and compound **II**.[27]

selectivity. The 2′-position tolerates a quite large of groups such as amine (ADAM[25], MADAM), alcohol (IDAM with a hydroxymethyl group) or ester. The nature of the function of the chemical group in this 2′-position seems to be important for the difference of selectivity observed between the derivatives.[23]

Furthermore, Kung et al. have studied the impact of alkyl or aryl acylation of the amino group on the phenyl ring B (**Ia–d**, Fig. 1) by in vitro evaluation[26]: *N* fluoroalkyl derivatives of ADAM produced compounds with good binding affinity.

In 2002, Wellsow and Kovar had made by molecular modeling around 100 predictions of binding affinity which were generated by CoMFA and CoMSIA.[27] They confirmed in silico that functionalization in 2′-positions should allow large substituent such as benzoyl moiety (see compound **II**, Fig. 1).

Based on these results, we have used a model of three-dimensional molecular structure of SERT, obtained from the lactose permease symporter (lac permease) crystal structure.[28] We have docked several potent ligands into putative binding site of SERT (Fig. 1) to validate the model, and then, we have predicted good potential binding affinity for new derivatives bearing a *p*-fluorobenzoyl or *p*-fluorobenzyl group on the 2′-position on a MADAM scaffold. These compounds as well as their precursors useable for C-11 or F-18 have been synthesized and evaluated in vitro for their binding potential at the SERT, DAT (dopamine transporter), and NET (noradrenalin transporter).

2. Results and discussion

2.1. Chemistry

The synthesis of derivatives **1** and **3** has been previously reported in literature.[23,29] Reaction of compound **1** with 2,2,2-trichloroethylchloroformate was realized to protect the secondary amine before acylation with *p*-fluorobenzoyl chloride. The nitro function is reduced by using tin chloride in an HCl/methanol/dimethylformamide solution. The addition of DMF is due to the insolubility of **1** in classical conditions. Acylation reactions were performed with derivatives **2** and **3** in THF, with *p*-(fluoro or nitro)benzoyl chloride to afford compounds **4**, **6**, and **7** in high yields (>90%). Derivative **5** was obtained in 44% yield by regeneration of the secondary amine function by treatment with glacial acetic acid and zinc. The reduction of the amide function of compounds **5**, **6**, and **7** occurred with borane–THF complex (1 M) and gave the fluoro(or nitro)benzylamines **8**, **10**, and **9**, respectively, in almost 70% yield (Scheme 1). All the compounds have been characterized by ^{1}H, ^{13}C NMR, and mass spectrometry.

2.2. Molecular modeling

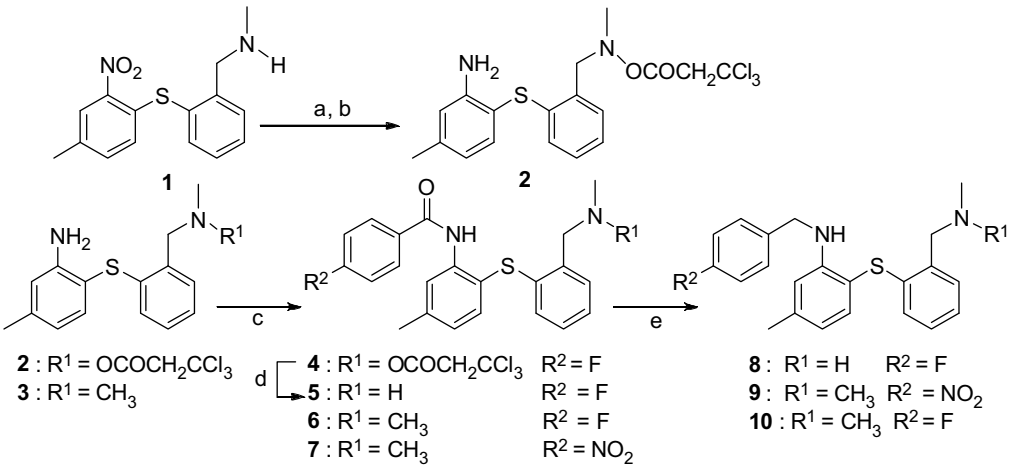
The rapid and accurate calculation of binding free energy of a putative protein–ligand complex is difficult to evaluate but important to consider in the structure-based drug design.

The LUDI program which is carried out in the InsightII environment (Accelrys Software Inc., San Diego, CA) has a simple scoring function to predict binding constants for protein–ligand complex of known three-dimensional structure.[30–33] The LUDI program positions small molecules into protein binding sites in such a way that hydrogen bonds are formed with the protein, and lipophilic groups are placed into hydrophobic pockets. Ionic interactions and the number of rotatable bonds in the ligand are also taken into account. It was shown that a very significant correlation between the sum of atom pair potentials and total binding free energy exists, and the sum is therefore a good measure for estimating binding affinities.[34] The binding affinities K_i are only a predictive value, and the 'score' is proportionate to K_i.

In attempt to develop a ligand-binding model for the SERT, the LUDI scoring program was used to identify a potential binding's site described by Ravna and colleagues,[28] by docking different potent ligands which bear different groups on the 2′-position (IDAM, MADAM, and **Ia–d**, see Fig. 1). Then, from the model obtained, we have investigated binding interactions of six diphenylsulfides **5–10** in the SERT pocket (Fig. 2) and we have obtained at physiological pH a 'score' (Table 1). To have a comparative level, we studied some known SERT ligands: IDAM, MADAM, diphenylsulfides **Ia–d**[26] (Fig. 1), compared to the new derivatives **5–10**. A higher LUDI score represents a higher potential affinity. The predictive affinities for **6** and **10** were the best compared to the other reference derivatives (Table 1). Their predictive affinity fall into the range of experimental values reported for potent diphenylsulfides (nanomolar range affinity). The low affinity of nitro and the *N*-desmethylated derivatives **7** and **9** and **5** and **8,** respectively, correlated with the fact that LUDI program failed to place these structures into the binding pocket ('no score'). Furthermore, the SERT amino acids involved in predictive ligand bindings are listed in Table 1. These results are in agreement with that of Ravna and colleagues study where they had shown that citalopram (SERT ligand) interacted, for example, with Val102, Trp103 (TMH1), Tyr121 (TMH2), Phe380 (TMH7) and Ile552 (TMH11).[28]

2.3. In vitro affinity, selectivity, and lipophilicity

As we have previously mentioned,[23] the *N*-desmethylation at 1-position does not improve the affinity for the SERT as proved by the low affinity of **5** and **8** (K_i > 1000 nM) and a dimethylaminomethyl group at this position is important for the cognition in the SERT binding site. Nitro derivatives **7** and **9** did not present any affinity for the SERT (K_i > 1000 nM). The two new fluorinated SERT radiotracers, *N,N*-dimethyl-2-[2-(*N*-4-fluorobenzamide)-4-tolylthio]- benzylamine **6** and the *N,N*-dimethyl-2-[2-(*N*-4-fluorobenzylamine)-4-tolylthio]benzylamine **10** displayed good affinity for the SERT as predicted by the docking study (K_i = 14.3 ± 1 and 10.1 ± 2.7 nM, respectively, Table 1). Moreover these two compounds are selective for the SERT as no DAT and NET affinities were obtained. ($K_{i\ DAT}$ > 1000 nM and $K_{i\ NET}$ > 1000 nM). Concerning the



Scheme 1. Synthesis of the fluoro-MADAM derivatives and their corresponding ^{11}C (R^1 = H) and ^{18}F (R^2 = NO_2) precursors. Reagents and conditions: (a) $ClOCOCH_2CCl_3$, reflux, 2 h; (b) MeOH, DMF, HCl, $SnCl_2$, below 10 °C, then rt overnight; (c) THF, pyridine, *p*-(fluoro or nitro)benzoyl chloride, reflux, 2 h; (d) AcOH/Zn, rt 48 h; (e) THF, borane–THF complex (1 M), reflux 5 h, rt overnight.

lipophilicities, the measured log$P_{7.4}$ from a reverse-phase HPLC method (see Table 2) gave a value greater than 4.5 for **10** (log$P_{7.4}$ = 4.70) indicating that this compound is probably less suitable for crossing the blood–brain barrier. Compound **6** showed an optimum log$P_{7.4}$ at 3.0 to get a good balance between brain penetrance and non-specific binding ($2 < \log P_{7.4} < 3.4$).[35,36] For these derivatives, *c*log*P* (ChemDraw®) was not able to predict the lipophilicity, on the other hand, by molecular modeling (Accelrys®), the log*D* predictions were quite accurate.

3. Conclusion

A docking study into the putative binding site of a 3D model of human serotonin transporter shown that fluorobenzamide or fluorobenzylamine on 2′-position on a diphenylsulfide scaffold should induce a good binding affinity for the SERT. This result is consistent with the in vitro data suggesting their potential application as imaging agents of serotoninergic nerve. Our work showed that this docking binding model is suitable to identify chemical structure with potential SERT affinity, especially to exclude from synthesis campaign compounds without SERT potency. In addition, because compounds **6** and **10** exhibit good in vitro SERT affinities, labeling and in vivo evaluation as PET tracers for the SERT imaging will be undertaken.

4. Experimental

4.1. Chemistry

General: NMR spectra were recorded on a Bruker DPX Advance 200 spectrometer (200 MHz for ^1H, 50.3 MHz for ^{13}C). $CDCl_3$ was used as solvent; chemical shifts are expressed in ppm relative to

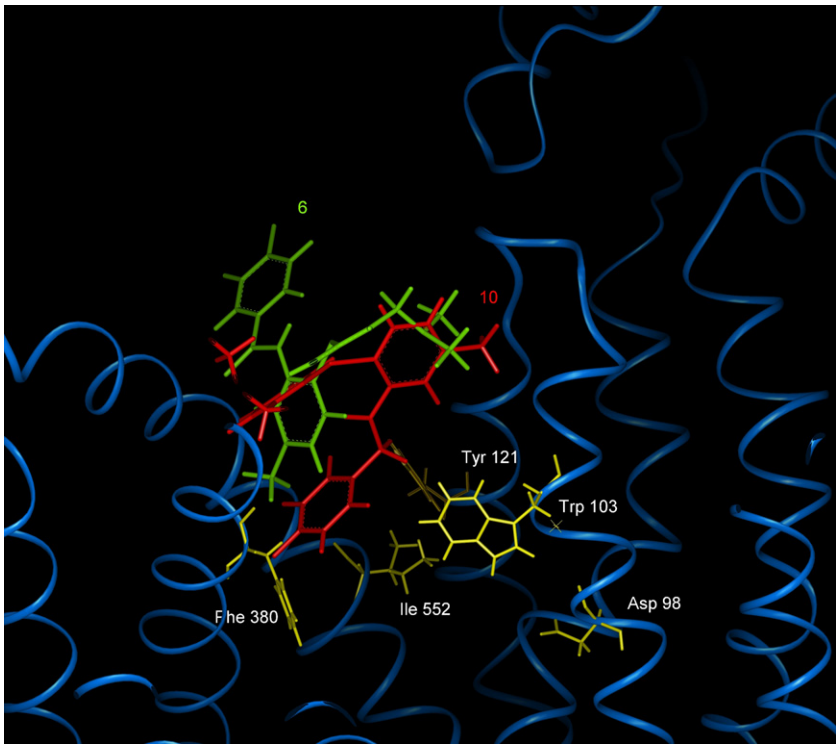


Figure 2. Binding model of SERT with residues which bind, in predictive studies, with **6** (in green) and **10** (in red).

Table 1
Inhibition constants of diphenylsulfide derivatives for the SERT and for human cloned monoamine transporters (DAT, NET); predictive binding of SERT–ligand complex via LUDI score, and SERT protein residues interacted in the binding site

Compound	K_i (nM) SERT	LUDI score pH 7.4	Residues of SERT		
IDAM	0.1[b]	364	Trp103, Tyr121, Ile552		
MADAM	1.65[c]	243	Val102, Tyr121, Ile552, Phe380		
Ia (X = H)	34[a]	131	Trp103, Tyr121, Pro560		
Ib (X = Cl)	0.9[a]	242	Phe380, Phe556, Leu563		
Ic (X = Br)	8.0[a]	154	Phe556, Leu563	K_i (nM)[e]	K_i (nM)[e]
Id (X = I)	193[a]	—[c]	—	hDAT	hNET
5	>500[e]	—[d]	—	>1000	>2500
6	14.3 ± 1[e]	413	Trp103, Pro106, Ser559	>1000	>5200
7	>100[e]	—[d]	—	>1000	>1000
8	>100[e]	—[d]	—	>2000	>1000
9	60 ± 17[e]	—[d]	—	>1000	>1900
10	10.1 ± 2.7[e]	521	Val102, Phe380, Gly384, Phe556	>1500	>1200

[a] Literature inhibition constants using [^{125}I]IDAM.[26]
[b] Literature inhibition constants using [^{125}I]IPT.[42]
[c] Literature inhibition constants using [^{3}H]paroxetine.[23]
[d] '—': no score obtained by LUDI studies.
[e] Inhibition constants (K_i) were obtained from means ± SD of four separate determination each in triplicate.

Table 2
Lipophilicity measurements and predictions for compounds **6** and **10** and references derivatives (ADAM and MADAM)

Compound	Experimental log$P_{7.4}$	Discovery Studio, Accelrys® log$D$[d]	ChemDraw® $c$log$P$[e]
ADAM	2.52[a]	2.29	4.51
MADAM	2.46[b]	2.20	3.59
6	2.99[c]	3.94	5.46
10	4.70[c]	4.54	5.90

[a] *n*-Octanol/0.02 M phosphate buffer partition.[22]
[b] *n*-Octanol/0.02 M phosphate buffer partition.[43]
[c] Reverse-phase HPLC experiments.
[d] Calculated log$D$, Discovery Studio, QSAR protocol, 2008, Accelrys®.
[e] Calculated $c$log$P$, ChemDraw Ultra 10.0, 2005, CambridgeSoft.

TMS as an internal standard. Mass spectra were obtained on a CG-MS Hewlett Packard 5989A spectrometer (electronic impact at 70 eV). The thin-layer chromatographic (TLC) analyses were performed using Merck 60 F_{254} silica gel plates. Flash chromatography was used for routine purification of reaction products using silica gel (230–400 mesh). For flash chromatography petroleum ether, ethyl acetate (EtOAc), methanol (MeOH), and triethylamine (TEA) were used. Visualization was accomplished under UV. All chemicals and solvents were of commercial quality and were purified following standard procedures. Elemental analyses of new compounds were within ±0.4% of theoretical values.

1 and **3** have been synthesized in the laboratory as previously described.[23,29]

4.1.1. *N*-Methyl-*N*-(3,3,3-trichloropropanoyloxy)-2-[2-amino-4-tolylthio]benzylamine (2)

Compound **1** (647 mg, 2.24 mmol) was heated in refluxing 2,2,2-trichloroethylchloroformate (3 mL, 21.79 mmol) for 2 h. The excess of 2,2,2-trichloroethylchloroformate was removed under reduce pressure. The crude product was poured in methanol (16 mL), dimethylformamide (16 mL), and HCl solution (33%) (8 mL). The solution was cooled and $SnCl_2$ (1.41 g, 7 mmol) was added by portion below 10 °C. The reaction mixture was stirred at ambient temperature overnight, treated with water (20 mL), basified with 10 N NaOH solution (to pH 10), and extracted with AcOEt (2× 20 mL). The organic phases were dried, removed under vacuo, and the residue was purified by flash chromatography (petroleum ether/EtOAc/TEA: 8/2/1) to give **2** in 52% yield. ^{1}H NMR δ: 2.34 (s, 3H), 3.07 (s, 3H), 4.22 (s, 2H), 4.76–4.87 (m, 4H), 6.61–6.71 (m, 1H), 7.13 (m, 1H), 7.13–7.32 (m, 5H). ^{13}C NMR δ: 21.4, 34.0, 50.3, 75.1, 95.7, 109.9, 115.0, 120.0, 125.4, 126.2, 127.0, 128.0, 133.4, 135.9, 137.1, 141.5, 148.6, 154.8.

4.1.2. *N*-Methyl-2-[2-(*N*-4-fluorobenzamide)-4-tolylthio]benzylamine (5)

To a solution of **2** (694 mg, 1.6 mmol) in THF (16 mL) and pyridine (252 mg, 3.2 mmol) was added at 0 °C 4-fluorobenzoyl chloride (349 mg, 2.2 mmol). The reaction mixture was heated to reflux for 2 h, the solvents were removed under reduced pressure and gave **4** as a crude compound. The crude **4** was dissolved to a solution of acetic acid (13 mL) and zinc (2 g, 30 mmol) and stirred for 2 days. The solution was filtrated on Celite, diluted with water (30 mL) and basified with concentrated NaOH. Extraction with methylene chloride (2× 30 mL) and flash chromatography (petroleum ether/EtOAc/MeOH/TEA 5:5:0.5:0.5) afforded compound **5** as a beige solid in a 44% total yield. ^{1}H NMR δ: 1.56 (s, 1H), 2.49 (s, 3H), 2.52 (s, 3H), 3.95 (s, 2H), 6.82 (dd, 1H, J = 7.1 Hz, J = 2.0 Hz), 7.01–7.23 (m, 5H), 7.37 (dd, 1H, J = 6.8 Hz, J = 1.7 Hz), 7.49–7.62 (m, 3H), 8.53 (d, 1H, J = 1.1 Hz), 8.93 (s, 1H). ^{13}C NMR δ: 21.6, 35.9, 53.8, 115.4 (d, 2C, J = 22.1 Hz), 116.6, 121.2, 125.4, 125.9, 126.7, 128.0, 129.1 (d, 2C, J = 9.1 Hz), 129.2, 130.9 (d, 1C, J = 2.5 Hz), 134.8, 136.3, 137.4, 139.4, 141.4, 164.0, 164.6 (d, J = 253.1 Hz). MS: m/e = 380 ($M^{+\cdot}$, 2), 150 (26), 123 (64), 120 (100), 95 (39), 42 (20).

4.1.3. *N,N*-Dimethyl-2-[2-(*N*-4-fluorobenzamide)-4-tolylthio]benzylamine (6)

To a solution of **3** (545 mg, 2 mmol) in THF (20 mL) and pyridine (0.32 mL 4 mmol) was added at 0 °C 4-fluorobenzoyl chloride (349 mg, 2.2 mmol). The reaction mixture was heated to reflux for 2 h, and solvents were removed under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/EtOAc/TEA 7:2:1). Compound **6** was obtained as a beige solid in 90% yield. ^{1}H NMR δ: 2.32, (s, 6H), 2.49 (s, 3H), 3.60 (s, 2H), 6.76–6.84 (m, 1H), 6.99–7.20 (m, 5H), 7.29–7.37 (m, 1H), 7.52–7.64 (m, 3H), 8.55 (d, 1H, J = 1.2 Hz), 8.93 (s, 1H). ^{13}C NMR δ: 21.8, 45.1 (2C), 62.4, 115.6 (d, 2C, J = 21.6 Hz), 117.1, 121.0, 125.5, 125.6, 126.5, 128.3, 129.2 (d, 2C, J = 8.6 Hz), 130.2, 131.1, (d, J = 2.5 Hz), 136.2, 136.6, 136.7, 136.9, 141.6, 164.1, 164.8 (d, J = 253.0 Hz). MS: m/e = 394 ($M^{+\cdot}$, 3), 165 (26), 164 (21), 150 (48), 134 (65), 132 (26), 123 (100), 95 (42), 58 (48), 46 (25), 44 (25). Anal. Calcd for $C_{23}H_{23}FN_2OS$: C, 70.02; H, 5.88; N, 7.10. Found: C, 69.76; H, 5.86; N, 7.14.

4.1.4. *N,N*-Dimethyl-2-[2-(*N*-4-nitrobenzamide)-4-tolylthio]benzylamine (7)

To a solution of **3** (200 mg, 0.73 mmol) in THF (5 mL) and pyridine (0.09 mL, 1.1 mmol) was added at 0 °C 4-nitrobenzoyl chloride (132 mg, 0.715 mmol). The reaction mixture was heated to reflux for 2 h, the solvents were removed under reduced pressure. Compound **7** was obtained after flash chromatography (petroleum ether/EtOAc/TEA 7:2:1) as a yellow powder in quantitative yield. ^{1}H NMR δ: 2.32 (s, 6H), 2.51 (s, 3H), 3.60 (s, 2H), 6.75–6.83 (m, 1H), 7.05–7.23 (m, 3H), 7.30–7.38 (m, 1H), 7.60 (d, 1H, J = 7.8 Hz), 7.68 (d, 2H, J = 8.8 Hz), 8.24 (d, 2H, J = 8.8 Hz), 8.54 (s, 1H), 9.02 (s, 1H). ^{13}C NMR δ: 21.8, 45.1 (2C), 62.5, 117.6, 121.2, 123.7 (2C), 125.7, 126.1, 126.5, 128.0 (2C), 128.4, 130.4, 136.0, 136.7, 139.0, 140.4, 141.7, 141.7, 149.6, 163.0. MS: m/e = 421 ($M^{+\cdot}$, 3), 165 (40), 164 (27), 150 (47), 134 (79), 132 (39), 120 (59), 92 (32), 91 (29), 58 (100), 46 (31), 44 (68), 42 (34). Anal. Calcd for $C_{23}H_{23}N_3O_3S$: C, 65.54; H, 5.50; N, 9.97. Found: C, 65.30; H, 5.49; N, 10.00.

4.1.5. *N*-Methyl-2-[2-(*N*-4-fluorobenzylamine)-4-tolylthio]benzylamine (8)

To a solution of compound **5** (228 mg, 0.60 mmol) in THF (2 mL) under nitrogen atmosphere was added drop by drop borane–THF (1 M, 1.9 mL) at 0 °C. The mixture was heated to reflux for 5 h, stirred at room temperature overnight, and quenched with HCl solution (10 N, 0.1 mL). The residue was then dissolved in water (5 mL), basified with NaOH solution (to pH 10), and extracted with methylene chloride (2× 10 mL). After evaporation of the solvent, the crude product was purified by flash chromatography (petroleum ether/EtOAc/TEA 5:5:0.5), **8** was obtained as a beige solid in 70% yield. ^{1}H NMR δ: 1.62 (s, 1H), 2.32 (s, 3H), 2.50 (s, 3H), 3.94 (s, 2H), 4.32 (d, 2H, J = 5.6 Hz), 5.74 (t, 1H, J = 5.6 Hz), 6.43 (d, 1H, J = 1.1 Hz), 6.58 (dd, 1H, J = 7.7 Hz, J = 1.1 Hz), 6.97–7.11 (m, 5H), 7.13–7.22 (m, 2H), 7.28–7.35 (m, 1H), 7.45 (d, 1H, J = 7.7 Hz). ^{13}C NMR δ: 21.6, 35.8, 46.1, 53.6, 110.9, 111.1, 114.9 (d, 2C, ^2J = 21.6 Hz), 117.7, 125.2, 127.0, 127.5, 128.1 (d, 2C, ^3J = 8.1 Hz), 129.0, 134.7 (d, ^4J = 2.5 Hz), 136.2, 137.1, 137.2, 141.2, 148.5, 161.4 (d, ^1J = 244.6 Hz). MS: m/e = 366 ($M^{+\cdot}$, 22), 335 (8), 240 (12), 226 (20), 212 (25), 150 (87), 120 (100), 109 (67), 83 (10), 42 (15).

4.1.6. *N,N*-Dimethyl-2-[2-(*N*-4-nitrobenzylamine)-4-tolylthio]benzylamine (9)

Compound **9** was prepared from derivative **7** (253 mg, 0.60 mmol) using the procedure described above. Compound **9** was obtained after purification by flash chromatography (petroleum ether/TEA 9.5:0.5) as a beige solid in 72% yield. ^{1}H NMR δ: 2.27 (s, 3H); 2.35 (s, 6H), 3.65 (s, 2H), 4.45 (d, 2H, J = 6.3 Hz), 6.24 (d, 1H, J = 1.0 Hz), 6.57 (dd, 1H, J = 7.7, J = 1.0 Hz), 6.73 (t, 1H, J = 6.3 Hz), 7.08–7.22 (m, 6 H), 7.55 (d, 1H, J = 7.6 Hz), 8.05 (d, 2H, J = 8.7 Hz). ^{13}C NMR δ: 21.7, 45.3 (2C), 46.0, 62.4, 110.9, 112.7, 117.8, 123.4 (2C), 125.4 (2C), 127.9 (2C), 128.9, 130.3, 136.6, 137.5 (2C), 141.1, 146.6, 147.6, 147.9. MS: m/e = 407 ($M^{+\cdot}$, 10), 273 (23), 136 (80), 134 (55), 120 (29), 58 (100), 44 (43). Anal. Calcd for $C_{23}H_{25}N_3O_2S$: C, 68.31; H, 5.99; N, 9.37. Found: C, 68.11; H, 6.00; N, 9.32.

4.1.7. *N,N*-Dimethyl-2-[2-(*N*-4-fluorobenzylamine)-4-tolylthio]benzylamine (10)

To a solution of compound **6** (600 mg, 1.52 mmol) in THF (3.8 mL) under nitrogen atmosphere was added drop by drop borane–THF (1 M, 3.8 mL) at 0 °C. The mixture was heated to reflux for 5 h, stirred at room temperature overnight, and quenched with HCl solution (10 N, 0.2 mL). The residue was then dissolved in water (10 mL), basified with NaOH solution (to pH 10), and extracted with methylene chloride (2× 20 mL). After evaporation of the solvent, the crude product was purified by flash chromatography (petroleum ether/TEA 9.5:0.5). Derivative **10** was obtained as a yellow solid in 71% yield. ^{1}H NMR δ: 2.29 (s, 3H); 2.31 (s, 6H), 3.60 (s, 2H), 4.30 (d, 2H, J = 5.8 Hz), 6.09 (t, 1H, J = 5.8 Hz), 6.36 (d, 1H, J = 1.2 Hz), 6.55 (dd, 1H, J = 7.7 Hz, J = 1.2 Hz), 6.85–7.04 (m, 5 H), 7.10–7.21 (m, 2H), 7.23–7.31 (m, 1H), 7.48 (d, 1H, J = 7.7 Hz). ^{13}C NMR δ: 21.8, 45.3 (2C), 46.2, 62.3, 111.2, 112.2, 115.2 (d, 2C, J = 21.1 Hz), 117.6, 125.2, 127.9 (d, 2C, J = 8.6 Hz), 128.1 (2C), 130.1, 134.9 (d, J = 2.5 Hz), 136.9, 137.5 (2C), 141.2, 148.6, 161.6 (d, J = 244.6 Hz). MS: m/e = 380 ($M^{+\cdot}$, 8), 226 (49), 212 (32), 211 (26), 194 (26), 165 (100), 164 (58), 150 (28), 134 (78), 132 (37), 109 (66), 58 (46). Anal. Calcd for $C_{23}H_{25}FN_2S$: C, 72.60; H, 6.62; N, 7.36. Found: C, 72.30; H, 6.63; N, 7.38.

4.2. Molecular modeling

The calculations and simulations were performed on a PC with linux workstation using the software modules Builder, Homology and LUDI in the InsightII environment (vers. InsightII 2005, Accelrys® Software Inc., San Diego, CA). The SERT model is described by Ravna and colleagues.[28,37] The different structures were improved by energy minimization and were also optimized using AM1 method. The center of the investigations in the binding site is obtained as the centroid of citalopram in the model of Ravna and colleagues.[28] Interaction sites were calculated within a radius of 7.0 Å. The fit was achieved with a maximum rms deviation of 0.45 Å from the interaction sites for each structure.

For the lipophilicities, $\log P_{\text{prediction}}$ was calculated with the QSAR protocol in the Discovery Studio environment (Accelrys® Software Inc., San Diego, CA).

4.3. In vitro binding studies

4.3.1. In vitro binding assays for SERT

Compounds were tested in duplicate in competition with [^{3}H]MADAM with a crude membrane fraction of homogenate of rat brain cortex. For these studies, each sample contained 0.2 mL of [^{3}H]MADAM at a concentration of 50 pM (K_d = 50 pM), 0.2 mL of competitors at various concentrations ranging from 10^{-5} M to 10^{-10} M, 0.5 mL containing 60 μg of membrane protein preparation in a total volume of 1 mL in the tris–HCl buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, pH 7.4). Non-specific binding was determined with 10^{-6} nM paroxetine. Samples were incubated at 22 °C for 90 mn, filtered on glass fiber filters (GF/B, Whatman), and washed with ice cold buffer and the residual radioactivity was measured with a beta counter (LKB, rack Beta 1215). The IC_{50} values were determined graphically for each compound and the K_i values were calculated according to Cheng and Prusoff.[38] The results (mean of four determinations) are expressed as inhibition constants (K_i) and are summarised in Table 1.

4.3.2. In vitro binding assays for NET and DAT

Candidate compounds were assayed for their affinities to the monoamine transporters (NET and DAT) in competitive binding experiments in vitro using cloned human transporters (hNET and hDAT) expressed on HEK-293 cells and the radioligands [^{3}H]nisoxetine (NET), and [^{3}H]GBR12935 (DAT), in accordance with the published procedures.[39]

4.4. Lipophilicity measurements

An indirect determination of octanol–water partition coefficients is to obtain $\log P_{7.4}$ by reverse-phase C-18 HPLC studies by comparison of their retention time (in triplicate) to that of seven reference compounds with known $\log P$ values as previously reported.[40,41] A Water XBridge, 5 μm 4.6 mm × 150 mm analytical

column was used with methanol/ 0.1 M phosphate buffer (pH 7.4) (50/50) at a flow rate of 1 mL/min. The standards used to produce a calibration equation were quinoline, phenol, benzamide, chlorpromazine, hexachlorobenzene, ADAM, MADAM, dissolved in the mobile phase. A calibration curve of retention time versus $\log P_{7.4}$ was obtained with an experimental calibration equation ($y = 1.438 \cdot \mathrm{Ln}(\text{retention time}) - 0.7301$) with R^2 of 0.93.

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