Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1297–1300

Synthesis and in vitro evaluation of novel derivatives of diphenylsulfide as serotonin transporter ligands

Johnny Vercouillie,[a] Sylvie Mavel,[a] Laurent Galineau,[a] Tiziana Ragusa,[a] Robert Innis,[b] Michael Kassiou,[c,d] Sylvie Chalon,[a] Frédéric Dollé,[e] Jean-Claude Besnard,[a] Denis Guilloteau[a] and Patrick Emond[a,*]

[a]*Université François Rabelais de Tours, INSERM U619, Laboratoire de Biophysique Médicale et Pharmaceutique, 31 avenue Monge, 37200 Tours, France*
[b]*Molecular Imaging Branch, National Institute of Mental Health, Bethesda, MD 20892, USA*
[c]*Department of PET and Nuclear Medicine, Royal Prince Alfred Hospital, Missenden Road, Camperdown, NSW, Sydney 2050, Australia*
[d]*Department of Pharmacology, University of Sydney, NSW 2006, Australia*
[e]*CEA-SHFJ, 4 place du Général Leclerc, 91401 Orsay Cdx, France*

Received 6 October 2005; revised 18 November 2005; accepted 18 November 2005
Available online 7 December 2005

Abstract—As the serotonin transporter (SERT) is involved in several neurodegenerative and psychiatric disorders, radiopharmaceuticals to image the SERT by PET or SPECT would be very valuable in studying these diseases. For the development of imaging agents, we have synthesized novel derivatives of recently reported diphenylsulfide SERT ligands, in which the sulfur atom linking the two phenyl rings was replaced by an oxygen, sulfinyl, sulfonyl, amino or carbon group. Three of these exhibited good to high in vitro affinity (0.5 nM < K_i < 11 nM) and selectivity for the SERT over the other monoamine transporters.

The serotonin transporter (SERT) plays a pivotal role in the regulation of serotoninergic neurotransmission by the reuptake of serotonin from the synaptic cleft into the presynaptic nerve.[1,2] Dysfunction of serotoninergic neurotransmission has been shown to be involved in several neurodegenerative[3,4] and psychiatric disorders.[5–7] In vivo SERT imaging in humans by positron emission tomography (PET) or by single photon emission computed tomography (SPECT) would assist in the early diagnosis and follow-up of treatment in these diseases.

The diphenylsulfide, 403U76 (Fig. 1), has been reported as a SERT and NET (norepinephrine transporter) inhibitor which provided the framework for the recent development of selective SERT imaging agents.[8] Its iodinated analog, 5-iodo-2-[2-(dimethylaminomethyl)phenylthio]-benzylalcohol (IDAM), displays high in vitro affinity

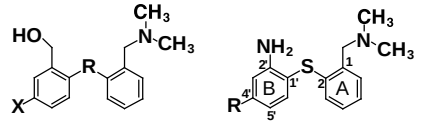


Figure 1. Potent SPECT or PET diphenylsulfide derivatives for SERT imaging.

(K_{iSERT} = 0.012 nM) and selectivity for SERT compared to the DAT (dopamine transporter) and NET.[9] However, its in vivo properties are far from ideal requiring further structural modifications to enhance its in vivo behavior such as reduced peripheral metabolism, increased brain uptake, and higher target to non-target ratio. The substitution of the sulfur atom of IDAM by an oxygen (ODAM, Fig. 1)[10] results in decreased in vitro affinity for the SERT (K_{iSERT} = 0.171 nM), increased brain uptake, slower kinetics, and peripheral metabolism.[10,11] These differences suggest that a minor change in the chemical structure can result in significant changes

Keywords: SERT; Diphenylsulfide derivatives; MADAM derivatives; Monoamine transporters.
* Corresponding author. Tel.: +33247367242; fax: +33247367224; e-mail: emond@univ-tours.fr

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.11.066

to binding affinity and in vivo biodistribution parameters.

Moreover, it has recently been found that an amino function at position 2′ of ring B in addition to the *N*,*N*-dimethylaminomethyl group in position 1 of the phenyl ring A yields compounds such as ADAM,[12] DASB,[13] or MADAM[14] (Fig. 1) with high in vitro and in vivo affinity and selectivity for the SERT. Preliminary in vivo results in monkeys with [^{11}C]MADAM[15] supported its use for in vivo SERT exploration by PET. Moreover, [^{123}I]ADAM[16] and [^{11}C]DASB[17] have been fully validated and, in view of their biological properties, proposed as potent radiopharmaceuticals to explore the SERT in humans by PET or SPECT.

On the basis of these results, we hypothesized that a series of derivatives of the diphenylsulfide MADAM with different bridge atoms should provide insight into SERT binding properties.[18] This hypothesis was supported by a molecular modeling study which predicted that substitution of the sulfur bridge by an amine function or a methylene bridge should generate SERT compounds with subnanomolar affinity.[19]

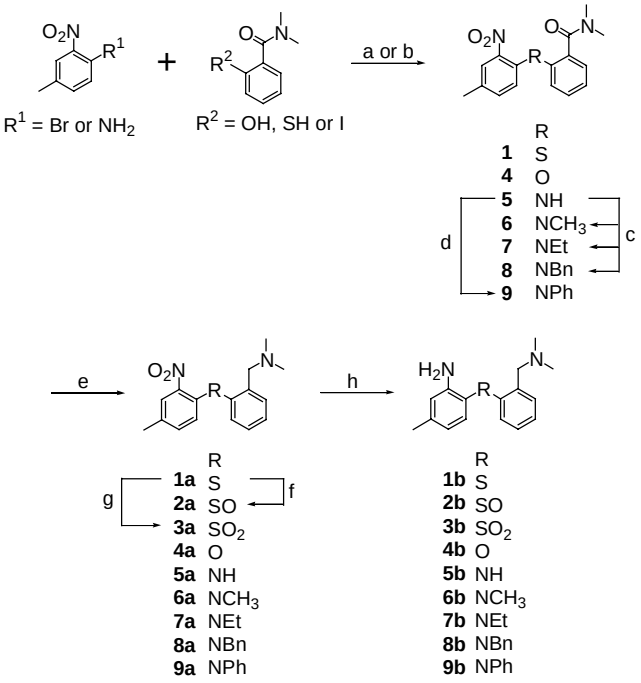
The pharmacological evaluation of 'O–' and 'CH_2–' bridge analogs of ADAM has recently been reported.[20] This study revealed that replacement of the sulfur atom of ADAM by an oxygen or methylene group differently affected SERT affinity and selectivity in vitro. All these findings suggest that the atom linking the two phenyl rings is critical in determining in vivo properties and may be involved in the SERT ligand recognition mechanism.

To explore this hypothesis, we have synthesized and evaluated in vitro eleven derivatives of MADAM with different linkers between the phenyl groups.

All of the compounds (**1b**–**13b**) were synthesized as outlined in Schemes 1–3.

Scheme 1 describes the preparation of compounds **1b**–**9b** which follows the same reaction sequence. The first step gave derivatives **1**, **4**, and **5** (R = S, O, and NH, respectively). Compounds **1**[15] and **4** were prepared by aromatic nucleophilic substitution, using 4-bromo-3-nitrotoluene, potassium carbonate as base, dimethylformamide (DMF), and 2-(mercapto or hydroxy)-*N*,*N*-dimethylbenzamide. To synthesize **5**, an Ullmann condensation was performed with 2-iodo-*N*,*N*-dimethylbenzamide, K_2CO_3, copper, DMF, and 4-methyl-2-nitroaniline (79% yield). The same reaction has been used to prepare the diphenyl derivative **13** (Scheme 3).

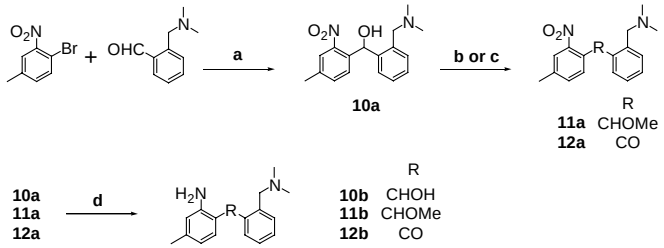
The amino compound **5** was used as starting material in the preparation of the corresponding alkylated derivatives: *N*-Me, *N*-Et, *N*-benzyl, and *N*-phenyl (**6**–**9**, respectively). To prepare compounds **6**–**8**, the reaction conditions were: sodium hydride, DMF, and the corresponding halo-derivatives (71–85% yield), whereas to prepare compound **9**, Ullmann reaction conditions were used which included reaction of **5**, iodobenzene, potassium carbonate, and copper in nitrotoluene (28% yield). Reduction of the amide function of compounds **1**, **4**–**9**, and **13** was achieved using diborane–THF complex to provide the amino derivatives **1a**,[15] **4a**–**9a**, and **13a**. The sulfinyl (**2a**) and sulfonyl (**3a**) derivatives were prepared by oxidation of compound **1a** in acetic acid using sodium perborate as oxidant[21] (63% and 60% yield, respectively). Scheme 2 describes the preparation of derivatives **10b**–**12b**. Compound **10a** was synthesized by nucleophilic addition of 4-lithio-3-nitrotoluene to 2-dimethylaminomethylbenzaldehyde. 4-Lithio-3-nitrotoluene was prepared by halogen-metal exchange using *n*-butyllithium in THF, as previously reported.[22] 2-Dimethylaminomethylbenzaldehyde was prepared by quenching 2-lithio-dimethylaminomethylbenzylamine with DMF.[23]



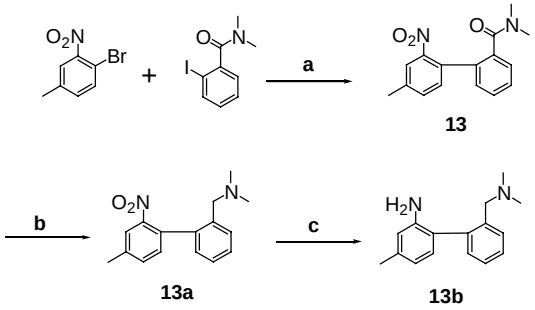
Scheme 1. Reagents and conditions: (a) DMF, K_2CO_3, 80 °C, 5 h (R=S and O); (b) DMF, K_2CO_3, Cu, reflux, 3 h (R=NH); (c) DMF, NaH, 0–75 °C, 45 min to overnight and corresponding halide (methyl iodide, ethyl iodide, and benzyl bromide); (d) iodobenzene, nitrotoluene, K_2CO_3, Cu, reflux, 7 h; (e) B_2H_6-THF, reflux 5 h and then rt overnight; (f) AcOH, $NaBO_3$ (1.2 equiv), reflux, 3 h; (g) AcOH, $NaBO_3$ (2.4 equiv), reflux, 3 h; (h) $SnCl_2$/HCl/MeOH, 5 °C to rt overnight.

The nitro group of derivatives **1a**–**13a** was finally reduced using tin(II) chloride in concentrated hydrochloric solution and methanol (Schemes 1–3) to give the corresponding amino derivatives **1b**–**13b** (25–94% yield).[24]

In vitro affinities of derivatives **1b**–**13b** were evaluated by in vitro competition studies using tritiated ligands of SERT ([^{3}H]paroxetine), DAT ([^{3}H]GBR12935), and NET ([^{3}H]nisoxetine) as previously detailed.[25,26] For each compound and each transporter, K_i values were only determined when 100 nM of a target compound inhibited at least 50% of tritiated ligand binding (IC_{50} < 100 nM). Replacement of the MADAM sulfur



Scheme 2. Reagents and conditions: (a) *n*-BuLi/THF/−100 °C; (b) DMF, NaH, methyl iodide, 0 °C, 45 min; (c) acetone, Jones reagent, 0 °C to rt, overnight; (d) $SnCl_2$/HCl/MeOH, 5 °C to rt overnight.



Scheme 3. Reagents and conditions: (a) DMF, Cu, reflux, 6 h; (b) B_2H_6/THF, reflux 5 h, RT overnight; (c) $SnCl_2$/HCl/MeOH, 5 °C to RT overnight.

bridge with other atoms, or groups, led to compounds without DAT or NET affinity ($IC_{50} > 100$ nM, Table 1). Because a *N*,*N*-dimethylaminomethyl group at the 1- and an amino group at 2′-positions of the diphenylsulfide structure have been found to be important for the SERT selectivity,[14] it can be suggested that these substituents are responsible for low DAT and NET affinities observed for compounds **1b**–**13b**. Compounds **2b**, **4b**, and **5b** exhibited moderate to high affinity to the SERT ($K_i = 4$, 0.53, and 10 nM, respectively, Table 1), whereas the remaining compounds showed poor SERT affinities ($IC_{50} > 100$ nM).

Table 1. Affinities of target compound for the SERT, NET, and DAT

Compound	R	K_i/IC_{50} (nM)[a]			Partial charge on R[b]
		SERT	NET	DAT	
1b	S	1.65 ± 0.10	>100	>100	0.19
2b	SO	4.15 ± 0.57	>100	>100	1.52
3b	SO_2	>100	>100	>100	2.89
4b	O	0.53 ± 0.04	>100	>100	−0.17
5b	NH	10.28 ± 3.60	>100	>100	−0.26
6b	NMe	>100	>100	>100	−0.26
7b	NEt	>100	>100	>100	−0.24
8b	NBn	>100	>100	>100	−0.23
9b	NPh	>100	>100	>100	−0.20
10b	CHOH	>100	>100	>100	−0.09
11b	CHOMe	>100	>100	>100	0.32
12b	CO	>100	>100	>100	−0.03
13b	—	>100	>100	>100	—

[a] K_i values have been determined when $IC_{50} < 100$ nM. K_i values are means of four experiments realized in duplicate. Radioligands (DuPont NEN) [^{3}H]paroxetine (SERT), [^{3}H]nisoxetine (NET), and [^{3}H]GBR12935 (DAT).

[b] After "Ampac/Mopac" (vers. Insight II 98.0) calculations, in MSI (San Diego, USA) package.

In addition, a MADAM analog was prepared in which the two phenyl rings were directly linked (**13b**, Scheme 3) resulting in no SERT affinity ($IC_{50} > 100$ nM). It could therefore be assumed that the bridge atom plays a critical role in the binding and is necessary to obtain ligands with high SERT affinity. These results contrast with recent molecular modeling predictions where Wellsow and Kovar[19] predicted that the replacement of the DASB sulfur bridge by an oxygen, an amino (NH or NR) or a ketone group should lead to SERT derivatives with subnanomolar affinity. Some of these predictions were confirmed by our results, such as compounds **4b** and **5b** (R=O and NH) which displayed K_i values of 0.53 and 10.28 nM, respectively. However, compounds **6b**–**9b** and **12b** (R = NR′ or CO), predicted to be SERT ligands with high affinity, displayed $IC_{50} > 100$ nM.

In addition, the O-bridged and CH_2-bridged derivatives of ADAM have recently been reported to display K_i values of 0.37 and 48.6 nM for the SERT, respectively, compared to 0.013 nM for ADAM.[20] These results clearly demonstrate that the bridge atom is a major component to be considered in designing new highly potent SERT ligands and that direct involvement of this atom in SERT-ligand recognition could be envisaged. To explore this hypothesis, we calculated the partial charge on the linking atom of the newly synthesized derivatives. As all these compounds interact at the same binding site, and positive and negative charges were found for both potent and non-potent compounds (Table 1), it could be assumed that this atom does not have a direct role in SERT binding site recognition. However, because groups at positions 1 and 2′ have been found to play an important role in SERT-ligand recognition,[14,19] active or inactive spatial conformations generated by the nature of the linking atom may explain the differences in SERT affinity.

Finally, comparison of SERT affinity of compound **1b** with that of compounds **2b** and **3b** could provide insights into in vivo pharmacological properties. As recently reported, diphenylsulfide has been shown to be oxidized by cytochrome P450 monooxygenase and/or by flavin-containing monooxygenase into the corresponding sulfoxide and thereafter into its sulfone.[27–29] MADAM could thus be oxidized in vivo into **2b** which also binds to the SERT and further oxidized into **3b** which does not bind to the SERT. This metabolic pathway for other diphenylsulfides could lead to potent and/or non-potent SERT metabolites, and thus must be considered in the development of any SERT imaging agent.

Based on these results, further development of the phenoxy derivative **4b** for radiolabeling with carbon-11 and in vivo pharmacological evaluation by PET is currently underway.

Acknowledgments

This work was supported by the Région Centre (France) and CEA (LRC 21V). Radioligand binding assays were performed by the National Institute of Mental Health Psychoactive Drug Screening Program, supported by NO1MH80005, at Case Western Reserve University's Biochemistry Department under the direction of B.L. Roth (supported by KO2MH01366). We thank SAVIT (Tours, France) for chemical analyses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.11.066.

References and notes

1. Masson, J.; Sagne, C.; Hamon, M.; El Mestikawy, S. *Pharmacol. Rev.* **1999**, *51*, 439.
2. Pineyro, G.; Blier, P. *Pharmacol. Rev.* **1999**, *51*, 533.
3. Chinaglia, G.; Landwehrmeyer, B.; Probst, A.; Palacios, J. M. *Neuroscience* **1993**, *54*, 691.
4. Tejani-Butt, S. M.; Yang, J.; Pawlyk, A. C. *NeuroReport* **1995**, *6*, 1207.
5. Owens, M. J.; Nemeroff, C. B. *Clin. Chem.* **1994**, *40*, 288.
6. Staley, J. K.; Malison, R. T.; Innis, R. B. *Biol. Psychiat.* **1998**, *44*, 534.
7. Stein, D. J. *Lancet* **2002**, *360*, 397.
8. Ferris, R. M.; Brieaddy, L.; Mehta, N.; Hollingsworth, E.; Rigdon, G.; Wang, C.; Soroko, F.; Wastila, W.; Cooper, B. *J. Pharm. Pharmacol.* **1995**, *47*, 775.
9. Oya, S.; Kung, M. P.; Acton, P. D.; Mu, M.; Hou, C.; Kung, H. F. *J. Med. Chem.* **1999**, *42*, 333.
10. Acton, P. D.; Mu, M.; Plossl, K.; Hou, C.; Siciliano, M.; Zhuang, Z. P.; Oya, S.; Choi, S. R.; Kung, H. F. *Eur. J. Nucl. Med.* **1999**, *26*, 26.
11. Acton, P. D.; Kung, M. P.; Mu, M.; Plossl, K.; Hou, C.; Siciliano, M.; Oya, S.; Kung, H. F. *Eur. J. Nucl. Med.* **1999**, *26*, 854.
12. Choi, S. R.; Hou, C.; Oya, S.; Mu, M.; Kung, M. P.; Siciliano, M.; Acton, P. D.; Kung, H. F. *Synapse* **2000**, *38*, 403.
13. Wilson, A. A.; Ginovart, N.; Schmidt, M.; Meyer, J. H.; Threlkeld, P. G.; Houle, S. *J. Med. Chem.* **2000**, *43*, 3103.
14. Emond, P.; Vercouillie, J.; Innis, R.; Chalon, S.; Mavel, S.; Frangin, Y.; Halldin, C.; Besnard, J. C.; Guilloteau, D. *J. Med. Chem.* **2002**, *45*, 1253.
15. Tarkianen, J.; Vercouillie, J.; Guilloteau, D.; Gulyas, B.; Sovago, J.; Cselényi, Z.; Emond, P.; Chalon, S.; Sandell, J.; Hiltunen, J.; Farde, L.; Halldin, C. *J. Labelled Compd. Radiopharm.* **2001**, *1*, S193.
16. Acton, P. D.; Choi, S. R.; Hou, C.; Plossl, K.; Kung, H. F. *J. Nucl. Med.* **2001**, *42*, 1556.
17. Wilson, A. A.; Ginovart, N.; Hussey, D.; Meyer, J.; Houle, S. *Nucl. Med. Biol.* **2002**, *29*, 509.
18. Emond, P.; Vercouillie, J.; Ragusa, T.; Mavel, S.; Dollé, F.; Innis, R. B.; Chalon, S.; Guilloteau, D. *J. Labelled Compd. Radiopharm.* **2003**, *46*, S160.
19. Wellsow, J.; Kovar, K. A.; Machulla, H. J. *J. Pharm. Pharm. Sci.* **2002**, *5*, 245.
20. Kung, H. F.; Newman, S.; Choi, S. R.; Oya, S.; Hou, C.; Zhuang, Z. P.; Acton, P. D.; Plossl, K.; Winkler, J.; Kung, M. P. *J. Med. Chem.* **2004**, *47*, 5258.
21. McKillop, A.; Tarbin, J. A. *Tetrahedron* **1987**, *43*, 1753.
22. Parham, W. E.; Picciliri, R. M. *J. Org. Chem.* **1977**, *42*, 257.
23. Viswanathan, C. T.; Wilkie, C. A. *J. Organomet. Chem.* **1973**, *54*, 1.
24. All new compounds gave analytical and spectroscopic results consistent with the assigned structure.
25. Glennon, R. A.; Lee, M.; Rangisetty, J. B.; Dukat, M.; Roth, B. L.; Savage, J. E.; McBride, A.; Rauser, L.; Hufeisen, S.; Lee, D. K. *J. Med. Chem.* **2000**, *43*, 1011.
26. Rothman, R. B.; Baumann, M. H.; Savage, J. E.; Rauser, L.; McBride, A.; Hufeisen, S. J.; Roth, B. L. *Circulation* **2000**, *102*, 2836.
27. Nnane, I. P.; Damani, L. A. *J. Pharm. Biomed. Anal.* **2002**, *27*, 315.
28. Nnane, I. P.; Damani, L. A. *Life Sci.* **2003**, *73*, 359.
29. Nnane, I. P.; Damani, L. A. *Biomed. Chromatogr.* **2004**, *19*, 87.