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New fluoro-diphenylchalcogen derivatives to explore the serotonin transporter by PET

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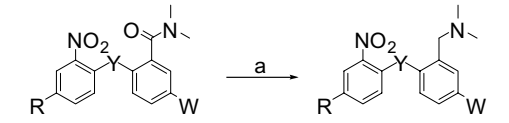
Abstract—A series of fluorinated diphenylchalcogen derivatives, possessing a sulfur or an oxygen bridge, has been prepared with the aim to get a suitable radiotracer to image the SERT in vivo using positron emission tomography (PET). The compounds were synthesized and assayed toward the serotonin (SERT), dopamine (DAT), and norepinephrine (NET) transporters. Among the developed series, five compounds display a high SERT affinity (K_i: 0.27–2.91 nM range) and can be labeled either with carbon-11 or fluorine-18.

The serotonin transporter (SERT), a pre-synaptic re-uptake system responsible for the clearance of the neurotransmitter serotonin after its release into the synaptic cleft, plays a key role in serotonergic signaling as it regulates the extracellular serotonin concentration.[1] Alterations of SERT densities have been reported in various psychiatric and neurological disorders.[1] Neuroimaging with SERT radiotracers is expected to provide information in diagnostics and therapy, in particular to evaluate properties of new drugs.[1]

During the last two decades, different classes of compounds were investigated for their suitability for the development of SERT radiotracers, for example, tropane, pyridine, and pyrroloisoquinoline derivatives.[1,2] Even though some of them have been proved to be useful for human studies, for example, the tropane derivatives [^{123}I]β-CIT or [^{123}I]nor β-CIT,[3–5] and the pyrroloisoquinoline derivative [^{11}C]McN5652,[6] all of them display certain limitations (Scheme 1).

Diphenylsulfide derivatives, initially proposed and synthesized as potential antidepressants,[7] are highly potent inhibitors of serotonin re-uptake.[8] A radioiodinated diphenylsulfide, 5-iodo-2-((2-((dimethylamino) methyl)phenyl)thio) benzyl alcohol (IDAM), has been anticipated to be a promising SPECT tracer for SERT imaging.[9] Based on this class of compounds, new SPECT agents called ODAM and ADAM were developed.[10,11] The interest in these compounds is based on their high SERT affinity (within the nanomolar range) and selectivity versus the dopamine transporter (DAT) and the norepinephrine transporter (NET). Among the three mentioned diphenylsulfide compounds, [^{123}I]ADAM appears to be the most suitable SPECT tracer,[12] due to its higher in vitro SERT affinity and selectivity in comparison to IDAM and ODAM. These better properties were attributed to the presence of the amino



1a	R = Me	Y = O	W=F	**1b**	R = Me	Y = O	W=F
2a	F	O	Cl	**2b**	F	O	Cl
3a	Me	S	F	**3b**	Me	S	F
4a	Cl	S	F	**4b**	Cl	S	F

Scheme 1. Reagents and conditions: (a) THF, addition of BH_3–THF (1 M in THF) at 0 °C, followed by 5 h at reflux and overnight at rt.

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group in *ortho*-position to the bridging atom for ADAM, which is substituted by a hydroxymethyl group in IDAM and ODAM.[13] Concerning the in vivo SPECT studies [^{123}I]ADAM displays an excellent uptake and a better target versus non-target contrast compared to the two other developed radioligands. However, critical assessment of the kinetics and metabolism of [^{123}I]ADAM, as well as acquisition protocols, is warranted.[1]

Regarding PET, there is still a need for alternative radiotracers to assess SERT densities in the human brain. We have recently prepared and evaluated in vivo a ^{11}C-labeled analog of ADAM.[14] It displays a slow kinetics which is incompatible with the short half-life of ^{11}C (~20 min). Based on the ADAM lead structure, numerous ^{11}C tracers were developed such as MADAM, DASB, SMe-ADAM, DAPA, EADAM, or AFM (Fig. 1).[15–19] In vitro, comparable properties concerning SERT affinity and selectivity were detected, but the in vivo evaluation of these tracers in non-human primates revealed that [^{11}C]DASB and [^{11}C]AFM are superior tracers compared to the others.[20,21] Although in vivo evaluation demonstrated that [^{11}C]DASB is a suitable tracer to image the SERT in humans,[22–24] there is still a potential for improvement such as reduction of non-specific binding and introduction of ^{18}F-label (half-life ~110 min) into the molecule to allow longer scanning protocols, radiotracer distribution within the satellite concept, and higher resolution due to the lower positron energy.[1]

Accordingly, several ^{18}F tracers based on the structures of ^{11}C radioligands, such as McN-derivatives[25,26] and diphenylsulfides, were prepared. First, the *S*-fluoromethyl and the *S*-fluoroethyl derivatives of McN5652 display SERT affinities similar to the parent ligand in vitro,[25,26] and have been evaluated in vivo to determine their potency to image the SERT. The *S*-fluoroethyl derivative presents a lower blood–brain penetration compared to [^{11}C]McN5652 and a low signal-to-noise ratio. The *S*-fluoromethyl derivative presents a higher potential than its fluoroethyl analog. Nevertheless, none of these tracers offer an improvement over [^{11}C]McN5652 for the in vivo PET imaging of the SERT except for the longer half-life.[27,28]

Second, attempts have been made to develop fluorodiphenylsulfide analogs based on the ADAM structure. It appears that the nature of the group on position 4′ in ring B of diphenylsulfides (see Fig. 1) has limited impact on the affinity and selectivity for the SERT.[13] Thus, at this position fluoro groups were introduced to obtain [^{18}F]AFA, [^{18}F]AFM, [^{18}F]AFE, and [^{18}F]AFP (fluorine linked either directly on the aryl, or via methyl-, ethyl-, and propyl-spacers, respectively; see Fig. 1). In vitro, all compounds display SERT affinity and specificity versus DAT similar to ADAM, but a lower selectivity toward the NET (K_i = 141 nM for AFA, for other derivatives between 500 and 1000 nM).[20] The potency of [^{18}F]AFP is reduced in vivo due to a low signal-to-noise ratio while [^{18}F]AFM displays the highest blood–brain penetration and specific binding (hypothalamus-to-cerebellum ratio: 6 at 60 min pi).[20] However, in vivo defluorination of [^{18}F]AFM may occur. Two other fluorinated analogs of ADAM, 5-[^{18}F]ADAM and [^{18}F]ACF, were synthesized and evaluated in vivo. 5-[^{18}F]ADAM displays a low brain uptake and significant defluorination,[29] while [^{18}F]ACF exhibits a good brain penetration but a moderate signal-to-noise ratio (hypothalamus-to-cerebellum ratio: ~3 at 60 min pi). So far, all fluorinated tracers developed for in vivo PET investigation of the SERT present critical limitations such as in vivo defluorination, low brain uptake, or a non-negligible NET affinity resulting in in vivo properties inferior to those of [^{11}C]DASB.

Herein we report the synthesis and in vitro evaluation of new diphenylsulfides and oxo analogs which can lead to ligands with high SERT affinity and selectivity.[30,31] To avoid in vivo defluorination of the radiotracers, the fluorine atom was linked directly to the aryl ring. For the better understanding of the molecular basis of the SERT–ligand interaction, the bridge atom was modified and the fluorine was introduced either at the aryl bearing the amino group (ring B, position 4′) or at the aryl bearing the dimethylaminomethyl group (ring A, position 5, Fig. 1). Moreover, these derivatives offer the possibility to be labeled either with ^{11}C or ^{18}F. While a standard ^{11}C methylation of a monomethylamine precursor is obvious, a ^{18}F fluorination in ring A is supposed to be possible via a nucleophilic substitution applying an electron deficient precursor followed by a reduction.[29]

Compounds **1a**–**4a** were obtained by nucleophilic aromatic substitution in yields of 60–92%, using 1-halo-2-nitrobenzenes (Br or Cl), 2-hydroxy (or 2-mercapto)-*N*,*N*-dimethylbenzamides and potassium carbonate in DMF. The reduction of the amide function to afford **1b**–**4b** was performed using diborane–THF complex (1 M) as reductive agent. The synthesis of the cyano analogs, **5b** and **6b**, is depicted in Scheme 2.

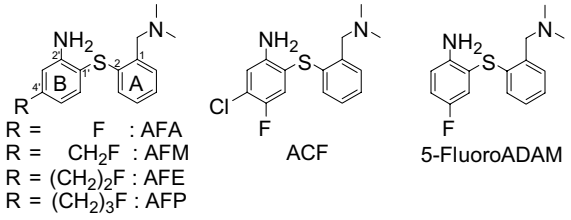
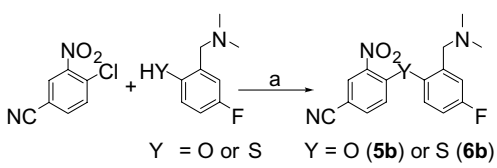


Figure 1. Potent fluorinated tracers developed to image the SERT by PET.



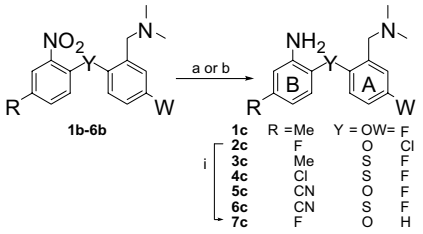
Scheme 2. Reagents and conditions: (a) DMSO, KOH, 160 °C, 8 h.

In contrast to **1b**–**4b**, compounds **5b** and **6b** could not be prepared from benzamides due to the presence of the cyano group and its incompatibility with reducing agents such as borane–THF complex. Based on similar coupling reaction for the preparation of diphenyl ether derivatives,[31] we synthesized **5b** and **6b** by nucleophilic aromatic substitution of 2-[(dimethylamino)methyl]-4-fluorophenol or 2-[(dimethylamino)methyl]-4-fluorobenzenethiol with 4-chloro-3-nitrobenzonitrile using potassium hydroxide in DMSO (yield >50%).

Two different methods were employed for the reduction of the nitro group of derivatives **1b**–**6b** (Scheme 3). For compounds **1b**–**4b** we used MeOH, hydrogen, and palladium/charcoal as catalyst. Under these conditions **1b**, **3b**, and **4b** led to **1c**, **3c**, and **4c**, respectively, in high yields (88–92%). However, with that method, concomitant to the reduction of the nitro group of **2b**, a hydrodechlorination was observed which led to derivative **7c**. Finally, compound **2c** was prepared from **2b** using tin (II) chloride under HCl–MeOH conditions.

The same method was also employed for the preparation of **5c** and **6c** using their correspondent precursors **5b** and **6b** to avoid the cyano group reduction.

Compounds **1c**–**7c** were tested to evaluate their affinity and selectivity for the SERT. [^{3}H]WIN 35,428 for DAT, [^{3}H]nisoxetine for NET, and [^{3}H]paroxetine for SERT, and rat cortical and striatal homogenates were used to determine the affinities for the different transporters.



Scheme 3. Reagents and conditions: (a) Pd/C, H_2, 5 h; (b) MeOH, HCl, $SnCl_2$, 0 °C to rt overnight. (i) Using palladium on charcoal with H_2 **2c** was not observed, dehydrochlorination occurred, concomitant to the reduction of the nitro group and afforded **7c**.

Table 1 shows that the compounds display a reasonable to high SERT affinity and selectivity. Among the tested compounds, three diphenylsulfide derivatives display subnanomolar affinities (K_i values **3c**: 0.27, **4c**: 0.35, and **6c**: 0.28 nM), whereas the oxo-compounds present higher K_i values (**1c**: 7.01, **2c**: 2.06, **5c**: 2.91, and **7c**:13.4 nM).

Although nitrobenzenes and nitroanilines have genotoxic properties,[32] we have not performed the Ames test[32] with our compounds because for most genotoxic carcinogenic drugs the cancer life-time risk is <0.0001% if <1.5 μg/d is applied (P. Kasper, unpublished). Three aspects are discussed concerning the structural modifications and their impact on the SERT ligand affinity and selectivity: (i) the effect of the nature of the substituent in position 4′ (R in ring B, Scheme 3), (ii) the bridge atom linking the phenyl rings, and (iii) the introduction of a halogen at position 5 in ring A.

The nature of the substituent at position 4′ does not affect the SERT affinity since the K_{iSERT} values of **3c**, **4c**, and **6c**, as well as **1c** and **5c**, are in the same range. Therefore, in the sulfide and oxo series, it seems that the nature of the group in position 4′ does not significantly affect the SERT–ligand interaction which is in accordance with the literature.[13,31] Second, by comparing the sulfur- and the respective oxo-bridged compounds (**1c**–**3c**, and **5c**–**6c**), the SERT affinities of the oxo-bridged compounds are generally lower. The same observation was made in the comparison of IDAM/ODAM and ADAM/OADAM (K_{iSERT}: 0.097 nM for IDAM, 0.12 nM for ODAM, 0.013 nM for ADAM, and 0.37 nM for OADAM).[31] By contrast, MADAM displays a slightly lower affinity than its oxo analog OMADAM (K_{iSERT}: 1.65 nM for MADAM and 0.53 nM for OMADAM).[30] It appears that both oxygen and sulfur bridges can lead to ligands with high SERT affinity and selectivity. Although the atom bridge linking the two phenyl rings seems to play a major role in the SERT–ligand interaction,[30] it does not directly interact with the transporter. The bridge atom may induce a different conformation of the two phenyl groups and thereby of the amino and dimethylaminomethyl groups, which could be essential in the SERT–ligand interaction.[13] In the oxo and diphenylsulfides series, the cyano

Table 1. Binding affinities of potential SERT–ligands (see Scheme 3) to the three biogenic amine transporters SERT, DAT, and NET isolated from rat brain

Compound	K_i in nM		
	SERT[b]	DAT[b]	NET[b]
1c	7.01 (4.82;9.20)	2790 (2330;3260)	518 (429;607)
2c	2.06[c] (1.84;2.28)	2530 (1800;3250)	2740 (1770;3700)
3c	0.27 (0.21;0.32)	3650 (5520;1780)	1040 (641;1430)
4c	0.35 (0.21;0.49)	1840 (1420;2270)	919 (487;1350)
5c	2.91 (1.69;3.47)	68,300 (4400;88,100)	5550 (1140;9970)
6c	0.28[c] (0.22;0.34)	14,100[a]	17,900[a]
7c	13.4 (6.26;20.5)	4360 (4300;4410)	1083 (686;1480)
MADAM	0.62 (0.62;0.62)	4160[a]	Not determined

K_i values are means of two experiments, each realized in triplicate.
[a] $n = 1$ (in triplicate).
[b] Radioligands: [^{3}H]paroxetine (SERT), [^{3}H]WIN35,428 (DAT), and [^{3}H]nisoxetine (NET).
[c] Radioligand: [^{3}H]citalopram (SERT).

derivatives **5c** and **6c** possess low affinities for the DAT and NET compared to the other ligands (Table 1). For compounds **5c** and **6c**, K_{iDAT} values of 68.3 and 14.1 μM, and K_{iNET} values of 5.55 and 17.9 μM, respectively, were determined. For the other derivatives, K_i values were in the range between 0.52 and 4.36 μM for both DAT and NET. Therefore, although the nature of the group in position 4 does not significantly affect the SERT affinity it influences the selectivity of the ligand. In a series of ADAM analogs with different groups in position 4′ such as a cyano (DASB), a chlorine, a methoxy (DAPP), and a trifluoro methyl group, developed by Wilson et al.,[33] the derivatives display similar affinity for DAT and NET (between 1.4 and 2.7 μM for the DAT and 1.2 and 2.0 μM for the NET), except the chlorine derivative with higher and non-negligible affinity for both transporters (K_{iDAT} = 115 nM and K_{iNET} = 230 nM). Comprehensive SAR studies are needed to draw a profound conclusion whether the cyano group in position 4′ (Scheme 3) affects the SERT selectivity of the compounds. Third, the introduction of a halogen in position 5 (ring A, Scheme 3) does not affect the SERT affinity of the ligands. Even if the electronic density has been modified, with the introduction of a chlorine or fluorine atom, all the compounds **1c**–**6c** display high affinity for the SERT, which is comparable to MADAM. Moreover, the introduction of an halogen at this position does not affect the SERT selectivity as shown by K_i values for DAT and NET higher than 500 nM. It appears that the introduction of a halogen at this position does not affect the SERT affinity and selectivity, and therefore this part of the molecule presumably does not play a critical role in the SERT–ligand interaction. It can be hypothesized, that at least small groups can be introduced at position 5 without significantly modifying SERT affinity and selectivity. This will allow chemical modifications which might result in improved SERT tracer properties.

In conclusion, we have synthesized a new series of fluorinated diphenylchalcogen derivatives with sub- to low nanomolar SERT affinity combined with low affinity toward the DAT and NET.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.02.082.

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