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FULL PAPER

Structure-affinity relationships of fluorinated spirocyclic σ2 receptor ligands with an exocyclic benzylamino moiety

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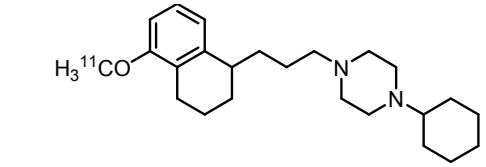
Abstract: In order to detect a potent and selective σ2 receptor ligand appropriate for the development as positron emission tomography (PET) tracer several fluorinated analogs of the spirocyclic lead compounds trans-6 and cis-6 (N-(2,4-dimethylbenzyl)-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine) were designed. In multi-step syntheses, an F-atom was introduced directly or as 2-fluoroethoxy moiety at the 2-benzopyran scaffold, at the dimethylbenzylamino moiety or at the central amino moiety. In receptor binding studies with radioligands the σ1 and σ2 receptor affinity was determined. With respect to σ2 affinity and σ2 : σ1 selectivity cis-N-(2,4-Dimethylbenzyl)-5-fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (cis-15c, Ki(σ2) = 51 nM) and cis-N-[4-(fluoromethyl)-2-methylbenzyl]-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (cis-28e, Ki(σ2) = 57 nM) represent the most promising ligands. Combination of both structural elements in one molecule cis-N-[4-(fluoromethyl)-2-methylbenzyl]-5-fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (cis-28c: Ki(σ2) = 874 nM) resulted in reduced σ2 and σ1 affinity. Methylation of secondary amines led to tertiary methylamines 29-31 with moderate affinity towards both σ receptor subtypes.

Introduction

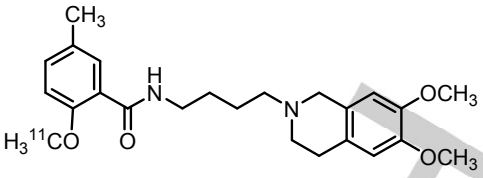
Since their discovery in 1976 both σ receptor subtypes came into the focus for the development of new drugs.1 In contrast to the σ1 receptor, which was cloned firstly in 19962 and crystallized 20 years later by Kruse and coworkers,3 the knowledge about the structure of the σ2 receptor was only limited for a long time. Very recently, the identity of the σ2 receptor and the transmembrane protein TMEM97 located in the endoplasmic reticulum was proven. Its structure shows four transmembrane helices with both the C- and N-termini located on the cytosolic side. Mutagenesis experiments revealed the importance of Asp29 and Asp56 for the binding of basic σ2 receptor ligands.4

Although the σ2 receptor is related to various diseases, its involvement in cancer is of particular importance. An overexpression of the σ2 receptor was observed for many different cancer cell lines.5 Moreover, the density of the σ2 receptor correlates with the proliferative status of cancer cells, since proliferating cancer cells show up to 10-fold higher expression of the σ2 receptor than quiescent cancer cells. Therefore, σ2 receptor ligands can be used in different imaging techniques to determine localization, size and proliferative status of tumors. Additionally, the application of σ2 receptor ligands in cancer therapy is an important object of research. The apoptotic effect of σ2 receptor ligands was demonstrated in vitro and in vivo on several cancer cell lines (reviewed in ref.6). Tumor cell growth was inhibited by single application of σ2 receptor ligands in combination with standard chemotherapeutics.7; 8

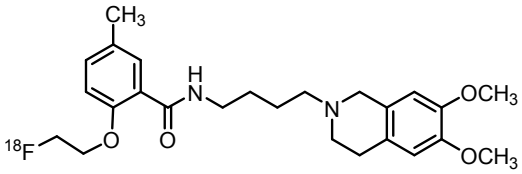
Due to the high expression of σ2 receptors in cancer cells, visualization of cancer tissue by imaging techniques like positron emission tomography (PET) using radiolabeled σ2 receptor ligands is of high interest. An overview of σ2 receptor PET tracers already used in preclinical studies is shown in Figure 1.



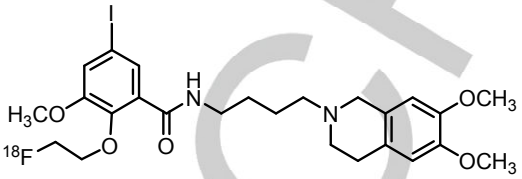
[^{11}C]**1**
([^{11}C]PB28)



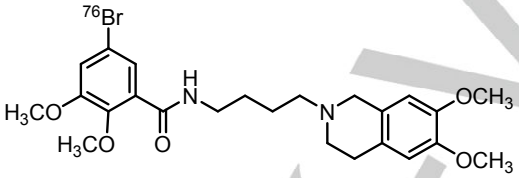
[^{11}C]**2**



[^{18}F]**3**
([^{18}F]ISO-1)



[^{18}F]**4**
([^{18}F]RHM-4)



[^{76}Br]**5**

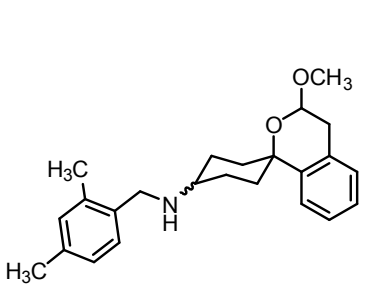
compd.	K_i (σ_1) [nM]	K_i (σ_2) [nM]	σ_2 : σ_1 selectivity
[^{11}C]**1** ([^{11}C]PB28)[9]	0.38	0.58	0.66
[^{11}C]**2**[10]	3078	10.3	300
[^{18}F]**3** ([^{18}F]ISO-1)[11]	330	7	50
[^{18}F]**4** ([^{18}F]RHM-4)[11]	2150	0.26	8300
[^{76}Br]**5**[12]	12900	8.2	1575

Figure 1. Radiolabeled σ2 receptor ligands in preclinical studies.

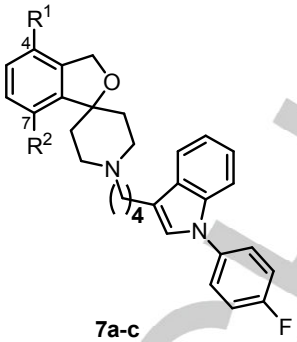
One of the first σ_2 receptor PET tracers was ^{11}C-labeled PB28 ([^{11}C]**1**), which was evaluated in an animal model. However, its missing selectivity over the σ_1 receptor led to problems in selective visualization of the σ_2 receptor.[9]

The class of benzamides shows promising affinity data rendering it well suited for imaging of σ_2 receptors. Several analogs were labeled with different radionuclides. The σ_2 selective benzamide [^{11}C]**2** displayed *in vivo* a high tumor uptake with low distribution in the surrounding tissue.[10] Since ^{18}F shows a longer half-life than ^{11}C, a low positron energy and a high positron decay ratio, it is more favorable for PET studies,[13] and therefore ^{18}F-labeled benzamides were synthesized. Replacement of the methoxy group of benzamide **2** by a [^{18}F]fluoroethoxy moiety led to [^{18}F]ISO-1 ([^{18}F]**3**) and further modifications of the substitution pattern to [^{18}F]RHM-4 ([^{18}F]**4**). Both **2** and **3** displayed high σ_2 receptor affinity and high selectivity over the σ_1 receptor. In animal models, high tumor uptake and appropriate biodistribution was demonstrated for both compounds.[11] [^{18}F]**3** has already been evaluated in a phase 0 clinical study with patients suffering from lymphoma, breast cancer, or head and neck cancer. A correlation between the uptake of [^{18}F]**3** and the proliferative status of tumors was observed.[14] Currently, a phase 1 study evaluating the properties of [^{18}F]**3** in patients with breast cancer is ongoing.

In addition to ^{18}F-labeled benzamides, the ^{76}Br-labeled benzamide [^{76}Br]**5** was synthesized and evaluated *in vivo*. In a mouse model, [^{76}Br]**5** revealed high and selective tumor uptake.[12] However, due to the limited availability and the high positron energy of [^{76}Br] its use as a radionuclide is not favorable.[6]



trans-**6**, *cis*-**6**



7a-c

compd.	R^1	R^2	$K_i(\sigma_1)$ ± SEM [nM]	$K_i(\sigma_2)$ ± SEM [nM]	$\sigma_2 : \sigma_1$ selectivity
trans-**6**[15]	-	-	736	7.6 ± 4.1	100
cis-**6**[15]	-	-	> 1000[#]	54 ± 15	> 20
7a (siramesine)[16]	H	H	17	0.12	140
7b[16]	F	H	290	1.8	160
7c[16]	H	F	150	0.58	260

[#] no correlation between concentration and σ_1 affinity.

Figure 2. Lead compounds with high σ2 receptor affinity.

In previous studies we have shown that the spirocyclic 2-benzopyran derivatives *trans*-**6** and *cis*-**6** with exocyclic amino moiety display high σ_2 receptor affinity and selectivity (Figure 2).[15] Therefore, these compounds served as lead compounds for the development of new σ_2 receptor ligands. Since fluorinated compounds with high σ_2 affinity and selectivity over related receptors could be used as PET tracers to visualize the σ_2 receptor, our interest was focused particularly on fluorinated σ_2 receptor ligands.

The indole derivative siramesine (**7a**) is known as high-affinity σ_2 receptor ligand with preference over the σ_1 receptor. Analogs of siramesine with a F-atom in 4- (**7b**) or 7-position (**7c**) of the 2-benzofuran scaffold show even higher selectivity over the σ_1 receptor than siramesine itself (Figure 2).[16] To improve the $\sigma_2 : \sigma_1$ selectivity of the spirocyclic 2-benzopyrans *trans*-**6** and *cis*-**6**, the concept of fluorination in aromatic position of the spirocyclic scaffold should be transferred to these compounds.

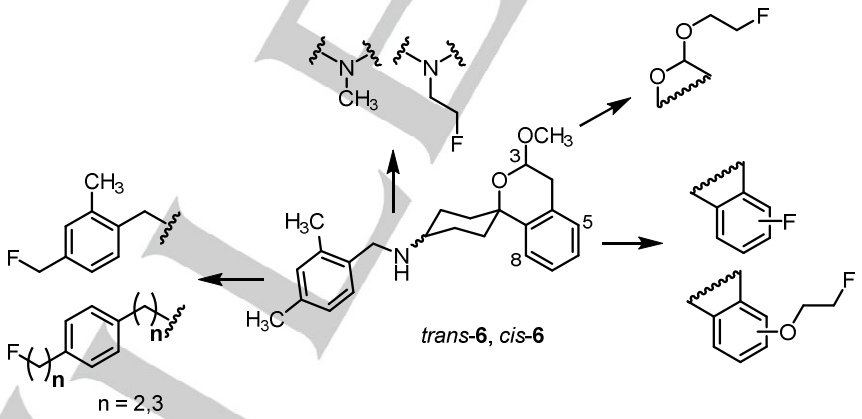


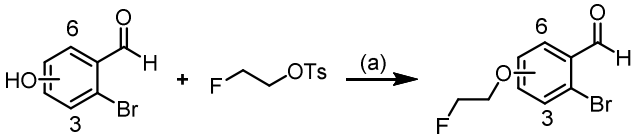
Figure 3. Spirocyclic lead compounds trans-6 and cis-6 and envisaged positions for the introduction of an F-atom.

In Figure 3 the ideas for the introduction of an F-atom into the lead compounds *trans*-**6** and *cis*-**6** are summarized. In addition to the synthesis of analogs with different fluorinated substituents in 5- and 8-position of the 2-benzopyran scaffold, exchange of the dimethylbenzylamino moiety by different fluorinated analogs, alkylation of the secondary amine and modification of the methyl acetal in 3-position of the 2-benzopyran ring should be performed. The affinity of all compounds towards σ_2 and σ_1 receptors will be determined to learn more about structure-affinity relationships of this compound class.

Synthesis

In order to develop ^{18}F-labeled PET tracers for imaging of σ_2 receptors, ^{19}F-labeled fluorinated σ_2 receptor ligands with promising pharmacological properties have to be developed first.

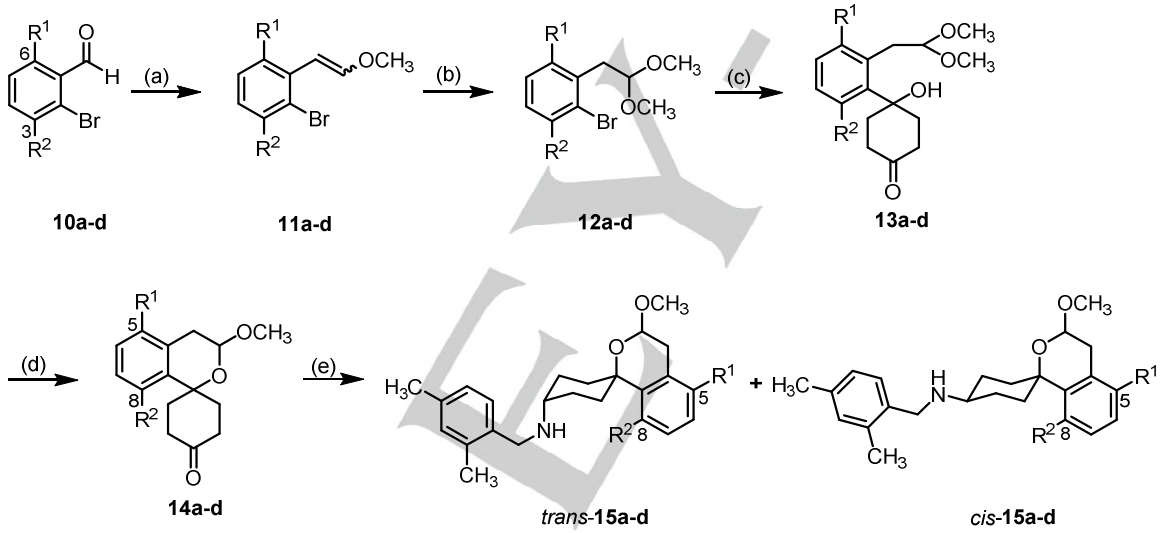
Since a fluorine atom in aromatic position of the spirocyclic scaffold seems to increase the σ_2 receptor selectivity (see siramesine analogs **7b** and **7c**),[16] analogs of *trans*-**6** and *cis*-**6** with a F-atom at the benzene ring were designed and prepared. Moreover, compounds with a larger fluoroethoxy moiety instead of the F-atom should be synthesized. The fluoroethoxy moiety was introduced at the very beginning of the synthesis. Nucleophilic substitution at 2-fluoroethyl tosylate (**9**) by phenols **8a** and **8b** provided the fluoroethoxy derivatives **10a** and **10b**, respectively (Scheme 1).



8a: 6-OH **9** **10a**: 6-OCH_2CH_2F
8b: 3-OH **10b**: 3-OCH_2CH_2F

Scheme 1. Synthesis of benzaldehydes **10a** and **10b** with fluoroethoxy moiety in 3- and 6-position. Reagents and reaction conditions: (a) K_2CO_3, DMF, 3 d, 50 - 70 °C; **10a**, 89 %; **10b**, 99 %.

The spirocyclic 2-benzopyrans with a F-atom or a fluoroethoxy moiety in 5- or 8-position were synthesized according to unsubstituted 2-benzopyrans (Scheme 2, $R^1 = R^2 = H$).[17] At first, a Wittig reaction of benzaldehydes **10a-d** with different substituents in 3- and 6-position using (methoxymethyl)triphenylphosphonium chloride and KO^{t}Bu afforded the enol ethers **11a-d** as mixtures of diastereomers (Scheme 2). For enol ethers **11b** and **11d** with a proton adjacent to the methoxyvinyl moiety (R^1 = H), the ratio of (*E*)- to (*Z*)-configured diastereomers was 50 : 50. However, enol ethers **11a** and **11c** with two substituents in *o*-position to the methoxyvinyl moiety were formed in the ratio (*E*) : (*Z*) = 80 : 20. A separation of the diastereomers by fc was only possible for the fluorinated enol ether **11c**. The electronegative Br- and F-atoms in both *o*-positions to the methoxyvinyl group in **11c** influenced the steric and electronic properties in such a way that (*E*)- and (*Z*)-configured diastereomers could form different interactions with the stationary phase resulting in separation.



10a-d **11a-d** **12a-d** **13a-d** **14a-d**

10 - 15	A	b	C	d
R^1	OCH_2CH_2F	H	F	H
R^2	H	OCH_2CH_2F	H	F

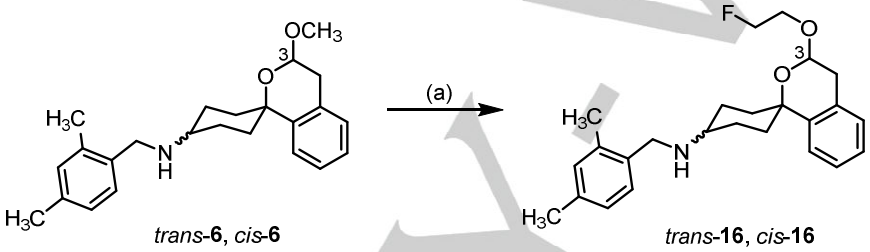
Scheme 2. Synthesis of spirocyclic amines trans- and cis-15a-d with an F-atom and a fluoroethoxy moiety in 5- or 8-position. Reagents and reaction conditions: (a) CH3OCH2PPh3Cl, KOtBu, THF, 5 - 24 h, -20 °C; 11a, 83 %; 11b, 85 %; 11c, 84 %; 11d, 50 %. (b) CH3OH, p-TsOH, 2 - 4 d, 75 °C; 12a, 82 %; 12b, 89 %; 12c, 88 %; 12d, 60 %. (c) 1. n-BuLi (2.5 M in hexane), THF, 20 min, -78 °C; 2. cyclohexane-1,4-dione, THF, 2 h, -78 °C → 30 min, rt; 13a, 48 %; 13b, 25 %; 13c, 44 %; 13d, 25 %. (d) HCl (2 M), CHCl3, 3.5 - 5 h, rt; 14a, 73 %; 14b, 73 %; 14c, 89 %; 14d, 93 %. (e) 2,4-dimethylbenzylamine, NaBH(OAc)3, HOAc, THF, 2.5 - 18 h, rt; trans-15a, 29 %; cis-15a, 58 %; trans-15b, 36 %; cis-15b, 52 %; trans-15c, 28 %; cis-15c, 59 %; trans-15d, 33 %; cis-15d, 18 %.

Acid catalyzed addition of methanol to the double bond of enol ethers **11a-d** yielded the acetals **12a-d**. Bromine-lithium exchange with *n*-BuLi and subsequent addition to cyclohexane-1,4-dione led to hydroxy acetals **13a-d** (Scheme 2). The formation of dimers by addition of another equivalent of lithiated compound to the second carbonyl group of cyclohexane-1,4-dione reduced the yield of hydroxy acetals **13a-d**. Moreover, the position of the additional substituent (F-, FCH_2CH_2O-) had a major influence on the yields: Two substituents in *o*-position to the Br-atom as in **12b** and **12d** lowered the reactivity of the aryllithium intermediates and thus reduced the yields of **13b** and **13d**. Aryl bromides **12a** and **12c** with only one substituent adjacent to the Br-atom gave higher yields of the hydroxy acetals **13a** and **13c**. This observation was explained by the strong electron withdrawing effects of the F- and O-atoms, which led to a lower polarization of the C-Br-bond and reduced nucleophilicity of the aryl intermediate. The lower the

distance between the Br-atom and the electron-withdrawing substituent, the stronger is this effect. Furthermore, two *o*-substituents inhibit sterically the approach of the electrophile (cyclohexanedione). As a result, hydroxy acetals **13b** and **13d** with two substituents in *o*-position to the hydroxycyclohexyl moiety were obtained in lower yields (25 % each) than hydroxy acetals **13a** and **13c** bearing a proton adjacent to the hydroxycyclohexyl moiety (48 % and 44 %).

Next, an intramolecular transacetalization of hydroxy acetals **13a-d** afforded the spirocyclic acetals **14a-d** in high yields (Scheme 2). The dimethylbenzylamino moiety was introduced by reductive amination of ketones **14a-d** using $NaBH(OAc)_3$ as a mild reducing agent in the presence of small amounts of HOAc. The resulting diastereomers *trans* **15a-d** and *cis*-**15a-d** were separated by fc. The first eluted diastereomers were termed *trans*-configured concerning the relative orientation of the N- and O-atoms within the cyclohexane ring. The second eluted diastereomers bearing the N- and O-atoms at the same side of the cyclohexane ring were termed *cis*-configured.

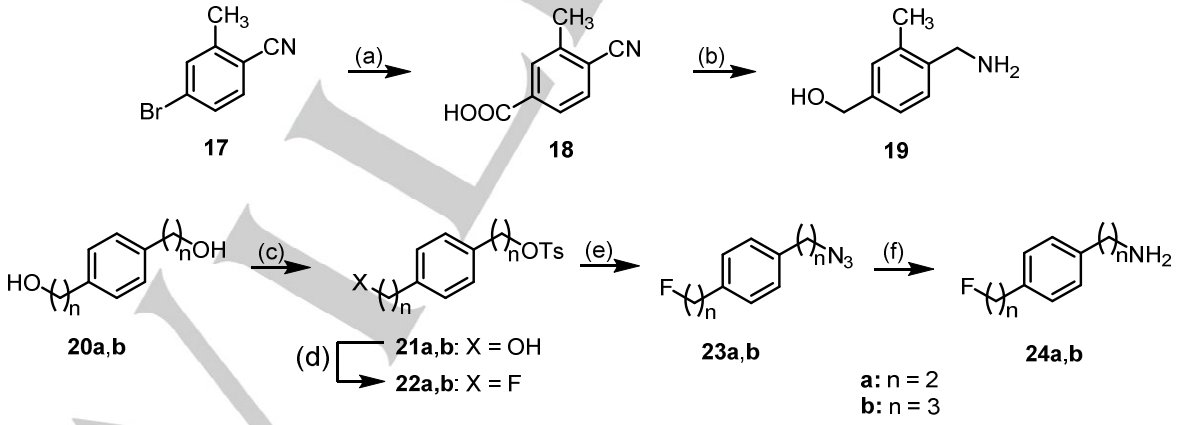
During the reductive amination, *cis*- and *trans*-configured amines were formed in different amounts. Stereoelectronic effects led to preferred hydride transfer in axial orientation to the iminium ion intermediate resulting in preferred formation of *cis*-configured diastereomers.[15; 18] This stereoselectivity was observed for both fluoroethoxy substituted derivatives **15a** and **15b** and the 5-fluoro derivatives **15c** (*trans*-**15a**, 29 %; *cis*-**15a**, 58 %; *trans*-**15b**, 36 %; *cis*-**15b**, 52 %; *trans*-**15c**, 28 %; *cis*-**15c**, 59 %). However, reductive amination of the ketone **14d** with the F-atom in 8-position led preferably to the *trans*-configured amine **15d** (*trans*-**15d**, 33 %; *cis*-**15d**, 18 %). Steric reasons were excluded to explain this observation, since *cis*-**15b** with the fluoroethoxy moiety in 8-position was formed in higher yield than its *trans*-configured diastereomer **15b** (*trans*-**15b**, 36 %; *cis*-**15b**, 52 %). In fact, the electronic properties of the F-atom at the phenyl ring shifted the preference of the hydride attack towards an equatorial approach and led to an increased formation of the *trans*-configured amine **15d**.



trans-**6**, *cis*-**6** *trans*-**16**, *cis*-**16**

Scheme 3. Synthesis of spirocyclic σ receptor ligands with fluoroethoxy moiety in 3-position. Reagents and reaction conditions: (a) *p*-TsOH, 2-fluoroethanol, 3 d, rt; *trans*-**16**, 73 %; *cis*-**16**, 73 %.

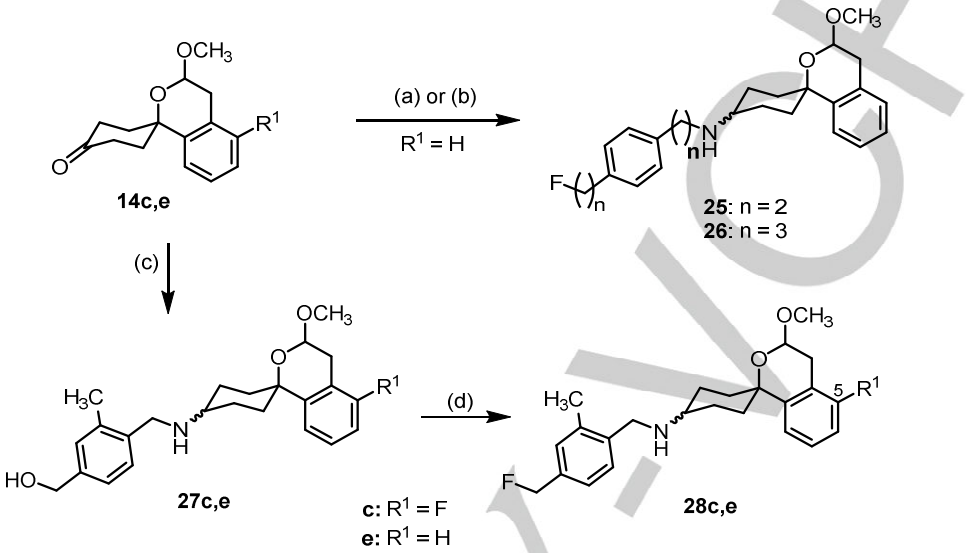
In a previous study it was shown that replacement of the methoxy moiety in 3-position of lead compounds *trans*-**6** and *cis*-**6** by a fluoroethyl moiety decreased the σ_2 affinity.[15] To investigate, if this effect was caused by the loss of the O-atom, compounds *trans*-**16** and *cis*-**16** with fluoroethoxy moiety in 3-position were synthesized. For this purpose, a transacetalization of methyl acetals *trans*-**6** and *cis*-**6** with 2-fluoroethanol as solvent was conducted (Scheme 3). The transformation was catalyzed by 1.1 equivalents of *p*-TsOH. Higher amounts of this acid did not accelerate the reaction but led to elimination of the acetalic group



Scheme 4. Synthesis of different amine derivatives. Reagents and reaction conditions: (a) *n*-BuLi, dry ice (CO_2), THF, 2 h, -78 °C → rt; 75 %. (b) $LiAlH_4$, THF, 2 d, 70 °C; 51 %. (c) *p*-TsCl, Et_3N, CH_2Cl_2, 16 h, rt; **21a**, 42 %; **21b**, 42 %. (d) DAST, CH_2Cl_2, 20 - 23 h, -78 °C → rt; **22a**, 48 %; **22b**, 72 %. (e) NaN_3, DMF, 17 h, 50 °C; **23a**, 72 %; **23b**, 92 %. (f) H_2, 10 % Pd/C, CH_3OH, 16 - 19 h, rt; **24a**, 49 %; **24b**, 65 %.

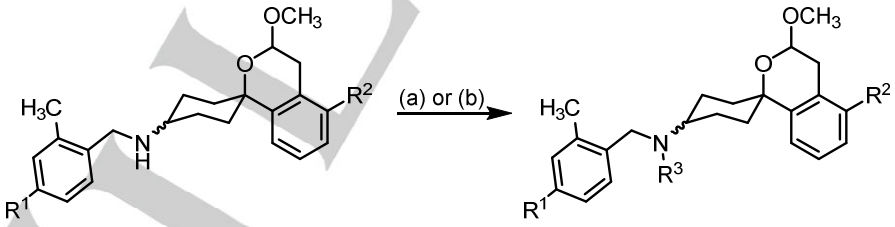
To obtain compounds with fluorinated benzylamino residues, amine **19** was synthesized. Carboxylation of nitrile **17** was conducted by halogen metal exchange using *n*-BuLi and subsequent addition of dry ice (CO_2) in THF. Afterwards, both the carboxy and cyano moieties were reduced with $LiAlH_4$ in one step to yield amine **19** (Scheme 4).

In order to investigate the influence of the size of the basic amino moiety on the σ receptor affinity, homologs of benzylamine **19** were synthesized (Scheme 4). In a first step, only one of two hydroxy moieties of symmetric diols **20a**[19] and **20b**[20; 21] were reacted with *p*-TsCl. To avoid ditosylation of diols **20a** and **20b**, only 0.9 equivalents of *p*-TsCl were used and Et_3N was added dropwise within 1 h. Under these conditions, both monotosylates **21a** and **21b** were obtained in 42 % yield. The reaction with DAST led to the fluorinated compounds **22a** and **22b**, still bearing a tosyloxy moiety. Afterwards, substitution of the tosyloxy group by sodium azide was conducted. In a last step, azides **23a** and **23b** were reduced with H_2 in the presence of the catalyst Pd/C to yield the primary amines **24a** and **24b** (Scheme 4).



Scheme 5. Synthesis of spirocyclic σ receptor ligands with an F-atom in the phenylalkyl side chain. Reagents and reaction conditions: (a) amine **24a**, $NaBH(OAc)_3$, HOAc, THF, 26 h, rt; *trans*-**25**, 29 %; *cis*-**25**, 35 %. (b) amine **24b**, $NaBH(OAc)_3$, HOAc, THF, 3 h, rt; *trans*-**26**, 29 %; *cis*-**26**, 38 %. (c) amine **19**, $NaBH(OAc)_3$, HOAc, THF, 6,5 h, rt; *trans*-**27c**, 24 %; *cis*-**27c**, 59 %; *trans*-**27e**, 10 %; *cis*-**27e**, 58 %. (d) DAST, CH_2Cl_2, 45 min -78 °C, 2 h rt; *trans*-**28c**, 70 %; *cis*-**28c**, 60 %; *trans*-**28e**, 50 %; *cis*-**28e**, 35 %.

Ketone **14e** was reductively aminated with amines **19**, **24a** and **24b** using $NaBH(OAc)_3$ as reducing agent and small amounts of HOAc. To obtain compounds bearing two F-atoms, ketone **14c** bearing an F-atom in 5-position of the 2-benzopyran scaffold was reacted with amine **19** in a reductive amination (Scheme 5). In each reaction, two diastereomers were obtained, which were separated by flash column chromatography. As observed in previous reactions, the yields of the first eluted *trans*-configured diastereomers were lower than the yields of the later eluted *cis*-configured diastereomers. DAST converted the alcohols *trans*-**27c**, *cis*-**27c**, *trans*-**27e** and *cis*-**27e** into the fluorinated compounds *trans*-**28c**, *cis*-**28c**, *trans*-**28e** and *cis*-**28e**, respectively. (Scheme 5).



starting material	product	R^1	R^2	R^3	yield
trans-**6**	*trans*-**29**	CH_3	H	CH_3	60 %
cis-**6**	*cis*-**29**	CH_3	H	CH_3	89 %
cis-**28c**	*cis*-**30**	CH_2F	F	CH_3	88 %
cis-**28e**	*cis*-**31**	CH_2F	H	CH_3	71 %
cis-**6**	*cis*-**32**	CH_3	H	CH_2CH_2F	42 %

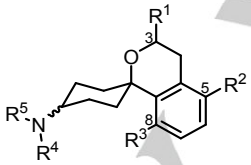
Scheme 6. Synthesis of N-alkylated spirocyclic amines. Reagents and reaction conditions: (a) formalin, $NaBH(OAc)_3$, CH_2Cl_2, 3 - 17 h, rt. (b) 1. 2-fluoroethanol, Dess-Martin-Periodinane (DMP), CH_2Cl_2, 18 h, rt; 2. addition of *cis*-**6** and $NaBH(OAc)_3$, HOAc, CH_2Cl_2, 3 h, rt.

Depending on the size of the N-residue, alkylation of secondary amines influences the σ receptor affinity. Small residues such as the CH_3 moiety increased the σ receptor affinity, whereas the propyl residues and larger groups led to reduced σ receptor affinity. Ethyl groups are tolerated by the σ receptors without changing the affinity.[22] Due to their high σ_2 receptor affinity, the lead compounds *trans*-**6** and *cis*-**6**, the monofluorinated amine *cis*-**28e** and amine *cis*-**28c** bearing two fluorine atoms were selected for further derivatization. Reductive alkylation of these amines with formalin and $NaBH(OAc)_3$ led to the tertiary methylamines *trans*-**29**, *cis*-**29**, *cis*-**31** and *cis*-**30** in high yields (Scheme 6). Introduction of a fluoroethyl group as third N-residue was achieved by reductive alkylation of the secondary amine *cis*-**6** with 2-fluoroacetaldehyde. For this purpose, 2-fluoroethanol was oxidized with Dess-Martin-Periodinane, the reaction mixture was filtered and directly used for the reductive alkylation of amine *cis*-**6** (Scheme 6).

Affinity data

Radioligand binding assays were used for the determination of the σ_1 and σ_2 receptor affinity of the synthesized compounds. For the σ_1 assay homogenates of guinea pig brains served as receptor material and [^{3}H]-(+)-pentazocine as σ_1 selective radioligand. The σ_2 assay was conducted with homogenates of rat liver as receptor material and with the non-selective radioligand [^{3}H]-1,3-di(o-tolyl)guanidine (DTG), since no σ_2 selective radioligand is available. An excess of non-tritiated (+)-pentazocine was added to selectively occupy σ_1 receptors.[23–25] Determined affinities of synthesized compounds and reference compounds towards both σ receptor subtypes are summarized in Table 1.

Table 1: σ_1 and σ_2 receptor affinity of spirocyclic amines and reference compounds, n = 3, if SEM is given, otherwise n = 1.



compd.	R^1	R^2	R^3	R^4	R^5	K_i ± SEM [nM]	
						σ_1	σ_2
(+)-pentazocine	-	-	-	-	-	5.4 ± 0.5	-
2 (siramesine)[26]	-	-	-	-	-	17	0.12
trans-**6**	OCH_3	H	H	H		736	7.6 ± 4.1
cis-**6**						> 1000*	54 ± 15
trans-**15a**	OCH_3	OCH_2CH_2F	H	H		> 1000	> 1000
cis-**15a**						310	469
trans-**15b**	OCH_3	H	OCH_2CH_2F	H		> 1000	> 1000
cis-**15b**						> 1000	> 1000
trans-**15c**	OCH_3	F	H	H		> 1000	178
cis-**15c**						403 ± 83	51 ± 6
trans-**15d**	OCH_3	H	F	H		> 1000	175
cis-**15d**						621	251
trans-**16**	OCH_2CH_2F	H	H	H		> 1000	599
cis-**16**						> 1000	> 1000
trans-**25**	OCH_3	H	H	H		> 1000	> 1000
cis-**25**						> 1000	> 1000
trans-**26**	OCH_3	H	H	H		> 1000	> 1000
cis-**26**						801	> 1000
trans-**28c**	OCH_3	F	H	H		84 ± 10	290 ± 45
cis-**28c**						> 1000	874

trans-**28e**	OCH_3	H	H	H	> 1000	803
cis-**28e**					513	57 ± 12
trans-**29**	OCH_3	H	H	CH_3	160	231
cis-**29**					74 ± 4	73 ± 30
cis-**30**	OCH_3	F	H	CH_3	29 ± 2	51 ± 6
cis-**31**	OCH_3	H	H	CH_3	31 ± 3	66 ± 5
cis-**32**	OCH_3	H	H	CH_2CH_2F	> 1000	> 1000

* No correlation between concentration and receptor affinity.

As already observed for siramesine analogs,[16] the introduction of a F-atom into the aromatic portion of the spirocyclic system leads to compounds with distinct preference for the σ_2 receptor subtype. Both, compounds with F-atom in 5- (**15c**) and 8-position of the 2-benzopyran scaffold (**15d**), bind with higher affinity to the σ_2 receptor than to the σ_1 receptor. The *cis*-configured amine *cis*-**15c** with F-atom in 5-position of the 2-benzopyran scaffold shows the highest σ_2 affinity of the compounds bearing the F-atom at an aromatic ring (K_i (σ_2) = 51 nM). Its affinity is comparable with the σ_2 affinity of the non-fluorinated *cis*-configured lead compound *cis*-**6** (K_i (σ_2) = 54 nM).

Replacement of the aromatic bound F-atom by a larger 2-fluoroethoxy group was not tolerated by the σ_2 receptor and led to decreased σ_2 affinity. Only amine *cis*-**15a** with the fluoroethoxy group in 5-position shows σ_2 receptor affinity in the high nanomolar range (K_i (σ_2) = 469 nM).

In order to investigate the role of the O-atom of the exocyclic 3-substituent of the 2-benzopyran system, fluoroethyl acetals *trans*-**16** and *cis*-**16** were prepared and included into this study. In comparison to the methyl acetals *trans*-**6** and *cis*-**6**, fluoroethyl acetals *trans*-**16** and *cis*-**16** show a significantly reduced affinity towards the σ_2 receptor. It was concluded that the σ_2 receptor affinity is more affected by the size and stereoelectronic properties of the residue in 3-position than by the presence of an O-atom in this position.

Variation of the dimethylbenzylamine part of lead compounds *trans*-**6** and *cis*-**6** had a remarkable impact on σ_2 receptor affinity. In contrast to its *trans*-configured diastereomer **28e**, amine *cis*-**28e** with a fluoromethyl group in *p*-position of the benzylamino moiety shows high σ_2 receptor affinity and selectivity (K_i (σ_2) = 57 nM; σ_2 : σ_1 selectivity = 10). However, elongation of the distance between the basic N-atom and the aromatic system and between the aromatic system and the F-atom, respectively, led to decreased σ_2 affinity. This was observed for amines *trans*-**25** and *cis*-**25** with ethylene spacer and for amines *trans*-**26** und *cis*-**26** with trimethylene spacer.

Combination of the structural elements of the most promising compounds *cis*-**15c** bearing an F-atom in 5-position of the 2-benzopyran and *cis*-**28e** with fluorinated dimethylbenzylamino moiety led to amines *trans*-**28c** and *cis*-**28c**. Surprisingly, amine *cis*-**28c** shows only low affinity towards both σ receptors. Its diastereomer *trans*-**28c** demonstrates a slightly higher σ_2 receptor affinity, but also a large increase in σ_1 affinity (K_i (σ_1) = 84 nM).

Methylation of the secondary benzylamine derivatives *trans*-**6** and *cis*-**6** led to tertiary amines *trans*-**29** and *cis*-**29** displaying moderate to high σ_1 and σ_2 receptor affinity. A preference for one of the two σ receptor subtypes could not be observed. Similar results were obtained by methylation of fluorinated amines *cis*-**28e** and *cis*-**28c**. The resulting tertiary amines *cis*-**31** and *cis*-**30** bind with high affinity to both σ receptors but show a slight preference for the σ_1 subtype. Comparable findings have already been described for methylation of various benzylamine derivatives.[17] However, introduction of the larger fluoroethyl moiety (*cis*-**32**) decreased the affinity towards both σ receptors.

Conclusion

The starting point for the development of fluorinated spirocyclic σ_2 receptor ligands was the spirocyclic 2-benzopyran *trans*-**6** showing high σ_2 affinity (K_i = 7.6 nM). Introduction of an F-atom at various positions of the lead compounds *cis*-**6** and *trans*-**6** resulted in considerably reduced σ_2 affinity. The highest σ_2 affinity was found for the analogous 5-fluoro derivative *cis*-**15c**. Although 8-fold σ_2 : σ_1 selectivity was found for *cis*-**15c**, its σ_2 affinity (K_i = 51 nM) is not high enough for the development as PET tracer.

Experimental

Chemistry, General

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Flash chromatography (fc): Silica gel 60, 40–64 µm (Merck); parentheses include: diameter of the column (d), length of the stationary phase (l), fraction size (V), eluent. MS: microTOF-Q II (Bruker Daltonics). Nuclear magnetic resonance (NMR) spectra were recorded on Agilent 600-MR (600 MHz for 1H, 151 MHz for 13C) or Agilent 400-MR

spectrometer (400 MHz for 1H, 101 MHz for 13C); δ in ppm related to tetramethylsilane.

HPLC methods to determine the purity of the compounds

HPLC: Pump: LPG-3400SD, degasser: DG-1210, autosampler: ACC-3000T, UV-detector: VWD-3400RS, interface: DIONEX UltiMate 3000, data acquisition: Chromeleon 7 (Thermo Fisher Scientific); column: LiChrospher® 60 RP-select B (5 µm), LiChroCART® 250-4 mm cartridge; guard column: LiChrospher® 60 RP-select B (5 µm), LiChroCART® 4-4 mm cartridge (No.: 1.50963.0001), manu-CART® NT cartridge holder; flow rate: 1.0 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: method 1: water with 0.05 % (v/v) trifluoroacetic acid; method 2: water; B: method 1: acetonitrile with 0.05 % (v/v) trifluoroacetic acid; method 2: acetonitrile: gradient elution: (A %): 0-4 min: 90 %, 4-29 min: 90 → 0 %, 29-31 min: 0 %, 31-31.5 min: 0 → 90 %, 31.5-40 min: 90 %. The purity of all compounds was determined by these methods. Unless otherwise mentioned, the purity of all test compounds is higher than 95 %.

Synthetic procedures

5-Fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cylohexan]-4'-one (14c)

A solution of acetal 13c (626 mg, 2.11 mmol) and 2 M HCl (0.3 mL, 0.60 mmol, 0.3 eq) in CHCl3 (50 mL) was stirred at rt for 4 h. CH2Cl2 (30 mL) was added and the mixture was washed with 1 M NaOH (2 x 20 mL) and H2O (20 mL). The organic layer was dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified by fc (d = 3 cm, l = 20 cm, V = 20 mL, cyclohexane/ethyl acetate 80:20 → 67:33). Colorless solid, mp 146 °C, yield 495 mg (89 %). C15H17FO3 (264.3 g/mol). Rf = 0.44 (cyclohexane/ethyl acetate 67:33). HR-MS (APCI, method 1): m/z = 233.0943 (calcd. 233.0972 for C14H14FO2 [M-CH3OH+H+]). 1H NMR (400 MHz, DMSO-d6): δ (ppm) = 1.95 – 2.03 (m, 1H, 2'-H), 2.11 – 2.24 (m, 3H, 3'-H, 5'-H, 6'-H), 2.28 – 2.37 (m, 1H, 6'-H), 2.43 – 2.54 (m, 1H, 2'-H), 2.61 – 2.76 (m, 2H, 4-H, 5'-H), 2.76 – 2.87 (m, 1H, 3'-H), 2.96 (ddd, J = 16.3/3.3/1.3 Hz, 1H, 4-H), 3.48 (s, 3H, OCH3), 5.04 (dd, J = 7.2/3.3 Hz, 1H, 3-H), 7.06 (ddd, J = 9.3/7.9/1.3 Hz, 1H, 6-H), 7.19 (dd, J = 8.0/1.3 Hz, 1H, 8-H), 7.25 (td, J = 7.9/5.8 Hz, 1H, 7-H). 13C NMR (101 MHz, DMSO-d6): δ (ppm) = 27.6 (d, J = 3.6 Hz, 1C, C-4), 35.2 (1C, C-6'), 36.79 (1C, C-5'), 36.84 (1C, C-3'), 37.6 (1C, C-2'), 55.5 (1C, OCH3), 74.9 (d, J = 2.1 Hz, 1C, C-1), 95.5 (1C, C-3), 112.9 (d, J = 21.2 Hz, 1C, C-6), 119.0 (d, J = 18.7 Hz, 1C, C-4a), 120.5 (d, J = 3.3 Hz, 1C, C-8), 127.5 (d, J = 8.6 Hz, 1C, C-7), 142.7 (d, J = 4.6 Hz, 1C, C-8a), 159.5 (d, J = 242.8 Hz, 1C, C-5), 209.6 (1C, C=O). FT-IR (neat): ν [cm-1] = 2963, 2928, 2835 (C-Halkyl), 1701 (C=O), 1462 (C=Carom). Purity (HPLC, method 1): 99.5 %, tR = 19.3 min.

trans-N-(2,4-Dimethylbenzyl)-5-fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (trans-15c) and

cis-N-(2,4-Dimethylbenzyl)-5-fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (cis-15c)

A solution of ketone 14c (84 mg, 0.22 mmol), 2,4-dimethylbenzylamine (66 mg, 0.49 mmol, 1.5 eq) and acetic acid (19 µL, 0.33 mmol, 1.0 eq) in THF (10 mL) was stirred under N2 atmosphere at rt. After 30 min, NaBH(OAc)3 (132 mg, 0.62 mmol, 1.9 eq) was added and the mixture was stirred for 2 h at rt. 1 M NaOH (10 mL) was added and the aqueous layer was extracted with Et2O (3 x 20 mL). The combined organic layers were dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 21 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % N,N-dimethylethanamine → 67:33 + 1 % N,N-dimethylethanamine). trans-15c was eluted first and cis-15c afterwards. trans-15c was purified again by fc (d = 2 cm, l = 23 cm, V = 5 mL, cyclohexane/ethyl acetate 90:10 + 1 % N,N-dimethylethanamine).

trans-15c: Colorless solid, mp 116 °C, yield 35 mg (28 %). C24H30FNO2 (383.5 g/mol). Rf = 0.54 (cyclohexane/ethyl acetate 67:33 + 1 % N,N-dimethylethanamine). HR-MS (APCI, method 1): m/z = 384.2299 (calcd. 384.2333 for C24H31FNO2 [MH+]). 1H NMR (400 MHz, CD3OD): δ (ppm) = 1.57 (dq, J = 13.5/3.0 Hz, 1H, 2'-Hequ), 1.72 – 1.80 (m, 2H, 3'-H, 5'-H), 1.81 – 1.90 (m, 1H, 6'-H), 1.96 – 2.06 (m, 2H, 5'-H, 6'-H), 2.06 – 2.16 (m, 1H, 3'-H), 2.24 – 2.35 (m, 1H, 2'-Hax), 2.29 (s, 3H, 4-CH3), 2.38 (s, 3H, 2-CH3), 2.62 (dd, J = 16.3/7.6 Hz, 1H, 4-H), 2.95 (ddd, J = 16.3/3.2/1.4 Hz, 1H, 4-H), 3.01 (quint, J = 3.0 Hz, 1H, 4'-Hequ), 3.55 (s, 3H, OCH3), 3.76 (s, 2H, ArCH2NH), 4.90 (dd, J = 7.6/3.2 Hz, 1H, 3-H), 6.90 (ddd, J = 9.3/6.4/2.8 Hz, 1H, 6-H), 6.96 – 7.03 (m, 2H, 3-Hbenzyl, 5-Hbenzyl), 7.15 – 7.20 (m, 2H, 7-H, 8-H), 7.22 (d, J = 7.6 Hz, 1H, 6-Hbenzyl). A signal for the NH proton is not observed in the spectrum. 13C NMR (101 MHz, CD3OD): δ (ppm) = 19.2 (1C, 2-CH3), 21.1 (1C, 4-CH3), 26.58 (1C, C-3'), 26.64 (1C, C-5'), 29.1 (d, J = 4.1 Hz, 1C, C-4), 31.4 (1C, C-6'), 34.2 (1C, C-2'), 50.2 (1C, ArCH2NH), 52.3 (1C, C-4'), 56.5 (1C, OCH3), 78.1 (1C, C-1), 97.1 (1C, C-3), 113.4 (d, J = 21.6 Hz, 1C, C-6), 120.2 (d, J = 18.4 Hz, 1C, C-4a), 121.9 (d, J = 3.3 Hz, 1C, C-8), 127.6 (1C, C-5benzyl), 128.4 (d, J = 8.7 Hz, 1C, C-7), 130.2 (1C, C-6benzyl), 132.1 (1C, C-3benzyl), 136.2 (1C, C-1benzyl), 137.4 (1C, C-2benzyl), 137.8 (1C, C-4benzyl), 146.1 (d, J = 4.5 Hz, 1C, C-8a), 161.5 (d, J = 243.0 Hz, 1C, C-5). FT-IR (neat): ν [cm-1] = 3298 (N-H), 2955, 2920, 2851 (C-Halkyl), 1458, 1443 (C=Carom). Purity (HPLC, method 1): 94.7 %, tR = 20.2 min.

cis-15c: Colorless solid, mp 118 °C, yield 74 mg (59 %). C24H30FNO2 (383.5 g/mol). Rf = 0.20 (cyclohexane/ethyl acetate 67:33 + 1 % N,N-dimethylethanamine). HR-MS (APCI, method 1): m/z = 384.2331 (calcd. 384.2333 for C24H31FNO2 [MH+]). 1H NMR (600 MHz, CD3OD): δ (ppm) = 1.66 – 1.73 (m, 1H, 2'-Hax), 1.74 – 1.94 (m, 5H, 3'-H, 5'-H, 6'-H), 1.96 – 2.02 (m, 1H, 6'-H), 2.13 (dq, J = 13.9/2.9 Hz, 1H, 2'-Hequ), 2.28 (s, 3H, 4-CH3), 2.33 (s, 3H, 2-CH3), 2.64 (dd, J = 16.2/7.4 Hz, 1H, 4-H), 2.74 (tt, J = 11.2/3.9 Hz, 1H, 4'-Hax), 2.95 (ddd, J = 16.3/3.3/1.2 Hz, 1H, 4-H), 3.57 (s, 3H, OCH3), 3.79 (s, 2H, ArCH2NH), 4.91 (dd, J = 7.5/3.2 Hz, 1H, 3-H), 6.91 (ddd, J = 9.2/8.1/1.0 Hz, 1H, 6-H), 6.97 – 7.03 (m, 3H, 8-H, 3-Hbenzyl, 5-Hbenzyl), 7.17 – 7.22 (m, 2H, 7-H, 6-Hbenzyl). A signal for the NH proton is not observed in the spectrum. 13C NMR (151 MHz, CD3OD): δ (ppm) = 19.1 (1C, 2-CH3), 21.1 (1C, 4-CH3), 28.95 – 29.04 (m, 3C, C-4, C-3', C-5'), 36.3 (1C, C-2'), 39.0 (1C, C-6'), 48.7 (1C, ArCH2NH), 56.6 (1C, OCH3), 57.1 (1C, C-4'), 77.4 (1C, C-1), 97.0 (1C, C-3), 113.6 (d, J = 21.6 Hz, 1C, C-6), 120.5 (d, J = 18.8 Hz, 1C, C-4a), 121.5 (d, J = 3.1 Hz, 1C, C-8), 127.6 (1C, C-5benzyl), 128.4 (d, J = 8.5 Hz, 1C, C-7), 129.9 (1C, C-6benzyl), 132.1 (1C, C-3benzyl), 135.8 (1C, C-1benzyl), 137.2 (1C, C-2benzyl), 137.8 (1C, C-4benzyl), 145.3 (d, J = 4.4 Hz, 1C, C-8a), 161.6 (d, J = 243.3 Hz, 1C, C-5). FT-IR (neat): ν [cm-1] = 3291 (N-H), 2936, 2855 (C-Halkyl), 1462, 1443 (C=Carom). Purity (HPLC, method 1): 99.4 %, tR = 20.0 min.

trans-(4-{[N-(5-Fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl)amino]methyl}-3-methylphenyl)methanol (trans-27c) and

cis-(4-{[N-(5-Fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl)amino]methyl}-3-methylphenyl)methanol (cis-27c)

A solution of ketone 14c (153 mg, 0.58 mmol), amine 19 (114 mg, 0.75 mmol, 1.3 eq), acetic acid (36 µL, 0.63 mmol, 1.1 eq) and NaBH(OAc)3 (220 mg, 1.04 mmol, 1.8 eq) in CH2Cl2 (30 mL) was stirred under N2 atmosphere at rt. After 6 h, 1 M NaOH (150 mL) was added and the aqueous layer was extracted with Et2O (3 x 30 mL). The combined organic layers were dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 21 cm, V = 10 mL,

cyclohexane/ethyl acetate 1:1 + 1 % N,N-dimethylethanamine). trans-27c was eluted first and cis-27c afterwards. trans-27c was purified again by fc (d = 2 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % N,N-dimethylethanamine → 67:33 + 1 % N,N-dimethyl-ethanamine).

trans-27c: Colorless solid, mp 130 °C, yield 56 mg (24 %). C24H30FNO3 (399.5 g/mol). Rf = 0.26 (cyclohexane/ethyl acetate 33:67 + 1 % N,N-dimethylethanamine). HR-MS (ESI): m/z = 400.2287 (calcd. 400.2282 for C24H31FNO3 [MH+]). 1H NMR (400 MHz, CD3OD): δ (ppm) = 1.60 (dq, J = 13.5/3.0 Hz, 1H, 2'-Hequ), 1.75 – 1.83 (m, 2H, 3'-H, 5'-H), 1.83 – 1.93 (m, 1H, 6'-H), 1.99 – 2.08 (m, 2H, 5'-H, 6'-H), 2.08 – 2.18 (m, 1H, 3'-H), 2.33 (td, J = 13.6/3.8 Hz, 1H, 2'-Hax), 2.45 (s, 3H, ArCH3), 2.65 (dd, J = 16.2/7.6 Hz, 1H, 4-H), 2.98 (ddd, J = 16.3/3.2/1.5 Hz, 1H, 4-H), 3.04 (quint, J = 2.9 Hz, 1H, 4'-Hequ), 3.57 (s, 3H, OCH3), 3.83 (s, 2H, ArCH2NH), 4.59 (s, 2H, ArCH2OH), 4.93 (dd, J = 7.6/3.3 Hz, 1H, 3-H), 6.93 (ddd, J = 9.3/7.1/2.1 Hz, 1H, 6-H), 7.17 – 7.26 (m, 4H, 7-H, 8-H, 2-Hbenzyl, 6-Hbenzyl), 7.36 (d, J = 7.5 Hz, 1H, 5-Hbenzyl). Signals for the OH and NH protons are not observed in the spectrum. 13C NMR (101 MHz, CD3OD): δ (ppm) = 19.3 (1C, ArCH3), 26.55 (1C, C-3'), 26.62 (1C, C-5'), 29.0 (d, J = 4.2 Hz, 1C, C-4), 31.4 (1C, C-6'), 34.2 (1C, C-2'), 50.1 (1C, ArCH2NH), 52.3 (1C, C-4'), 56.5 (1C, OCH3), 65.0 (1C, ArCH2OH), 78.1 (d, J = 1.8 Hz, 1C, C-1), 97.1 (1C, C-3), 113.5 (d, J = 21.6 Hz, 1C, C-6), 120.2 (d, J = 18.6 Hz, 1C, C-4a), 121.8 (d, J = 3.2 Hz, 1C, C-8), 125.7 (1C, C-6benzyl), 128.4 (d, J = 8.6 Hz, ,1C, C-7), 130.1 (1C, C-2benzyl), 130.2 (1C, C-5benzyl), 137.7 (1C, C-3benzyl), 138.3 (1C, C-4benzyl), 141.6 (1C, C-1benzyl), 146.0 (d, J = 4.3 Hz, 1C, C-8a), 161.5 (d, J = 243.1 Hz, 1C, C-5). FT-IR (neat): ν [cm-1] = 3275 (N-H/O-H), 2928, 2851 (C-Halkyl), 1462, 1443 (C=Carom). Purity (HPLC, method 1): 87.4 %, tR = 16.8 min.

cis-27c: Colorless solid, mp 137 °C, yield 137 mg (59 %). C24H30FNO3 (399.5 g/mol). Rf = 0.09 (cyclohexane/ethyl acetate 33:67 + 1 % N,N-dimethylethanamine). HR-MS (APCI, method 1): m/z = 400.2297 (calcd. 400.2282 for C24H31FNO3 [MH+]). 1H NMR (600 MHz, CD3OD): δ (ppm) = 1.69 (td, J = 13.7/3.4 Hz, 1H, 2'-H), 1.73 – 1.79 (m, 1H, 3'-H), 1.79 – 1.89 (m, 2H, 5'-H, 6'-H), 1.89 – 1.96 (m, 2H, 3'-H, 5'-H), 2.00 (td, J = 14.2/3.5 Hz, 1H, 6'-H), 2.12 – 2.17 (m, 1H, 2'-H), 2.39 (s, 3H, ArCH3), 2.65 (dd, J = 16.2/7.4 Hz, 1H, 4-H), 2.75 (tt, J = 11.0/3.9 Hz, 1H, 4'-Hax), 2.96 (dd, J = 16.2/3.1 Hz, 1H, 4-H), 3.59 (s, 3H, OCH3), 3.84 (s, 2H, ArCH2NH), 4.57 (s, 2H, ArCH2OH), 4.92 (dd, J = 7.3/3.0 Hz, 1H, 3-H), 6.90 – 6.95 (m, 1H, 6-H), 7.02 (d, J = 7.9 Hz, 1H, 8-H), 7.16 – 7.22 (m, 3H, 7-H, 2-Hbenzyl, 6-Hbenzyl), 7.32 (d, J = 7.6 Hz, 1H, 5-Hbenzyl). Signals for the OH and NH protons are not observed in the spectrum. 13C NMR (151 MHz, CD3OD): δ (ppm) = 19.2 (1C, ArCH3), 28.9 – 29.1 (m, 3C, C-4, C-3', C-5'), 36.3 (1C, C-2'), 39.0 (1C, C-6'), 48.8 (1C, ArCH2NH), 56.6 (1C, OCH3), 57.1 (1C, C-4'), 65.0 (1C, ArCH2OH), 77.4 (d, J = 2.1 Hz, 1C, C-1), 97.0 (1C, C-3), 113.6 (d, J = 22.0 Hz, 1C, C-6), 120.5 (d, J = 18.5 Hz, 1C, C-4a), 121.5 (d, J = 3.1 Hz, 1C, C-8), 125.7 (1C, C-6benzyl), 128.4 (d, J = 8.6 Hz, 1C, C-7), 129.9 (1C, C-5benzyl), 130.1 (1C, C-2benzyl), 137.4 (1C, C-4benzyl), 137.9 (1C, C-3benzyl), 141.6 (1C, C-1benzyl), 145.3 (d, J = 4.5 Hz, 1C, C-8a), 161.6 (d, J = 243.3 Hz, 1C, C-5). FT-IR (neat): ν [cm-1] = 3264 (O-H/N-H), 2932, 2820 (C-Halkyl), 1458, 1447 (C=Carom). Purity (HPLC, method 1): 99.4 %, tR = 16.9 min.

trans-(4-{[N-(3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl)amino]methyl}-3-methylphenyl)methanol (trans-27e) and

cis-(4-{[N-(3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl)amino]methyl}-3-methylphenyl)methanol (cis-27e)

A solution of ketone 14e (251 mg, 1.02 mmol), amine 19 (201 mg, 1.33 mmol, 1.3 eq), acetic acid (64 µL, 1.13 mmol, 1.1 eq) and NaBH(OAc)3 (390 mg, 1.84 mmol, 1.8 eq) in THF (30 mL) was stirred under N2 atmosphere at rt. After 6.5 h, 1 M NaOH (15 mL) was added and the aqueous layer was extracted with Et2O (3 x 30 mL). The combined organic layers were dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified by fc (d = 3 cm, l = 20 cm, V = 20 mL, cyclohexane/ethyl acetate 33:67 + 1 % N,N-dimethylethanamine). trans-27e was eluted first and cis-27e afterwards. trans-27e was purified again by fc (d = 2 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 67:33 + 1 % N,N-dimethylethanamine).

trans-27e: Colorless solid, mp 130 °C, yield 40 mg (10 %). C24H31NO3 (381.5 g/mol). Rf = 0.44 (cyclohexane/ethyl acetate 33:67 + 1 % N,N-dimethylethanamine). HR-MS (APCI, method 1): m/z = 382.2357 (calcd. 382.2377 for C24H32NO3 [MH+]). 1H NMR (600 MHz, CD3OD): δ (ppm) = 1.57 (dq, J = 13.5/3.1 Hz, 1H, 2'-Hequ), 1.75 – 1.80 (m, 2H, 3'-H, 5'-H), 1.83 (dq, J = 13.5/3.1 Hz, 1H, 6'-Hequ), 1.99 – 2.07 (m, 2H, 5'-H, 6'-Hax), 2.07 – 2.17 (m, 1H, 3'-H), 2.31 (td, J = 13.7/4.0 Hz, 1H, 2'-Hax), 2.44 (s, 3H, ArCH3), 2.80 (dd, J = 15.6/7.7 Hz, 1H, 4-H), 2.91 (dd, J = 15.6/3.1 Hz, 1H, 4-H), 3.02 (quint, J = 3.0 Hz, 1H, 4'-Hequ), 3.55 (s, 3H, OCH3), 3.82 (s, 2H, ArCH2NH), 4.58 (s, 2H, ArCH2OH), 4.90 (dd, J = 7.7/3.1 Hz, 1H, 3-H), 7.07 (dd, J = 7.5/1.3 Hz, 1H, 5-H), 7.13 (td, J = 7.4/1.4 Hz, 1H, 6-H), 7.16 – 7.21 (m, 3H, 7-H, 2-Hbenzyl, 6-Hbenzyl), 7.32 (dd, J = 7.8/1.3 Hz, 1H, 8-H), 7.35 (d, J = 7.6 Hz, 1H, 5-Hbenzyl). Signals for the NH and OH protons are not observed in the spectrum. 13C NMR (151 MHz, CD3OD): δ (ppm) = 19.3 (1C, ArCH3), 26.6 (1C, C-3'), 26.8 (1C, C-5'), 31.6 (1C, C-6'), 34.3 (1C, C-2'), 36.3 (1C, C-4), 50.2 (1C, ArCH2NH), 52.4 (1C, C-4'), 56.3 (1C, OCH3), 65.1 (1C, ArCH2OH), 78.3 (1C, C-1), 97.8 (1C, C-3), 125.7 (1C, C-6benzyl), 126.1 (1C, C-8), 127.47 (1C, C-7), 127.53 (1C, C-6), 130.0 (1C, C-5), 130.1 (1C, C-2benzyl), 130.2 (1C, C-5benzyl), 132.4 (1C, C-4a), 137.7 (1C, C-3benzyl), 138.4 (1C, C-4benzyl), 141.5 (1C, C-1benzyl), 143.5 (1C, C-8a). FT-IR (neat): ν [cm-1] = 3271, 3044 (O-H/N-H), 2932, 2859 (C-Halkyl), 1469, 1443 (C=Carom). Purity (HPLC, method 1): 99.1 %, tR = 16.4 min.

cis-27e: Colorless solid, mp 116 °C, yield 227 mg (58 %). C24H31NO3 (381.5 g/mol). Rf = 0.11 (cyclohexane/ethyl acetate 33:67 + 1 % N,N-dimethylethanamine). HR-MS (APCI, method 1): m/z = 382.2340 (calcd. 382.2377 for C24H32NO3 [MH+]). 1H NMR (600 MHz, CD3OD): δ (ppm) = 1.70 (td, J = 13.7/3.6 Hz, 1H, 2'-Hax), 1.75 – 1.83 (m, 1H, 3'-H), 1.84 – 1.90 (m, 2H, 5'-H, 6'-H), 1.90 – 1.95 (m, 2H, 3'-H, 5'-H), 1.99 (ddd, J = 14.4/13.9/3.7 Hz, 1H, 6'-H), 2.12 (dq, J = 14.0/3.0 Hz, 1H, 2'-Hequ), 2.39 (s, 3H, ArCH3), 2.75 (tt, J = 11.1/4.0 Hz, 1H, 4'-Hax), 2.80 (dd, J = 15.6/7.5 Hz, 1H, 4-H), 2.92 (dd, J = 15.7/3.1 Hz, 1H, 4-H), 3.58 (s, 3H, OCH3), 3.85 (s, 2H, ArCH2NH), 4.57 (s, 2H, ArCH2OH), 4.91 (dd, J = 7.5/3.1 Hz, 1H, 3-H), 7.08 (d, J = 7.3 Hz, 1H, 5-H), 7.12 – 7.20 (m, 5H, 6-H, 7-H, 8-H, 2-Hbenzyl, 6-Hbenzyl), 7.32 (d, J = 7.6 Hz, 1H, 5-Hbenzyl). Signals for the NH and OH protons are not observed in the spectrum. 13C NMR (151 MHz, CD3OD): δ (ppm) = 19.2 (1C, ArCH3), 29.05 (1C, C-5'), 29.14 (1C, C-3'), 36.2 (1C, C-4), 36.6 (1C, C-2'), 39.1 (1C, C-6'), 48.8 (1C, ArCH2NH), 56.5 (1C, OCH3), 57.2 (1C, C-4'), 65.0 (1C, ArCH2OH), 77.5 (1C, C-1), 97.8 (1C, C-3), 125.68 (1C, C-8), 125.73 (1C, C-6benzyl), 127.5 (1C, C-7), 127.6 (1C, C-6), 129.9 (1C, C-5benzyl), 130.08 (1C, C-2benzyl), 130.13 (1C, C-5), 132.6 (1C, C-4a), 137.4 (1C, C-3benzyl), 137.9 (1C, C-4benzyl), 141.6 (1C, C-1benzyl), 142.7 (1C, C-8a). FT-IR (neat): ν [cm-1] = 3264, 3132 (O-H/N-H), 2932, 2835, 2820 (C-Halkyl), 1466, 1443 (C=Carom). Purity (HPLC, method 1): 98.9 %, tR = 16.3 min.

trans-N-[4-(Fluoromethyl)-2-methylbenzyl]-5-fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (trans-28c)

A solution of alcohol trans-27c (46 mg, 0.11 mmol) in CH2Cl2 (3 mL) was added dropwise to a solution of DAST (0.04 mL, 0.30 mmol, 2.7 eq) in CH2Cl2 (5 mL) under N2 atmosphere at -78 °C. After 45 min, the mixture was warmed to rt and stirred for 2 h. H2O (20 mL) was added and the aqueous layer was extracted with CH2Cl2 (4 x 10 mL). The combined organic layers were dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 21 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % N,N-dimethylethanamine). Colorless oil, yield 31 mg (70 %). C24H29F2NO2 (401.5 g/mol). Rf = 0.36 (cyclohexane/ethyl acetate 80:20 + 1 % N,N-dimethylethanamine). HR-MS (ESI): m/z = 402.2245 (calcd. 402.2239 for C24H30F2NO2 [MH+]). 1H

NMR (400 MHz, CD3OD): δ (ppm) = 1.66 (dq, J = 12.7/3.2 Hz, 1H, 2'-Hequ), 1.83 – 1.97 (m, 3H, 3'-H, 5'-H, 6'-Hequ), 2.03 (td, J = 13.6/3.2 Hz, 1H, 6'-Hax), 2.09 – 2.20 (m, 1H, 5'-H), 2.20 – 2.37 (m, 2H, 2'-Hax, 3'-H), 2.48 (s, 3H, ArCH3), 2.67 (dd, J = 16.3/7.4 Hz, 1H, 4-H), 2.99 (ddd, J = 16.3/3.3/1.4 Hz, 1H, 4-H), 3.17 – 3.22 (m, 1H, 4'-Hequ), 3.58 (s, 3H, OCH3), 3.98 (s, 2H, ArCH2NH), 4.95 (dd, J = 7.5/3.3 Hz, 1H, 3-H), 5.36 (d, J = 48.0 Hz, 2H, ArCH2F), 6.94 (ddd, J = 9.3/7.6/1.7 Hz, 1H, 6-H), 7.17 – 7.25 (m, 2H, 7-H, 8-H), 7.25 – 7.31 (m, 2H, 3-Hbenzyl, 5-Hbenzyl), 7.47 (d, J = 7.6 Hz, 1H, 6-Hbenzyl). A signal for the NH proton is not observed in the spectrum. 13C NMR (101 MHz, CD3OD): δ (ppm) = 19.3 (1C, ArCH3), 25.95 (1C, C-3'), 26.00 (1C, C-5'), 29.0 (d, J = 4.2 Hz, 1C, C-4), 31.4 (1C, C-6'), 34.1 (1C, C-2'), 49.7 (1C, ArCH2NH), 53.1 (1C, C-4'), 56.5 (1C, OCH3), 77.7 (1C, C-1), 85.3 (d, J = 164.5 Hz, 1C, ArCH2F), 97.2 (d, J = 1.1 Hz, 1C, C-3), 113.6 (d, J = 21.7 Hz, 1C, C-6), 120.3 (d, J = 18.4 Hz, 1C, C-4a), 121.7 (1C, C-8), 126.4 (d, J = 5.6 Hz, 1C, C-5benzyl), 128.4 (d, J = 8.7 Hz, 1C, C-7), 130.6 (1C, C-6benzyl), 130.8 (d, J = 5.6 Hz, 1C, C-3benzyl), 137.5 (d, J = 14.9 Hz, 1C, C-4benzyl), 138.0 (1C, C-1benzyl), 138.4 (1C, C-2benzyl), 145.7 (d, J = 4.1 Hz, 1C, C-8a), 161.5 (d, J = 243.3 Hz, 1C, C-5). FT-IR (neat): ν [cm-1] = 3306 (N-H), 2920, 2839 (C-Halkyl), 1458, 1447 (C=Carom). Purity (HPLC, method 1): 96.3 %, tR = 19.4 min.

cis-N-[4-(Fluoromethyl)-2-methylbenzyl]-5-fluoro-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (cis-28c)

A solution of alcohol cis-27c (60 mg, 0.15 mmol) in CH2Cl2 (3 mL) was added dropwise to a solution of DAST (0.05 mL, 0.38 mmol, 2.5 eq) in CH2Cl2 (8 mL) under N2 atmosphere at -78 °C. After 45 min, the mixture was warmed to rt and stirred for 2 h. H2O (20 mL) was added and the aqueous layer was extracted with CH2Cl2 (4 x 10 mL). The combined organic layers were dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 21 cm, V = 10 mL, cyclohexane/ethyl acetate 50:50 + 1 % N,N-dimethylethanamine). Pale yellow solid, mp 140 °C, yield 36 mg (60 %). C24H29F2NO2 (401.5 g/mol). Rf = 0.19 (cyclohexane/ethyl acetate 50:50 + 1 % N,N-dimethylethanamine). HR-MS (ESI): m/z = 402.2245 (calcd. 402.2239 for C24H30F2NO2 [MH+]). 1H NMR (400 MHz, CD3OD): δ (ppm) = 1.68 – 1.79 (m, 2H, 2'-H, 3'-H), 1.80 – 1.98 (m, 4H, 3'-H, 5'-H, 6'-H), 2.03 (td, J = 13.6/3.6 Hz, 1H, 6'-H), 2.13 – 2.20 (m, 1H, 2'-H), 2.42 (s, 3H, ArCH3), 2.67 (dd, J = 16.3/7.4 Hz, 1H, 4-H), 2.79 (tt, J = 10.9/3.8 Hz, 1H, 4'-Hax), 2.99 (ddd, J = 16.3/3.4/1.3 Hz, 1H, 4-H), 3.61 (s, 3H, OCH3), 3.89 (s, 2H, ArCH2NH), 4.95 (dd, J = 7.4/3.2 Hz, 1H, 3-H), 5.34 (d, J = 48.1 Hz, 2H, ArCH2F), 6.94 (ddd, J = 9.2/8.1/1.0 Hz, 1H, 6-H), 7.04 (d, J = 7.7 Hz, 1H, 8-H), 7.19 – 7.27 (m, 3H, 7-H, 3-Hbenzyl, 5-Hbenzyl), 7.38 – 7.42 (m, 1H, 6-Hbenzyl). A signal for the NH proton is not observed in the spectrum. 13C NMR (101 MHz, CD3OD): δ (ppm) = 19.1 (1C, ArCH3), 28.9 – 29.1 (m, 3C, C-4, C-3', C-5'), 36.3 (1C, C-2'), 38.9 (1C, C-6'), 48.7 (1C, ArCH2NH), 56.6 (1C, OCH3), 57.1 (1C, C-4'), 77.4 (d, J = 1.9 Hz, 1C, C-1), 85.4 (d, J = 164.2 Hz, 1C, ArCH2F), 97.1 (1C, C-3), 113.6 (d, J = 22.0 Hz, 1C, C-6), 120.5 (d, J = 18.5 Hz, 1C, C-4a), 121.5 (d, J = 3.3 Hz, 1C, C-8), 126.5 (d, J = 5.7 Hz, 1C, C-5benzyl), 128.4 (d, J = 8.5 Hz, 1C, C-7), 130.0 (d, J = 1.6 H, 1C, C-6benzyl), 130.7 (d, J = 5.4 Hz, 1C, C-3benzyl), 136.8 (d, J = 16.9 Hz, 1C, C-4benzyl), 137.8 (1C, C-2benzyl), 139.5 (1C, C-1benzyl), 145.3 (d, J = 4.3 Hz, 1C, C-8a), 161.6 (d, J = 243.4 Hz, 1C, C-5). FT-IR (neat): ν [cm-1] = 3260 (N-H), 2924, 2855 (C-Halkyl), 1462, 1443 (C=Carom). Purity (HPLC, method 1): 95.1 %, tR = 19.2 min.

trans-N-[4-(Fluoromethyl)-2-methylbenzyl]-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (trans-28e)

A solution of alcohol trans-27e (24 mg, 0.06 mmol) in CH2Cl2 (3 mL) was added dropwise to a solution of DAST (0.02 mL, 0.15 mmol, 2.5 eq) in CH2Cl2 (4 mL) under N2 atmosphere at -78 °C. After 45 min, the mixture was warmed to rt and stirred for 2 h. H2O (10 mL) was added and the aqueous layer was extracted with CH2Cl2 (4 x 5 mL). The combined organic layers were dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified by fc (d = 1 cm, l = 20 cm, V = 3 mL, cyclohexane/ethyl acetate 80:20 + 1 % N,N-dimethylethanamine). Pale yellow oil, yield 12 mg (50 %). C24H30FNO2 (383.5 g/mol). Rf = 0.41 (cyclohexane/ethyl acetate 80:20 + 1 % N,N-dimethylethanamine). HR-MS (APCI, method 1): m/z = 384.2344 (calcd. 384.2333 for C24H31FNO2 [MH+]). 1H NMR (600 MHz, CD3OD): δ (ppm) = 1.58 (dq, J = 13.6/3.1 Hz, 1H, 2'-Hequ), 1.75 – 1.81 (m, 2H, 3'-H, 5'-H), 1.83 (dq, J = 13.2/2.6 Hz, 1H, 6'-Hequ), 2.00 – 2.08 (m, 2H, 5'-H, 6'-Hax), 2.08 – 2.17 (m, 1H, 3'-H), 2.32 (td, J = 13.6/3.9 Hz, 1H, 2'-Hax), 2.45 (s, 3H, ArCH3), 2.80 (dd, J = 15.6/7.6 Hz, 1H, 4-H), 2.91 (dd, J = 15.6/3.1 Hz, 1H, 4-H), 3.02 (quint, J = 2.9 Hz, 1H, 4'-Hequ), 3.55 (s, 3H, OCH3), 3.84 (s, 2H, ArCH2NH), 4.90 (dd, J = 7.7/3.1 Hz, 1H, 3-H), 5.33 (d, J = 48.2 Hz, 2H, ArCH2F), 7.07 (d, J = 7.5 Hz, 1H, 5-H), 7.14 (td, J = 7.4/1.3 Hz, 1H, 6-H), 7.16 – 7.20 (m, 1H, 7-H), 7.21 – 7.26 (m, 2H, 3-Hbenzyl, 5-Hbenzyl), 7.32 (dd, J = 7.8/1.2 Hz, 1H, 8-H), 7.42 (d, J = 7.5 Hz, 1H, 6-Hbenzyl). A signal for the NH proton is not observed in the spectrum. 13C NMR (151 MHz, CD3OD): δ (ppm) = 19.2 (1C, ArCH3), 26.7 (1C, C-3'), 26.8 (1C, C-5'), 31.6 (1C, C-6'), 34.3 (1C, C-2'), 36.3 (1C, C-4), 50.2 (1C, ArCH2NH), 52.5 (1C, C-4'), 56.3 (1C, OCH3), 78.3 (1C, C-1), 85.4 (d, J = 164.2 Hz, 1C, ArCH2F), 97.8 (1C, C-3), 126.1 (1C, C-8), 126.4 (d, J = 5.7 Hz, 1C, C-5benzyl), 127.5 (1C, C-7), 127.5 (1C, C-6), 130.0 (1C, C-5), 130.2 (d, J = 1.7 Hz, 1C; C-6benzyl), 130.7 (d, J = 5.2 Hz, 1C, C-3benzyl), 132.4 (1C, C-4a), 136.7 (d, J = 16.8 Hz, 1C; C-4benzyl), 138.1 (d, J = 1.7 Hz, 1C; C-2benzyl), 140.1 (1C; C-1benzyl), 143.5 (1C, C-8a). FT-IR (neat): ν [cm-1] = 3318 (N-H), 2924, 2835 (C-Halkyl), 1443, 1381 (C=Carom). Purity (HPLC, method 1): 93.5 %, tR = 18.6 min.

cis-N-[4-(Fluoromethyl)-2-methylbenzyl]-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (cis-28e)

A solution of alcohol cis-27e (60 mg, 0.16 mmol) in CH2Cl2 (4 mL) was added dropwise to a solution of DAST (0.04 mL, 0.30 mmol, 1.9 eq) in CH2Cl2 (5 mL) under N2 atmosphere at -78 °C. After 1 h, the mixture was warmed to rt and stirred for 2 h. H2O (10 mL) was added and the aqueous layer was extracted with CH2Cl2 (4 x 8 mL). The combined organic layers were dried (Na2SO4), filtered, concentrated in vacuo and the residue was purified twice by fc (d = 2 cm, l = 21 cm, V = 5 mL, cyclohexane/ethyl acetate 80:20 + 1 % N,N-dimethylethanamine → 67:33 + 1 % N,N-dimethylethanamine; d = 2 cm, l = 24 cm, V = 10 mL, CH2Cl2/CH3OH 98:2 + 1 % N,N-dimethylethanamine). Colorless solid, mp 99 °C, yield 21 mg (35 %). C24H30FNO2 (383.5 g/mol). Rf = 0.31 (CH2Cl2/CH3OH 95:5 + 1 % N,N-dimethylethanamine). HR-MS (APCI, method 1): m/z = 384.2330 (calcd. 384.2333 for C24H31FNO2 [MH+]). 1H NMR (600 MHz, CD3OD): δ (ppm) = 1.72 (td, J = 13.7/3.5 Hz, 1H, 2'-Hax), 1.76 – 1.84 (m, 1H, 3'-H), 1.84 – 1.90 (m, 2H, 5'-H, 6'-H), 1.91 – 1.96 (m, 2H, 3'-H, 5'-H), 2.00 (td, J = 13.4/3.0 Hz, 1H, 6'-H), 2.12 (dq, J = 14.0/3.1 Hz, 1H, 2'-Hequ), 2.41 (s, 3H, ArCH3), 2.74 – 2.78 (m, 1H, 4'-Hax), 2.80 (dd, J = 15.7/7.4 Hz, 1H, 4-H), 2.92 (dd, J = 15.6/3.2 Hz, 1H, 4-H), 3.58 (s, 3H, OCH3), 3.88 (s, 2H, ArCH2NH), 4.92 (dd, J = 7.5/3.1 Hz, 1H, 3-H), 5.33 (d, J = 48.1 Hz, 2H, ArCH2F), 7.07 – 7.10 (m, 1H, 5-H), 7.13 – 7.19 (m, 3H, 6-H, 7-H, 8-H), 7.21 – 7.25 (m, 2H, 3-Hbenzyl, 5-Hbenzyl), 7.39 (d, J = 7.7 Hz, 1H, 6-Hbenzyl). A signal for the NH proton is not observed in the spectrum. 13C NMR (151 MHz, CD3OD): δ (ppm) = 19.1 (1C, ArCH3), 29.0 (1C, C-5'), 29.1 (1C, C-3'), 36.2 (1C, C-4), 36.6 (1C, C-2'), 39.1 (1C, C-6'), 48.7 (1C, ArCH2NH), 56.5 (1C, OCH3), 57.3 (1C, C-4'), 77.5 (1C, C-1), 85.4 (d, J = 164.1 Hz, 1C, ArCH2F), 97.8 (1C, C-3), 125.7 (1C, C-8), 126.5 (d, J = 5.8 Hz, 1C, C-5benzyl), 127.5 (1C, C-7), 127.6 (1C, C-6), 130.0 (d, J = 1.5 Hz, 1C, C-6benzyl), 130.1 (1C, C-5), 130.7 (d, J = 5.3 Hz, 1C, C-3benzyl), 132.6 (1C, C-4a), 136.8 (d, J = 16.4 Hz, 1C, C-4benzyl), 137.8 (d, J = 1.7 Hz, 1C, C-2benzyl), 139.5 (d, J = 3.2 Hz, 1C, C-1benzyl), 142.7 (1C, C-8a). FT-IR (neat): ν [cm-1] = 3298 (N-H), 2940, 2859 (C-Halkyl), 1443, 1373 (C=Carom). Purity (HPLC, method 1): 96.8 %, tR = 18.7 min.

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Keywords

σ receptors; spirocyclic ligands; cis-trans-configuration; structure-affinity relationships; receptor selectivity; fluorinated PET tracer;

Supporting Information

Supporting Information contains the Experimental, Chemistry, the Experimental, Receptor Binding Studies and all ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Conflict of interests

The authors declare no conflict of interest.

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