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In vitro affinities of various halogenated benzamide derivatives as potential radioligands for non-invasive quantification of D_2-like dopamine receptors

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Abstract—Benzamide derivatives as radiotracers have played an important role in diagnosing malfunction in dopaminergic neurotransmission. A variety of halogenated and two unsubstituted benzamide derivatives were synthesised and their in vitro affinities to dopaminergic, serotonergic and adrenergic receptors and their lipophilicities were determined. As references IBZM (**3**), raclopride (**4**) and FLB457 (**5**) were tested as well. The two iodinated compounds NAE (**27**) and NADE (**28**) displayed K_i values of 0.68 and 14 nM for the D_2 receptor. The well-established radiotracers FP (**1**) and DMFP (**2**) showed affinities in the same range as did the brominated compounds NABrE (**29**) and NABrDE (**30**). The log $D_{7.4}$ values of 2.91 for NAE (**27**) and of 2.81 for NADE (**28**) are in the range of those found for IBZM (**3**), FP (**1**) and DMFP (**2**). These facts allow to expect good properties for the two iodinated compounds NAE (**27**) and NADE (**28**) regarding in vivo imaging with SPECT.

1. Introduction

Abnormalities of dopaminergic neurotransmission have been implicated in a variety of neuropsychiatric diseases such as Parkinson's disease, schizophrenia and drug abuse. Pharmaceuticals for the treatment of these conditions target dopamine receptors (D_1-like receptors: D_1, D_5; D_2-like receptors: D_2, D_3, D_4), dopamine transporters and dopamine synthesis. D_2 receptor antagonists have been used for decades for the treatment of schizophrenia and related disorders. They belong to a broad array of chemical classes such as butyrophenones (e.g., haloperidol), thioxanthenes (e.g., flupenthixole) and benzamides (e.g., amisulpride). Among those, the benzamides stand out due to their high selectivity for D_2-like dopamine receptors, their reversibility in binding and their high affinity.[1]

Especially their extraordinarily high selectivity for D_2/D_3 receptors led to the development of various benzamide derivatives as radioactively labelled compounds for positron emission tomography (PET) and single photon emission computed tomography (SPECT) (Fig. 1).[2–5] [^{11}C]raclopride for PET and [^{123}I]iodobenzamide ([^{123}I]IBZM) for SPECT have been used for almost two decades for quantification of D_2/D_3 receptors in a broad range of scientific and clinical applications.[6,7] Recently, substituted benzamides with a fluorine-18 label have been developed for broader clinical use, because the fluorine-18 label offers the advantage of a longer half-life compared to the carbon-11 label of e.g., [^{11}C]raclopride. Two of the most promising compounds are [^{18}F]fallypride ([^{18}F]FP) and [^{18}F]desmethoxyfallypride ([^{18}F]DMFP).[8,9] However, due to their only moderate affinity, most of these tracers, including [^{11}C]raclopride, [^{123}I]IBZM, or [^{18}F]DMFP, allow for quantification of D_2/D_3 receptors only in brain regions with high D_2 receptor densities, like the striatum. [^{18}F]fallypride on the other hand, due to its very high affinity, seems to be an ideal tracer for the study of both striatal and extrastriatal receptors in a single PET scan. Tracer affinity is of crucial importance for its kinetics in

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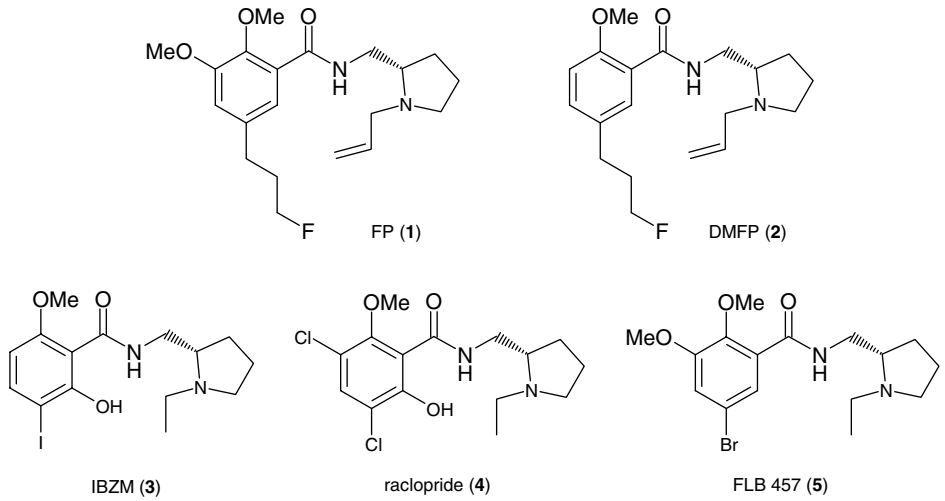


Figure 1. Structure of FP (**1**), DMFP (**2**), IBZM (**3**), raclopride (**4**) and FLB 457 (**5**).

brain, and tracers with very high affinity give new insights into the role of extrastriatal dopamine systems and antipsychotic drug action.[9,10]

A first intent was to make these favourable properties of FP and DMFP available to SPECT. Therefore the fluoropropyl moiety at the 5-position of the aromatic ring was substituted by iodine. The obtained compounds *N*-allyl-epidepride (NAE, **27**)[11,12] and the new compound *N*-allyl-desmethoxyepidepride (NADE, **28**)[13,14] were tested in vitro. For the racemate of NAE (**27**) in vitro binding affinities towards D_2 receptors have already been reported (K_D = 0.033 nM, log $P_{7.4}$ = 2.72).[15]

To investigate the influence of other halogen substituents at the five position of the aromatic ring analogue bromine derivatives, *N*-allyl-bromo-epidepride (NABrE, **29**)[12] and *N*-allyl-bromo-desmethoxyepidepride (NABrDE, **30**),[13,14] chlorine derivatives, *N*-allyl-chloro-epidepride (NAClE, **31**) and *N*-allyl-chloro-desmethoxyepidepride (NAClDE, **32**) and the unsubstituted derivatives, *N*-allyl-benzamide (NAB, **33**) and *N*-allyl-desmethoxybenzamide (NADB, **34**) were synthesised. In first pharmacological experiments the lipophilicities and affinities of these benzamide derivatives to the different dopamine receptors, serotonin and adrenergic receptors were determined in receptor binding assays.

2. Chemistry

The molecules **1** and **2**, and **27**–**34** were synthesised by reacting the respective benzoic acid derivatives **13**–**17** and **22**–**26** with (*S*)-1-allyl-2-aminomethylpyrrolidine (**9**). [16] The pyrrolidinyl compound was prepared following a stereo-conservative route established by Högberg et al. (Scheme 1).[17,18] In three steps, starting with the amino acid L-proline (**6**), (*S*)-1-allyl-2-aminomethylpyrrolidine (**9**) was prepared. The alkylation of **6** with allyl bromide was followed by ammonolysis of the so obtained allyl ester, (*S*)-allyl-1-allylpyrrolidine-2-carboxylate (**7**), with ammonia in methanol employing sodium cyanide as catalyst. The obtained (*S*)-1-allyl-pyrrolidine-2-carboxamide (**8**) was reduced with sodium bis(2-methoxyethoxy)aluminium dihydride (SDMA) to give the desired compound **9**.[19]

The required benzoic acid derivatives were synthesised from their benzaldehydes or benzoic acid derivatives following the pathways illustrated for the dimethoxy (Scheme 2) and monomethoxy derivatives (Scheme 3). The benzoic acid derivatives **13**, **16** and **25** are commercially available. 5-Iodo-2-methoxybenzoic acid (**22**) was obtained by methylation of the respective 5-iodo-2-hydroxybenzoic acid (**18**) (Scheme 3).[20,21] The bromine analogue benzoic acid derivatives **14** and **23** were obtained by methylation and oxidation of the respective aldehydes **10** and **19** (Schemes 2 and 3).[20,22] 5—Chloro-2,3-dimethoxybenzoic acid (**15**) was synthesised by oxidising the aldehyde derivative **11**. 5-Chloro-2-methoxybenzoic acid (**24**) was synthesised by methylating 5-chloro-2-hydroxybenzoic acid (**20**) and hydrolysing the ester intermediate. For the synthesis of 5-(3-hydroxypropyl)-2,3-dimethoxybenzoic acid (**17**) and 5-(3-hydroxypropyl)-2-methoxybenzoic acid (**26**) a procedure described by Mukherjee[23] was used starting from 3-(3,4-dimethoxyphenyl)propan-1-ol (**12**) and 3-(4-methoxyphenyl)propan-1-ol (**21**) (Schemes 2 and 3).

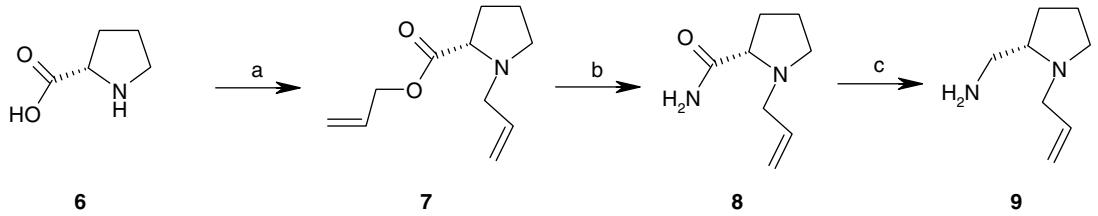
The benzoic acid derivatives **13**–**17** and **22**–**26** were coupled with (*S*)-1-allyl-2-aminomethylpyrrolidine (**9**) via their mixed anhydrides using ethyl chloroformiate (Scheme 4).

The 3-fluoropropyl derivatives **1** and **2** were synthesised as described elsewhere (Scheme 4).[23]

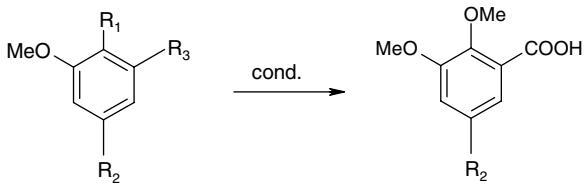
3. Results and discussion

3.1. In vitro evaluation

3.1.1. Affinities. To determine the binding affinities of the synthesised compounds NAE (**27**), NADE (**28**),

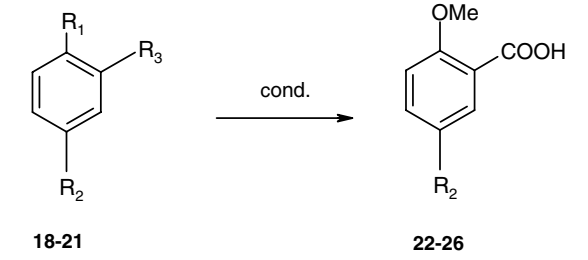


Scheme 1. Synthesis of **9** according to the route established by Hoegberg et al.[17,18] Reagents : (a) allyl bromide, K_2CO_3, DMSO; (b) NH_3/methanol, NaCN; (c) SDMA/toluene.



Cpd.	R_1	R_2	R_3	Cond.	Cpd.	R_2
-	-	-	-		**13**	I
10	OH	Br	CHO	a	**14**	Br
11	OMe	Cl	CHO	b	**15**	Cl
-	-	-	-		**16**	H
12	OMe	$(CH_2)_3OH$	H	c	**17**	$(CH_2)_3OH$

Scheme 2. Synthesis of dimethoxybenzoic acids. Reagents: (a) K_2CO_3, MeI, 2-butanone; acetone, $KMnO_4$, H_2O; (b) acetone, $KMnO_4$, H_2O; (c) *n*-butyllithium, THF, CO_2; **13** and **16** are commercially available.



Cpd.	R_1	R_2	R_3	Cond.	Cpd.	R_2
18	OH	I	CHO	a	**22**	I
19	OMe	Br	CHO	b	**23**	Br
20	OH	Cl	COOH	c	**24**	Cl
-	-	-	-		**25**	H
21	OMe	$(CH_2)_3OH$	H	d	**26**	$(CH_2)_3OH$

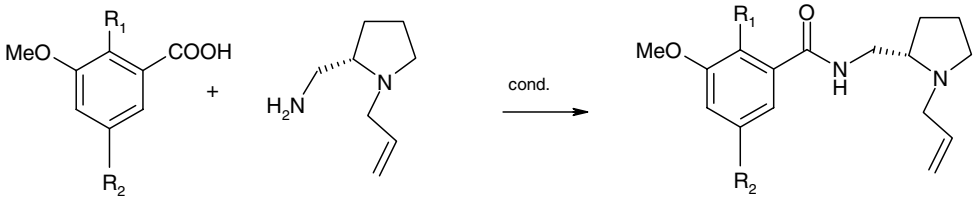
Scheme 3. Synthesis of monomethoxybenzoic acids. Reagents: (a) MeI, NaH, DMF, LiOH, acetone, $KMnO_4$, H_2O; (b) acetone, $KMnO_4$, H_2O; (c) NaOH, CH_2Cl_2, $(C_4H_9)_4NI$, DMSO, NaOH; (d) *n*-butyllithium, THF, CO_2; **25** is commercially available.

NABrE (**29**), NaBrDE (**30**), FP (**1**) and DMFP (**2**) as well as for the reference substances IBZM (**3**), raclopride (**4**) and FLB 457 (**5**) to the receptors of the dopamine D_2-family and the related GPCRs we established a radioligand binding assay.[24] Utilizing stably transfected CHO cells expressing the human dopamine receptor subtypes $D_{2\ short}$, D_{2long},[25] D_3[26] and $D_{4.4}$[27] and the radioligand [^{3}H]spiperone we examined the affinities to the D_2-family. Furthermore, binding to the D_1 receptor was investigated using porcine striatal membranes and the selective radioligand [^{3}H]SCH 23390. To get evidences for the selectivity of the test compounds the affinities to the related serotonin receptor subtypes 5-HT_{1A}, 5-HT_2 and the adrenergic receptor α_1 were examined with porcine cortical membranes and the selective radioligands [^{3}H]8-OH-DPAT, [^{3}H]ketanserin and [^{3}H]prazosin, respectively.[28] The resulting K_i values are depicted in Table 1.

3.1.2. Selectivity. The figures displayed in Table 1 and the graph in Figure 2 clearly show that the compounds synthesised have a low affinity for the D_1 receptor. The K_i values of all tested compounds for the D_1 subtype ranged from 10 to 40 μM (Table 1). The affinities for compounds **1** and **2**, and **27**–**30** are within the range of those found for IBZM (**3**), raclopride (**4**) and FLB457 (**5**), which have been applied as radiotracers in research to examine D_2-like receptor related issues with PET and SPECT. For the D_1 receptor, affinities are up to 10,000-fold lower than for the D_2-like receptors and between 10- and 100-fold lower than for the serotonin and adrenergic receptor subtypes.

K_i values for all examined compounds for the dopaminergic receptor subtype $D_{4.4}$, the serotonergic receptor subtypes 5-HT_{1A}, 5-HT_2 and the adrenergic receptor subtype α_1 range from 100 nM to 4.5 μM (Fig. 2). The halogenated compounds NAE (**27**), NADE (**28**), NABrE (**29**), NABrDE (**30**) display K_i values for the 5-HT_{1A} receptor in the range of 140–225 nM. K_i values for the $D_{4.4}$ receptor subtype range from 210 to 1700 nM for these compounds. Affinities for the D_4 receptor subtype are in the high nM to low μM range.

The affinities for the D_{2short}, D_{2long} and D_3 receptor subtype are in the range of 0.4–35 nM for all examined compounds (Table 1 and Fig. 2). For these three receptor subtypes K_i values are up to 25,000-fold higher than those found for the D_1 receptor. Of the compounds synthesised, NAE (**27**) and NABrE (**29**) show the highest affinity for the D_{2short}/D_{2long} receptor with K_i values of 0.7 nM, and 0.9 nM, respectively, and for the D_3 receptor with K_i values of 0.5 nM and 0.6 nM, respectively. To sum up, the results of the studies concerning the selectivity of the benzamides it can be clearly said that



13-17 and 22-26 9 1-2 and 27-34

Cpd.	R_1	Cond.	R_2	Cpd.	R_1	Cond.	R_2
27	OMe	a	I	**34**	H	a	H
28	H	a	I	35	OMe	a	$CH_2CH_2CH_2OH$
29	OMe	a	Br	36	OMe	b	$CH_2CH_2CH_2OTs$
30	H	a	Br	**1**	OMe	c	$CH_2CH_2CH_2F$
31	OMe	a	Cl	37	H	a	$CH_2CH_2CH_2OH$
32	H	a	Cl	38	H	b	$CH_2CH_2CH_2OTs$
33	OMe	a	H	**2**	H	c	$CH_2CH_2CH_2F$

Scheme 4. Synthesis of the compounds **1** and **2**, and **27**–**34** employing chloroethyl formiate and triethylamine. Reagents: (a) ethyl chloroformiate $(C_2H_5)_3N$, **9**; (b) 4-methylbenzene-1-sulfonylchloride, pyridine, CH_2Cl_2, **35** or **37**; (c) KF, Kryptofix®2.2.2, K_2CO_3, MeCN, **36** or **38**.

Table 1. Binding affinities of the synthesised benzamide derivatives **1** and **2**, **27**–**30**, and the reference compounds **3**–**5** to dopaminergic, serotonergic and adrenergic receptor subtypes (K_i values in nM)

Compound		K_i[a] (nM ± SEM)							
		pD_1 [^{3}H]SCH23990	hD_{2short} [^{3}H]spiperone	hD_{2long} [^{3}H]spiperone	hD_3 [^{3}H]spiperone	$hD_{4.4}$ [^{3}H]spiperone	p5-HT_{6A} [^{3}H]8-HO-DPAT	p5-HT_2 [^{3}H]ketanserin	pα_6 [^{3}H]prazosin
27	NAE	18,000 ± 500	0.74 ± 0.17	0.68 ± 0.065	0.46 ± 0.010	250 ± 30	150 ± 15	490 ± 65	1100 ± 170
28	NADE	13,000 ± 1000	15 ± 0.50	14 ± 4.0	11 ± 1.0	630 ± 11	170 ± 98	2400 ± 1200	2000 ± 150
29	NABrE	13,000 ± 0	0.88 ± 0.12	0.85 ± 0.015	0.60 ± 0.075	210 ± 10	140 ± 40	1100 ± 640	1600 ± 100
30	NABrDE	17,000 ± 0	27 ± 2.5	23 ± 1.0	22 ± 2.5	1700 ± 100	230 ± 5.0	2100 ± 800	2000 ± 0
1	FP	18,000 ± 2000	2.1 ± 0.15	2.2 ± 0.050	1.6 ± 0.30	1300 ± 250	1100 ± 120	3700 ± 300	970 ± 340
2	DMFP	30,000 ± 1500	30 ± 5.0	30 ± 4.0	33 ± 3.6	3000 ± 400	2300 ± 750	4500 ± 50	1900 ± 100
3	IBZM	12,000 ± 1500	4.2 ± 0.75	4.2 ± 0.45	4.2 ± 0.69	570 ± 30	1100 ± 50	2000 ± 900	1900 ± 50
4	Raclopride	37,000 ± 0	31 ± 2.2	17 ± 1.7	15 ± 1.5	3100 ± 150	4400 ± 600	3500 ± 500	3000 ± 100
5	FLB457	15,000 ± 0	1.6 ± 0.33	0.65 ± 0.0090	0.42 ± 0.045	210 ± 3.4	2200 ± 50	1200 ± 0	830 ± 95

[a] K_i values in nM ± SEM are based on the means of 2–4 experiments each done in triplicate.

all compounds display a high affinity for the D_2 and D_3 receptor subtypes in the low nM to high pM range.

The selectivity for the serotonergic system of all examined compounds represented by two subtypes is less than for the D_2-like receptors by a factor of about 10 for the 5-HT_{1A} and by a factor of about and over 100 to the 5-HT_2 receptor subtype. Looking at the adrenergic α_1 receptor, affinities are about a factor of 100 lower than those found for the D_2-like receptors.

3.2. Structure–affinity relationship

Regarding the affinities of compounds **1** and **2**, **27**–**30** and **3**–**5** for the D_{2short}/D_{2long} and D_3 receptor subtypes some tendencies can be seen that are related to their structure (cf. Fig. 3).

Different studies in the literature[4] have shown that the addition of a second methoxy group at the 3-position of the aromatic ring (position A, Fig. 3) results in a strong increase in affinity for D_2-like receptors. This trend can clearly be seen from the K_i values for the fluorinated and iodinated compound pairs for the D_2 receptor (Table 1). The K_i values for the fluorinated compounds FP (**1**) (two methoxy groups) and DMFP (**2**) (one methoxy group) are 2.2 and 30 nM, respectively. The K_i value for **1** is 13-fold lower than for **2**. The K_i values gained for the iodinated compounds NAE (**27**) and NADE (**28**) are 0.7 and 19 nM. The affinity of the compound carrying two methoxy groups, **27**, is 27-fold higher than the affinity of the compound carrying just one methoxy group.

Compounds carrying two methoxy groups (**1**, **27**, **29**) show the best affinities for those receptor subtypes. K_i values are 0.7, 0.9 and 2.2 nM, respectively, for both D_2 subtypes, D_{2short} and D_{2long}. FLB 457 (**5**) follows this rule as well.

Another tendency described in literature[4] concerns the change of the substituent at the nitrogen atom of the pyrrolidinyl ring (position B, Fig. 3). Changing an ethyl group into an allyl moiety enhances the affinity for the

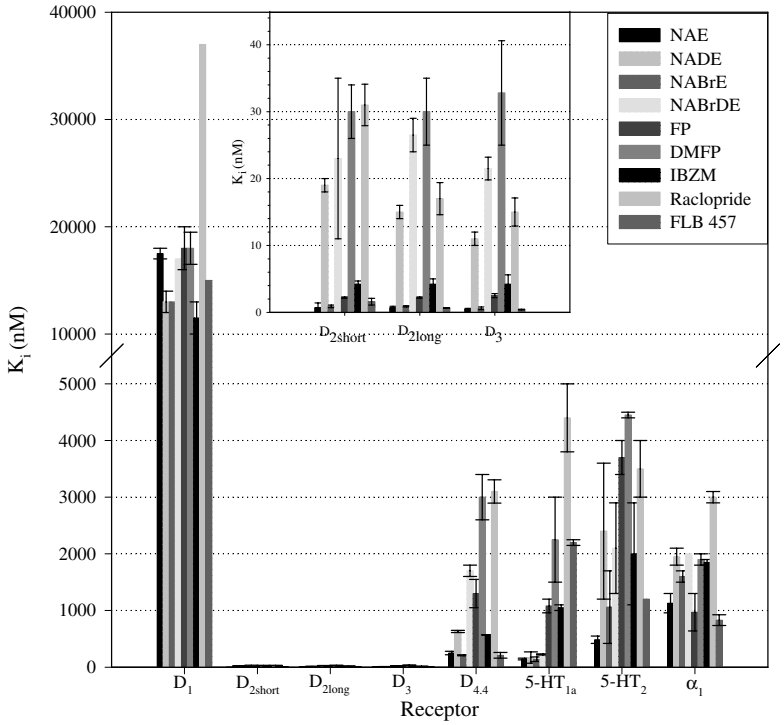


Figure 2. Affinities to various receptor subtypes for the synthesised benzamide derivatives (K_i in nM).

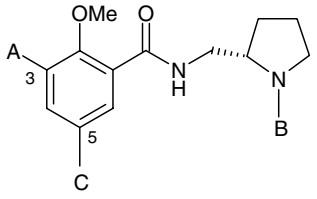


Figure 3. Modification sites of the benzamide lead structure (A, 3-position of aromatic ring; B, nitrogen of pyrrolidinyl moiety; C, 5-position of aromatic ring).

D_2 receptor. The K_i values found for FLB 457 (**5**) and for NABrE (**29**) to the D_{2short}/D_{2long} receptor (1.6/0.65 nM and 0.9 nM) show this effect (Table 1). The two compounds differ only in the moiety attached to the nitrogen atom of the pyrrodinyl ring. The affinity for D_{2short} of **29** is almost twice as high as the affinity of **5**, to D_{2long} the affinity of **29** is about a third lower compared to the value for **5**. From the K_i values obtained for the allyl compounds **27**–**30** for the D_2 receptor one can conclude that the ethyl compounds **3**–**5** show lower affinities than corresponding allyl compounds. In a more general scope the difference in substitution does not seem to have an extensive effect on the affinities for the D_2 receptor subtypes.

The substitutions at the 5-position of the aromatic ring (position C, Fig. 3) seem to exert less influence on the binding for the D_2 receptor. Looking at the influence on the K_i values of the three different halogen atoms directly placed at the 5-position of the aromatic ring (iodine, bromine, chlorine and unsubstituted) no trend can be seen. Even the 3-fluoropropane moiety at the 5-position does not have dramatic effects on the affinities of the compounds. The difference in the K_i values is negligible.

3.3. Lipophilicities

The lipophilicities of **1**–**5** and **27**–**34** were determined using the HPLC method according to the OEC guideline for the testing of chemicals.[29] Soerenson buffer was used as eluent and $\log D_{7.4}$ values calculated from retention times of the respective substances.

The calculated $\log D_{7.4}$ values are displayed in Table 2. All compounds examined showed $\log D_{7.4}$ values which are within the range of those found for already established radiotracers like IBZM (**3**), FP (**1**) and DMFP (**2**).

Table 2. Lipophilicities/$\log D_{7.4}$ values of synthesised benzamide derivatives **1**–**5** and **27**–**34**

Compound	Name	$\log D_{7.4}$
27	NAE	2.91
28	NADE	2.81
29	NaBrE	2.83
30	NaBrDE	2.71
31	NaClE	n/a
32	NaClDE	n/a
33	NAB	n/a
34	NADB	n/a
1	FP	2.66
2	DMFP	2.63
3	IBZM	3.13
4	Raclopride tartrate	2.18
5	FLB457	n/a

This fact gives rise to the assumption that those compounds will have similarly good properties for the use as radioligands for molecular imaging.

4. Conclusions

For the two iodinated compounds of which NAE (**27**) displays a 3-fold higher affinity for the D_2 receptor than FP (**1**) (K_i = 2.2 nM) and NADE (**28**) with a 1.5-fold higher affinity than DMFP (**2**) (K_i = 30 nM), excellent properties for molecular imaging can be expected.

Comparing the values obtained for the iodinated compounds with the ones obtained for FP (**1**) and DMFP (**2**) allows to expect similar properties for the two iodinated compounds NAE (**27**) and NADE (**28**) regarding selectivity and in vivo imaging potential. The brominated compounds NABrE (**29**) and NABrDE (**30**) show K_i values for the D_2 receptor which are in the same range as those for the iodinated analogues NAE (**27**) and NADE (**28**).

Of the structural elements having an influence on the affinities of benzamides for the D_2 receptor the addition of a second methoxy group at the 3-position of the aromatic ring has the most noticeable effect. The enhancements in affinities that can be related to changes at the nitrogen atom of the pyrrolidinyl ring or the 5-position of the aromatic ring are less obvious.

The log $D_{7.4}$ values of 2.91 for NAE (**27**) and of 2.81 for NADE (**28**) are in the range of those found for IBZM (**3**), FP (**1**) and DMFP (**2**).

The affinities for D_2-like receptors determined for IBZM (**3**) are 6-fold lower than the value found for NAE (**27**) and about five times higher than the value obtained for NADE (**28**). This fact gives reason to the assumption that better resolution and specificity can be achieved in SPECT by using the new iodinated compounds.

5. Experimental

5.1. Chemistry

Unless otherwise noted all chemicals were used without further purification. Moisture sensitive reactions were carried out under an argon or nitrogen atmosphere using dry solvents over molecular sieve (Fluka). 5-Iodo-2,3-dimethoxybenzoic acid (**13**) was purchased from Apin Chemicals. *S*-(−)-IBZM (**3**) was purchased from Sigma RBI, FLB 457 (**5**) from ABX and *S*-(−)-raclopride(+)-tartrate salt (**4**) from Sigma. All other chemicals, including the benzoic acid derivatives **16** and **25**, were purchased from either Merck, Aldrich, Acros or Fluka.

Two-hundred, three-hundred and four-hundred megahertz NMR spectra were recorded on a Bruker 200-MHz FT NMR Spectrometer AC 200, a Bruker 300-MHz FT NMR Spectrometer AC 300 or a Bruker-Biospin DRX 400 MHz spectrometer. Chemical shifts were reported in parts per million (ppm). FD mass spectrometry was performed on a Finnigan MAT90-Spectrometer.

All synthesised benzamide derivatives were purified by HPLC (system: Sykam S 1100 Solvent Delivery System, S 8110 Low Pressure Gradient Mixer, Rheodyne 9725i Inject Valve; Linear UVIS-205 Absorbance Detector; Axxiom Chromatography 900-200 Pyramid; software: Pyramid version 2.07; column: LiChrospher 100RP 18 EC-5μ, 250 × 20 mm; loop, 100 μL; flow, 5 mL/min) before carrying out the receptor binding studies.

5.1.1. *N*-(*S*)-Allyl-1-allylpyrrolidine-2-carboxylate (7). Twenty-one grams (0.18 mol) L-proline (**6**) and 61 g (0.44 mol) K_2CO_3 were suspended in 200 mL of dry DMSO. Under stirring 53 g (0.43 mol) of allyl bromide was added dropwise at ambient temperature over 4 h. After the addition was completed the reaction mixture was stirred overnight at room temperature and then poured onto ice water. The aqueous phase was extracted with diethyl ether (4× 250 mL). The organic extracts were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The obtained viscous liquid was purified through column chromatography on silica gel (eluent: *n*-hexane/ethyl acetate, 4:1). Thirty grams of crude product yielded 9.82 g (0.05 mol; 28%) of a colourless liquid. ^{1}H NMR (400 MHz, $CDCl_3$)δ 5.91–5.70 (m, 2H, —C*H*=CH_2); 5.29–4.96 (m, 4H, —CH=CH_2); 4.57–4.49 (d, 2H, —O—CH_2—CH=CH_2); 3.35 (dd, 1H, —C*H* (2)); 3.04–2.81 (m, 2H, N—CH_2—CH=CH_2); 2.59–2.50 (m, 2H, CH_2 (5)); 2.30–1.97 (m, 2H, CH_2 (3)); 1.81–1.60 (m, 2H, CH_2 (4)); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.82 (C=O); 135.33 (O—CH_2—*C*H=CH_2); 132.12 (N—*C*H=CH_2); 118.36 (O—CH_2—CH=*C*H_2); 117.43 (N—CH_2—CH=*C*H_2); 65.17 (O—*C*H_2—CH CH_2); 65.13 (C (2)); 57.63 (N—*C*H_2—CH=CH_2); 53.42 (C (5)); 29.51 (C (5)); 23.07 (C (3)); 23.07 (C (4)); MS (FD) *m*/*z* (% rel int.) 195.7 (100.0 $[M]^+$); 431.1 (11.92 $[M+NPM]^+$); 196.7 (0.79 $[M+1]^+$).

5.1.2. *N*-(*S*)-1-Allylpyrrolidine-2-carboxamide (8). Five grams (26 mmol) of (*S*)-1-allylpyrrolidine-2-carboxylate (**7**) and 135 mg of NaCN were dissolved in 100 mL of methanolic ammonia (9 M). The reaction mixture was kept at 35 °C for 45 h. Methanol was then removed under vacuum, the residue taken up in diethyl ether and washed with brine. The organic phase was dried over Na_2SO_4, the solvent removed under vacuum and the residue was recrystallised from diisopropyl ether. 2.42 g (16 mmol; 60%) of a white crystalline solid was obtained. Mp 79–80 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.24 (br s, 1H, NH—*H*); 5.93–5.73 (m, 2H, NH-*H*, —C*H*=CH_2); 5.22–5.04 (m, 2H, —CH=CH_2); 3.68–2.97 (m, 3H, N—CH_2 —CH=CH_2,—C*H*— (2)); 2.39–1.63 (m, 6H, CH_2 (3, 4, 5)); MS (FD) *m*/*z* (% rel int.) 154.7 (100.0 $[M]^+$); 155.6 (13.04 $[M+1]^+$); 309.9 (0.80 $[2M]^+$); 110.6 (0.14 $[M-CONH_2]$.

5.1.3. *N*-(*S*)-1-Allyl-2-aminomethylpyrrolidine (9). In a three-necked round bottomed flask 70 mL (0.25 mol) SDMA (70% in toluene) was added to 280 mL of dry toluene and heated to 70 °C. Twelve grams (79 mmol)

of (*S*)-1-allylpyrrolidine-2-carboxamide (**8**) were dissolved in 60 mL of dry THF and added dropwise to the SDMA solution over the period of 1 h. The reaction mixture was stirred for four additional hours at 70 °C and then cooled to room temperature. The mixture was acidified with 2 M HCl (pH 2–3) and then washed with diethyl ether. The pH of the aqueous phase was adjusted to 10 by addition of NaOH solution (45%). The aqueous phase was then extracted with ethyl acetate (5× 200 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The remaining crude product was distilled under vacuum (approx. 65 mbar) to yield 2 g (14 mmol, 18%, bp 103–112 °C) of a colourless liquid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.96–5.76 (m, 1H, —C*H*=CH_2); 5.18–4.99 (m, 2 H, —CH=CH_2); 3.60–2.57 (m, 5H, C*H* (2), CH_2 (7), —CH_2—CH=CH_2); 2.43–2.10 (m, 2H, NH_2); 1.89–1.32 (m, 6 H, —CH_2—CH_2—CH_2— (3–5)); MS (FD): *m*/*z* (% rel int.) = 141.7 (100.0 $[M+1]^+$); 140.7 (96,13 $[M]^+$); 110.7 (2.81 $[M-CH_2NH_2]$).

5.1.4. 5-Iodo-2-methoxybenzoic acid (22). 8.3 g (30 mmol) of 2-hydroxy-5-iodobenzoic acid (**18**) was dissolved in 20 mL of dry DMF under argon. 2.5 g (63 mmol) NaH (60% adsorbed on mineral oil) were added. The mixture was cooled to room temperature, 9.85 g (4.3 mL; 70 mol) of iodomethane was added and the mixture was stirred at 70 °C for 48 h. The solvent was removed under vacuum, the residue was taken up in water and 35 mL of a 1-M LiOH solution was added. The mixture was stirred at 70–80 °C until the cleavage was completed (~2 h). The mixture was cooled down to room temperature, filtered and the filtrate was acidified (pH 3–4) with dilute HCl (10%). The precipitate was filtrated, dried and purified through column chromatography on silica gel with ethyl acetate/*n*-hexane, 95:5 as eluent to yield 3 g (12 mmol, 42%) of a white crystalline solid. ^{1}H NMR (200 MHz, d_6-DMSO) δ 12.89 (br s, 1H, —COO*H*); 7.86 (d, 1H, arom. H (6)); 7.77 (dd, 1H, arom. H (4)); 6.96 (d, 1H, arom. H (3)); 3.79 (s, 3H, —OCH_3), MS (FD) *m*/*z* (% rel int.) 278.6 (100.0 $[M]^+$); 279.6 (8.57 $[M+1]^+$); 264.6 (0.77 $[M-CH_3]^+$).

5.1.5. 5-Bromo-2,3-dimethoxybenzoic acid (14). Six grams (26 mmol) 5-bromo-2-hydroxy-3-methoxy-benzaldehyde (**10**) was dissolved in 50 mL of 2-butanone. 5.4 g (39 mmol) of K_2CO_3 and 2.5 mL of iodomethane (39 mmol) were added. The mixture was kept at 80 °C for 22 h and then concentrated to dryness. The residue was dissolved in 100 mL of ethyl acetate and washed with 1 M NaOH and brine. The organic phase was dried over $MgSO_4$, the solvent removed under reduced pressure and the crude product (6.75 g) was further purified by column chromatography on silica gel with ethyl acetate/*n*-hexane, 2:8 as eluent. 2.3 g (9 mmol; 36%) of 5-bromo-2,3-dimethoxybenzaldehyde was obtained. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.32 (s, 1H, —CO*H*); 7.51 (d, 1H, arom. H (6)); 7.21 (d, 1H, arom. H (4)); 3.95 (s, 3H, —OCH_3 (2)); 3.88 (s, 3H, —OCH_3 (3)); MS (FD) *m*/*z* (% rel int.) 247.0 (100.0 $[M]^+$); 245.0 (96.91 $[M]^+$).

1.6 g (7 mmol) 5-bromo-2,3-dimethoxybenzaldehyde was dissolved in 100 mL of acetone and 2.1 g (13 mmol) $KMnO_4$ and 40 mL of water was added. After 22 h of stirring at room temperature the reaction mixture was filtered over Celite and the filtrate acidified with concentrated HCl. The precipitate was filtered, dried and purified through recrystallisation from water to yield 643 mg (2.5 mmol; 35%) of a white solid. ^{1}H NMR (200 MHz, d_6-DMSO) δ 7.36 (d, 1H, arom. H (6)); 7.28 (d, 1H, arom. H (4)); 3.84 (s, 3H, —OCH_3 (2)); 3.72 (s, 3H, —OCH_3 (3)); MS (FD) *m*/*z* (% rel int.) 263.0 (100.0 $[M]^+$); 261.7 (97.40 $[M]^+$).

5.1.6. 5-Bromo-2-methoxybenzoic acid (23). The procedure for **14** was followed with 6.5 g (30 mmol) 5-bromo-2-methoxybenzaldehyde (**19**) as starting compound. Yield: 2.1 g (9 mmol; 30%) of a white solid. ^{1}H NMR (200 MHz, d_6-DMSO) δ 12.95 (br s, 1H, —COO*H*); 7.72 (d, 1H, arom. H (6)); 7.66 (d, 1H, arom. H (4)); 7.09 (d, 1H, arom. H (3)); 3.80 (s, 3H, —OCH_3); MS (FD) *m*/*z* (% rel int.) 230.7 (100.0 $[M]^+$); 232.7 (80.81 $[M]^+$).

5.1.7. 5-Chloro-2,3-dimethoxybenzoic acid (15). The procedure for **14** was followed with 5.2 g (26 mmol) of 5-chloro-2,3-dimethoxybenzaldehyde (**11**) as starting compound. Yield: 750 mg (3 mmol, 14%) of a white solid was obtained. ^{1}H NMR (300 MHz, d_6-DMSO) δ 7.27 (s, 1 H, arom. H (6)); 7.15 (s, 1H, arom. H (4)); 3.80 (s, 3 H, —OCH_3(2)); 3.67 (s, 3 H, —OCH_3(3)); MS (FD) *m*/*z* (% rel int.) 216.2 (100.0 $[M]^+$); 218.2 (30.88 $[M]^+$).

5.1.8. 5-Chloro-2-methoxybenzoic acid (24). 7.6 g of NaOH was dissolved in 590 mL of water. Five hundred and eighty millilitres of CH_2Cl_2 was added, followed by 4.4 g of tetrabutyl ammonium iodide and 20 g of 5-chloro-2-hydroxybenzoic acid (**20**). Under vigorous stirring 33 mL of DMSO was added dropwise and the mixture was stirred at room temperature for 4 h. Distillation gave 9.9 g (46%, 49 mmol) of a colourless liquid. 50 mL of 1 M NaOH was added to 2.0 g of the ester. After stirring at room temperature overnight 2 M HCl was added until a precipitate formed. The aqueous phase was extracted with ether (four portions of 50 mL). The organic phase was dried with $MgSO_4$, the solvent removed under in vacuo and 1.6 g (83%, 8 mmol) of a white solid was isolated. ^{1}H NMR (300 MHz, d_6-DMSO) δ 7,66 (dd, 1H, arom. H (6)); 7,59 (dd, 1H, arom. H (4)); 7,06 (dd, 1H, arom. H (3)); 3,80 (s, 3 H, —OCH_3); MS (FD) *m*/*z* (% rel int.) 186.2 (100.0 $[M]^+$); 188.2 (28.39 $[M]^+$).

5.1.9. 5-(3-Hydroxypropyl)-2,3-dimethoxybenzoic acid (17). 3-(3,4-Dimethoxyphenyl)propan-1-ol (**12**) was treated according to the procedure described for **14** to yield 62% of the title compound **17**. ^{1}H NMR (200 MHz, $CDCl_3$/d_4-MeOH) δ 6.98 (d, 1H, arom. H (6)); 6.90 (d, 1H, arom. H (4)); 3.88 (s, 3H, OCH_3 (2)); 3.86 (s, 3H, OCH_3 (3)); 3.60 (t, 2H, Ar—CH_2—CH_2—CH_2OH), 2.68 (t, 2H, Ar—CH_2—CH_2—CH_2OH), 1.97–1.81 (m, 2H, Ar—CH_2—CH_2—CH_2OH).

5.1.10. 5-(3-Hydroxypropyl)-2-methoxybenzoic acid (26). Two grams 3-(4-methoxyphenyl)propan-1-ol (**21**) (12 mmol) was dissolved in 20 mL dry THF and cooled to −78 °C. Under a nitrogen atmosphere 14.6 mL of an *n*-butyllithium solution in *n*-hexane (15%; 24 mmol) was

added and the mixture was stirred at −78 °C for 30 min. The mixture was allowed to warm to room temperature, stirred for 30 min and poured into a suspension of dry ice in diethyl ether. The solvent was removed under reduced pressure, the residue dissolved in water and extracted with diethyl ether. The aqueous phase was acidified, extracted with ethyl acetate and the solvent evaporated under reduced pressure to give 2.15 g (85%, 10 mmol) of a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.99 (d, 1H, arom. H (6)); 7.37 (dd, 1H, arom. H (4); 6.99 (d, 1H, arom. H (3)); 4.04 (s, 3H, OCH_3); 3.64 (t, 2H, Ar—CH_2—CH_2—CH_2OH), 2.70 (t, 2H, Ar—CH_2—CH_2—CH_2OH), 1.93–1.77 (m, 2H, Ar—CH_2—CH_2—CH_2OH).

5.1.11. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-5-iodo-2,3-dimethoxybenzamide (NAE) (27). Synthesis followed the same procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)-methyl)-5-iodo-2-methoxybenzamide (NADE, **28**) using 5-iodo-2,3-dimethoxybenzoic acid (**13**) as the benzoic acid derivative. The title compound was obtained in 82% yield as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$)δ 8.23 (br s, 1H, N—*H*); 7.97 (d, 1H, arom. H (6)); 7.22 (d, 1H, arom. H (4)); 5.88–5.78 (m, 1H, —CH_2—C*H*=CH_2); 5.16–5.01 (m, 2H, —CH_2—CH=CH_2); 3.81 (s, 6H, 2× OCH_3); 3.71–3.63 (m, 1H, —NH—CH_2—C*H*—); 3.43–3.23 (m, 2H, —NH—CH_2—CH); 3.07–2.78 (m, 2H, —CH_2—CH=CH_2); 2.64–2.15 (m, 2H, CH_2—CH_2—CH_2—N (11)); 1.68–1.55 (m, 4H, —CH_2—CH_2—CH_2—N (9,10)); MS (FD) *m*/*z* (% rel int.) 430.7 (100.0 $[M]^+$); 431.8 (29.08 $[M+1]^+$).

5.1.12. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (28). Two hundred and forty grams (0.84 mmol) 5-iodo-2-methoxybenzoic acid (**22**) was dissolved in 6 mL CH_2Cl_2 and cooled to 0 °C (ice bath). Subsequently 0.15 mL (1.10 mmol) triethyl amine and 0.1 mL (1.0 mmol) ethyl chloroformiate in 2 mL of CH_2Cl_2 were added under stirring at 0 °C. After 1 h a mixture of 140 mg (1 mmol) *N*-(*S*)-1-allyl-2-aminomethylpyrrolidine (**9**) and 0.15 mL (1.1 mmol) triethyl amine in 5 mL CH_2Cl_2 was added to the reaction mixture and stirred for another 1.5 h at 0 °C. The solvent was evaporated under reduced pressure; the residue taken up in CH_2Cl_2, the organic phase was washed with water and was dried over Na_2SO_4. After removal of the solvent the crude product was purified by column chromatography yielding 306 mg (0.76 mmol, 76%) of a yellow oil using ethyl acetate/methanol 8/2 as eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, 1H, arom. H (6)); 8.22 (br s, 1H, N-*H*); 7.66 (dd, 1H, arom. H (4)); 6.70 (d, 1H, arom. H (3)); 5.91–5.80 (m, 1H, —CH_2—C*H*=CH_2); 5.20–5.03 (m, 2H, —CH_2—CH=CH_2); 3.89 (s, 3H, OCH_3); 3.70–3.66 (m, 1H, —NH—CH_2—C*H*—); 3.45–3.28 (m, 2H, -NH—CH_2—CH); 3.10–2.84 (m, 2H, —CH_2—CH=CH_2); 2.68–2.18 (m, 2H, CH_2—CH_2—CH_2—N (11)); 1.76–1.55 (m, 4H, —CH_2—CH_2—CH_2—N (9, 10)); MS (FD) *m*/*z* (% rel int.) 400.9 (100.0 $[M]^+$); 802.4 (30.89 $[2M]^+$); 401.9 (23.26 $[M+1]^+$).

5.1.13. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-5-bromo-2,3-dimethoxybenzamide (NABrE) (29). Synthesis followed the same procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (**28**) using **14** as the benzoic acid derivative. The title compound was obtained in 86% yield as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (br s, 1H, N-*H*); 7.69 (d, 1H, arom. H (6)); 7.10 (d, 1H, arom. H (4)); 6.14–6.03 (m, 1H, —CH_2—C*H*=CH_2); 5.43–5.38 (m, 2H, —CH_2—CH=CH_2); 4.04 (s, 3H, OCH_3 (2)); 3.84 (s, 3H, OCH_3 (3)); 3.96–3.91 (m, 2H, —NH—CH_2—CH); 3.89–3.86 (m, 1H, —NH—CH_2—C*H*—); 3.77–3.69 (m, 1H, —CH_2—CH=CH_2); 3.50–3.42 (m, 1H, —CH_2—CH=CH_2); 2.94–2.88 (m, 2H, CH_2—CH_2—CH_2—N (11)); 2.24–1.86 (m, 4H, —CH_2—CH_2—CH_2—N (9, 10)); MS (FD) *m*/*z* (% rel int.) 385.0 (100.0 $[M]^+$); 383.0 (98.29 $[M]^+$).

5.1.14. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-5-bromo-2-methoxybenzamide (NABrDE) (30). Synthesis followed the same procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (**28**) using **23** as the benzoic acid derivative. The title compound was obtained in 86% yield as a yellow oil. ^{1}H NMR (200 MHz, d_6-DMSO) δ 8.31 (br s, 1H, N-*H*); 7.88 (d, 1H, arom. H (6)); 7.63 (dd, 1H, arom. H (4)); 7.13 (d, 1H, arom. H (3)); 5.93–5.77 (m, 1H, —CH_2—C*H*=CH_2); 5.23–5.04 (m, 2H, —CH_2 —CH=CH_2); 3.88 (s, 3H, OCH_3); 3.54–2.47 (m, 5H, —NH—CH_2—C*H*—, —NH—CH_2—CH—, —CH_2—CH=CH_2); 2.23–1.46 (m, 6H, —CH_2—CH_2 —CH_2— (9–11)); MS (FD) *m*/*z* (% rel int.) 352.7 (100.0 $[M]^+$); 354.7 (96.13 $[M]^+$); 353.7 (18.61 $[M+1]^+$); 355.7 (13.31 $[M+1]^+$).

5.1.15. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-5-chloro-2,3-dimethoxybenzamide (NAClE) (31). Synthesis followed the same procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (**28**) using **15** as the benzoic acid derivative. The title compound was obtained in 55% yield as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (br s, 1H, N-*H*); 7.66 (d, 1H, arom. H (6)); 6.96 (d, 1H, arom. H (4)); 5.89–5.88 (m, 1H, —CH_2—C*H*=CH_2); 5.22–5.07 (m, 2H, —CH_2—CH=CH_2); 3.81 (s, 6H, OCH_3); 3.78–3.71 (m, 1H, —NH—CH_2—C*H*); 3.45–3.12 (m, 2H, —NH—CH_2—CH—); 2.92–2.74 (m, 2H, —CH_2—CH=CH_2); 2.74–1.25 (m, 2H, CH_2—CH_2—CH_2—N (11)); 1.94–1.65 (m, 4H, —CH_2—CH_2—CH_2—N (9, 10)).

5.1.16. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-5-chloro-2-methoxybenzamide (NAClDE) (32). Synthesis followed the same procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (**28**) using **24** as the benzoic acid derivative. The title compound was obtained in 39% yield as colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (br s, 1H, N—*H*); 7.74 (d, 1H, arom. H (6)); 7.51 (dd, 1H, arom. H (4)); 7.18 (d, 1H, arom. H (3)); 5.94–5.80 (m, 1H, —CH_2—C*H*=CH_2); 5.22–5.06 (m, 2H, —CH_2—CH=CH_2); 3.88 (s, 3H, OCH_3); 3.40 (m, 1H, —NH—CH_2—C*H*—); 3.20–2.99 (m, 2H, —NH—CH_2—CH—); 2.88–2.61 (m, 2H, —CH_2—CH=CH_2); 2.22–1.76 (m, 2H, —CH_2 (11); 1.67–1,45 (m, 4H, —CH_2—CH_2—CH_2— (9, 10)).

5.1.17. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-2,3-dimethoxybenzamide (NAB) (33). Synthesis followed the same

procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (**28**) using **16** as the benzoic acid derivative. The title compound was obtained in 36% yield as a colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (br s, 1H, N-*H*); 7.76 (d, 1H, arom. H (6)); 7.11 (t, 1H, arom. H (5)); 7.01 (d, 1H, arom. H (4)); 5.92–5.90 (m, 1H, —CH_2—C*H* =CH_2); 5.23–5.08 (m, 2H, —CH_2—CH=C*H*$_2$); 3.88 (s, 6H, OC*H*$_3$); 3.83–3.77 (m, 1H, —NH—CH_2 —C*H*); 3.52–3.36 (m, 2H, —NH—C*H*$_2$—CH—); 3.19–2.88 (m, 1H, —C*H*$_2$—CH=CH_2); 2.32–1.91 (m, 2H, CH_2—CH_2—C*H*$_2$—N (11)); 1.72–1.69 (m, 4H, —C*H*$_2$—C*H*$_2$—CH_2—N (9, 10)).

5.1.18. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-2-methoxy benzamide (NADB) (34). Synthesis followed the same procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (**28**) using **25** as the benzoic acid derivative. The title compound was obtained in 62% yield as a colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (br s, 1H, N-*H*); 8.17 (d, 1H, arom. H (6)); 7.43 (t, 1H, arom. H (5)); 7.04 (t, 1H, arom. H (4)); 6.94 (d, 1H, arom. H (3)); 5.91–5.89 (m, 1H, —CH_2—C*H*=CH_2); 5.22–5.08 (m, 2H, —CH_2—CH=C*H*$_2$); 3.93 (s, 3H, OC*H*$_3$); 3.77–3.70 (m, 1H, —NH—CH_2—C*H*—); 3.51–3.16 (m, 2H, -NH—C*H*$_2$—CH—); 3.10–2.84 (m, 2H, —C*H*$_2$—CH=CH_2); 2.81–2.21 (m, 2H, —C*H*$_2$—C*H*$_2$—C*H*$_2$— (11)); 1.95–1.64 (m, 4H, —C *H*$_2$—C*H*$_2$—C*H*$_2$— (9, 10)).

5.1.19. *N*-(((*S*)-1-Allylpyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2,3-dimethoxybenzamide (FP) (1). Synthesis was carried out in analogy to the synthesis of *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2-methoxybenzamide (**2**), starting out from 3-(3,4-dimethoxyphenyl)propan-1-ol (**12**), coupled alcohol **35**, tosylated compound **36** and finally FP (**1**) affording comparable yields.

5.1.19.1. Compound 35. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, 1H, arom. H (6)); 6.80 (d, 1H, arom. H (4)); 5.78–5.89 (m, 1H, —CH_2—C*H*=CH_2); 5.00–5.15 (m, 2H, —CH_2—CH=C*H*$_2$); 3.81 (s, 3H, *OCH*$_3$ (2)); 3.80 (s, 3H, OC*H*$_3$ (3)); 3.67–3.74 (m, 1H, —NH—C *H*$_2$—CH—); 3.59 (t, 2H, Ar—CH_2—CH_2—C*H*$_2$OH); 3.42–2.78 (m, 4H; —NH—C*H*$_2$—CH—; —NH—CH_2—C*H*—; —C*H*$_2$—CH=CH_2); 2.63 (t, 2H, Ar—C*H*$_2$—CH_2—CH_2OH); 2.15–2.18 (m, 1H, —CH_2—CH_2—C*H*$_2$— (11)); 1.61–1.87 (m, 7H, —CH_2—CH_2—C*H*$_2$— (11); —C*H*$_2$—C*H*$_2$—CH_2— (9, 10); Ar—CH_2—C *H*$_2$—CH_2OH).

5.1.19.2. Compound 36. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (d, 2H, tos.); 7.44 (d, 1H, arom. H (6));7.32 (d, 2H, tos.); 6.82 (d, 1H, arom. H (4)); 5.78–5.98 (m, 1H, —CH_2—C*H*=CH_2); 5.01–5.25 (m, 2H, —CH_2—CH=C*H*$_2$); 3.99 (t, 2H, Ar—CH_2—CH_2—C*H*$_2$OTos); 3.86 (s, 3H, OC*H*$_3$ (2)); 3.84 (s, 3H, OC*H*$_3$ (3)); 3.80–2.77 (br, 5H, —NH—CH_2—C*H*—; —NH—C*H*$_2$—CH—; —C*H*$_2$—CH=CH_2); 2.64 (t, 2H, Ar—C*H*$_2$—CH_2—CH_2OTos); 2.39 (s, 3H, tos-CH_3); 2.13–2.28 (m, 1H, —CH_2—CH_2—C*H*$_2$— (11)); 1.59–2.03 (m, 7H, —CH_2—CH_2—C*H*$_2$— (11); —C*H*$_2$—C*H*$_2$—CH_2— (9, 10); Ar—CH_2—C*H*$_2$—CH_2OTos); MS (FD) *m*/*z* (% rel int.) 516.9 (100.0 $[M]^+$); 1032.9 (47.00 $[2M]^+$); 1033.9 (26.78 $[2M+1]^+$).

5.1.19.3. Compound 1. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49 (d, 1H, arom. H (6)); 6.80 (d, 1H, arom. H (4)); 5.87–5.95 (m, 1H, —CH_2—C*H*=CH_2); 5.02–5.20 (2d, 2H, —CH_2—CH=C*H*$_2$); 4.31–4.47 (dt, 2H, Ar—CH_2—CH_2—C*H*$_2$F, J = 48.0 Hz); 3.82 (2s, 6H, OC*H*$_3$); 3.75–2.78 (m, 5H, —NH—CH_2—C*H*—; —C*H*$_2$—CH=CH_2; —NH—C*H*$_2$—CH—); 2.67 (t, 2H, Ar—C*H*$_2$—CH_2—CH_2F); 2.13–2.2 (m, 2H, —CH_2—CH_2—C*H*$_2$— (11)); 1.90–2.03 (m, 2H, Ar—CH_2—C*H*$_2$—CH_2F; J = 26 Hz); 1.52–1.89 (m, 4H, —C*H*$_2$—C*H*$_2$—CH_2— (9, 10)): MS (FD): *m*/*z* (% rel int.) = 364.8 (100.0 $[M]^+$); 365.8 (57.3 $[M]^+$).

5.1.20. *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-(3-hydroxypropyl)-2-methoxybenzamide (37). Synthesis followed the same procedure as for *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-iodo-2-methoxybenzamide (NADE) (**28**) using **26** as the benzoic acid derivative yielding 81% of the title compound **37**.

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 8.00 (d, 1H, arom. H (6)); 7.22 (dd, 1H, arom. H (4)); 6.87 (d, 1H, arom. H (3)); 5.72–5.98 (m, 1H, —CH_2—C*H*=CH_2); 4.99–5.19 (m, 2H, —CH_2—CH=C*H*$_2$); 3.90 (s, 3H, OC*H*$_3$); 3.61 (t, 2H, Ar—CH_2—CH_2—C*H*$_2$OH); 3.53–2.75 (br, 6H, —NH—C*H*$_2$—CH—; —C*H*$_2$—CH=CH_2; —NH—CH_2—C*H*—; —CH_2—CH_2—C*H*$_2$- (11)); 2.64 (t, 2H, Ar—C*H*$_2$—CH_2—CH_2OH); 2.10–2.29 (m, 1H, —CH_2—CH_2—C*H*$_2$— (11)); 1.52–2.00 (m, 6H, —C*H*$_2$—C*H*$_2$—CH_2— (9, 10); Ar—CH_2—C*H*$_2$—CH_2OH).

5.1.21. 3-(3-(((*S*)-1-allylpyrrolidin-2-yl)methylcarbamoyl)-4-methoxyphenyl)propyl-4-methylbenzenesulfonate (38). Two hundred and eighty grams *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-(3-hydroxypropyl)-2-methoxybenzamide **37** and 130 μL pyridine were dissolved in 2 mL dichloromethane and the solution was cooled to 0–5 °C. One hundred and seventy milligrams (0.85 mmol) of 4-methylbenzene-1-sulfonyl chloride was added and the reaction mixture was stirred for 2 h at 0–5 °C and then for 3 h at room temperature. The solution was washed with water and the aqueous phase was extracted with ethyl acetate. The solvent was removed under reduced pressure. The residue was taken up in diethyl ether and washed with potassium hydroxide solution (10%) and water. The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel with ethyl acetate/methanol, 4:1 as eluent. The product was obtained as viscous yellow oil in 48% yield (183 mg). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.93 (d, 1H, arom. H (6)); 7.76 (d, 2H, tos.), 7.23 (d, 2H, tos.); 7.15 (dd, 1H, arom. H (4)); 6.83 (d, 1H, arom. H (3)); 5.78–5.95 (m, 1H, —CH_2—C*H*=CH_2); 5.05–5.22 (m, 2H, —CH_2—CH=C*H*$_2$); 3.98 (t, 2H, Ar—CH_2—CH_2—C*H*$_2$OTos); 3.89 (s, 3H, OC*H*$_3$); 3.49–2.74 (br, 6H, —CH_2—CH_2—C*H*$_2$— (11); —C*H*$_2$—CH=CH_2; —NH—C*H*$_2$—CH—); 2.61 (t, 2H, Ar—C*H*$_2$—CH_2—CH_2OTos); 2.38 (s, 3H, tos—CH_3); 2.15–2.29 (m, 1H, —NH—CH_2—C*H*—); 1.56–1.98 (m, 6H, —C*H*$_2$—C*H*$_2$—CH_2— (9, 10); Ar—CH_2—C*H*$_2$—

CH_2OTos); MS (EI, 70 eV): *m/z* (% rel int.) 468 (0.49, $[m]^+$), 457 (1.29, $[M-C_2H_5]^+$), 347 (2.26, $[M-C_7H_7O_2S]^+$), 110 (100.0, $[C_7H_{11}N]^+$); FD: *m/z* (% rel int.) 487 (100.0, $[M+1]^+$), 333 (6.45, $[(M+1)-C_7H_7O_2S]^+$).

5.1.22. *N*-(((*S*)-1-allylpyrrolidin-2-yl)methyl)-5-(3-fluoropropyl)-2-methoxybenzamide (DMFP) (2). One hundred milligrams 3-(3-(((*S*)-1-allylpyrrolidin-2-yl)methyl-carbamoyl)-4-methoxyphenyl)propyl 4-methylbenzene-sulfonate **38** (0.19 mmol), 16 mg potassium fluoride (0.28 mmol), 26 mg dehydrated potassium carbonate (0.12 mmol) and 70 mg Kryptofix®2.2.2. (0.28 mmol) were dissolved in 4 mL of dry acetonitrile and refluxed for 16 h. The reaction mixture was allowed to cool down, the solvent was removed under reduced pressure and the obtained crude product purified by column chromatography on silica gel using methanol/ethyl acetate, 1:1 as eluent. The product was obtained as a brownish viscous mass with a yield of 79% (44 mg). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm): 7.71 (d, 1H, arom. H (6)); 7.27 (dd, 1H, arom. H (4)); 6.98 (d, 1H, arom. H (3)); 5.75–5.97 (m, 1H, $-CH_2-CH=CH_2$); 5.02–5.24 (m, 2H, $-CH_2-CH=CH_2$); 4.17–4.47 (dt, 2H, $Ar-CH_2-CH_2-CH_2F$, J = 47.3 Hz); 3.85 (s, 3H, OCH_3); 3.59–2.86 (br, 6H, $-CH_2-CH_2-CH_2-$ (11); $-CH_2-CH=CH_2$; $-NH-CH_2-CH-$); 2.62 (t, 2H, $Ar-CH_2-CH_2-CH_2F$); 2.21–2.37 (m, 1H, $-NH-CH_2-CH-$); 1.52–2.00 (m, 6H, $-CH_2-CH_2-CH_2-$ (9, 10); $Ar-CH_2-CH_2-CH_2F$); MS (FD): *m/z* (% rel int.) 334.3 (100.0, $[M]^+$), 670.1 (10.6, $[2M+2]^+$).

5.2. Binding assays

Receptor binding studies were carried out as described in the literature.[24] In brief, the dopamine D_1 receptor assay was done with porcine striatal membranes at a final protein concentration of 40 μg/assay tube and the radioligand [^{3}H]SCH 23390 at 0.3 nM (K_d = 0.95 nM). Competition experiments with the human D_{2long}, D_{2short}, D_3 and $D_{4.4}$ receptors were run with preparations of membranes from CHO cells expressing the corresponding receptor and [^{3}H]spiperone at a final concentration of 0.1 nM. The assays were carried out with a protein concentration of 3–20 μg/assay tube and K_d values of 0.06 nM for D_{2long}, 0.08–0.15 nM for D_{2short}, 0.13–0.35 nM for D_3 and 0.28 nM for $D_{4.4}$. The investigation of serotonergic 5-HT_{1A} and 5-HT_2 and adrenergic α_1 binding was performed as described in the literature.[27] In brief, porcine cortical membranes were subjected to the binding assay at a concentration of 80–115 μg/assay tube for determination of 5-HT_{1A} and 5-HT_2 binding utilizing [^{3}H]8-OH-DPAT and [^{3}H]ketanserin each at a final concentration of 0.5 nM with K_d values of 2.0–3.0 nM (for 5-HT_{1A}) and 1.9–2.1 nM (for 5-HT_2). Cortical membranes at 55–80 μg/assay tube and the radioligand [^{3}H]prazosin at a final concentration of 0.4 nM were applied to determine adrenergic α_1 binding with K_d values of 0.06 nM.

Protein concentration was established by the method of Lowry using bovine serum albumin as standard.[30]

Data analysis of the resulting competition curves was accomplished by non-linear regression analysis using the algorithms in PRISM (GraphPad Software, San Diego, CA). K_i values were derived from the corresponding EC_{50} data utilizing the equation of Cheng and Prusoff.[31]

5.3. Lipophilicity

Lipophilicities were determined using the HPLC system described above with a LiChrospher 100 RP 18 EC-5μ column. Soerensen buffer was used as eluent. Retention times for compounds **1**–**4** and **27**–**30** were determined. Retention times for a number of reference substances of known log *P* were assessed as well. From the obtained retention times the capacity factor *k* was calculated for all compounds. Plotting log *k* against log *P* gives the reference curve which is used to find the log *P* values for compounds **1**–**4** and **27**–**30**. Log $D_{7.4}$ values of the synthesised compounds were calculated using this reference curve and the determined retention times.

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