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Synthesis and in vitro affinities of various MDL 100907 derivatives as potential ^{18}F-radioligands for 5-HT$_{2A}$ receptor imaging with PET

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ABSTRACT

Radiolabelled piperidine derivatives such as [^{11}C]MDL 100907 and [^{18}F]altanserin have played an important role in diagnosing malfunction in the serotonergic neurotransmission. A variety of novel piperidine MDL 100907 derivatives, possible to label with ^{18}F-fluorine, were synthesized to improve molecular imaging properties of [^{11}C]MDL 100907. Their in vitro affinities to a broad spectrum of neuroreceptors and their lipophilicities were determined and compared to the clinically used reference compounds MDL 100907 and altanserin. The novel compounds MA-1 (**53**) and (*R*)-MH.MZ (**56**) show K_i-values in the nanomolar range towards the 5-HT$_{2A}$ receptor and insignificant binding to other 5-HT receptor subtypes or receptors. Interestingly, compounds MA-1 (**53**), MH.MZ (**55**) and (*R*)-MH.MZ (**56**) provide a receptor selectivity profile similar to MDL 100907. These compounds could possibly be preferable antagonistic ^{18}F-tracers for visualization of the 5-HT$_{2A}$ receptor status. Medium affine compounds (VK-1 (**32**), (**51**), (**52**), (**54**)) were synthesized and have K_i values between 30 and 120 nM. All promising compounds show log*P* values between 2 and 3, that is, within the range of those for the established radiotracers altanserin and MDL 100907. The novel compounds MA-1 (**53**) and (*R*)-MH.MZ (**56**) thus appear to be promising high affine and selective tracers of ^{18}F-labelled analogues for 5-HT$_{2A}$ imaging with PET.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT), its transporter and various receptors are of central interest in the field of medicinal chemistry.[1–3] Seven major families of transmembrane receptors (5-HT$_{1-7}$) and one transporter (SERT) are known to control 5-HT function by three different structures (transporters, ligand-gated ion channels and G-protein-coupled receptors).[4] The role of 5-HT$_{2A}$ receptors in the regulation of a number of processes of the central nervous system (CNS) such as mood, appetite, sexual behaviour, learning and memory and their dysfunctions such as psychosis, depression and anxiety has been well documented.[5,6] In particular, the 5-HT$_{2A}$ receptors have been implicated in the beneficial effects of some antidepressants as well as antipsychotics.[7] All clinically approved atypical antipsychotic drugs are also potent 5-HT$_{2A}$ receptor antagonists.[8,9] Moreover, many hallucinogens including LSD function as agonists at 5-HT$_{2A}$ receptors.[7]

Consequently, in vivo studies of 5-HT$_{2A}$ receptor availability would significantly advance the understanding of the biological principles of mentioned disorders and contribute to the development of appropriate therapies. Positron emission tomography (PET) is an appropriate tool to measure in vivo directly, non-invasively and repetitively the relevant pharmacologic parameters of ligand–neuroreceptor interactions.

Supposed adequate radiolabeled neurotransmitter analogues are available for the molecular imaging of the 5-HT$_{2A}$ receptor. To date, in vivo studies have been performed with several 5-HT$_{2A}$ selective antagonists. Of these tracers, [^{11}C]MDL 100907 (a) and [^{18}F]altanserin (b) represent the radioligands of choice for in vivo 5-HT$_{2A}$ PET imaging because of their high affinity and selectivity for the 5-HT$_{2A}$ receptor. In addition to their high binding affinities to the 5-HT$_{2A}$ receptor (altanserin: K_i = 0.13 nM; MDL 100907 K_i = 0.36 nM) their binding affinities are more than 30-fold lower for other relevant receptors such as 5-HT$_{1A}$, 5-HT$_{2C}$, α_1, D$_1$ and D$_2$ (Table 1).[10–14]

In in vitro and in in vivo experiments, both tracers revealed high affinity, selectivity and a good ratio of specific to non-specific

Table 1
Affinities (K_i (nM)) of altanserin and MDL 100907[10–14]

	5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_{2c}$	D$_2$	α_1
Altanserin	1570	0.13	40	62	4.55
(*R*)-MDL 100907	>10,000	0.36	107	2250	128

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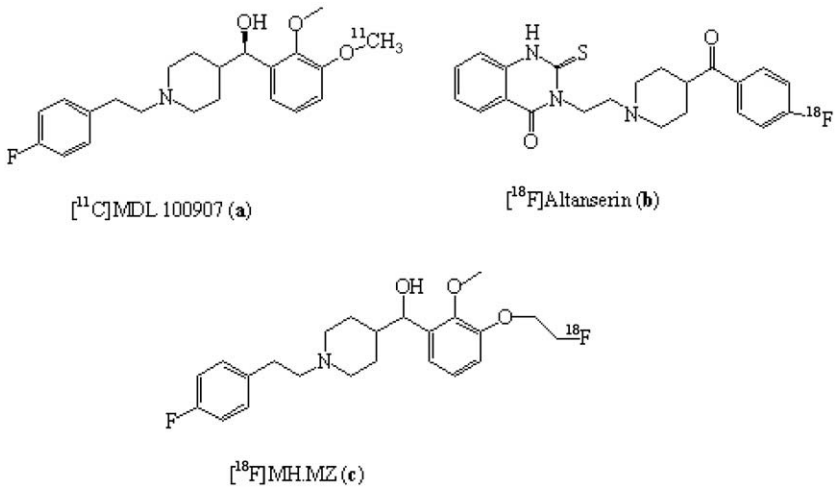


Figure 1. Structures of [^{11}C]MDL 100907 (a), [^{18}F]altanserin (b) and [^{18}F]MH.MZ (c).

binding for 5-HT$_{2A}$ receptors.[15,16] However, Table 1 indicates that the selectivity of [^{11}C]MDL 100907 for the 5-HT$_{2A}$ receptor is slightly higher than that of [^{18}F]altanserin.[15] Tracer affinity and selectivity are of crucial importance for uptake kinetics in brain, and tracers with very high affinity and selectivity give new insights into the role of the status of the serotonergic system and antipsychotic drug action. For example, Mintun et al. measured in vivo with [^{18}F]altanserin a decreased hippocampel 5-HT$_{2A}$ receptor binding in major depressive patients.[17]

Concerning molecular imaging, the advantage of [^{18}F]altanserin (b) over [^{11}C]MDL 100907 (a) is the possibility to perform equilibrium scans lasting several hours and to transport the tracer to other facilities based on the 110 min half-life of ^{18}F-fluorine.

A drawback of [^{18}F]altanserin is its rapid and extensive metabolism. Four metabolites are formed in humans that cross the blood-brain-barrier,[13] whereas metabolites of [^{11}C]MDL 100907 do not enter the brain to any larger extent.[18]

The aim of this study was to synthesize a ligand combining the reported better selectivity and in vivo stability of MDL 100907 as compared to those of altanserin and the superior isotopic properties of an ^{18}F-label as compared to those of a ^{11}C-label.

Recently, we have reported the synthesis, first in vitro, ex vivo and in vivo evaluations of an ^{18}F-analogue of MDL 100907, [^{18}F]MH.MZ (c) (Fig. 1).

The promising results obtained in ex vivo and in vivo experiments[19,20] encouraged us to study structure–activity relationships of MDL 100907 analogues in more detail aiming at even improving affinity and selectivity of new compounds. Unfortunately, no detailed structure–activity relationship has been reported yet for this class of compound. A rudimental pharmacophore model has been published by Andersen et al.[21] It describes the binding of various arylpiperidines at 5-HT$_2$ receptors. The model requires two aryl substituents, separated by distance *a* and located distances b and *c* from an amine moiety (Fig. 2). Distances suggested by Anderson et al. for *a*, b and *c* are 5.1, 7.5 and 8.1 Å, respectively.

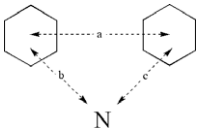


Figure 2. A general pharmacophore model to account for the binding of 5-HT$_2$ antagonists.[21]

Heinrich et al.[22] described an optimization of the structure and the discovery of a selective 5-HT$_{2A}$ antagonist, but no attempts have been made to optimize these MDL 100907 analogues for in vivo PET imaging.

Herein, we report the syntheses of new MDL 100907 analogues to enlighten structure–activity relationships. In addition, some new derivatives may be considered as potential ^{18}F-radioligands for 5-HT$_{2A}$ receptor PET imaging.

2. Results and discussion

2.1. Chemistry

Organic synthesis of MDL 100907 has been described in the literature by Huang et al.[16] and Ullrich and Ice.[23] Both methods depended upon the formation of a Weinreb amide, as a key intermediate, which is then reacted with *ortho*-lithiated veratrole derivatives to afford the ketone matrix of the MDL 100907 lead structure.

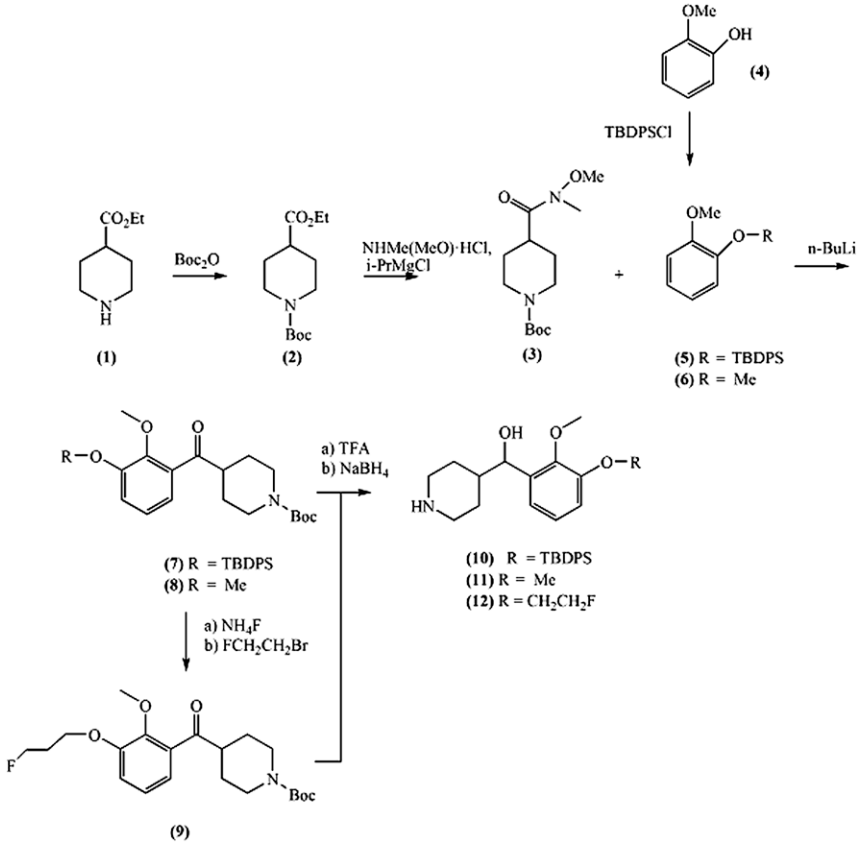
Huang et al.[16] synthesized the racemic phenolic precursor molecule first, and subsequently separated the isomers by chiral derivatization with (*S*)-(+)-α-methoxyphenyl-acetic acid and flash chromatography. Whereas Ullrich and Ice[23] performed the optical resolution earlier, in particular before adding the *p*-fluorophenethyl substituent to the piperidine moiety. This strategy also allows introducing a variety of N-substituents.

We decided to follow a similar synthesis route, but in contrast to Ullrich and Ice[23] the ketone bodies (**7**) and (**8**) were isolated (Scheme 1). Those compounds permit structure–affinity studies of the ketone group versus the racemic or enantioselective aliphatic secondary alcohol.

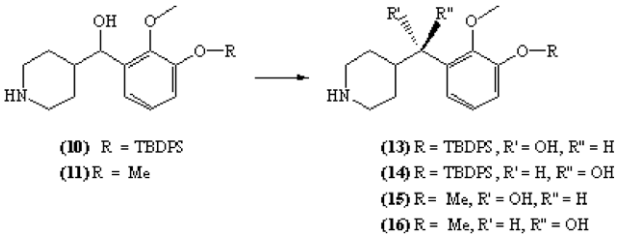
In addition, deprotection of the phenolic hydroxyl group is performed earlier to get versatile access to fluoroethylated reference compounds.

According to the literature,[23] ethylisonipecotate (**1**) was *t*-boc-protected and afterwards transformed to the corresponding Weinreb amide (**3**). Regioselective *ortho*-lithiated veratrole derivatives (**5**/**6**) were reacted with the mentioned amide (**3**) to afford the matrix ketone body (**7**/**8**). Either deprotection of the TBDPS-group with NH_4F followed by fluoroalkylation was performed or direct deprotection of the piperidine moiety with TFA ensued by reduction with $NaBH_4$ to the racemic alcohol was carried out. Compounds (**9**)–(**12**) were prepared in this way (Scheme 1).

The racemic alcohol could be separated by means of salt formation with (+)- and (−)-mandelic acid (Scheme 2) as reported by Ullrich and Ice.[23]



Scheme 1. Synthesis of MDL 100907 key intermediates.



Scheme 2. Optical resolution of (**10**) and (**11**).

The diastereomeric salts were isolated and recrystallized twice from methanol. Aqueous work up afforded the enantiomers in high purity, as determined via optical resolution (Table 2).

Enantiomeric excess (ee) of compounds (**13**)–(**16**) was not examined via chiral HPLC. Instead, enantioselective reference compounds and precursors were tested on (ee) via chiral HPLC later on.

In addition, N-alkylation of piperidine derivatives was performed in DMF in yields of 60–90% and provided compounds (**17**)–(**32**) that enable structure–activity studies of the *p*-substituent of the aromatic ring and of the chain length [$(-CH_2-)_n$ with $n = 1,2$] between the piperidine and phenyl moiety (Scheme 3).

Table 2
Optical resolution of (**13**)–(**16**)

Compd#	$[\alpha]_D$	
	This work	Ref. 23
(**13**)	+46.86 (*c* 0.1, MeOH)	+45.5 (*c* 0.1, MeOH)
(**14**)	−53.34 (*c* 0.1, MeOH)	−46.8 (*c* 0.1, MeOH)
(**15**)	−6.32 (*c* 0.13; MeOH)	−7.1 (*c* 0.1, MeOH)
(**16**)	+7.04 (*c* 0.12, MeOH)	+6.6 (*c* 0.1, MeOH)

Longer chain lengths lead to a higher α_1 and D_2 affinity as reported by Schmidt et al.,[24] that is, n > 2 were not considered.

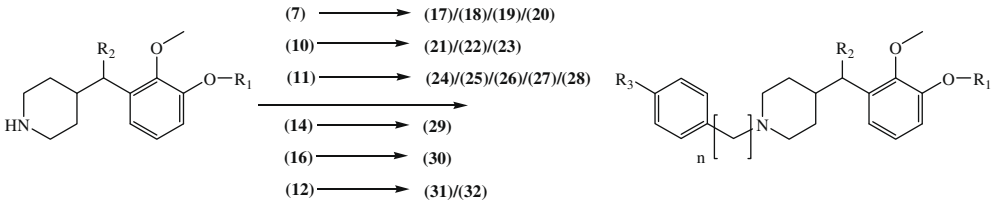
Commercially unavailable (2-bromoethyl)-4-fluorobenzene (**36**) and (1-bromomethyl)-4-fluorobenzene (**35**) were obtained by treatment of 4-fluorophenethyl alcohol (**34**) or (4-fluorophenyl) methanol (**33**) with PBr_3 (Scheme 4). The latter resulted in a heavy lachrymatory substance.

To determine the influence of a carbonyl group between the piperidine and the phenyl moiety, preparation of the three compounds (**39**)–(**41**) were tried via N-alkylation (Scheme 5).

In contrast to (**39**), both ketone derivatives (**40**)–(**41**) were synthetically not accessible. Even Finkelstein-conditions, variation of solvent and temperature could not alter the synthetic behaviour. Modified reaction characteristics could possibly be due to the electronic withdrawing carbonyl group and could therefore lead to styrene derivatives. These reactive by-products could interact with themselves or other reactants thus forming a broad spectrum of compounds. However, protection of the carbonyl moiety by forming 2-(bromomethyl)-2-(4-fluorophenyl)-1,3-dioxolane (**37**), and thus reducing the electronic withdrawing effect, could not overcome the problem. N-Alkylation to (3-(2-fluoroethoxy)-2-methoxyphenyl)(1-((2-(4-fluorophenyl)ethyl)-1,3-dioxolan-2-yl) methyl)piperidin-4-yl)methanol (**38**) was not accessible probably due to steric effects.

Deprotection of the phenolic hydroxy moiety with NH_4F or with K_2CO_3 overnight leads to high yields of >75%. Precursors (**42**)–(**49**) were prepared in such a way. In addition, reference compounds (**50**)–(**56**) were synthesized by fluoroalkylation with 1,2-bromofluoroethane (Scheme 6).

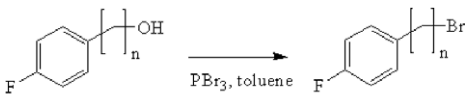
Alternatively, MH.MZ (**55**) and (**54**) could be prepared by reduction of their carbonyl derivatives with $NaBH_4$. Similarly, compound (**57**) could also be obtained via that reaction route (Scheme 7).



cmpd#	R_1	R_2
7	TBDPS	= O
10	TBDPS	- OH
11	Me	- OH
14	TBDPS	◀ OH
16	Me	◀ OH
12	-$[CH_2]_2$F	- OH

cmpd#	R_1	R_2	R_3	n
17	TBDPS	= O	- CH_3	2
18	TBDPS	= O	- NO_2	2
19	TBDPS	= O	- OCH_3	2
20	TBDPS	= O	- F	2
21	TBDPS	- OH	- NO_2	2
22	TBDPS	- OH	- OCH_3	2
23	TBDPS	- OH	- F	2
24	Me	- OH	- F	1
25	Me	- OH	- NO_2	2
26	Me	- OH	- OCH_3	2
27	Me	- OH	- CH_3	2
28	Me	- OH	- F	2
29	TBDPS	◀ OH	- F	2
30	Me	◀ OH	-F	2
31	-$[CH_2]_2$F	- OH	-H	2
32 (VK-1)	-$[CH_2]_2$F	- OH	-NO_2	2

Scheme 3. N-Alkylation of piperidine derivatives (general reaction conditions: alkylating agent, DMF, $NaHCO_3$, 85 °C, 90 min).

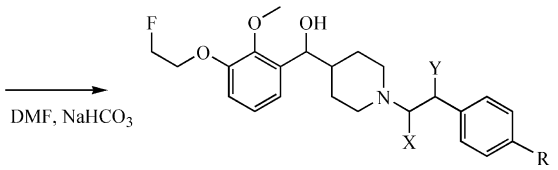


(33) n = 1
(34) n= 2
(35) n = 1
(36) n= 2

Scheme 4. Synthesis of bromo-alkyl-4-fluorobenzenes.

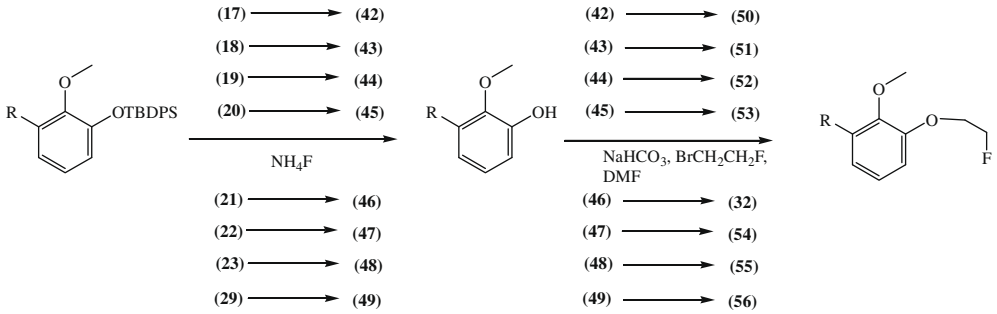
Finally, an amine MDL 100907 derivative (**58**) was synthesized by reduction of the NO_2-compound (**32**) in a microwave oven (CEM LabMate) (Scheme 8).

Enantiomeric excess of (*R*)-MH.MZ (**56**) and (*R*)-MDL 100907 (**30**) was determined by chiral HPLC analysis (Fig. 3). Purities of ee >98% were detected.



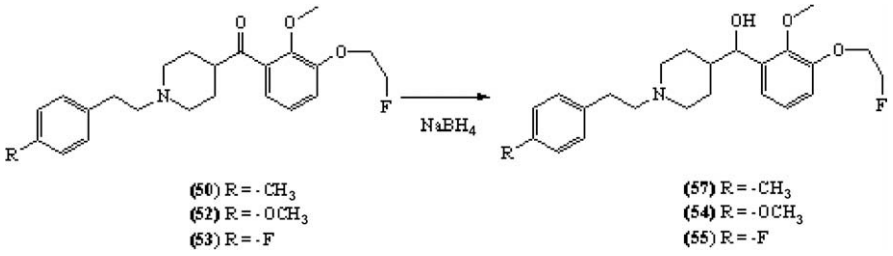
(39) X = =O, Y = H, R = H
(40) X = H, Y = =O, R = H
(41) X = H, Y = =O, R = F

Scheme 5. Synthesis to carbonyl derivatives of MDL 100907 via N-alkylation.

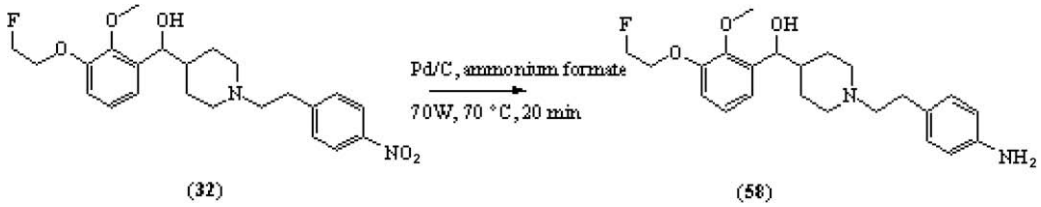


cmpd#	R	R_1	R_2	cmpd#	R	R_1	R_2
42		= O	$-CH_3$	**50**		= O	$-CH_3$
43		= O	$-NO_2$	**51**		= O	$-NO_2$
44		= O	$-OCH_3$	**52**		= O	$-OCH_3$
45		= O	-F	**53 [MA-1]**		= O	-F
46		-OH	$-NO_2$	**32 [VK-1]**		-OH	$-NO_2$
47		-OH	$-OCH_3$	**54**		-OH	$-OCH_3$
48		-OH	-F	**55 [MH.MZ]**		-OH	-F
49		◀ OH	-F	**56 [(R)-MH.MZ]**		◀ OH	-F

Scheme 6. Deprotection and fluoroalkylation of various MDL 100907 derivatives.



Scheme 7. Reduction of carbonyl-compounds to their corresponding alcohol derivatives.



Scheme 8. Synthesis of (**58**) by reduction of (**32**) in a microwave oven.

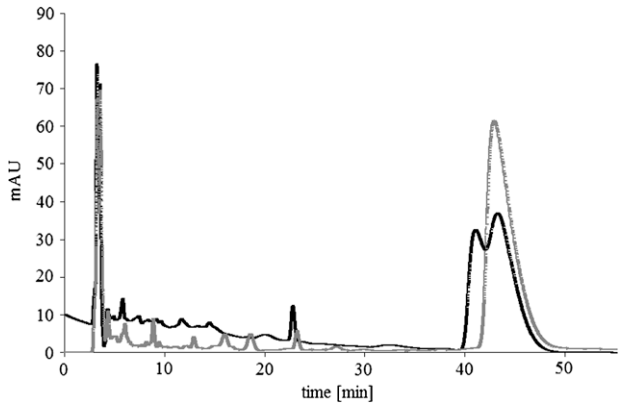


Figure 3. Chiral HPLC analyses of (*R*)-MH.MZ: The black line represents the racemat (MH.MZ); in contrast, the grey line shows the enantioselective pure compound (*R*)-MH.MZ.

2.2. Lipophilicities

The lipophilicities of promising compounds were determined using the HPLC method according to Krass et al.[25] Soerensen buffer was used as eluent and log*P* values were calculated from retention times of the respective substances. The calculated log*P* values are displayed in Table 3.

All new fluoroethylated reference compounds besides (**39**), (**50**) and (**57**) examined showed log*P* values between 2 and 3, ranging within those found for already established radiotracers such as altanserin and MDL 100907 (Table 3). Rowley et al. pointed out that a log*P* between 2 and 3 presents the ideal interval for small molecules for penetrating the blood-brain-barrier (BBB).[26] This fact gives rise to the assumption that the new compounds may have similarly good properties for molecular imaging.

As expected, log*P* values of precursors (**46**) and (**48**) are reduced by 0.4 compared to their corresponding reference compounds VK-1 (**32**) and MH.MZ (**55**) probably resulting in less BBB penetrating ligands, which are formed by the metabolic cleavage of the ether. For compound (**48**), MDL 105725, this behaviour has already been observed.[18] Comparison of ketone- versus hydroxyl moieties shows a 0.3 higher lipophilicity for carbonyl compounds, see, for example, (**53**)–(**55**). MDL 100907 (**28**), MH.MZ (**55**) and MDL 105725 (**48**) indicate the expected descending order of lipophilicity. However, log*P* values of MDL 100907 (**28**) and MH.MZ (**55**) differ not much and therefore, similar lipophilicities should be expected. Influence of *p*-substitution of the aryl moiety was examined and showed the expected ascending tendency $-NO_2$ < –OMe < –F < –H < –Me.

In conclusion, almost all reference compounds besides (**39**), (**50**) and (**57**) demonstrated favourable lipophilicity and therefore should have good in vivo properties. In comparison, relevant labelling precursors, which could be possibly formed via metabolism of the parent, should penetrate the BBB less because of their reduced log*P* values.

Table 3
Lipophilicities/log*P* values of altanserin, MDL 100907 and new MDL 100907 derivatives[a]

Reference compounds			Precursors	
Name	Compd#	Log*P*	Cmpd#	Log*P*
Altanserin	—	2.15	(**48**)	2.27
(*R*)-MDL 100907	(**30**)	2.98	(**46**)	1.90
MDL 100907	(**28**)	2.98		
MH.MZ	(**55**)	2.80		
(*R*)-MH.MZ	(**56**)	2.80		
MA-1	(**53**)	3.08		
VK-1	(**32**)	2.37		
	(**57**)	3.40		
	(**50**)	3.64		
	(**54**)	2.72		
	(**51**)	2.71		
	(**52**)	3.02		
	(**39**)	1.57		
	(**31**)	2.92		

[a] Log*P* values are based on the means of three experiments.

2.3. Receptor characterization

2.3.1. Affinity towards the 5-HT_{2A} receptor

5-HT_{2A} receptor affinity was determined by a radioligand competition binding assay with GF-62 cells, a clonal cell line expressing high amounts (5–7 pmol/mg) of the 5-HT_{2A} receptor. The test tubes contained [^{3}H]MDL (0.2 nM) and seven different concentrations of the test compounds (1 μM–1 pM) in a total of 1 mL assay buffer. Ketanserin (1 μM) was added to determine non-specific binding. Binding affinities of the tested compounds are shown in Table 4.

The tested compounds had affinities to the 5-HT_{2A} receptor in the nanomolar range, except (**24**) and (**39**). Both compounds are varied between the piperidine and phenyl ring. Shortening the chain length from $n = 2$ to $n = 1$ leads to a total loss of affinity towards the 5-HT_{2A} receptor and is therefore in accordance with the receptor model suggested by Andersen et al.[21] It describes the ideal distance between the phenyl moieties to be 8.1 Å. Introducing an amide between the piperidine and phenyl ring reduced the affinity by a factor of 2000. This is probably due to the lower basic characteristic properties of the resulting compound than the original tertiary amine structure.

Moreover, replacing the 3-methoxy- by a fluoroethoxy group slightly reduces the 5-HT_{2A} affinity but the remaining compound is still within the nanomolar range of the parent, for example, (**28**)–(**55**), (**30**)–(**56**) and (**27**)–(**57**). However, 3-hydroxy derivatives (**48**)–(**55**) had a better affinity. In contrast, introducing a methoxy- or a nitro-moiety to the phenethyl group resulted in a reduced affinity by a factor of ~20 for methoxy- and by a factor of ~40 for nitro-derivatives, for example, (**28**)–(**25**)–(**26**) or (**53**)–(**52**)–(**51**). One exception to the mentioned results was found by analyzing compounds (**32**) and (**54**). Indeed, replacing the fluorine atom of MH.MZ (**55**) by a nitro- or a methoxy group leads to a decreased affinity, but only by factor ~3 for the nitro- and by factor ~7 for the methoxy compound. Interestingly, reduction of affinity appears not so harsh and vice versa in that case compared with (**28**)–(**25**)–(**26**) and (**53**)–(**52**)–(**51**). Therefore, we speculate that (**32**) and (**54**) could fit differently in the binding pocket. Nevertheless, all compounds of these series show medium affinity towards the 5-HT_{2A} receptor. Besides, replacing the *p*-substituent of the phenethyl moiety by a methyl group, an amine or a single proton

Table 4
Affinities of tested ligands towards the 5-HT_{2A} receptor[a]

Name	Compd#	K_i (nM)	Compd#	K_i (nM)
Altanserin	—	0.72 ± 0.19	(**31**)	1.63 ± 5.50
(*R*)-MDL 100907	(**30**)	0.38 ± 0.05	(**39**)	1987 ± 360
MDL 100907	(**28**)	2.10 ± 0.13	(**45**)	1.34 ± 0.48
MH.MZ	(**55**)	9.02 ± 2.11	(**48**)	1.24 ± 0.23
(*R*)-MH.MZ	(**56**)	0.72 ± 0.21	(**49**)	0.25 ± 0.06
MA-1	(**53**)	3.23 ± 0.18	(**50**)	4.56 ± 0.73
VK-1	(**32**)	26.0 ± 6.70	(**51**)	138 ± 20.0
	(**24**)	>1000	(**52**)	153 ± 93.0
	(**25**)	90.0 ± 71.0	(**54**)	59 ± 54.0
	(**26**)	55 ± 12.0	(**57**)	1.83 ± 0.43
	(**27**)	0.31 ± 0.06	(**58**)	2.06 ± 0.96

[a] K_i values in nM ± SEM are based on the means of four experiments.

leads to a slightly increased affinity, for example, (**28**)–(**27**) and (**55**)–(**58**)–(**57**). This may probably be due to the slightly different space required. Changing the racemic secondary hydroxy group to a ketone group [(**28**)–(**53**); (**57**)–(**50**)] showed no dramatic effects but as expected, the enantioselective pure product (**56**) showed increased affinity than the carbonyl (**53**) and racemic compounds (**28**).

2.3.2. Selectivity of promising ligands

Promising 5-HT_{2A} affinity compounds (**32**) and (**50**)–(**57**) were assayed for their selectivity for other 5-HT receptor subtypes (Table 5).

Besides a medium affinity of MH.MZ (**55**) towards the 5-HT_7 (K_i = 116 ± 14 nM), and the 5-HT_{2C} receptor (K_i = 71 ± 10 nM), all other tested receptors showed affinities in the μM range. Thus, MH.MZ (**55**) (5-HT_{2A}: K_i = 9.02 ± 2.11 nM) is a selective ligand for the 5-HT_{2A} receptor. Furthermore, the enantioselective derivative (*R*)-MH.MZ (**56**) even showed a better selectivity profile, whereas MA-1 (**53**) showed a good affinity towards the 5-HT_{2A} receptor (K_i = 3.23 ± 0.18 nM) and a medium affinity towards the 5-HT_{2C} receptor (K_i = 128 ± 14 nM). Moreover, the introduced carbonyl group within MA-1 (**53**) leads to a slightly affine compound of the 5-HT_{1A} receptor (K_i = 1681 ± 97 nM) compared to its structural related hydroxyl group derivatives MH.MZ (**55**) and (*R*)-MH.MZ (**56**). But still MA-1 (**53**) is a selective 5-HT_{2A} ligand. All other tested compounds (**32**), (**50**), (**51**), (**52**), (**54**) and (**57**) showed a similar behaviour regarding the 5-HT_{2A} selectivity.

Moreover, high 5-HT_{2A} affinity compounds (**50**), (**53**), (**56**) and (**57**) were also assayed for a broad spectrum of receptors and monoamine transporters in competitive binding experiments in vitro using cloned human receptors or transporters (Table 6). Interestingly, new compounds, (**50**), (**53**), (**56**) and (**57**), have no affinity to Beta1-3, H4, M1-M5, DOR, KOR receptors. Minor affinity towards the other tested receptors and transporters (D1-D5, H1-H2, DAT, SERT, MOR, sigma 2, alpha 1A-2B) is summarized in Table 6.

Table 6
Affinities to a broad spectrum of neuroreceptors and transporters of MDL 100907 derivatives

	K_i[a] (nM)				
	MA-1 (**53**)	(*R*)-MH.MZ (**56**)	MH.MZ (**55**)	(**50**)	(**57**)
D1	1410	3828	899	1930	1759
D2	3828	2686	>10,000	>10,000	>10,000
D4	174	442	544	923	2708
D5	7398	7419	n.d.	n.d.	n.d.
Beta3	>10,000	>10,000	>10,000	>10,000	>10,000
H1	944	509	374	665	439
H2	1757	2428	3178	827	1156
H3	>10,000	>10,000	>10,000	9033	>10,000
Sigma 2	86	923	n.d.	228	676
Alpha 1A	146	335	n.d.	n.d.	n.d.
Alpha 1B	550	500	325	n.d.	>10,000
Alpha 2A	863	>10,000	>10,000	n.d.	>10,000
Alpha 2C	333	n.d.	148	154	143
DAT	>10,000	>10,000	>10,000	n.d	>10,000
SERT	3087	2207	3516	1009	2646
MOR	>10,000	>10,000	>10,000	2009	>10,000

[a] K_i values in nM are based on the means of four experiments; n.d. (not determined).

3. Conclusion

A series of novel MDL 100907 derivatives containing a fluorine atom were synthesized and evaluated for their in vitro behaviour. Structure–activity relationships (SARs) studies suggested that the tested compounds had affinities to the 5-HT_{2A} receptor in the nanomolar range except (**24**) and (**39**), which are varied between the piperidine and phenyl ring. This is in accordance with the rudimental pharmacophore model that has been published by Andersen et al.[21] Replacing the 3-methoxy by a fluoroethoxy group slightly reduces the 5-HT_{2A} affinity but still the affinity is within the nanomolar range of the parent, for example, (**28**)–(**55**), (**30**)–(**56**) and (**27**)–(**57**). The 3-hydroxy derivatives (**48**)–(**55**) had a better affinity. In contrast, introducing a methoxy or a nitro moiety to the phenethyl group results in a reduction of affinity to medium affine compounds ($K_i \sim 1$ μM). Besides, replacing the *p*-substituent of the phenethyl moiety by a methyl group, an amine or a single proton slightly increases the affinity, for example, (**28**)–(**27**) and (**55**)–(**58**)–(**57**). This could be due to the slightly different space required. Changing the racemic secondary hydroxyl group to a ketone group [(**28**)–(**53**); (**57**)–(**50**)] causes no dramatic effects but as expected, the enantioselective pure product (**56**) enhances the affinity than the carbonyl (**53**) and the racemic compound (**28**). Furthermore, 5-HT_{2A} affine compounds (Tables 5 and 6) were tested for selectivity. All tested ligands thereby showed a reasonable receptor profile and determined them as 5-HT_{2A} selective compounds.

Except for (**39**), (**50**) and (**57**), all our fluoroethylated compounds had log*P* values between 2 and 3, that is, within the range of those of already established radiotracers such as altanserin and MDL 100907.

The novel compounds MA-1 (**53**) and (*R*)-MH.MZ (**56**) seem to be promising high affinity compounds for ^{18}F-PET-tracer due to their K_i-values in the nanomolar range of 1–10 towards the 5-HT_{2A} receptor and their insignificant binding to other 5-HT receptor subtypes or receptors. Interestingly, compounds MA-1 (**53**), MH.MZ (**55**) and (*R*)-MH.MZ (**56**) showed a receptor selectivity profile similar to MDL 100907, which is slightly improved

Table 5
Ligand affinities to 5-HT receptors

	K_i[a] (nM)								
	MA-1 (**53**)	(*R*)-MH.MZ (**56**)	MH.MZ (**55**)	(**50**)	(**57**)	(**51**)	VK-1 (**32**)	(**52**)	(**54**)
5-HT_{1A}	1681 ± 97	>10,000	>10,000	1883 ± 158	>10,000	1723 ± 239	>10,000	991 ± 135	>10,000
5-HT_{1B}	2174 ± 336	884 ± 106	1846 ± 426	3359 ± 676	4886 ± 1081	>10,000	>10,000	>10,000	>10,000
5-HT_{1D}	713 ± 124	1062 ± 186	706 ± 109	n.d.	1440 ± 253	1835 ± 259	3418 ± 512	1436 ± 196	>10,000
5-HT_{1E}	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
5-HT_{2A}	3.23 ± 0.18	0.72 ± 0.21	9.02 ± 2.11	4.56 ± 0.73	1.83 ± 0.43	118 ± 20.0	26.0 ± 6.70	153 ± 93.0	59 ± 54
5-HT_{2B}	960 ± 113	320 ± 27	299 ± 27	559 ± 58	466 ± 51	1052 ± 111	1844 ± 183	1972 ± 181	2751 ± 289
5-HT_{2C}	128 ± 14	53 ± 7	71 ± 10	497 ± 72	508 ± 70	>10,000	>10,000	9417 ± 2798	>10,000
5-HT_3	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
5-HT_{5A}	1584 ± 270	6814 ± 982	>10,000	1684 ± 287	>10,000	>10,000	>10,000	>10,000	>10,000
5-HT_6	>10,000	3842 ± 623	3890 ± 1921	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
5-HT_7	210 ± 38	59 ± 11	116 ± 14	731 ± 117	498 ± 77	6241 ± 847	5170 ± 642	2862 ± 430	>10,000

[a] K_i values in nM ± SEM are based on the means of four experiments; n.d. (not determined).

compared to that of altanserin. Therefore, the mentioned compounds could possibly be preferable antagonistic ^{18}F-tracers for visualization of the 5-HT_{2A} status. Medium affine compounds (VK-1 (**32**), (**51**), (**52**) and (**54**)) have K_i values of 30–120 nM which are in the range of endogenous serotonin (according to PDSP). Challenging experiments with those compounds to measure changes in the endogenous serotonin level could be possible and allow a deeper insight in the serotonin system.

In extension to the in vitro data discussed concerning the SAR of the new fluorine containing MDL 100907 derivatives, ^{18}F-fluoroethylation and small μPET studies are planned to exam the in vivo characteristics of the potential 5-HT_{2A} ligands. In particular, a comparison of [^{18}F]MH.MZ to [^{18}F]altanserin and other ^{18}F-labelled MDL 100907 derivatives should be done.

4. Experimental

4.1. General

4.1.1. Chemicals, flash chromatographies and TLC

Chemicals were purchased from ABX, Acros, Aldrich, Fluka, Merck or Sigma. 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.

Chromatographic purifications were conducted on Silica Gel 60 (0.040–0.063 mm, Acros) columns. TLCs were run on pre-coated plates of Silica Gel 60F_{254} (Merck).

4.1.2. Analytical HPLC

Systems were equipped with a 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; Pyramid 2.07; loop: 20 μL.

4.1.3. Spectroscopy

300 and 400 MHz NMR spectra were recorded on a Bruker 300 MHz-FT-NMR-spectrometer AC 300 or on 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. Optical rotations were determined with a polarimeter Perkin–Elmer 241 using 10 cm, 1 mL cell.

4.1.4. Microwave

Syntheses were carried out in a commercially available microwave oven (CEM LabMate).

4.2. Chemistry

4.2.1. 4-(*N*-Methoxy-*N*-methyl-carboxamido)-1-piperidinecarboxylic acid *t*-butyl ester (3)

4-(*N*-Methoxy-*N*-methyl-carboxamido)-1-piperidinecarboxylic acid *t*-butyl ester (**3**) was synthesized as described by Carr et al.[27]

4.2.2. *t*-Butyldiphenylsilyl-guaiacol (5)

t-Butyldiphenylsilyl-guaiacol (**5**) was synthesized as described by Mathis et al.[28]

4.2.3. 4-(2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-benzoyl)-1-piperidinecarboxylic acid *t*-butyl ester (7) and 4-(2,3-Bismethoxy-benzoyl)-1-piperidinecarboxylic acid *t*-butyl ester (8)

n-Butyllithium (64.5 mL of a 2.5 M solution in hexane, 161 mmol) was added to a stirred solution of *t*-butyldiphenylsilyl-guaiacol (**5**) or veratrole (**6**) (161 mmol) under nitrogen at 0 °C. The ice bath was removed and the solution was allowed to stir for 2 h. After cooling to −50 °C, 4-(*N*-methoxy-*N*-methyl-carboxamido)-1-piperidinecarboxylic acid *t*-butyl ester (**3**) (153 mmol) was added dropwise to the stirred solution, followed by warming to room temperature and stirring for 2 h. Saturated aqueous NH_4Cl was added; the layers were separated and the aqueous layer was extracted 3× with ether. The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and evaporated to afford the crude product. Chromatography of the residue gave the pure product.

4.2.3.1. 4-(2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-benzoyl)-1-piperidinecarboxylic acid *t*-butyl ester (7). 21 g (**5**) (58 mmol), *n*-BuLi (120 mmol), 15.9 g (**3**) (58 mmol) and 150 mL dry THF yielded 14.8 g of (**7**) (25.8 mmol; 44%). R_f 0.43 (silica gel, 5:1 PE/EtOAc). ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.702 (dd, 4H); 7.421–7.322 (m, 6H); 6.832 (t, 1H); 6.685 (t, 1H); 6.617 (dd, 1H); 4.053 (d, 2H); 3.901 (s, 3H); 3.176 (tt, 1H); 2.827 (dt, 2H); 1.764 (dd, 2H); 1.527 (dd, 2H); 1.444 (s, 9H); 1.113 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 573.4 (100.0 $[M]^+$); 574.4 (44.8 $[M+1]^+$); 575.4 (14.2 $[M+2]^+$).

4.2.3.2. 4-(2,3-Bismethoxy-benzoyl)-1-piperidinecarboxylic acid *t*-butyl ester (8). 10.65 g veratrole (**6**) (58 mmol), *n*-BuLi (77 mmol), 20.63 g (**3**) (74.3 mmol) and 200 mL dry THF yielded 18 g of (**8**) (51.72 mmol; 68%). R_f 0.27 (silica gel, 4.5:1 PE/EtOAc). ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.087–6.922 (m, 3H); 4.095–4.048 (m, 2H); 3.861 (s, 3H); 3.832 (s, 3H); 3.241–3.167 (m, 1H); 2.839–2.747 (m, 2H); 1.843–1.789 (dd, 2H); 1.611–1.490 (m, 2H); 1.414 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 349.4 (100.0 $[M]^+$); 350.4 (20.69 $[M+1]^+$).

4.2.4. General procedure for TBDPS-deprotection

A solution of TBDPS-protected substance (4 mmol) and NH_4F (9 mmol) in anhydrous MeOH (30 mL) was stirred for 15 min at 70 °C. After evaporation of the solvent, the residue was taken up in NH_4OH and extracted 3× with $CHCl_3$. The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and evaporated. Chromatography of the residue gave the pure product.

4.2.4.1. 4-(3-Hydroxy-2-methoxy-benzoyl)-1-piperidinecarboxylic acid *t*-butyl ester (9a). 0.75 g (**7**) (1.31 mmol), 0.41 g NH_4F (5.54 mmol) and 15 mL MeOH yielded 0.27 g of (**9a**) (0.8 mmol; 61%). R_f 0.32 (silica gel, 1:1 PE/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.079–6.917 (m, 3H); 6.151 (br s, 1H); 4.065 (d, 2H); 3.779 (s, 3H); 3.211 (tt, 1H); 2.816 (t, 2H); 1.787 (dd, 2H); 1.639–1.496 (m, 2H); 1.427 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 473.3 (100.0 $[M]^+$); 474.3 (56.7 $[M+1]^+$); 475.3 (12.5 $[M+2]^+$).

4.2.4.2. 4-(3-Hydroxy-2-methoxy-benzoyl)-1-(2-*p*-toluylethyl)-piperidine (42). 0.88 g (**17**) (1.49 mmol), 0.2 g NH_4F (2.64 mmol) and 30 mL MeOH yielded 185 mg of (**42**) (0.52 mmol; 35%). R_f 0.45 (silica gel, 8:1 $CHCl_3$/MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.173–6.882 (m, 7H); 3.789 (s, 3H); 3.200–3.154 (m, 1H); 3.077–3.038 (m, 2H); 2.889–2.852 (m, 3H); 2.436 (br s, 1H); 2.277 (s, 3H); 2.038–2.005 (m, 2H); 1.934–1.812 (m, 2H) MS (FD) *m*/*z* (% rel Int.): 354.3 (100.0 $[M]^+$); 353.3 (98.37 $[M-1]^+$); 475.3 (12.5 $[M+2]^+$).

4.2.4.3. 4-(3-Hydroxy-2-methoxy-benzoyl)-1-(2-*p*-nitrophenyl-ethyl)-piperidine (43). 900 mg (**18**) (1.45 mol), 300 mg NH_4F (8 mmol) and 16 mL dry MeOH yielded 475 mg of (**43**) (1.23 mmol; 86%). R_f 0.55 (silica gel, 8:1 $CHCl_3$/MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 8.118 (dt, 2H); 7.320 (dt, 2H); 7.073–6.994 (m, 2H); 6.930 (dd, 1H); 3.905 (t, 1H); 3.784 (s, 3H); 3.095 (t, 1H); 2.998–2.864 (m, 4H); 2.635 (t, 2H); 2.204 (t, 2H); 1.884 (br d,

2H); 1.826–1.689 (m, 2H) MS (FD) *m/z* (% rel Int.): 384.2 (100.0 $[M]^+$); 385.2 (66.1 $[M+1]^+$); 386.2 (13.3 $[M+2]^+$).

4.2.4.4. 4-(3-Hydroxy-2-methoxy-benzoyl)-1-(2-*p*-methoxyphenylethyl)-piperidine (44). 591 mg (**19**) (0.88 mmol), 0.9 g NH_4F (24 mmol) and 20 mL MeOH yielded 290 mg of (**44**) (0.78 mmol; 80%). R_f 0.53 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.193–7.076 (m, 3H); 7.005 (t, 1H); 6.915 (dd, 1H); 6.801 (dt, 2H); 3.790 (s, 3H); 3.751 (s, 3H); 3.298 (br s, 1H); 3.092 (br s, 2H); 2.918 (dt, 4H); 2.884–2.594 (m, 2H); 2.174 (br s, 2H); 2.044–1.905 (m, 2H) MS (FD) *m/z* (% rel Int.): 369.2 (100.0 $[M]^+$); 370.2 (62.1 $[M+1]^+$); 371.2 (11.7 $[M+2]^+$).

4.2.4.5. 4-(3-Hydroxy-2-methoxy-benzoyl)-1-(2-*p*-fluorophenylethyl)-piperidine (45). 2.99 g (**20**) (5 mmol), 0.91 g NH_4F (12.43 mmol) and 30 mL MeOH yielded 1.65 g of (**45**) (4.63 mmol; 92%). R_f 0.64 (silica gel, 5:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.144–6.906 (m, 7H); 3.785 (s, 3H); 3.108–2.967 (m, 3H); 2.814–2.759 (m, 2H); 2.611–2.557 (m, 2H); 2.192 (br s, 2H); 1.935–1.766 (m, 4H) MS (FD) *m/z* (% rel Int.): 358.2 (100.0 $[M]^+$); 359.2 (17.19 $[M+1]^+$).

4.2.4.6. (3-Hydroxy-2-methoxyphenyl)-(1-(2-*p*-nitrophenylethyl)-piperidine-4-yl)-methanol (46). 932 mg (**21**) (1.5 mmol), 300 mg NH_4F (8 mmol) and 20 mL MeOH yielded 515 mg of (**46**) (1.33 mmol; 89%). R_f 0.52 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 9.202 (s, 1H); 8.111 (d, 2H); 7.490 (d, 2H); 6.889–6.679 (m, 2H); 4.891 (d, 1H); 4.552 (t, 1H); 3.684 (s, 3H); 3.333 (br s, 1H); 3.039–2.795 (m, 4H); 2.589–2.487 (m, 4H); 2.015–1.689 (m, 2H); 1.429 (br s, 1H); 1.330–1.119 (m, 2H) MS (FD) *m/z* (% rel Int.): 386.9 (100.0 $[M]^+$); 387.9 (20.5 $[M+1]^+$).

4.2.4.7. (3-Hydroxy-2-methoxyphenyl)-(1-(2-*p*-methoxyphenylethyl)-piperidine-4-yl)-methanol (47). 420 mg (**22**) (0.69 mmol), 600 mg NH_4F (16 mmol) and 15 mL MeOH yielded 223 mg of (**47**) (0.6 mmol; 87%). R_f 0.26 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.065 (dt, 2H); 6.985–6.752 (m, 5H); 4.635 (d, 1H); 3.788 (s, 3H); 3.743 (s, 3H); 3.177 (dd, 2H); 2.887 (d, 1H); 2.779 (dt, 4H); 2.221 (q, 1H); 2.108 (d, 2H); 1.709 (q, 1H); 1.535 (q, 1H); 1.318 (d, 1H) MS (FD) *m/z* (% rel Int.): 371.0 (100.0 $[M]^+$); 372.0 (71.9 $[M+1]^+$).

4.2.4.8. (3-Hydroxy-2-methoxyphenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol (48). 1 g (**23**) (1.82 mmol), 0.71 g NH_4F (9.6 mmol) and 30 mL MeOH yielded 0.65 g of (**48**) (1.8 mmol; 95%). R_f 0.64 (silica gel, 5:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.083–7.181 (m, 2H); 6.811–7.073 (m, 5H); 4.649 (d, 1H); 3.810 (s, 3H); 3.063–3.175 (m, 1H); 2.884–3.004 (m, 1H); 2.768 (t, 2H); 2.542 (t, 2H); 1.855–2.147 (m, 3H); 1.624–1.797 (m, 1H); 1.197–1.586 (m, 3H) MS (FD) *m/z* (% rel Int.): 360.5 (100.0 $[M]^+$); 359.5 (55.81 $[M-1]^+$); 361.4 (14.00 $[M+1]^+$]; 358.5 (11.09 $[M-2]^+$).

4.2.4.9. (*R*)-(3-Hydroxy-2-methoxyphenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol (49). 1 g (**29**) (1.82 mmol), 0.71 g NH_4F (9.6 mmol) and 30 mL MeOH yielded 0.64 g of (**49**) (1.75 mmol; 93%). R_f 0.6 (silica gel, 5:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.087–7.180 (m, 2H); 6.814–7.079 (m, 5H); 4.643 (d, 1H); 3.816 (s, 3H); 3.066–3.172 (m, 1H); 2.881–3.008 (m, 1H); 2.767 (t, 2H); 2.541 (t, 2H); 1.852–2.145 (m, 3H); 1.623–1.792 (m, 1H); 1.197–1.586 (m, 3H) MS (FD) *m/z* (% rel Int.): 360.5 (100.0 $[M]^+$); 359.5 (52.98 $[M-1]^+$); 361.4 (11.77 $[M+1]^+$); 358.5 (11.34 $[M-2]^+$) $[\alpha]_D$ = +16.65 (c 0.1; MeOH).

4.2.5. Alkylating procedure for 1,2-bromofluoroethane

NaH (0.42 mmol) was gradually added to the corresponding phenolic derivatives (0.42 mmol) diluted in 20 mL of dry, cold DMSO (0 °C) and stirred for 30 min. To the resulted mixture 1,2-bromofluoroethane (0.42 mmol) was injected slowly and afterwards stirred for 20 h at 60 °C. After evaporation of the solvent, the residue was taken up in EtOAc, washed with H_2O and brine and finally extracted 3× with EtOAc. The combined organic extracts were dried (Na_2SO_4), filtered and evaporated. Chromatography of the residue gave the pure product.

4.2.5.1. 4-(3-(2-Fluoroethoxy)-2-methoxybenzoyl)-1-piperdinecarboxylic acid *t*-butyl ester (9). 6.27 g (**9a**) (20 mmol), 481 mg NaH (20 mmol), 2.54 g 1,2-bromofluoroethane (20 mmol) and 100 mL dry DMF yielded 7.77 g of (**9**) (20 mmol; 100%). R_f 0.79 (silica gel, 20:1 $CHCl_3$/MeOH). ^1H NMR (300 MHZ, $CDCl_3$) δ [ppm] = 7.083–6.978 (m, 3H); 4.856 (t, 1H); 4.698 (t, 1H); 4.296 (t, 1H); 4.201 (t, 1H); 4.047 (br d, 2H); 3.882 (s, 3H); 3.215 (tt, 1H); 2.800 (t, 2H); 1.806 (dd, 2H); 1.623–1.478 (m, 2H); 1.423 (s, 9H) MS (FD) *m/z* (% rel Int.): 381.3 (100.0 $[M]^+$); 382.3 (17.49 $[M+1]^+$); 383.3 (1.57 $[M+2]^+$).

4.2.5.2. (3-(2-Fluorethoxy)-2-methoxyphenyl)-(1-(2-*p*-nitrophenylethyl)-piperidine-4-yl)-methanol (VK-1) (32). 415 mg (**46**) (1 mmol), 24 mg NaH (1 mmol), 0.13 g 1,2-bromofluoroethane (1 mmol) and 10 mL dry DMF yielded 430 mg of (**32**) (0.93 mmol; 93%). R_f 0.64 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 8.095–8.123 (d, 2H); 7.314–7.343 (d, 2H); 7.010 (t, 1H); 6.907 (d, 1H); 6.822 (d, 1H); 4.845 (q, 1H); 4.688 (q, 1H); 4.611 (d, 1H); 4.274 (t, 1H); 4.180 (t, 1H); 3.888 (s, 3H); 3.078 (d, 1H); 2.918 (t, 3H); 2.602 (t, 2H); 1.938–2.120 (m, 3H); 1.612–1.750 (m, 1H); 1.205–1.573 (m, 4H) MS (FD) *m/z* (% rel Int.): 432.0 (100.0 $[M]^+$); 433.0 (45.8 $[M+1]^+$); 434.0 (8.0 $[M+2]^+$).

4.2.5.3. 4-(3-(2-Fluoroethoxy)-2-methoxy-benzoyl)-1-(2-*p*-tolulylethyl)-piperidine (50). 150 mg (**42**) (0.42 mmol), 10.1 mg NaH (0.42 mmol), 53.45 mg 1,2-bromofluoroethane (0.45 mmol) and 20 mL dry DMF yielded 150 mg of (**50**) (0.38 mmol; 91%). R_f 0.69 (silica gel, 12:1 $CHCl_3$/MeOH).^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.094–6.997 (m, 7H); 4.868–4.841 (m, 1H); 4.710–4.683 (m, 1H); 4.307–4.280 (m, 1H); 4.214–4.187 (m, 1H); 3.889 (s, 3H); 3.323 (br s, 1H); 3.097–2.694 (m, 7H); 2.349–2.164 (s, 5H); 2.071–1.900 (br s, 3H) MS (FD) *m/z* (% rel Int.): 399.3 (100.0 $[M]^+$); 400.3 (29.86 $[M+1]^+$).

4.2.5.4. 4-(3-(2-Fluoroethoxy)-2-methoxy-benzoyl)-1-(2-*p*-nitrophenylethyl)-piperidine (51). 360 mg (**43**) (0.94 mmol), 23 mg NaH (0.94 mmol), 25 μL 1,2-bromofluoroethane (0.94 mmol) and 15 mL dry DMF yielded 203 mg of (**51**) (0.47 mmol; 50%). R_f 0.86 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 8.094 (d, 2H); 7.317 (d, 2H); 7.082–7.007 (t, 3H); 4.857 (t, 1H); 4.699 (t, 1H); 4.296 (t, 1H); 4.203 (t, 1H); 3.882 (s, 3H); 3.077 (t, 1H); 2.971–2.849 (q, 2H); 2.918 (t, 2H); 2.587 (t, 2H); 2.120 (t, 2H); 1.871 (d, 2H); 1.727 (dt, 2H) MS (FD) *m/z* (% rel Int.): 429.9 (100.0 $[M]^+$); 430.9 (2.64 $[M+1]^+$).

4.2.5.5. 4-(3-(2-Fluoroethoxy)-2-methoxy-benzoyl)-1-(2-*p*-methoxyphenylethyl)-piperidine (52). 20 mg (**44**) (0.54 mmol), 13 mg NaH (0.54 mmol), 14 μL 1,2-bromofluoroethane (0.54 mmol) and 15 mL dry DMF yielded 223 mg of (**52**) (0.53 mmol; 98%). R_f 0.71 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.126–7.036 (m, 5H); 6.821 (d, 2H); 4.854 (t, 1H); 4.696 (t, 1H); 4.294 (t, 1H); 4.200 (t, 1H); 3.892 (s, 3H); 3.749 (s, 3H); 3.416 (s, 1H); 3.052 (m, 4H); 2.329 (m, 2H);

2.122 (m, 2H); 2.027 (s, 2H); 1.220 (s, 2H) MS (FD) *m*/*z* (% rel Int.): 415.2 (100.0 $[M]^+$); 416.2 (35.5 $[M+1]^+$); 417.3 (2.84 $[M+2]^+$).

4.2.5.6. 4-(3-(2-Fluoroethoxy)-2-methoxy-benzoyl)-1-(2-*p*-fluorophenylethyl)-piperidine (MA-1) (53). 0.6 g (**45**) (1.68 mmol), 44.25 mg NaH (1.68 mmol), 124 μL 1,2-bromofluoroethane (1.68 mmol) and 20 mL dry DMF yielded 420 mg of (**53**) (1.04 mmol; 63%). R_f 0.8 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.155–6.908 (m, 7H); 4.872–4.845 (m, 1H); 4.712–4.687 (m, 1H); 4.312–4.285 (m, 1H); 3.885 (s, 3H); 3.128–3.078 (m, 1H); 3.032–2.931 (m, 2H); 2.823–2.771 (m, 2H); 2.611–2.539 (m, 2H); 2.021–1.929 (m, 2H); 1.840–1.686 (m, 2H) MS (FD) *m*/*z* (% rel Int.): 403.1 (100.0 $[M]^+$); 404.1 (26.75 $[M+1]^+$); 402.1 (26.17 $[M-1]^+$).

4.2.5.7. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-*p*-methoxyphenylethyl)-piperidine-4-yl)-methanol (54). 150 mg (**47**) (0.4 mmol), 9.8 mg NaH (0.4 mmol), 11 μL 1,2-bromofluoroethane (0.4 mmol) and 15 mL dry DMF yielded 71 mg of (**54**) (0.17 mmol; 43%). R_f 0.4 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.080 (d, 2H); 7.088 (t, 1H); 6.926 (dd, 1H); 6.809 (t, 3H); 4.840 (m, 1H); 4.688 (m, 1H); 4.634 (d, 1H); 4.161–4.283 (dt, 2H); 3.884 (s, 3H); 3.749 (s, 3H); 3.026–3.276 (dd, 2H); 2.635–2.864 (dt, 4H); 2.016–2.038 (m, 3H); 1.708 (m, 2H); 1.575 (t, 1H); 1.366 (d, 2H) MS (FD) *m*/*z* (% rel Int.): 417.2 (74.1 $[M]^+$); 418.2 (100.0 $[M+1]^+$); 419.3 (20.6 $[M+2]^+$).

4.2.5.8. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol (MH.MZ) (55). 0.2 g (**48**) (0.55 mmol), 20 mL DMF, 13.2 mg NaH (0.55 mmol) and 70 mg 1,2-bromofluoroethane (0.041 mL, 0.55 mmol) yielded 90 mg of (**55**) (0.22 mmol; 40%). R_f 0.36 ($CHCl_3$/MeOH/concd NH_3 9:1:0.2). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.107–7.061 (m, 2H); 7.017–6.873 (m, 4H); 6.810–6.784 (d, 1H); 4.839–4.812 (m, 1H); 4.668–4.653 (m, 1H); 4.611–4.584 (d, 1H), 4.260–4.233 (m, 1H); 4.167–4.140 (m, 1H); 3.861 (s, 3H); 3.122–3.086 (d, 1H); 2.989–2.923 (d, 1H); 2.811–2.765 (m, 2H); 2.580–2.526 (m, 2H); 2.065–1.916 (m, 3H); 1.694–1.629 (m, 1H); 1.583–1.367 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 405.2 (100.0 $[M]^+$); 406.2 (35.64 $[M+1]^+$).

4.2.5.9. (*R*)-(3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol ((*R*)-MH.MZ) (56). 0.1 g (**49**) (0.275 mmol), 20 mL DMF, 6.6 mg NaH (0.275 mmol) and 35 mg 1,2-bromofluoroethane (0.020 mL, 0.275 mmol) yielded 56 mg of (**56**) (0.15 mmol; 52%). R_f 0.36 ($CHCl_3$/MeOH/concd NH_3 9:1:0.2). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.107–7.061 (m, 2H); 7.017–6.873 (m, 4H); 6.810–6.784 (d, 1H); 4.839–4.812 (m, 1H); 4.668–4.653 (m, 1H); 4.611–4.584 (d, 1H), 4.260–4.233 (m, 1H); 4.167–4.140 (m, 1H); 3.861 (s, 3H); 3.122–3.086 (d, 1H); 2.989–2.923 (d, 1H); 2.811–2.765 (m, 2H); 2.580–2.526 (m, 2H); 2.065–1.916 (m, 3H); 1.694–1.629 (m, 1H); 1.583–1.367 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 405.5 (100.0 $[M]^+$); 406.5 (57.17 $[M+1]^+$) $[\alpha]_D$ = +6.84 (c 0.16; $CHCl_3$).

4.2.6. General procedure for boc-deprotection

Ketones (**7**), (**8**) and (**9**) (70 mmol) were carefully and gradually dissolved in trifluoroacetic acid (160 mL). After 2 h of stirring at room temperature, the solutions were diluted with 500 mL of ether and carefully neutralized with NH_4OH and ice bath cooling. The layers were separated and the aqueous layer was extracted 3× with ether. The combined organic extracts were washed with water, dried (Na_2SO_4), filtered and evaporated to afford a viscous oil. Chromatography of the residues gave the pure products.

4.2.6.1. 4-(2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-benzoyl)-piperidine (10a). 5.2 g (**7**) (9.1 mmol) and 100 mL TFA yielded 3.23 g of (**10a**) (6.8 mmol; 75%). R_f 0.33 ($CHCl_3$/MeOH 5:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.727–7.670 (m, 4H); 7.466–7.319 (m, 6H); 6.881 (dt, 1H); 6.751–6.631 (m, 2H); 6.417 (br s, 1H); 3.908 (s, 3H); 3.383 (br s, 2H); 3.083 (br d, 2H); 2.098 (br s, 2H); 2.029–1.839 (m, 2H); 1.114 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 473.3 (100.0 $[M]^+$); 474.3 (56.7 $[M+1]^+$); 475.3 (12.5 $[M+2]^+$).

4.2.6.2. 4-(2,3-Bismethoxy-benzoyl)-piperidine (11a). 18 g (**8**) (51 mmol) and 120 mL TFA yielded 7.7 g of (**11a**) (31 mmol; 62%). R_f 0.57 ($CHCl_3$/MeOH 5:1). ^1H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 7.077–6.917 (m, 3H); 3.851 (s, 3H); 3.823 (s, 3H); 3.235–3.068 (m, 3H); 2.897 (br s, 2H); 1.903–1.788 (d, 2H); 1.621–1.489 (m, 2H) MS (FD) *m*/*z* (% rel Int.): 249.4 (100.0 $[M]^+$); 250.4 (23.31 $[M+1]^+$).

4.2.6.3. 4-(3-(2-Fluoroethoxy)-2-methoxybenzoyl)-piperidine (12a). 7.68 g (**9**) (20 mmol) and 100 mL TFA yielded 5.15 g of (**12a**) (18.3 mmol; 92%). R_f 0.15 ($CHCl_3$/MeOH 10:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.075–6.964 (m, 3H); 4.852 (t, 1H); 4.694 (t, 1H); 4.294 (t, 1H); 4.198 (t, 1H); 3.879 (s, 3H); 3.197 (tt, 1H); 3.100 (dt, 2H); 2.659 (td, 2H); 2.538 (br s, 1H); 1.834 (dd, 2H); 1.622–1.480 (m, 2H) MS (FD) *m*/*z* (% rel Int.): 281.1 (100.0 $[M]^+$); 282.1 (16.6 $[M+1]^+$); 283.1 (1.56 $[M+2]^+$).

4.2.7. General procedure for reduction of carbonyl compounds via $NaBH_4$

30 mmol of the corresponding hydroxyl derivative was dissolved in 150 mL dry MeOH. Under nitrogen atmosphere 55 mmol $NaBH_4$ was added gradually at 25–30 °C, and the solution was stirred until no more gas evolved, generally overnight. After evaporation of the solvent, the residue was taken up in NH_4OH and extracted 3× with $CHCl_3$. The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and evaporated to afford pure product.

4.2.7.1. (2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-phenyl)-(piperidine-4-yl)-methanol (10). 4.22 g (**10a**) (8.9 mmol), 800 mg $NaBH_4$ (21 mmol) and 40 mL MeOH yielded 3.45 g of (**10**) (7.3 mmol; 81%). R_f 0.36 ($CHCl_3$/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.756–7.676 (m, 4H); 7.450–7.286 (m, 6H); 6.767 (dd, 1H); 6.614 (t, 1H); 6.408 (dd, 1H); 4.595 (d, 1H); 3.964 (s, 3H); 3.059 (dd, 2H); 2.616–2.435 (m, 2H); 2.314–1.956 (m, 2H); 1.720 (br s, 1H); 1.352–1.136 (m, 4H); 1.102 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 475.2 (100.0 $[M]^+$); 476.2 (64.6 $[M+1]^+$); 477.2 (18.6 $[M+2]^+$).

4.2.7.2. (2,3-Dimethoxyphenyl)-(piperidine-4-yl)-methanol (11). 7.7 g (**11a**) (31 mmol), 2.3 g $NaBH_4$ (62 mmol) and 200 mL dry MeOH yielded 6.7 g of (**11**) (27 mmol; 87%). R_f 0.39 ($CHCl_3$/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.042–6.990 (t, 1H); 6.877–6.790 (q, 2H); 4.595–4.568 (d, 1H); 3.834 (s, 6H); 3.104–3.062 (d, 1H); 2.984–2.942 (d, 1H); 2.566–2.405 (m, 2H); 2.242 (br s, 2H); 2.030–1.999 (d, 1H); 1.788–1.658 (m, 1H); 1.333–1.091 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 251.4 (100.0 $[M]^+$); 252.4 (16.48 $[M+1]^+$).

4.2.7.3. (3-(2-Fluoroethoxy)-2-methoxy-phenyl)-(piperidine-4-yl)-methanol (12). 4.24 g (**12a**) (15.1 mmol), 1.15 g $NaBH_4$ (30.2 mmol) and 70 mL dry MeOH yielded 2.4 g of (**12**) (8.4 mmol; 56%). R_f 0.2 ($CHCl_3$/MeOH 8:1). ^1H NMR: (300 MHz, DMSO-d_6) δ [ppm] = 7.027–6.873 (m, 3H); 4.913 (br s, 1H); 4.832 (t, 1H); 4.671 (t, 1H); 4.570 (dd, 1H); 4.270 (t, 1H); 4.170 (t, 1H); 3.720 (s, 3H); 3.328 (br s, 2H); 2.906–2.782 (m, 2H); 2.334–2.220 (m, 2H); 1.663 (d, 1H); 1.482 (br s, 1H); 1.094 (q, 2H) MS (FD) *m*/*z* (% rel Int.): 283.0 (100.0 $[M]^+$); 284.0 (19.1 $[M+1]^+$).

4.2.7.4. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol (MH.MZ) (55). 168 mg (**53**) (0.42 mmol), 100 mg $NaBH_4$ (2.6 mmol) and 20 mL MeOH yielded 158 mg of (**55**) (0.38 mmol; 93%). R_f 0.36 ($CHCl_3$/MeOH/conc NH_3 9:1:0.2). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.107–7.061 (m, 2H); 7.017–6.873 (m, 4H); 6.810–6.784 (d, 1H); 4.839–4.812 (m, 1H); 4.668–4.653 (m, 1H); 4.611–4.584 (d, 1H), 4.260–4.233 (m, 1H); 4.167–4.140 (m, 1H); 3.861 (s, 3H); 3.122–3.086 (d, 1H); 2.989–2.923 (d, 1H); 2.811–2.765 (m, 2H); 2.580–2.526 (m, 2H); 2.065–1.916 (m, 3H); 1.694–1.629 (m, 1H); 1.583–1,367 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 405.5 (100.0 $[M]^+$); 406.5 (57.17 $[M+1]^+$).

4.2.7.5. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-*p*-toluylphenylethyl)-piperidine-4-yl)-methanol (57). 90 mg (**50**) (0.15 mmol), 11.5 mg $NaBH_4$ (0.3 mmol) and 6 mL dry MeOH yielded 31 mg of (**57**) (0.08 mmol; 52%). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.135–6.815 (m, 7H); 4.860–4.832 (m, 1H); 4.709–4.673 (m, 2H); 4.283–4.255 (m, 1H); 4.189–4.162 (m, 1H); 3.888 (s, 3H); 3.674–3.618 (d, 1H); 3.550–3.511 (d, 1H); 3.164 (br s, 2H); 3.083 (br s, 2H); 2.239–1.985 (m, 6H); 1.894 (m, 1H) MS (FD) *m*/*z* (% rel Int.): 401.2 (100.0 $[M]^+$); 402.3 (46.56 $[M+1]^+$).

4.2.7.6. (2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-phenyl)-(1-(2-*p*-methoxyphenylethyl)-piperidine-4-yl)-methanol (22). 600 mg (**19**) (0.99 mmol), 150 mg $NaBH_4$ (4 mmol) and 20 mL dry MeOH yielded 520 mg of (**22**) (0.86 mmol; 87%). R_f 0.54 ($CHCl_3$/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.718–7.646 (m, 4H); 7.412–7.270 (m, 6H); 7.131 (d, 2H); 6.816 (q, 3H); 6.615 (t, 1H); 6.433 (d,1H); 4.695 (d, 1H); 3.946 (s, 3H); 3.753 (s, 3H); 3.523 (s, 1H); 3.242–3.012 (m, 4H); 2.662–2.4305 (m, 2H); 2.285–2.052 (m, 2H); 1.911–1.671 (m, 2H); 1.422–1.176 (m, 3H); 1.093 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 607.4 (100.0 $[M]^+$); 608.4 (54.8 $[M+1]^+$); 609.4 (16.5 $[M+2]^+$).

4.2.8. Optical resolution

*Optical resolution of (**10**)/(**11**)*: Optical resolution was carried out as described by Ullrich and Ice.[23] NMR and mass spectral data for both enantiomers are identical with (**10**). Enantiomeric purity was determined via chiral HPLC separation.

4.2.9. General procedure for N-alkylation

Method A: A suspension of the appropriate amine (21.4 mmol), $NaHCO_3$ (32.1 mmol) and the corresponding bromo-derivative (21.4 mmol) in anhydrous DMF (100 mL) was stirred for 90 min at 85 °C. After evaporation of the solvent, the residue was taken up in NH_4OH and extracted 3× with EtOAc. The combined organic extracts were washed 3× with brine, dried (Na_2SO_4) and evaporated.

Method B [Finkelstein conditions]: Conducted as *Method (A)*, but besides mentioned raw materials NaI (21.4 mmol) was added.

4.2.9.1. 4-(2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-benzoyl)-1-(2-*p*-toluylethyl)-piperidine (17). *(Method A)* 1.41 g (**7**) (2.98 mmol), 0.60 g 1-(2-bromoethyl)-4-methyl-benzene (2.98 mmol), 10 mL DMF, and 0.37 g $NaHCO_3$ (4.47 mmol) yielded 0.88 g of (**17**) (1.49 mmol; 50%) as colourless crystals. R_f 0.53 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.731–7.695 (m, 4H); 7.473–7.302 (m, 6H); 6.923–7.281 (m, 7H); 3.793 (s, 3H); 3.146–3.275 (m, 1H); 3.046–2.858 (m, 2H); 2.744–2.633 (m, 2H); 2.381–2.559 (m, 1H); 2.3 (s, 3H), 1.992–2.182 (m, 2H); 1.800–1.933 (m, 2H); 1.211 (s, 9H) MS (FD) *m*/*z* (% rel Int.): MS (FD) *m*/*z* (% rel Int.): 591.5 (100.0 $[M]^+$); 592.5 (22.8 $[M+1]^+$).

4.2.9.2. 4-(2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-benzoyl)-1-(2-*p*-nitrophenylethyl)-piperidine (18). *(Method A)* 2.4 g (**10a**) (5 mmol), 0.65 g $NaHCO_3$ (7.5 mmol), 1.5 g *p*-nitrophenethylbromide (5 mmol) and 25 mL dry DMF yielded 1 g of (**18**) (1.6 mmol; 32%). R_f 0.9 ($CHCl_3$/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 8.195–8.121 (q, 2H); 7.712–7.695 (m, 4H); 7.458–7.317 (m, 8H); 6.888 (dd, 1H); 6.738–6.635 (m, 2H); 3.784 (s, 3H); 3.095 (tt, 1H); 2.998–2.864 (m, 4H); 2.635 (t, 2H); 2.204 (t, 2H); 1.884 (br d, 2H); 1.826–1.689 (m, 2H); 1.106 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 622.4 (100.0 $[M]^+$); 623.4 (50.1 $[M+1]^+$); 624.4 (9.3 $[M+2]^+$).

4.2.9.3. 4-(2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-benzoyl)-1-(2-*p*-methoxyphenylethyl)-piperidine (19). *(Method A)* 2.4 g (**10a**) (5 mmol), 0.65 g $NaHCO_3$ (7.5 mmol), 1.5 g *p*-methoxyphenethylbromide (5 mmol) and 25 mL dry DMF yielded 1.28 g of (**19**) (2.1 mmol; 42%). R_f 0.83 ($CHCl_3$/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.709–7.653 (m, 4H); 7.457–7.302 (m, 6H); 7.116 (q, 2H); 6.885 (m, 1H); 6.862–6.803 (m, 4H); 4.431 (d, 1H); 3.858 (s, 3H); 3.761 (s, 3H); 3.623 (br s, 1H); 3.382 (br d, 2H); 3.235–3.023 (m, 4H); 3.003–2.885 (m, 2H); 2.729–2.534 (m, 2H); 2.374–2.150 (m, 1H); 2.141 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 607.4 (100.0 $[M]^+$); 608.4 (54.8 $[M+1]^+$); 609.4 (16.5 $[M+2]^+$).

4.2.9.4. 4-(2-Methoxy-3-(t-butyldiphenylsilyloxy)-benzoyl)-1-(2-*p*-fluorophenylethyl)-piperidine (20). *(Method B)* 4.91 g (**10a**) (8.14 mmol), 0.64 g $NaHCO_3$ (9 mmol), 1.02 g *p*-fluorophenethylbromide (8.14 mmol), NaI (8.14 mmol) and 50 mL dry DMF yielded 2.99 g of (**20**) (5.02 mmol; 61%). R_f 0.5 (EtOAc). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.719–7.663 (m, 4H); 7.457–7.302 (m, 6H); 7.114–7.055 (m, 2H); 6.962–6.921 (m, 2H); 6.806–6.748 (dd, 1H), 6.666–6.405 (t, 2H); 3.995 (s, 3H); 3.131–2.983 (m, 2H), 2.87 (m, 3H); 2.201 (m, 2H); 1.955–1.735 (m, 4H); 1.211 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 595.5 (100.0 $[M]^+$); 596.5 (72.30 $[M+1]^+$); 597.5 (21.48 $[M+2]^+$); 594.5 (18.92 $[M-1]^+$).

4.2.9.5. (2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-phenyl)-(1-(2-*p*-nitrophenylethyl)-piperidine-4-yl)-methanol (21). *(Method A)* 1.33 g (**10**) (2.8 mmol), 0.93 g $NaHCO_3$ (4.2 mmol), 0.84 g *p*-nitrophenethylbromide (2.8 mmol) and 25 mL dry DMF yielded 1 g of (**21**) (1.57 mmol; 56%). R_f 0.65 ($CHCl_3$/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 8.121 (d, 2H); 7.704 (dt, 5H); 7.454–7.304 (m, 10H); 3.970 (s, 3H); 3.895 (s, 1H); 3.071 (d, 1H); 2.965–2.765 (m, 4H); 2.594 (t, 2H); 2.124–1.889 (m, 3H); 1.509–1.185 (m, 3H); 1.101 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 624.3 (100.0 $[M]^+$); 625.3 (44.7 $[M+1]^+$); 626.3 (8.7 $[M+2]^+$).

4.2.9.6. (2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-phenyl)-(1-(2-*p*-methoxyphenylethyl)-piperidine-4-yl)-methanol (22). *(Method A)* 2 g (**10**) (4.2 mmol), 0.4 g 4-methoxyphenethylbromide (4.2 mmol), 20 mL dry DMF and 0.53 g $NaHCO_3$ (6.3 mmol) yielded 0.38 g of (**22**) (0.63 mmol; 15%). R_f 0.45 (CH_3Cl/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.732–7.681 (m, 4H); 7.416–7.301 (m, 6H); 7.095–6.996 (m, 3H); 6.893–6.776 (m, 4H); 4.648–4.622 (d, 1H); 3.839 (s, 3H); 3.187–3.152 (d, 1H); 3.055–3.014 (d, 1H); 2.818–2.744 (m, 2H); 2.637–2.583 (m, 2H); 2.131–1.963 (m, 3H); 1.756–1.429 (m, 1H); 1.345–1.281 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 609.5 (100.0 $[M]^+$), 610.5 (30.12 $[M-1]^+$).

4.2.9.7. (2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-phenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol (23). *(Method B)* 2 g (**10**) (4.2 mmol), 0.85 g *p*-fluorophenethylbromide (4.2 mmol), NaI (4.2 mmol), 15 mL dry DMF and 0.53 g $NaHCO_3$ (6.3 mmol) yielded 2.52 g (4.6 mmol; 100%) of (**23**). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.732–7.681 (m, 4H); 7.416–7.301 (m, 6H); 7.156–7.110 (m, 2H); 6.969–6.911 (t, 2H); 6.787–6.762 (d, 1H); 6.642–6.589 (d, 1H); 6.418–6.391 (d, 1H); 4.622–4.595 (d, 1H); 3.968 (s, 3H); 3.113–3.077 (d, 1H); 2.975–2.932 (d, 1H); 2.806–2.752 (m, 2H); 2.571–2.517 (m, 2H); 2.101–1.924 (m, 3H);

1.650–1.639 (m, 1H); 1.554–1.215 (m, 3H); 1.102 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 597.6 (100.0 $[M]^+$); 598.6 (77.39 $[M+1]^+$); 599.6 (24.91 $[M+2]^+$); 595.6 (24.91 $[M-1]^+$).

4.2.9.8. (2,3-Dimethoxyphenyl)-(1-(*p*-fluorobenzyl)-piperidine-4-yl)-methanol (24). *(Method A)* 100 mg (**11**) (0.38 mmol), 72 mg 1-bromomethyl-4-fluorobenzene (0.38 mmol), 10 mL dry DMF and 40 mg $NaHCO_3$ (0.56 mmol) yielded 82 mg of (**24**) (0.22 mmol; 58%) R_f 0.48 (CH_3Cl/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.300–7.240 (m, 2H); 7.739–6.937 (m, 3H); 6.859–6.798 (m, 2H); 4.616–4.589 (d, 1H); 3.836 (s, 6H); 3.481 (br s, 2H); 2.983–2.945 (d, 1H); 2.861–2.817 (d, 1H); 2.345–2.326 (br s, 1H); 2.050–1.868 (m, 3H); 1.683–1.596 (m, 1H); 1.579–1.310 (m, 2H) MS (FD) *m*/*z* (% rel Int.): 359.2 (100.0 $[M]^+$); 360.23 (26.57 $[M+1]^+$).

4.2.9.9. (2,3-Dimethoxyphenyl)-(1-(2-*p*-nitrophenylethyl)-piperidine-4-yl)-methanol (25). *(Method A)* 1.5 g (**11**) (5.95 mmol), 1.35 g 2-(4-nitrophen)-1-bromethane (5.95 mmol), 50 mL DMF and 0.75 g $NaHCO_3$ (8.93 mmol) yielded 2.41 g of (**25**) (5.91 mmol; 99%) as an orange oil. R_f 0.62 ($CHCl_3$/MeOH 5:1 + 5% formic acid). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 8.173–8.081 (d, 2H); 7.323–7.294 (d, 2H); 7.046–6.993 (t, 1H); 6.878–6.803 (q, 2H); 4.621–4.594 (d, 1H); 3.836 (s, 6H); 3.049–2.982 (d, 1H); 2.923–2.836 (m, 3H); 2.614–2.483 (m, 2H); 2.066–1.854 (m, 3H); 1.649 (br s, 1H); 1.466–1.179 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 400.5 (100.0 $[M]^+$), 401.5 (27.80 $[M-1]^+$).

4.2.9.10. (2,3-Dimethoxyphenyl)-(1-(2-*p*-methoxyphenylethyl)-piperidine-4-yl)-methanol (26). *(Method A)* 50 mg (**11**) (0.19 mmol), 18 mg 4-methylphenethylbromide (0.19 mmol), 10 mL dry DMF and 20 mg $NaHCO_3$ (0.28 mmol) yielded 18 mg of (**26**) (0.05 mmol; 26%). R_f 0.50 (CH_3Cl/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.095–6.996 (m, 3H); 6.893–6.776 (m, 4H); 4.648–4.622 (d, 1H); 3.839 (s, 3H); 3.749 (s, 3H); 3.187–3.152 (d, 1H); 3.055–3.014 (d, 1H); 2.818–2.744 (m, 2H); 2.637–2.583 (m, 2H); 2.131–1.963 (m, 3H); 1.756–1.429 (m, 1H); 1.345–1.281 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 385.1 (100.0 $[M]^+$), 384.1 (91.67 $[M-1]^+$).

4.2.9.11. (2,3-Dimethoxyphenyl)-(1-(2-*p*-toluylethyl)-piperidine-4-yl)-methanol (27). *(Method B)* 50 mg (**11**) (0.19 mmol), 38 mg 4-methylphenethylbromide (0.19 mmol), NaI (0.19 mmol), 10 mL dry DMF and 20 mg $NaHCO_3$ (0.28 mmol) yielded 42 mg of (**27**) (0.11 mmol; 58%). R_f 0.4 (CH_3Cl/MeOH 8:1). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.128–6.993 (m, 5H); 6.890–6.805 (m, 2H); 4.638–4.612 (d, 1H); 3.839 (s, 6H); 3.159–3.115 (d, 1H); 3.014–2.978 (d, 1H); 2.804–2.748 (m, 2H); 2.610–2.556 (m, 2H); 2.280 (s, 3H); 2.084–1.908 (m, 3H); 1.711–1.597 (m, 1H); 1.322–1.232 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 369.1 (100.0 $[M]^+$), 370.15 (77.30 $[M+1]^+$) $[\alpha]_D = -12.49$ (*c* 0.12; MeOH).

4.2.9.12. (2,3-Dimethoxyphenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol (28). *(Method A)* 0.7 g (**11**) (2.78 mmol), 0.56 g (**36**) (2.78 mmol), 50 mL DMF and 0.35 g $NaHCO_3$ (4.17 mmol) yielded 786 mg of (**28**) (2.33 mmol; 84%) as a colourless powder. R_f 0.24 ($CHCl_3$/MeOH 10:1). ^1H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.128–6.801 (m, 7H); 4.633–4.606 (d, 1H); 3.840 (s, 3H); 3.086–3.046 (d, 1H); 2.946–2.914 (d, 1H); 2.791–2.686 (m, 2H); 2.538–2.455 (m, 2H); 2.061–1.864 (m, 3H); 1.715–1.600 (m, 1H), 1.549–1.232 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 373.5 (100.0 $[M]^+$); 374.5 (37.03 $[M+1]^+$).

4.2.9.13. (*R*)-(2-Methoxy-3-(*t*-butyldiphenylsilyloxy)-phenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol (29). *(Method A)* 2 g (**14**) (4.2 mmol), 0.85 g *p*-fluorophenethylbromide (4.2 mmol), 15 mL dry DMF and 0.53 g $NaHCO_3$ (6.3 mmol) yielded 2.3 g (4.2 mmol; 91%) of (**29**). ^1H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.732–7.681 (m, 4H); 7.416–7.301 (m, 6H); 7.156–7.110 (m, 2H); 6.969–6.911 (t, 2H); 6.787–6.762 (d, 1H); 6.642–6.589 (d, 1H); 6.418–6.391 (d, 1H); 4.622–4.595 (d, 1H); 3.968 (s, 3H); 3.113–3.077 (d, 1H); 2.975–2.932 (d, 1H); 2.806–2.752 (m, 2H); 2.571–2.517 (m, 2H); 2.101–1.924 (m, 3H); 1.650–1.639 (m, 1H); 1.554–1.215 (m, 3H); 1.102 (s, 9H) MS (FD) *m*/*z* (% rel Int.): 597.5 (100.0 $[M]^+$); 598.5 (68.22 $[M+1]^+$); 595.5 (17.39 $[M-1]^+$) $[\alpha]_D = -12.07$ (*c* 0.9; $CHCl_3$).

4.2.9.14. (*R*)-(2,3-Dimethoxyphenyl)-(1-(2-*p*-fluorophenylethyl)-piperidine-4-yl)-methanol ((*R*)-MDL 100907)) (30). *(Method A)* 0.7 g (**15**) (2.78 mmol), 0.56 g (**36**) (2.78 mmol), 50 mL DMF and 0.35 g $NaHCO_3$ (4.17 mmol) yielded 786 mg of (**30**) (2.33 mmol; 84%) as a colourless powder. ^1H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.128–6.801 (m, 7H); 4.633–4.606 (d, 1H); 3.840 (s, 3H); 3.086–3.046 (d, 1H); 2.946–2.914 (d, 1H); 2.791–2.686 (m, 2H); 2.538–2.455 (m, 2H); 2.061–1.864 (m, 3H); 1.715–1.600 (m, 1H), 1.549–1.232 (m, 3H) MS (FD) *m*/*z* (% rel Int.): 373.5 (100.0 $[M]^+$); 374.5 (37.03 $[M+1]^+$) $[\alpha]_D = +15.67$ (*c* 0.06; MeOH).

4.2.9.15. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-phenylethyl)-piperidine-4-yl)-methanol (31). *(Method A)* 200 mg (**12**) (0.71 mmol), 90 mg $NaHCO_3$ (1.1 mmol), 130 mg phenethylbromide (0.71 mmol) and 10 mL dry DMF yielded 246 mg of (**31**) (0.64 mmol; 90%). R_f 0.43 ($CHCl_3$/MeOH 8:1). ^1H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.270–7.131 (m, 5H); 7.006 (t, 1H); 6.922 (dd, 1H); 6.812 (dd, 1H); 4.839 (t, 1H); 4.686 (t, 1H); 4.599 (d, 1H); 4.268 (t, 1H); 4.174 (t, 1H); 3.883 (s, 3H); 3.067 (d, 1H); 2.825 (s, 1H); 2.795–2.498 (dt, 4H); 2.074–1.983 (m, 2H); 1.961–1.835 (m, 2H); 1.531–1.357 (m, 2H); 1.300–1.200 (m, 2H) MS (FD) *m*/*z* (% rel Int.): 387.2 (100.0 $[M]^+$); 388.2 (36.0 $[M+1]^+$); 389.3 (8.0 $[M+2]^+$).

4.2.9.16. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-*p*-nitrophenylethyl)-piperidin-4-yl)-methanol (VK-1) (32). *(Method A)* 200 mg (**12**) (0.71 mmol), 90 mg $NaHCO_3$ (1.1 mmol), 130 mg *p*-nitrophenethylbromide (0.71 mmol) and 10 mL dry DMF yielded 276 mg of (**32**) (0,65 mmol; 91%). R_f 0.64 (silica gel, 8:1 $CHCl_3$/MeOH). ^1H NMR (300 MHz, $CDCl_3$), δ [ppm] = 8.095–8.123 (d, 2H); 7.314–7.343 (d, 2H); 7.010 (t, 1H); 6.907 (d, 1H); 6.822 (d, 1H); 4.845 (q, 1H); 4.688 (q, 1H); 4.611 (d, 1H); 4.274 (t, 1H); 4.180 (t, 1H); 3.888 (s, 3H); 3.078 (d, 1H); 2.918 (t, 3H); 2.602 (t, 2H); 1.938–2.120 (m, 3H); 1.612–1.750 (m, 1H); 1.205–1.573 (m, 4H) MS (FD) *m*/*z* (% rel Int.): 432.0 (100.0 $[M]^+$); 433.0 (45.8 $[M+1]^+$); 434.0 (8.0 $[M+2]^+$).

4.2.9.17. 4-(3-(2-Fluoroethoxy)-2-methoxybenzoyl)-1-(2-*p*-fluorophenylethyl)-piperidine (MA-1) (53). *(Method A)* 400 mg (**12a**) (1.42 mmol), 180 mg $NaHCO_3$ (2.2 mmol), 287 mg *p*-fluorophenethylbromide (1.42 mmol) and 15 mL dry DMF yielded 466 mg of (**53**) (1.15 mmol; 81%). R_f 0.66 ($CHCl_3$/MeOH 8:1). ^1H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.148–7.077 (m, 2H); 7.031–6.903 (m, 5H); 4.857 (t, 1H); 4.699 (t, 1H); 4.296 (t, 1H); 4.203 (t, 1H); 3.883 (s, 3H); 3.077 (tt, 1H); 2.953 (dt, 2H); 2.644 (dt, 4H); 2.109 (t, 2H); 1.918 (d, 2H); 1.799–1.658 (m, 2H) MS (FD) *m*/*z* (% rel Int.): 402.8 (100.0 $[M]^+$); 403.8 (25.8 $[M+1]^+$); 404.8 (3.1 $[M+2]^+$).

4.2.10. General procedure for the bromination of *p*-fluorophenylalkyl alcohols

To a stirred solution of the corresponding *p*-fluorophenalkyl alcohol (0.4 mol) dissolved in 40 mL toluene 0.3 mol PBr_3 was slowly added, heated to 100 °C and then cooled, treated with ice water and washed with saturated Na_2CO_3 solution and water. The aqueous phase was extracted with toluene (3 × 250 mL), the organic extracts

dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The products were obtained via distillation.

4.2.10.1. *p*-Fluorophenylmethyl bromide (35). The crude product was purified via distillation (85 °C, 20 mbar) to yield 55.16 g (0.29 mol; 75%) of (**35**) as a colourless oil. ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.413–7.382 (m, 2H); 7.034 (m, 2H); 4.555 (s, 2H) MS (FD) m/z (% rel Int.): 189.9 (100.0 $[M]^+$); 187.9 (33.96 $[M-2]^+$).

4.2.10.2. 2-*p*-Fluorophenylethyl bromide (36). The crude product was purified via distillation (105 °C, 15–20 mbar) to yield 45.68 g (0.22 mol; 81%) of (**36**) as a colourless oil. ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.114 (t, 2H); 7.034 (t, 2H); 3.567 (t, 2H); 3.188 (t, 2H) MS (FD) m/z (% rel Int.): 202.0 (100.0 $[M]^+$); 204.0 (63.77 $[M+2]^+$).

4.2.11. 2-Bromoethyl-2-(4-fluorophenyl)-(1,3)dioxo-lane (37)

Four grams of 2-bromo-4-fluoroacetophenone (18 mmol), 8.92 g ethylenglycol (144 mmol) and 0.76 *p*-toluenesulfonic acid (4 mmol) were dissolved in 50 mL benzene and refluxed for 16 h. After evaporating the solvent, the residue was taken up with CH_2Cl_2, washed with K_2CO_3 and then extracted with CH_2Cl_2, and dried with $MgSO_4$. The solvent was reduced to afford 2.2 g of a yellowish oil (16.1 mmol; 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.484–7.429 (m, 2H); 7.036–6.970 (m, 2H); 4.172–4.092 (m, 2H); 3.877–3.802 (m, 2H); 3.596 (s, 2H) MS (FD) m/z (% rel Int.): 167.6 (100.0 $[M]^+$); 168.6 (6.37 $[M+1]^+$); 260.4 (9.95 $[M+3]^+$).

4.2.12. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-(2-*p*-aminophenylethyl)-piperidine-4-yl)-methanol (58)

Three hundred and fifty milligrams of **32** (0.81 mmol) were dissolved in 20 mL isopropanol. 500 mg Pd/C and 204.2 mg ammonium formate (3.24 mmol) were added and then heated to 70 °C for 20 min (70 W) in a microwave oven. Afterwards the mixture was filtered, the solvent reduced, the residue taken up with 1 M HCl and 3× extracted with EtOAc. Organic layers were dried (NA_2SO_4) and the solvent removed to yield 248 mg of (**58**) (0.62 mmol; 76%) as colourless crystals. R_f 0.33 ($CHCl_3$/MeOH 8:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 7.030–6.890 (m, 4H); 6.817 (d, 1H); 6.586 (d, 2H); 4.840 (t, 1H); 4.688 (t, 1H); 4.608 (d, 1H); 4.267 (t, 1H); 4.173 (t, 1H); 3.885 (s, 3H); 3.528 (br s, 2H); 2.983 (dd, 2H); 2.565 (dt, 4H); 2.021 (t, 1H); 1.941–1.816 (m, 2H); 1.719–1.579 (m, 1H); 1.517–1.324 (m, 2H); 1.260 (d, 2H) MS (FD) m/z (% rel Int.): 402.3 (100.0 $[M]^+$); 403.3 (46.1 $[M+1]^+$); 404.3 (7.2 $[M+2]^+$).

4.2.13. (3-(2-Fluoroethoxy)-2-methoxyphenyl)-(1-phenylacetyl-piperidine-4-yl)-methanol (39)

To 200 mg (**12**) (0.71 mmol) dissolved in 10 mL of dry THF 100 μL of phenacetylchloride (0.84 mmol) was added while cooling to 0 °C. The resulting mixture was then stirred for 2 h and afterwards the solvent evaporated. The residue was taken up with EtOAc, washed with water, extracted 3× with EtOAc and finally dried (Na_2SO_4). The solvent was removed to give the crude product which was purified via chromatography yielding 180 mg of (**39**) (0.45 mmol; 64%). R_f 0.71 ($CHCl_3$/MeOH 8:1). ^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] = 7.325–7.158 (m, 5H); 7.000 (t, 1H); 6.887–6.787 (m, 2H); 4.841 (t, 1H); 4.684 (t, 1H); 4.552 (d, 1H); 4.267 (t, 1H); 4.173 (t, 1H); 3.956–3.671 (m, 2H); 3.874 (s, 3H); 3.692 (d, 2H); 2.960–2.740 (m, 1H); 2.566–2.361 (m, 1H); 2.044.1.722 (m, 3H); 1.367–0.948 (m, 3H) MS (FD) m/z (% rel Int.): 401.2 (100.0 $[M]^+$); 402.2 (23.3 $[M+1]^+$); 403.2 (3.1 $[M+2]^+$).

4.3. Lipophilicity

Lipophilicities were determined using the HPLC system described above with a LiChrospher 100 RP 18 EC-5μ (250 × 7.8 mm) and a 20 μL loop. Soerensen buffer was used as eluent with a flow rate of 4 mL/min. Retention times for all tested compounds and for reference substances (ascorbic acid, benzaldehyde, anisol, toluene, 4-bromoanisol and 4-iodoanisol) of known log*P* were assessed which enabled the calculation of the capacity factor *k*. A plot of these reference values against their known log*P* values gave a reference curve which was used to calculate log*P* values for synthesized compounds.

4.4. In vitro pharmacological evaluation

4.4.1. Binding assays

4.4.1.1. In vitro radioligand competition binding assay of ^{18}F-tracers on high 5-HT_{2A} expressing GF-62 cells. *Cell membrane homogenate.* NIH3T3 cells, derived from mouse fibroblasts, were stably transfected with the rat 5-HT_{2A} receptor cDNA before isolation of GF-62, a clonal cell line expressing higher amounts (5–7 pmol/mg) of the 5-HT_{2A} receptor. Cells were homogenized in buffer (5 mM Tris-base, 5 mM EDTA, pH 7.4, 4 °C) and incubated for 10 min at 4 °C. Centrifuged for 10 min (33,000*g*) at 2–4 °C, the pellet was suspended in 4 mg/mL wet weight assay buffer (50 mM Tris-base, 120 mM NaCl, 50 mM KCl, 1% bovine serum albumin, 0.1% ascorbic acid, pH 7.4, 37 °C) and 50 μL/mL protease inhibitor cocktail was added (4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), aprotinin, leupeptin, bestatin, pepestatin A and E-64), aliquoted in NUNC tubes and stored at −80 °C until use.

Radioligand binding assay. Competition binding experiments were carried out in test tubes containing [^{3}H]MDL (0.2 nM), seven different concentrations of the test compound (1 μM–1 pM) and 10–20 μg GF-62 clonal cells in a total of 1 mL assay buffer (50 mM Tris-base, 120 mM NaCl, 50 mM KCl, 1% bovine serum albumin, 0.1% ascorbic acid, pH 7.4, 37 °C). Ketanserin (1 μM) was used to determine non-specific binding. Incubation was carried out for 1 h at 37 °C and terminated by rapid filtration over glass fibre GF/C filters presoaked in 1% polyethyleneimine, using a Brandel cell harvester. Filters were washed with 300 mL cold assay buffer (tritrated to pH 7.4 at 4 °C). Filters were placed in scintillation vials and 2.5 mL Ultima Gold scintillation fluid was added. The scintillation cocktails were placed in cold and dark overnight and counted for 4 min in a Tri-Carb 2900TR Liquid Scintillation Analyser from Packard. K_i and error values were calculated with Graphpad Prism 5.

4.4.1.2. In vitro radioligand binding assays through NIMH-Psychoactive Drug Screening Programme (PDSP). Binding assays were performed by the NIMH Psychoactive Drug Screening Programme at the Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio, USA (Bryan Roth, Director). Compounds (**32**), (**50**)–(**53**) and (**55**)–(**57**) were assayed for their affinities for a broad spectrum of receptors and transporters in competitive binding experiments in vitro using cloned human receptors. Reported values of the inhibition coefficient (K_i) are mean ± SD of four separate determinations.

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