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Structural Combination of Established 5-HT$_{2A}$ Receptor Ligands: New Aspects of the Binding Mode

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MH.MZ, MDL 100907, and altanserin are structurally similar 4-benzoyl-piperidine derivatives and are well accommodated to receptor interaction models. We combined structural elements of different high-affinity and selective 5-HT$_{2A}$ antagonists, as MH.MZ, altanserin, and SR 46349B, to improve the binding properties of new compounds. Three new derivatives were synthesized with a 4-benzoyl-piperidine moiety as the lead structure. The *in vitro* affinity of the novel compounds was determined by a [^{3}H]MDL 100907 competition binding assay. The combination of MH.MZ and SR 46349B resulted in a compound (8) with a moderate affinity toward the 5-HT$_{2A}$ receptor (K_i = 57 nM). The remarkably reduced affinity of other compounds (4a), (4b), and (4c) (K_i = 411, 360 and 356 nM respectively) indicates that MH.MZ can only bind to the 5-HT$_{2A}$ receptor with the *p*-fluorophenylethyl residue in a sterically restricted hydrophobic binding pocket.

Key words: chemical structure, drug design, G-protein coupled receptor, receptor and ligands (agonist/antagonist)

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Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter implicated in almost every conceivable physiologic or behavioral function as affect, aggression, appetite, cognition, endocrine function, gastrointestinal function, motor function, neurotrophism, sex, sleep, and vascular function (1). Moreover, most drugs that are currently used for the treatment of psychiatric disorders (e.g., Alzheimer's disease, depression, mania, schizophrenia, autism, obsessive compulsive, alcoholism, disorder, and anxiety disorders) are thought to act, at least partially, through serotoninergic mechanisms (2). Most if not all clinically approved atypical antipsychotic drugs are potent 5-HT$_{2A}$ receptor antagonists (3,4).

In particular, it was shown by [^{18}F]altanserin and positron emission tomography (PET) that 5-HT$_{2A}$ receptor binding in patients with mild cognitive impairment is reduced and hippocampal 5-HT$_{2A}$ receptor availability is decreased in major depressive patients (5).

To date, high-affinity and selective 5-HT$_{2A}$ receptor antagonists are well known, such as MDL 100907, MH.MZ, altanserin, and SR 46349B (Figure 1) (6,7). MH.MZ, MDL 100907, and altanserin are structurally similar 4-benzoyl-piperidine derivatives, whereas SR 46349B contains no piperidine but a dimethylaminoethyl oxime ether moiety.

Especially, MH.MZ, MDL 100907 and altanserin are in accordance regarding their structural body with a rudimentary pharmacophore model published by Andersen *et al.* (1) Two aryl substituents, separated by distance a, located distances b and c from an amine moiety (Figure 2) are required in this model for effective 5-HT$_{2A}$ receptor ligands. Distances suggested by Anderson *et al.* for a, b, and c are 5.1, 7.5, and 8.1 Å, respectively.

Recently, Herth *et al.* (6,8,9) described ^{18}F-labelable MDL 100907 derivatives, MH.MZ, and the corresponding ketone derivative DD-1, retaining the favorable characteristics of the parent compound (Table 1). This is surprising because of the fact that the affinity is not retained by changing the ketone body of altanserin to the secondary alcohol altanserinol as shown by Tan *et al.* (10).

Therefore, the purpose of this study was to combine structural elements of MH.MZ, MDL 100907, altanserin, and SR 46349B to study their structure–activity relationship (SAR), and thereby the influence on the 5-HT$_{2A}$ affinity of different structural substitutions on the carbonyl moiety of altanserin (Figure 3).

For 3-(2-(4-(3-(2-fluoroethoxy)-2-methoxybenzoyl)-piperidin-1-yl)-ethyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-on (**4a**) the *p*-fluorophenyl ring of altanserin was replaced by the 3-fluoroethoxy-2-methoxyphenyl ring of MH.MZ. 3-(2-(4-(3-(2-Fluoroethoxy)-2-methoxy-α-hydroxybenzyl)-piperidin-1-yl)-ethyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-on (**4b**) contains a quinazolinone ring instead of the *p*-fluorophenyl substituent of MH.MZ. To investigate SAR about the size of the aromatic

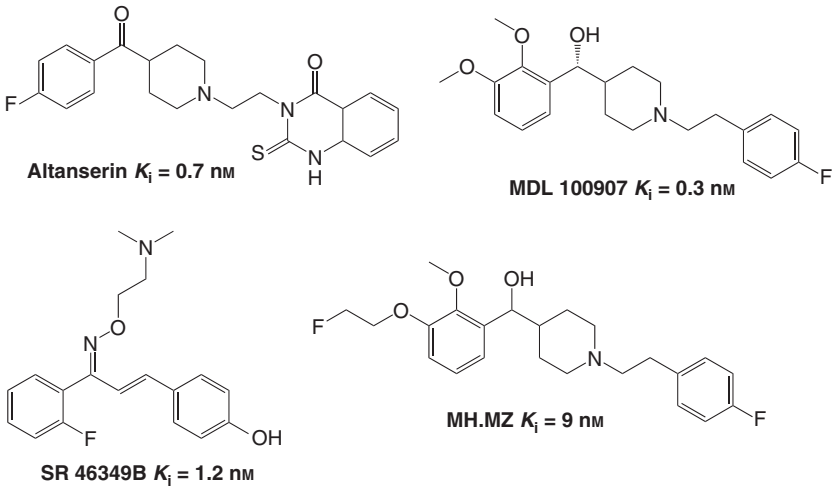


Figure 1: Structure and affinities of selective 5-HT_{2A} antagonists.

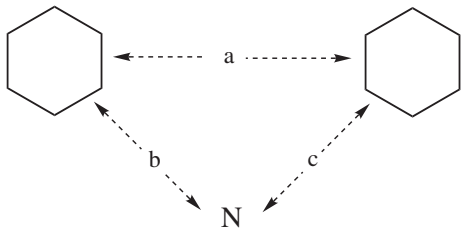


Figure 2: A general pharmacophore model to account for the binding of 5-HT_{2A} antagonists (1).

Table 1: Affinities toward 5-HT_{2A} receptors of various antagonists and the new compounds. Data presented as K_i [nM] ± SD (n = 4) (5, 7, 10)

Compound	K_i [nM]
Altanserin	0.74 ± 0.88
Altanserinol	186
MDL 100907	0.3 ± 0.1
MH.MZ	9.02 ± 2.11
DD-1	3.23 ± 0.18
SR 46349B	1.2
(**4a**)	411 ± 347
(**4b**)	390 ± 60
(**4c**)	356 ± 56
(**8**)	57 ± 18

ether substituents, the 2-fluoroethyl substituent of compound (**4b**) was replaced by a methoxy group in 3-(2-(4-(2,3-dimethoxy benzoyl)-piperidin-1-yl)-ethyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-on (**4c**). Moreover, the influence of the ketone, the secondary hydroxyl or the dimethylaminoethyl oxime ether moiety of SR 46349B, toward the affinity profile of these compounds is determined by the comparison of compound (**8**), MH.MZ and DD-1 (Figure 3).

Organic syntheses of key intermediates (**1a**), (**1b**), and (**1c**) were carried out similar to a route described by Ullrich *et al.* (11) Synthesis of the quinazolinone derivatives was achieved by a route similar to that reported by Tan *et al.* (12) Therefore, reaction with boc-protected bromoethyl-amine, deprotection, and ring closure with methyl-2-isothiocyanatobenzoate[a] resulted in compounds (**4a**), (**4b**), and (**4c**) (Figure 4).

The 4-(3-(2-fluoroethoxy)-2-methoxybenzyl)-piperidine moiety of MH.MZ and the dimethylaminoethyl oxime ether residue of SR 46349B were combined following a condensation reaction of ketone **DD-1** and amine (**7**).[b] The necessary educts were produced as reported by Herth *et al.* (6) and Villani *et al.* (13), respectively. Figure 5 illustrates the synthetic route to (**8**).

To study whether the new compounds are full antagonists as MDL 100907 and altanserin, their activity toward the 5-HT_{2A} receptor was determined by a functional assay that assesses the activation of the receptor by measuring the accumulation of formed inositolphosphate (IP).[c] The results (Figure 6) demonstrate that all new compounds, as well as MH.MZ, and DD-1 are full antagonists.

5-HT_{2A} receptor affinity (K_i) 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 100907 (0.3 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 and reference compounds are summarized in Table 1.[d]

The combination of structural elements of MH.MZ and SR 46349B led to the medium affine compound E-dimethylaminoethoxy-[3-(2-fluoroethoxy)-2-methoxy-phenyl]-1-[2-(*p*-fluorophenyl)-ethyl]-pipe-ridin-4-yl-methanonoxim (**8**). It has a ~6 fold lower K_i-value (57 nM) compared to MH.MZ. This probably is attributed to the additional space required by the dimethylaminoethyl oxime residue. However, the retained affinity indicates that substitution with larger substituents in that position is tolerated to a certain point. There might also be a positive hydrogen bond interaction between the oxime nitrogen and a serine residue. The same interaction was also observed for the carbonyl residue of altanserin (14,15). Therefore, compound **(8)** could be used as a lead structure for further

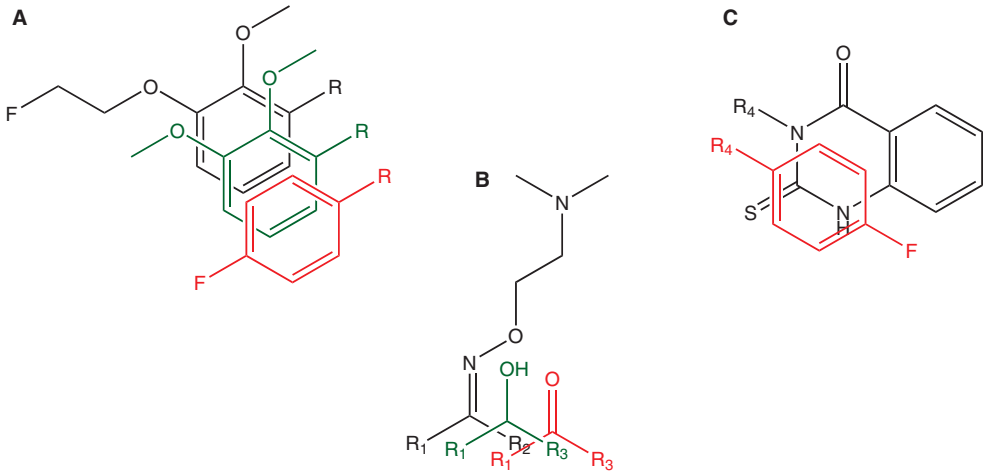
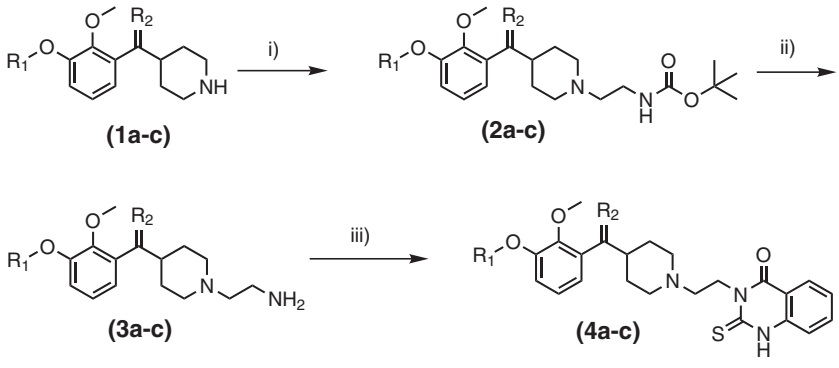


Figure 3: A: Variation of the benzoyl moiety: compound (**4a**) and (**4b**) (black), compound (**4c**) (green), and altanserin (red); B: Variation of the α-benzyl position: compound (**8**) (black), MH.MZ (green) and DD-1 (red); C: Variation of the phenethyl group of MH.MZ: compound (**4b**) (black) and MH.MZ (red).



(a) R_1 = Me; R_2 = = O

(b) R_1 = Me; R_2 = -OH

(c) R_1 = Fluorethyl; R_2 = -OH

Figure 4: Synthesis of (**4a**), (**4b**), and (**4c**): reagents and conditions: (i) tertiary-butyl-2-bromethylcarbamate, KI, K_2CO_3, DMF, rt, 51–77%; (ii) TFA, rt, 83–95%; (iii) methyl-2-isothiocyanatobenzoate, THF, rt, 30–32%.

optimization. Changing the secondary hydroxyl group of MH.MZ and compound (**4b**) to a carbonyl group in DD-1 and (**4a**) showed no dramatic effects on the binding profile. The affinity of (**4a**), (**4b**), and (**4c**) is 411, 390, and 356 nM, respectively (Table 1). The descending affinity in the order (**4a**) > (**4b**) > (**4c**) is in accordance with the increasing bulk by the fluoroethoxy group.

The binding properties in the receptor–altanserin complex are described in a 3D-QSAR study published by Dezi *et al.* (16) Therein, altanserin binds to the 5-HT$_{2A}$ receptor with the *p*-fluorobenzoyl moiety in a hydrophobic binding pocket. This result is in good agreement with experimental data from previously published mutagenesis experiments (14,15). The size of the binding pocket is restricted by the amino acids Trp6.48, Phe6.51, and Phe6.52 from which Phe6.52 participates in π–π interactions with the aromatic ring of the ligands (15).

The remarkably reduced affinity of compounds (**4a**), (**4b**), and (**4c**) indicates that the additional space required by the fluoroethoxy group and the methoxy group is not tolerated by the receptor binding pocket. We suggest that the benzyl alcohol of MH.MZ and the benzoyl ring of altanserin cannot bind to the same site at the 5-HT$_{2A}$ receptor. Two different binding modes might be possible for antagonists with a 4-benzoylpiperidin lead structure. MH.MZ may bind to the 5-HT$_{2A}$ receptor with the *p*-fluorophenylethyl residue at the same hydrophobic binding pocket as the benzoyl ring of altanserin. This result totally reflects the slightly reduced affinity for compounds with larger substituents at the phenyl ring of MH.MZ (Table 2) (5).

The two different binding modes of altanserin and MH.MZ also account for the retained affinity of MH.MZ compared to altanserinol. By varying size and hydrophobic properties of the substituent in position 4 of the phenylethyl substituent, it should be possible to improve the binding characteristics of MH.MZ.

The lipophilicities of the reference compounds were determined using the HPLC method according to Krass *et al.* (17) Soerensen buffer was used as eluent, and log p values were calculated from

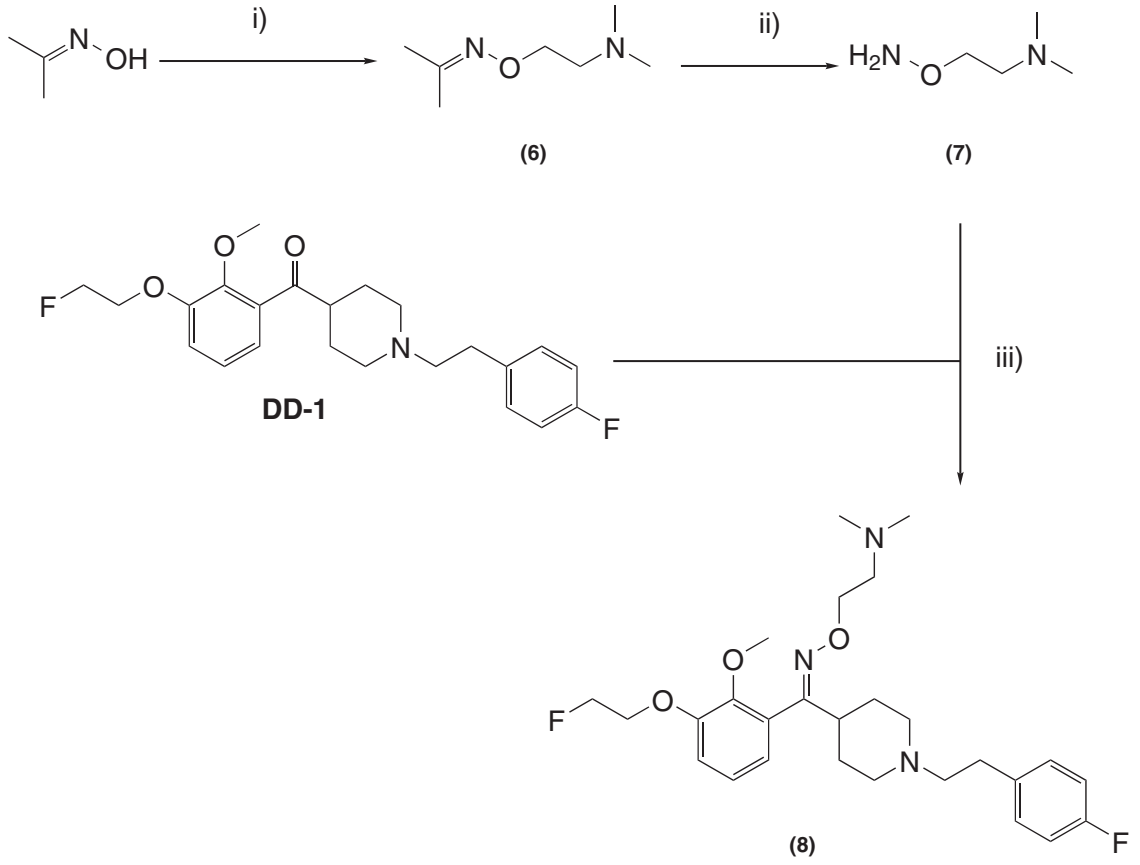


Figure 5: Synthesis of (**8**): reagents and conditions: (i) dimethylamino-ethylchloride hydrochloride, K_2CO_3, benzene, reflux, 54%; (ii) hydrochloric acid 10%, reflux, 74%; (iii) 1.25 M hydrochloric acid in ethanol, reflux, 23%.

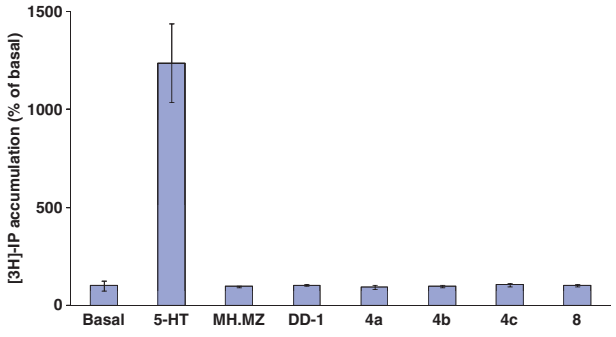
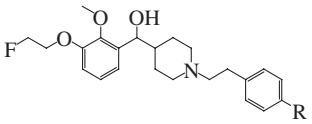


Figure 6: Functional properties of the new compounds toward the 5-HT_{2A} receptor. The full agonist 5-HT activates 5-HT_{2A} robustly ($p < 0.01$, one-way ANOVA with Tukeys *post-hoc* test), while the new compounds all exert antagonistic properties. Data presented as % of basal ± SD (n = 3–6).

retention times of the respective substances.[e] The calculated log p values are displayed in Table 3.

The lipophilicities of altanserin, MDL 100907, and MH.MZ are in the range of log p = 2–3 and indicate a good possibility to penetrate the blood–brain barrier. The increased lipophilicity of (**8**) can be explained by the dimethylaminoethyl oxime residue. Compounds (**4a**), (**4b**), and (**4c**), in contrast, are more hydrophilic than altanserin because of the two additional aromatic ether functions. These derivatives are more polar and may not be able to cross the blood–brain barrier.

Table 2: Affinities toward human 5-HT_{2A} receptor of different substituted antagonists (5)



Compound	R	K_i [nM]
MH.MZ	F	9.02 ± 2.11
Nitro-MH.MZ	-NO_2	26 ± 6,7
Methoxy-MH.MZ	-OMe	59 ± 57

In conclusion, four new 5-HT_{2A} antagonists based on a 4-(benzoyl)-piperidine moiety were synthesized in good chemical yields. The agonistic activity was determined by a functional assay, and the affinity to the 5-HT_{2A} receptor was determined in a [^{3}H]MDL 100907

Table 3: Lipophilicities/log p values of altanserin, MDL 100907, and new MDL 100907 derivatives. Data presented as K_i [nM] ± SD (n = 3)

Compound	Log p-values
Altanserin	2.15 ± 0.01
MDL 100907	2.98 ± 0.01
MH.MZ	2.80 ± 0.01
DD-1	3.08 ± 0.1
(**4a**)	1.65 ± 0.02
(**4b**)	1.31 ± 0.01
(**4c**)	1.53 ± 0.01
(**8**)	3.80 ± 0.01

binding assay. The combination of MH.MZ and SR 46349B represents a compound (**8**) with a moderate affinity toward the 5-HT$_{2A}$ receptor (K_i = 57 nM). This is possible because of the increased bulky group required by the dimethylaminoethyl oxime residue. The affinity of (**4a**), (**4b**), and (**4c**) in contrast is reduced significantly. This indicates that MH.MZ binds to the 5-HT$_{2A}$ receptor with the *p*-fluorophenylethyl residue in a sterically restricted hydrophobic binding pocket. Herth *et al.* (6) could already demonstrate that an improved binding characteristic of MH.MZ is accessible by variation of the substituent in the para-position. Small lipophilic residues such as a methyl group or a hydrogen may lead to higher affinities, whereas larger substituents lead to medium affine compounds.

Acknowledgments

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Notes

[a]3-(2-(4-(3-(2-Fluoroethoxy)-2-methoxybenzo-yl)-piperidin-1-yl)-ethyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-on (**4a**); 3-(2-(4-(3-(2-Fluoroethoxy)-2-methoxy-α-hydroxybenzyl)-piperidin-1-yl)-ethyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-on (**4b**) and 3-(2-(4-(2,3-dimethoxy-α-hydroxybenzyl)-piperidin-1-yl)-ethyl)-2-thioxo-2,3-dihydro-1H-quinazolin-4-on (**4c**): (0,61 mmol) (**3a**), (**3b**) or (**3c**) and (2 mmol) methyl-2-isothiocyanatobenzoate were dissolved in 10 mL dry methanole and stirred 2 h at room temperature. Evaporation of the solvent and CC (ethylacetate) gave the product as colorless crystals.

(**4a**): (0.18 mmol; 30%) ^{1}H-NMR (300 MHZ, DMSO-d_6) δ [ppm] = 12.908 (bs, 1H); 7.939 (d, 1H); 7.722 (t, 1H); 7.399-7.292 (m, 2H); 7.206 (d, 1H); 7.100 (t, 1H); 6.952 (d, 1H); 4.851 (t, 1H); 4.695 (t, 1H); 4.510 (t, 2H); 4.338 (t, 1H); 4.238 (t, 1H); 3.802 (s, 3H); 3.321 (s, 1H); 3.025-2.881 (m, 3H); 2.601 (t, 2H); 2.107 (t, 2H); 1.709 (d, 2H); 1.541-1.377 (m, 2H); MS (FD) *m/z* (% rel Int.): 485.4 (100 $[M]^+$); 486.4 (69.5 $[M + 1]^+$); 487.4 (24.3 $[M + 2]^+$);

(**4b**): (0.19 mmol; 32%) ^{1}H-NMR: (300 MHZ, DMSO-d_6) δ [ppm] = 12.875 (bs, 1H); 7.932 (d, 1H); 7.717 (t, 1H); 7.391-7.275 (m, 2H); 7.031-6.872 (m, 3H); 4.938 (d, 1H); 4.832 (t, 1H); 4.675 (t, 1H); 4.582 (t, 1H); 4.487 (t, 2H); 4.272 (t, 1H); 4.172 (t, 1H); 3.722 (s, 3H); 3.320 (bs, 1H); 3.001-2.829 (m, 2H); 2.548 (t, 2H); 1.879 (q, 2H); 1.781 (d, 1H); 1.411 (bs, 1H); 1.314-1.130 (m, 2H); MS (FD) *m/z* (% rel Int.): 487.4 (43.6 $[M]^+$); 488.4 (100 $[M + 1]^+$); 489.4 (25.9 $[M + 2]^+$); (**4c**): (0.26 mmol; 43%) ^{1}H-NMR: (300 MHZ, DMSO-d_6) δ [ppm] = 7.641 (m, 1H); 7.521 (d, 1H); 7.432 (m, 1H); 7.377-7.277 (m, 2H); 6.921-6.728 (m, 2H); 5.007 (d, 1H); 4.769 (m, 2H); 4.172 (t, 1H); 3.829 (s, 3H); 3.806 (s, 3H); 3.320 (bs, 1H); 3.262 (m, 2H); 2.799-2.648 (m, 2H); 2.104 (m, 2H); 1.859 (m, 2H); 1.555-1.253 (m, 2H); MS (ESI) *m/z* (% rel Int.): 455.6 (32.4 $[M]^+$); 456.6 (100 $[M + 1]^+$); 457.6 (21.2 $[M + 2]^+$).

[b]E-Dimethylaminoethoxy-[3-(2-fluoroethoxy)-2-methoxy-phenyl]-1-[2-(*p*-fluorophenyl)-ethyl]-pipe-ridin-4-yl-methanonoxim (**8**): 2 mmol **MA-1** and 2 mmol dimethylaminoethoxy-amine (**7**) were dissolved in ethanol containing 1.25 M HCl and heated under reflux for 20 h. After evaporation of the solvent and extraction with chloroform, the residue was taken up in concentrated ammonia solution. Extraction with chloroform, evaporation and CC (chloroform/methanol 5:1) gave the product as colorless crystals (0.5 mmol; 25%). ^{1}H-NMR (300 MHZ, $CDCl_3$) δ [ppm] = 7.148-7.072 (m, 3H); 7.026-6.829 (m, 4H); 6.581 (dd, 1H); 4.841 (t, 1H); 4.683 (t, 1H); 4.274 (t, 1H); 4.181 (t, 1H); 4.142 (t, 2H); 3.806 (s, 3H); 3.048-2.951 (m, 3H); 2.771-2.688 (m, 2H); 2.628 (t, 2H); 2.580-2.483 (m, 2H); 2.240 (s, 6H); 2.085 (q, 2H); 1.909-1.792 (m, 2H); 1.778-1.596 (m, 2H); MS (FD) *m/z* (% rel Int.): 490.5 (100 $[M + 1]^+$); 491.5 (27.9 $[M + 2]^+$).

[c]PI hydrolysis assay: GF-62 cells (1.5×10^6 cells/mL) were cultured overnight in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, California, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen), 1 mM sodium pyruvate (Sigma-Aldrich, St. Louis, Missouri, USA), penicillin (P: 100 units/mL; Invitrogen), and streptomycin (S: 100 μg/mL; Invitrogen) at 37 °C and 5% CO_2. Subsequently, cells were incubated with 4 μCi/well of myo-(1,2)-[^{3}H]-inositol (Amersham, Little Chalfont, Buckinghamshire, United Kindom) in labeling medium [inositol-free DMEM (US Biological) containing 10% dFBS and P/S] overnight at 37 °C and 5% CO_2. The cells were washed once with incubation buffer (20 mM HEPES, pH 7.4; 20 μM LiCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$; all from Sigma) and incubated at 37 °C in the same buffer for 30 min. The solutions were removed, and test compounds diluted in incubation buffer were added to the wells for 30 min at 37 °C. The formed inositol phosphates were extracted with 10 mM ice-cold formic acid (Sigma) for 30 min at 4 °C. Supernatants were transferred to columns with AG 1-X8 anion exchange resin (Bio-Rad, Hercules, California, USA) and eluted directly into Ultima-FLO AF scintillation liquid (Packard, Waltham, Massachusetts, USA) using a 2 M ammonium formate/0.1 M formic acid solution. Accumulated [^{3}H]inositol phosphates were measured with a Tri-Carb 2900TR liquid scintillation counter (Packard Instruments) after 1-h incubation at room temperature. Statistics were performed with GraphPad Prism 5 (GraphPad Software, San Diego, California, USA).

[d]Competition binding experiments were carried out in test-tubes containing [^{3}H]MDL 100907 (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 albumine, 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 fiber GF/C filters presoaked in 1% polyethyleneimine, using a Brandel cell harvester. Filters were washed with 300 mL cold assay buffer (titrated 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.

[e]Lipophilicities were determined using the HPLC system described previously 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 and enabled the calculation of the capacity factor *k*. A plot of these reference values against their known log p values gave a reference curve that was used to calculate log p values for synthesized compounds.