Dopamine/Serotonin Receptor Ligands. 9.[1] Oxygen-Containing Midsized Heterocyclic Ring Systems and Nonrigidized Analogues. A Step toward Dopamine D_5 Receptor Selectivity

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Abstract: Eleven-membered heterocycles (dibenz[*g*,*j*]-1-oxa-4-azacycloundecenes) and open-chain analogues were synthesized and investigated for affinities to human dopamine receptor subtypes. The moderately rigidized rings displayed nanomolar and subnanomolar K_i values at D_1-like receptors with a significant D_1 to D_2 and a slight D_5 to D_1 selectivity. The open-chain analogues showed lower affinities but significant D_1 to D_2 selectivities. Compound **3** ($K_i(D_5)$ = 0.57 nmol) showed antagonistic or inverse agonistic binding characteristics in a functional Ca assay.

Dopamine receptor mediated neurotransmission plays a key role in psychiatric, motoric, and endocrinological disorders. While a limited number of D_1-like receptor ligands such as the antagonist LE 300 (**1**)[1−3] are known, none of them show a significant binding selectivity to the D_1 or the D_5 receptor. Hence, knowledge about the physiological impact of activation or inhibition of these single receptors, especially the D_5 receptor, is limited or not existent.[4]

Originating from lead LE 300 (**1**) (Figure 1), our interests are focused on dibenzo derivatives. We synthesized compounds with an enlarged central ring system (Figure 1, Scheme 1) and an isosteric replacement of the methylene group in position 5 against an oxygen atom (therefore resulting in resorcine mono- and diethers), leading to a lower degree of rigidization and a different electronic situation in the central ring system as well as induction of a different electrostatic field in the respective benzene moiety (Figure 1). To investigate the effect of rigidization on the binding affinities of these substances, we also synthesized (2-benzylphenoxy)alkylamines (**5**−**14**) representing open-chain analogues of the 11-membered heterocycles varying in their substitution patterns at the benzyl C atom and at the N atom (Scheme 2).

The dibenz[*g*,*j*]-1-oxa-4-azacycloundecenes were synthesized as 3-monomethoxylated (**2**) and 3-monohydroxylated (**3**) compounds. 2-[3-(Benzyloxy)phenoxy]-ethylamine (**4**) was obtained by monobenzylation of resorcine,[5] reaction with 2-methyl-2-oxazoline to the acetamide, and hydrolysis in ethanolic 6.3 M NaOH solution (superior to phosphoric acid, which led to debenzylation).[6]

Compound **3** was obtained following a similar synthetic protocol as described for the 3-methoxylated **2**[6] but using a benzyl group to protect the phenolic function, which could be removed in the final reaction step under Birch conditions together with the cleavage of the central C−N bond (Scheme 1). The synthesis of the nonrigidized analogue **5** could be carried out by performing a Mitsunobu etherification of 2-benzoylphenol with *N*-(2-hydroxyethyl)pyrrolidine yielding **5** (2-benzylphenol yielded **9** in a one-step procedure). A two-step procedure via phenoxyalkylbromide intermediates and subsequent substitution reactions with different cyclic and noncyclic alkylamines yielded **7**−**13** (Scheme 2). Compounds **7**−**11** were reported in the literature[7] as potential antihistaminergic agents but were synthesized in a different, less efficient and less comfortable way.

Benzophenone **5** was further reduced to the alcohol **6** by using lithium aluminum hydride. Additionally to

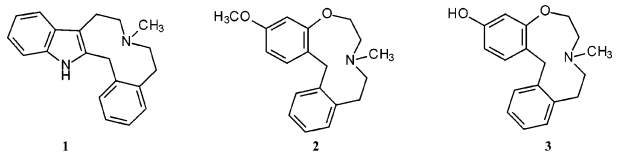
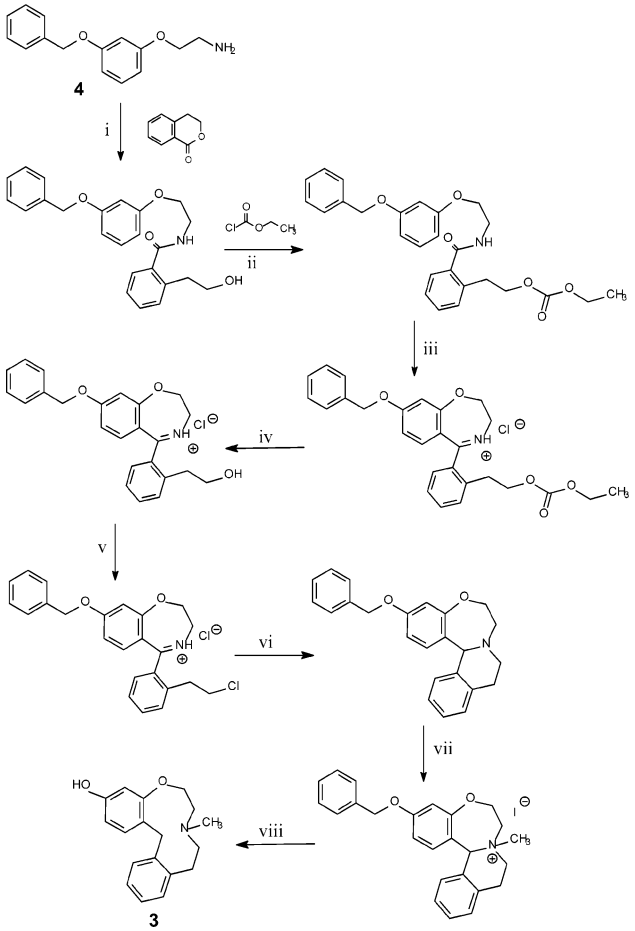


Figure 1. Condensed midsize heterocyclic ring systems with antidopaminergic activity.

Scheme 1. Synthesis of **3**[a]



[a] Reagents and conditions: (i) 120 °C, 8 h; (ii) $CHCl_3$, pyridine, room temp, 1 h; (iii) $POCl_3$, CH_3CN, reflux, 6 h; (iv) ethanolic KOH, room temp, 16 h; (v) $POCl_3$, 60 °C, 1 h; (vi) $NaBH_4$, ice-cooling, MeOH, 1 h; (vii) MeI, toluene, 90 °C, 16 h; (viii) Na^0, liquid NH_3, −40 °C, 7 min, termination with NH_4Cl.

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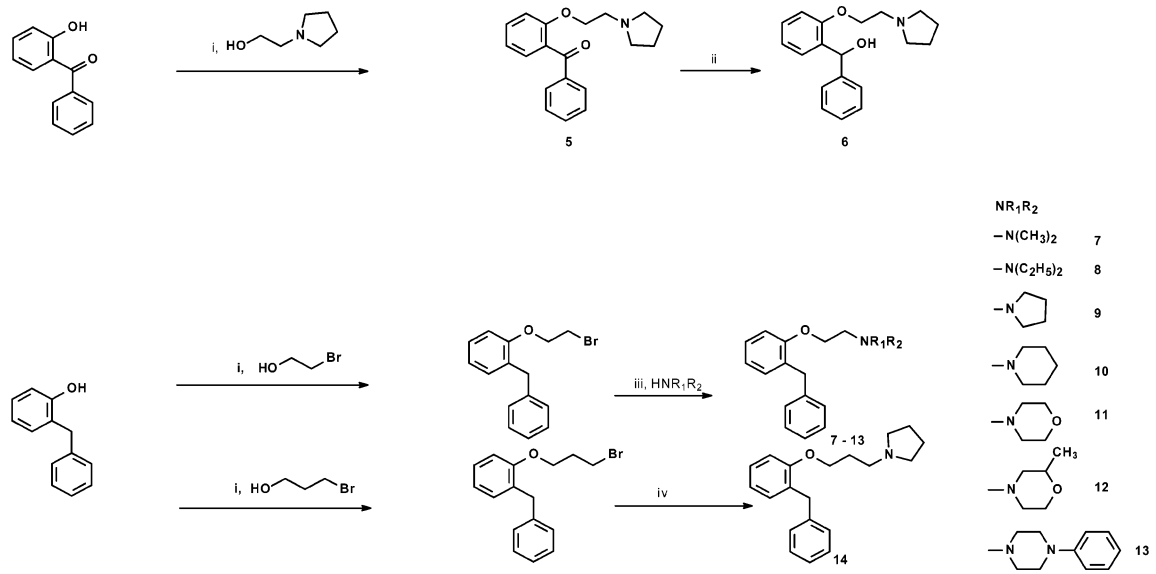
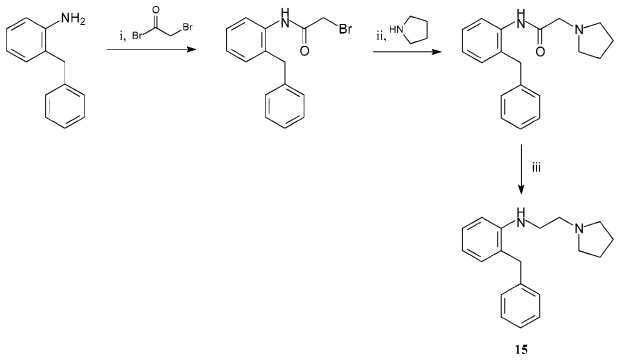
Scheme 2. Synthesis of Nonrigidized Analogues[a]



[a] Reagents and conditions: (i) diisopropylazodicarboxylate (DIAD), triphenylphosphine (TPP), dry THF, 0 °C, then room temp, 48 h; (ii) $LiAlH_4$, dry ether, ice-cooling, then room temp, 2 h; (iii) K_2CO_3, toluene, reflux, 16 h; (iv) pyrrolidine, K_2CO_3, toluene, reflux, 16 h.

Scheme 3. Synthesis of Anilino Derivative **15**[a]



[a] Reagents and conditions: (i) dry ether, ice-cooling, then room temp, 2 h; (ii) K_2CO_3, toluene, reflux, 16 h; (iii) $LiAlH_4$, dry THF, ice-cooling, then reflux, 3 h.

the (2-benzylphenoxy)ethylamines, a (2-benzylphenoxy)-propylamine containing a pyrrolidine ring (**14**) was synthesized to evaluate the influence of side chain elongation on the binding characteristics of these compounds (Scheme 2). Finally an anilino compound of 1-[2-(2-benzylphenoxy)ethyl]pyrrolidine (**15**) was synthesized, resulting in a compound in which the phenolic oxygen is replaced by a secondary amine function (Scheme 3). Synthesis of **15** was performed starting from 2-benzylaniline and 2-bromoacetyl bromide yielding the bromoacetamide, followed by a substitution reaction with pyrrolidine and reduction of the amide function to the secondary amine (Scheme 3).

Compounds **2**, **3**, and **5**−**15** were screened for their binding affinities to human cloned dopamine receptor subtypes by in vitro radioligand binding studies following a protocol so far previously described[8] but in 96-well format.

D_1, D_{2L}, D_3, $D_{4.4}$, and D_5 receptors were stably expressed in HEK 293 or CHO cells. [^{3}H]SCH 23390 and [^{3}H]spiperone were used as radioligands for experiments at the D_1-like and D_2-like receptor family, respectively. Incubations at 27 °C were terminated after 90 min by rapid filtration with a Perkin-Elmer Mach III harvester. At least two independent experiments were carried out in triplicate each. K_i values were calculated from IC_{50} values by applying the equation of Cheng and Prusoff[9] and are given in nanomolar units (Table 1).

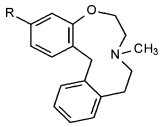
The oxaazacycloundecenes **2** and **3** show nanomolar affinities at the hD_1 receptor subtype with a 10-fold higher affinity of the 3-hydroxylated compound. Furthermore, **3** binds with subnanomolar affinity to the hD_5 receptor. The D_5/D_1 selectivity ratio (1:6) is moderate. The 3-methoxylated derivative **2** on the other hand shows lower affinities but a D_5/D_1 affinity ratio of 1:20 (Table 1).

Both oxygen-containing midsize heterocycles display weaker affinities at the D_2-like receptor family (D_{2L}, D_3, $D_{4.4}$) in the range 10^{-7}−10^{-6} M for their K_i values. Concerning D_5 over D_{2L} binding selectivity, the hydroxylated **3** shows a significant ratio of almost 1:500, while the methoxylated derivative **2** still reaches a ratio of 1:88. While affinities to the D_2 and D_4 receptor subtype are very similar, the binding affinity to the D_3 receptor turned out to be the lowest with both compounds, showing only micromolar K_i values. For comparison, the well-established antipsychotic haloperidol shows nanomolar affinities to all hD receptor subtypes with around 10 times higher affinities to the D_2-like family: hD_1, 82 nM; hD_2, 2.2; hD_3, 7.8; $hD_{4.2}$, 7.3; hD_5, 58 nM (representative values).[10] Compared to the heterocycles, all of the nonrigidized analogues **5**−**15** are compounds with lower affinities to all of the binding sites.

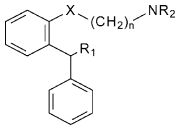
The K_i values range from 10^{-8} to 10^{-6} M for D_1 and D_5 and from 10^{-6} to 10^{-5} M for D_{2L}, D_3, and $D_{4.4}$. Generally, these open-chain analogues show consistently higher D_1 over D_2 binding affinities with the exception of **8**.

The D_1 receptor affinity of the 2-benzylphenoxyethylamines reaches a maximum with **10**, carrying a piperidine ring as the alkylamino moiety, while D_1/D_2 selectivity reaches its maximum with **11**, carrying a morpholine ring. Interestingly enough, affinity to the

Table 1. K_i Values of Dibenz[*g,j*]-1-oxa-4-azycycloundecenes and Open-Chain Analogues at Dopamine Receptor Subtypes



		K_i (nM)[a]				
compd	R	D_1	D_{2L}	D_3	$D_{4.4}$	D_5
2	OCH_3	35.5 ± 11.7	158 ± 38	1601 ± 306	546 ± 110	1.8 ± 0.2
3	OH	3.2 ± 0.8	274 ± 0.5	1384 ± 454	375 ± 106	0.57 ± 0.1



					K_i (nM)[a]				
compd	X	*n*	R_1	NR_2	D_1	D_{2L}	D_3	$D_{4.4}$	D_5
5	O	2	=O	pyrrolidinyl	1651 ± 408	5317 ± 1038	2408 ± 1139	>10000	nd
6	O	2	−OH	pyrrolidinyl	2621 ± 1121	6280 ± 1621	>10000	>10000	nd
7	O	2	H	$N(CH_3)_2$	343 ± 29	2740 ± 415	>10000	2765 ± 469	414 ± 18
8	O	2	H	$N(C_2H_5)_2$	1959 ± 140	2024 ± 7	4667 ± 993	>10000	nd
9	O	2	H	pyrrolidinyl	379 ± 157	>10000	1818 ± 156	1428 ± 198	951 ± 334
10	O	2	H	piperidinyl	33.7 ± 0.4	1015 ± 516	7804 ± 2731	4476 ± 2496	43 ± 5
11	O	2	H	morpholinyl	201 ± 98	>10000	>10000	6484 ± 462	366 ± 8
12	O	2	H	2-methylmorpholinyl	1676 ± 429	>10000	>10000	>10000	nd
13	O	2	H	phenylpiperazinyl	1480 ± 197	4188 ± 302	2860 ± 1094	>10000	620 ± 255
14	O	3	H	pyrrolidinyl	113 ± 3	1006 ± 342	2923 ± 1448	1261 ± 84	113 ± 26
15	NH	2	H	pyrrolidinyl	>10000	>10000	4654 ± 988	>10000	nd

[a] K_i values are the mean of at least two experiments ± SEM (performed in triplicate).

D_1 receptor subtype and thereby D_1/D_2 selectivity are dramatically lowered by introduction of a methyl group next to the oxygen in the morpholine ring. By comparison of **9** and **14**, side chain elongation to a phenoxypropyl derivative leads to higher affinity to the D_1, D_2, and D_5 receptors. The isosteric replacement of the phenolic oxygen atom by a secondary amine function leads to dramatic loss of affinity with K_i > 10000 nM, only at the D_3 subtype showing micromolar affinity.

Compound **3** was investigated for its functional behavior at the hD_1 receptor in an intracellular Ca^{2+} assay developed in our group.[11] HEK293 cells stably expressing the hD_1 receptor were loaded with a fluorescent dye (Oregon Green), and after preincubation with rising concentrations of **3** an agonist (SKF 38393) was injected and fluorescence was measured with a NOVOSTAR microplate reader. In this assay, **3** was able to suppress agonist-induced Ca^{2+} influx with rising concentrations, indicating an antagonistic or inverse agonistic behavior at the D_1 receptor subtype (Figure 2). From the inhibition curve, a K_i value could also be defined (12.3 ± 1.3 nM), which is slightly higher than that obtained by radioligand binding studies (3.2 ± 0.8 nM).

Compound **2** revealed high binding affinities to D_1-like receptors in the nanomolar range and represents the first step to a structurally new class of dopaminergic ligands with binding selectivity to the hD_5 receptor subtype, thus giving chances for evaluating the properties of D_5 receptors in the brain. The 3-hydroxylated oxaazacycloundecene derivative **3** binds with even higher affinity to the D_5 receptor, reaching a subnanomolar K_i value, but lower selectivity to the D_5 receptor.

Compound **3** was identified as an inhibitory ligand at the hD_1 receptor subtype. Hence, it could be an

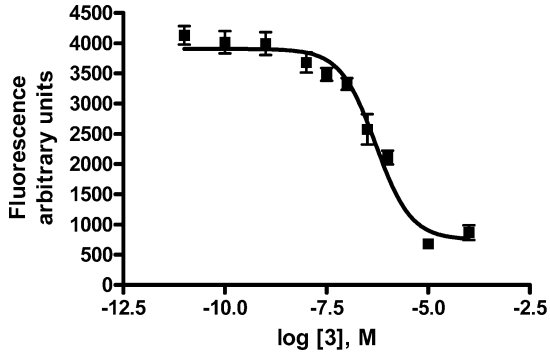


Figure 2. Suppression of agonist-induced receptor-mediated Ca^{2+} influx by rising concentrations of **3**.

interesting candidate for further pharmacological characterization as a potential neuroleptic drug candidate with a novel receptor binding profile, especially concerning the D_2 receptor, which is responsible for extrapyramidal motoric effects.

The investigated open-chain analogues represent dopamine receptor ligands with lower affinities but remarkable selectivities at dopamine receptor subtypes. Almost all derivatives showed significant D_1 binding affinitiy over D_2 binding affinity. The phenolic oxygen atom in the compounds presented here seems to play an essential role for receptor binding because isosteric replacement against a secondary amine function and thereby a switch from a proton acceptor to a proton donor moiety result in a nearly complete loss of binding potential to dopamine receptors except for the D_3 subtype. This finding helps in the design of new compounds with stronger proton acceptor properties, which is on the way.

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Supporting Information Available: Synthetic procedures, spectral characterization, and elemental analysis results for **3**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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