Brief Articles

Influence of the 5-HT$_6$ Receptor on Acetylcholine Release in the Cortex: Pharmacological Characterization of 4-(2-Bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a Potent and Selective 5-HT$_6$ Receptor Antagonist†

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A small series of aryl pyridyl sulfones has been prepared and investigated for its 5-HT$_6$ receptor binding properties. Thereof, pyrrolidinyl derivative **11** proved to be a very potent (pK_i 9) and selective 5-HT$_6$ receptor antagonist. By means of in vivo microdialysis in the frontal cortex and a passive avoidance paradigm, where **11** reversed a scopolamine induced retention deficit, a functional correlation between 5-HT$_6$ receptors and cholinergic neurotransmission could be shown, supporting the therapeutic potential of 5-HT$_6$ receptors in the treatment of cognitive deficits.

The 5-HT$_6$ receptor is the most recently discovered and cloned member of the serotonin receptor family, which comprises currently a total of 14 distinct receptors with a variety of different functions and diverse localization patterns. It is positively coupled to adenylate cyclase and rather unique in its structure, exhibiting only 30−40% sequence homology versus all the other serotonin receptor subtypes. Immunological methods revealed high levels of expression in the olfactory tubercle, striatum, frontal cortex, and hippocampus with almost no localization in the periphery.[1−7]

Though a variety of antipsychotics such as clozapine, as well as classical antidepressants, are potent antagonists of this serotonin receptor subtype, its physiological relevance is still under debate.[8] Treatment of rats with antisense oligonucleotides evoked behavioral syndromes, which could only be antagonized by atropine indicating a possible involvement of 5-HT$_6$ receptors in the modulation of cholinergic neurotransmission.[9] These findings were confirmed by use of the first selective 5-HT$_6$ receptor antagonist, RO-04-6790.[9,10]

During the past few years, the discovery and development of a series of novel ligands for the 5-HT$_6$ receptor has been reported, introducing various new classes of compounds as potent and selective binders for this serotonin receptor subtype.[11−15]

The in vivo pharmacology of these ligands and their relevance for the treatment of CNS-related disorders has been discussed and summarized in detail in a series of reviews.[16]

In vivo microdialysis studies in freely moving rats with SB-271046,[12] a potent (pK_i 8.9) and selective 5-HT$_6$ receptor antagonist, revealed a significant increase of the excitatory neurotransmitters aspartate and glutamate in the frontal cortex and hippocampus at doses of 10 mg/kg sc.[17] The observed increase of glutamate in the frontal cortex has been substantially diminished by coinfusion of the voltage dependent Na^+ channel blocker tetrodotoxin (10 μM) and not by coadministration of the muscarinic antagonist atropine (3 mg/kg sc), indicating a tonic serotonergic modulation of glutamatergic neurons via the 5-HT$_6$ receptor without a direct participation of cholinergic pathways.[18]

Recently we reported on the optimization and biological evaluation of *N*-heteroaryl and *N*-aryl sulfonamides as 5-HT$_6$ receptor selective antagonists.[19]

Some representatives thereof reversed a scopolamine induced retention deficit in a passive avoidance paradigm with minimal effective doses (MED) below 10 mg/kg po.[20]

To further optimize these compounds and simultaneously extend the structural scope of 5-HT$_6$ receptor ligands, the synthesis and evaluation of corresponding sulfone congeners has been anticipated. In a very focused approach based on the SAR derived from our sulfonamide series, a limited number of sulfone analogues have been synthesized and investigated.[21] The pyridyl sulfone derivative **2** exhibited a 10-fold higher affinity for the 5-HT$_6$ receptor as compared to its sulfonamide analogue **1**,[19] indicating a significantly improved recognition by the receptor for sulfones as opposed to sulfonamides. The superb selectivity profile

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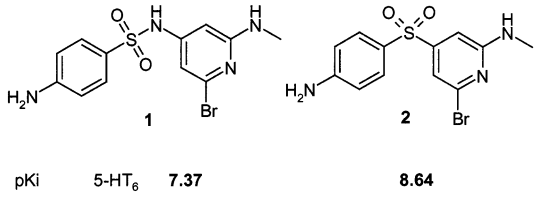
Table 1. Serotonin Subtype Receptor Affinities of Sulfones **2**, **10**−**12**[a]

		pK_i (±SEM)				
compd	Y	5-HT$_6$	5-HT$_{1D}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_7$
2	NHMe	8.64 ± 0.01	<5	nd	<5	<5
10	Br	7.27 ± 0.02	nd	nd	nd	nd
11	pyrrolidinyl	9.00 ± 0.02	<4	5.84	<5	<4
12	piperazinyl	9.94 ± 0.02	5.95	nd	7.69	<5
	Ro 04-6790[11]	7.26 ± 0.06	<5	<5	<5	<5

[a] The following receptors and radioligands were used in the binding assays: 5-HT$_6$ (human recombinant receptors expressed in HeLa cells, [^{3}H]-LSD); 5-HT$_{1D}$ (human recombinant receptors expressed in HEK 293 cells, [^{3}H]-LSD); 5-HT$_{2A}$ (human recombinant receptors expressed in 3T3 cells, [^{3}H]-DOB); 5-HT$_{2C}$ (human recombinant receptors expressed in 3T3 cells, [^{3}H]-5-HT); 5-HT$_7$ (human recombinant receptors expressed in CHO cells, [^{3}H]-LSD).[11] 5-HT6 results are the mean ± SEM of the three independent determinations performed in triplicate. Selectivity data presented versus other serotonin receptor subtypes are screening data.

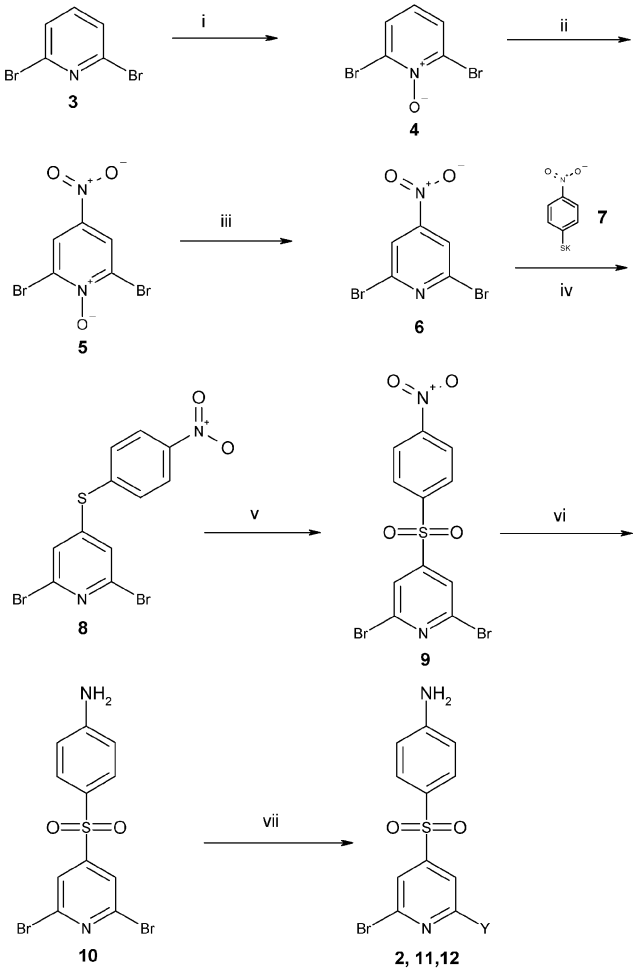
of **2**, at least 1000-fold selectivity over 5-HT$_{1D,2C,7}$ receptors (Table 1), corroborated our new approach even more.



The synthetic approach to this series was based on the known 2,6-dibromo-4-nitropyridine *N*-oxide **5** as a key intermediate (Scheme 1). Commercially available 2,6-dibromopyridine **3** was converted into the 4-nitro *N*-oxide **4** as described by den Hertog.[22,23] Chemoselective reduction with PBr_3 in CH_3CN under reflux conditions for 14 h yielded 2,6-dibromo-4-nitropyridine **6** quantitatively.[24] Nucleophilic displacement of the 4-nitro group by 4-nitro-thiophenol potassium salt **7**[25] in DMF led to sulfide **8** in 86% yield. Conversion of the sulfide moiety to the sulfone by means of mCPBA followed by reduction of the nitro group to the 4-amino functionality with Fe and NH_4Cl in H_2O/MeOH gave rise to the dibromo key intermediate **10** in high yield. Replacement of one bromo substituent by several primary and secondary amines yielded a variety of potent 5-HT$_6$ receptor antagonists. The most potent compounds thereof are summarized in Table 1, exhibiting the cyclic amines **11** and **12** as the best substituents for the 5-HT$_6$ receptor. The piperazinyl derivative **12** with picomolar affinity unfortunately displays significant affinity for the 5-HT$_{2C}$ receptor, which can be attributed to the mCPP (*m*-chlorophenylpiperazine)-like substructure of the molecule. Substitution with various other cyclic as well as open chain amines did not result in compounds with improved affinity (data not shown).

Due to its high affinity for the 5-HT$_6$ receptor and high selectivity within the serotonin receptor family (>1000-fold), pyrrolidine **11** has been selected for further profiling. A broad screen revealed no further binding affinity for a subset of more than 50 neuroreceptors and proteins, including muscarinic, purinergic, dopaminergic, opiate, gabaergic, histaminergic, adrenergic, nicotinergic, and tachykinergic receptors, as well as various calcium and potassium ion channels in the submicromolar range (data not shown).

Scheme 1. Synthesis of Pyridyl Sulfones **2** and **10**−**12**[a]



[a] Reagents: (i) H_2O_2 30%, CF_3COOH, 100 °C, 3 h, 79%; (ii) HNO_3/H_2SO_4, 100 °C, 1.5 h, 80%; (iii) PBr3, CH_3CN, reflux, 14 h, 99%; (iv) DMF, 60 °C, 3 h, 86%; (v) mCPBA, CH_2Cl_2, rt, 2 h, 87%; (vi) Fe, NH_4Cl, H_2O/MeOH, reflux, 1.5 h, 80%; (vii) amine, dioxane, rt, 2 h, (70−90%).

The intrinsic properties of **11** have been assessed in a functional cAMP-binding assay using 5-CT to stimulate human 5-HT$_6$ receptors stably expressed in HEK-293 cells. As it can be seen in Figure 1, increasing concentrations of **11** shifted the 5-CT concentration response curve parallel rightward without affecting the maximal dose effect. Schild analysis yielded a slope of 1.031, confirming competitive antagonism and a pA_2 of 8.5 in good agreement with the binding value (Figure 1, inset). Compound **11** alone exhibited no intrinsic efficacy suggesting a profile as a silent competitive antagonist.

Table 2 summarizes the physicochemical and DMPK properties of **11**. The in vitro clearance in human and rat liver microsomes is low to intermediate (data not shown). The pharmacokinetic profile of **11** was assessed in rats after both oral and intravenous administration. When administered by oral gavage (10 mg/kg, in PEG/PG/WIP 40/40/20), compound **11** was rapidly absorbed, and sustained plasma concentrations were measured over 8 h (C_{max} = 490 ng/mL, T_{max} = 1 h). Bioavailability was around 50%. After intravenous administration of the same dose and formulation, compound **11** showed a

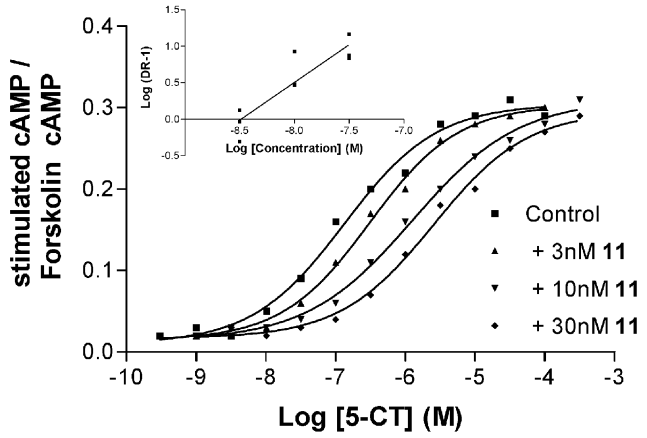


Figure 1. Influence in concentration of compound **11** on 5-CT stimulated adenylate cyclase activity. Inset: Schild analysis of compound **11**, $n = 3$.

Table 2. Physicochemical and Pharmacokinetic Properties of Compound **11**

Physicochemical Properties		
solubility at pH 6.5	log *D* at pH 7.4	pK_a
1 μg/mL	3.3	<2

Pharmacokinetics in Vivo Rat at 10 mg/kg[a]		
	iv values (average ± SEM)	po values (average ± SEM)
CL_P (mL/min/kg)	20 ± 5	–
$t_{1/2}$ (h)	3.7 ± 1.6	3.0 ± 0.4
F (%)	NA	46 ± 10
V_{ss} (L/kg)	1.4 ± 0.2	NA
brain/plasma ratio %	Nd	24 ± 7

[a] CL_P, plasma clearance; $t_{1/2}$, apparent terminal half-life; *F*, bioavailability; V_{ss}, volume of distribution at steady-state; NA, not applicable.

low to intermediate systemic plasma clearance (in agreement with the in vitro data) and an apparent terminal half-life of 4 h.

CNS penetration studies were also performed with compound **11** in rats after oral administration. According to log *D* (3.3) and pK_a values (<2) a reasonable brain penetration could have been anticipated (Table 2).

To establish a direct link between 5-HT_6 receptors and cholinergic neurotransmission in the frontal cortex, microdialysis studies in rats have been conducted with **11**.[26]

Oral administration of **11** at the dose of 30 mg/kg produced a clear 2-fold increase of the extracellular level of acetylcholine (ACh) in the rat frontal cortex (Figure 2). The cortical extracellular levels of ACh were maximally increased 20−40 min after administration of **11** and, thereafter, slowly declined and returned to basal levels 2 h after administration. The rapid onset of action of **11** confirms that this compound is rapidly absorbed and able to readily enter the brain, which is in agreement with our assumptions derived from its physicochemical properties (cf. Table 2). **11** did not modify the cortical levels of choline, the precursor for the synthesis of ACh.

The ability of **11** to increase ACh suggests that 5-HT_6 receptors mediate an inhibitory serotoninergic input to the cholinergic inervation of the frontal cortex. Blockade of 5-HT_6 receptors by **11** reduces such inhibitory input.

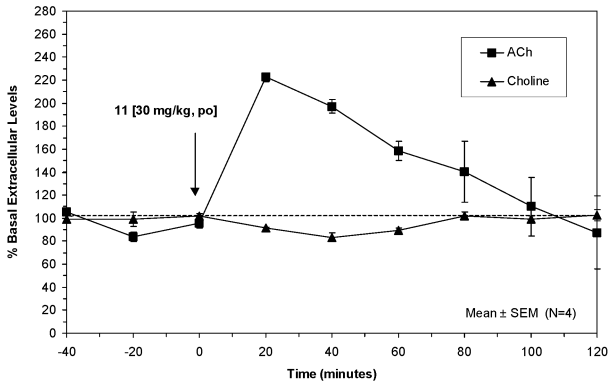


Figure 2. Compound **11** increases the extracellular levels of acetylcholine (ACh), but not choline, in the rat frontal cortex. Each point represents mean ± SEM of 4 animals.

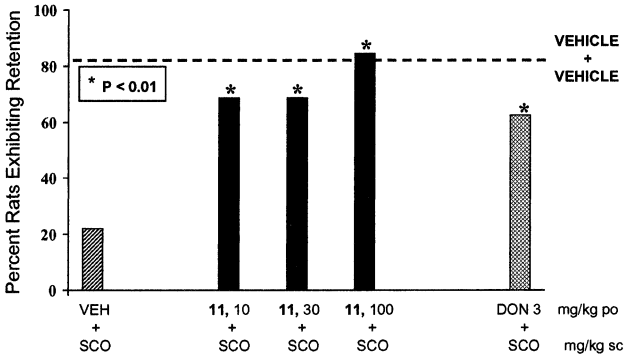


Figure 3. Reversal of a scopolamine (SCO)-induced retention deficit in a passive avoidance task by a single post-training oral administration of either compound **11** (solid bar) or donepezil (DON) in comparison with vehicle (VEH).

This release of ACh is in good agreement with the activity of 5-HT_6 receptor antagonists in reversing a scopolamine-induced passive avoidance retention deficit in rats.[19,20] Compound **11** was tested under both conditions of acute and repeated treatment. In these experiments, the reference AChE inhibitor donepezil was included as an active control condition.

Following a single oral administration, **11** was found to statistically significantly reverse a scopolamine-induced passive avoidance retention deficit at 10−100 mg/kg (Figure 3). In a subsequent experiment in which the treatments were administered orally on 10 successive days followed by evaluation for reversal of a scopolamine-induced passive avoidance deficit, 3 and 10 mg/kg of **11** exhibited a significant ameliorative effect compared to the vehicle condition (Figure 4). In both the acute and chronic experiments, the optimal effect of **11** was approximately that achieved with a single optimal dose of the reference AChE inhibitor donepezil.

Aryl pyridyl sulfone **11** proved to be a highly potent and selective 5-HT_6 receptor antagonist with a very good overall DMPK profile allowing for its use as an ideal pharmacological tool in the elucidation of the functional role of this particular serotonin receptor subtype in a variety of in vivo studies. By means of in vivo microdialysis studies in the frontal cortex and in a passive avoidance paradigm a potential relevance of the 5-HT_6 receptor for cognition and memory related effects has been shown. These experimental results contribute further evidence supporting the therapeutic potential

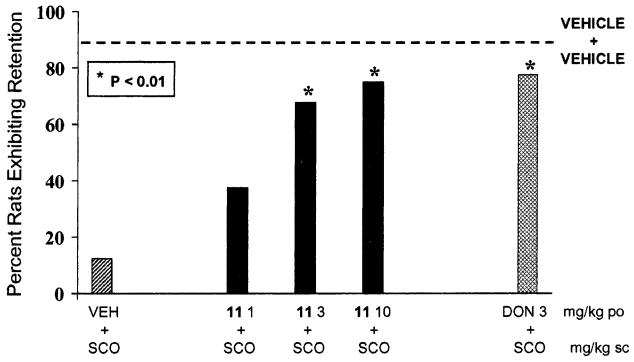


Figure 4. Reversal of a scopolamine (SCO)-induced retention deficit in a passive avoidance task by repeated daily oral administration of either compound **11** (solid bar) or donepezil (DON) in comparison with vehicle (VEH).

for 5-HT_6 receptor antagonists for the treatment of memory disorders.

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Supporting Information Available: Experimental procedures and spectral characterization data for compounds **2** and **10**−**12** are available free of charge via the Internet at http://pubs.acs.org.

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