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Original article

5-HT_6 receptor antagonists: lead optimisation and biological evaluation of *N*-aryl and *N*-heteroaryl 4-amino-benzene sulfonamides

Michael Bös[a]*, Andrew J. Sleight[b], Thierry Godel[b], James R. Martin[b], Claus Riemer[b], Heinz Stadler[b]

[a]Boehringer Ingelheim (Canada) Ltd, Research & Development, 2100 Cunard Street, Laval, Québec, Canada H7S 2G5
[b]F. Hoffmann-LaRoche, Pharma Division, Preclinical Research, CH-4070 Basel, Switzerland

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Abstract – RO-04-6790 (**6a**) has been identified in a random screen for 5-HT_6 receptor antagonists. In a medicinal chemistry optimisation program a series of analogs comprising *N*-heteroaryl- and *N*-arylbenzenesulfonamides have been synthesised and investigated for their binding affinity. Compounds with a log *D* profile indicative of brain penetration have been subjected to in vivo testing for reversal of a scopolamine-induced retention deficit in a passive avoidance paradigm. © 2001 Éditions scientifiques et médicales Elsevier SAS

sulfonamides / serotonin / passive avoidance paradigm / brain penetration / lipophilicity

1. Introduction

The effects of the neurotransmitter 5-hydroxytryptamine (5-HT) are mediated through at least 14 distinct receptors. At present, these comprise of 13 G-protein coupled receptors and a ligand-gated ion channel (the 5-HT_3 receptor) [1, 2]. G-protein coupled receptors can be divided into a number of different families based on their structure, function and pharmacology. The 5-HT_1 family is negatively coupled to adenylyl cyclase, the 5-HT_2 family to phosphoinositol hydrolysis, and the 5-HT_4, 5-HT_6 and 5-HT_7 receptors are positively coupled to adenylyl cyclase [1, 2]. At present, the functional coupling of the 5-HT_5 receptor is not known.

Both the rat and human 5-HT_6 receptors have been isolated, and in rats, the highest levels of 5-HT_6 receptor mRNA are present in the olfactory tubercle, nucleus accumbens, striatum and hippocampus [3–6]. In addition to these regions, 5-HT_6-like immunoreactivity was also identified in the frontal and entorhinal cortex and the molecular layer of the cerebellum [7].

5-HT_6 receptors can be radiolabeled with [^{125}I]-LSD, [^{3}H]-LSD and [^{3}H]-5-HT [8]. Many non-selective compounds such as tricyclic antidepressants, antipsychotic agents, tryptamine and ergoline derivatives bind to the 5-HT_6 receptor with high affinity [8]. The first study exploring the functional significance of the receptor in vivo used antisense oligonucleotides, which should abolish or reduce expression of the 5-HT_6 receptor protein. Treatment with these antisense probes produced a sequence specific behavioural syndrome of yawning, stretching and chewing, which could be antagonised by atropine but not by haloperidol [9].

In order to further study 5-HT_6 receptors and their physiological function, screening of the Roche compound library for potent and selective ligands was initiated and yielded 4-amino-*N*-(2,4-bis-methylamino-pyrimidin-4-yl)benzene sulfonamide **6a**. This selective 5-HT_6 receptor antagonist was shown to induce a behavioural syndrome similar to that seen with 5-HT_6 receptor antisense treatment [9].

SB-271046 [10], a potent (pK_i 8.9) and selective 5-HT_6 receptor antagonist of low brain penetration but very good oral bioavailability, was shown to

* Correspondence and reprints.
E-mail address: mboes@lav.boehringer-ingelheim.com (M. Bös).

exhibit anticonvulsant activity in the rat MEST test [11], suggesting an antiepileptic potential for 5-HT_6 receptor antagonists. In vivo microdialysis studies in freely moving rats with this compound showed no effect on basal levels of 5-HT, DA and NA in the striatum and frontal cortex. Levels of the excitatory neurotransmitters aspartate and glutamate were also not altered in the striatum. However, in the frontal cortex a significant increase at a dose of 10 mg kg^{-1} s.c. has been observed for these excitatory amino acids, adding further evidence to the therapeutic potential of the 5-HT_6 receptor antagonist for the treatment of cognitive dysfunction and memory impairment [12].

Very recently, a series of arylsulfonyl tryptamines and -indoles has been reported as novel 5-HT_6 receptor antagonists without disclosing any in vivo relevant data [13, 14].

In this paper we report the synthesis of *N*-pyrimidin-4-yl, *N*-pyridin-4-yl, *N*-phenyl and *N*-indolyl 4-amino-benzene sulfonamides as novel 5-HT_6 receptor antagonists, and the evaluation of some of these ligands in the passive avoidance paradigm, an animal model of cognitive function.

2. Chemistry

N-(Pyrimidin-4-yl)-4-amino-benzenesulfonamides **6c–6t** were prepared according to *figure 1*. Sequential substitution of 2,4,6-trichloro-pyrimidine by an amine [15–19], the potassium salt of sulfonamide **3** and finally a second amine yielded after hydrolysis of the acetyl group the products in 30–60% yield. Alternatively, **6a** and **6h** were obtained from commercially available sulfadimethoxine **1** in one step with methyl- or ethylamine, respectively.

The synthesis of the corresponding *N-(pyridin-4-yl)-4-amino-benzenesulfonamides* **11a–11b** and **12a–12c** is outlined in *figure 2*. Sulfonamide formation with 4-amino-2,6-dibromo-pyridine **7** [20, 21] and sulfonylchloride **8** followed by deprotection gave the precursor **10**. Treatment of **10** with an ethanolic solution of an amine yielded the monobromo derivatives **11a** and **11b** [22, 23]. Aminolysis of these compounds led to the sulfonamides **12a–12c**.

Figure 3 and 4 summarise the preparation of the *N-phenyl-4-amino-benzenesulfonamides* **20a–20f**. Nitro-phenyl derivatives **14** and **18a–c** were synthesised via acetylation and alkylation or dialkylation of

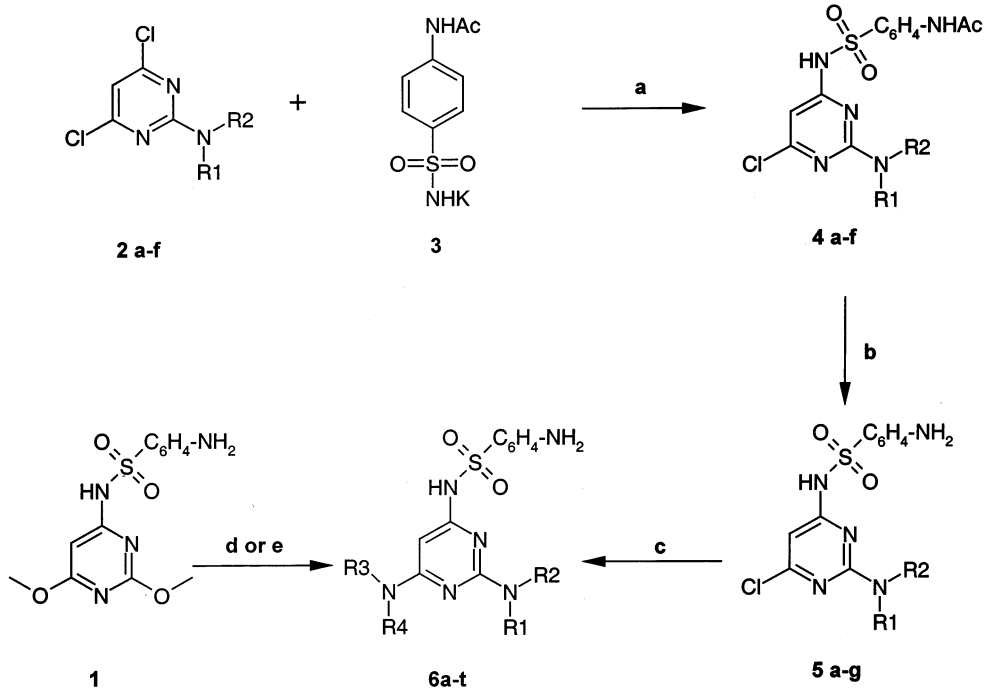

Figure 1. (a) NMP, 140°C; (b) 1 N NaOH, 1 N HCl; (c) 8 M HNR_3R_4–EtOH, 130°C; (d) NH_2CH_3 in ethanol, 150°C; (e) $NH_2C_2H_4$ 150°C.

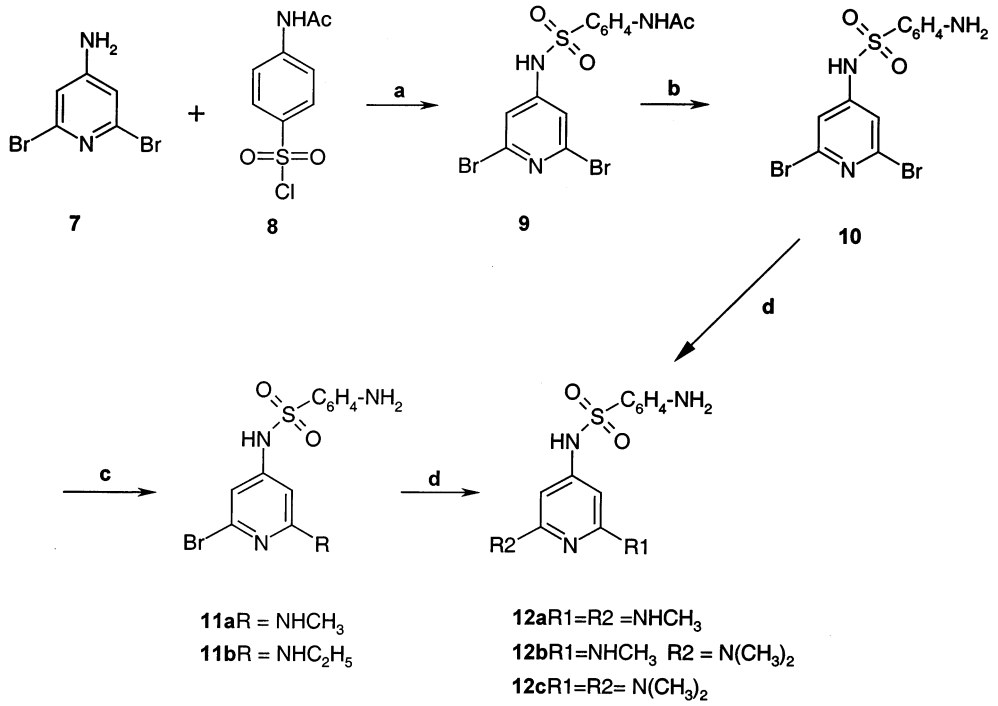

Figure 2. (a) Pyridine, 60°C; (b) 1 N NaOH, 1 N HCl; (c) HNR_1R_2, 130°C; (d) HNR_3R_4, 160°C.

known precursors [24–26] followed by reduction of the nitro group (H_2, Pd/C for **15, 23** and **19c**, $SnCl_2$ for **19a,b** and **d**). Coupling of these anilines with sulfonylchloride **8** followed by hydrolysis yielded the sulfonamides **20a**–**f**.

N-(Indol-4-yl)-4-amino-benzenesulfonamide **29** and *N-(indol-6-yl)-4-amino-benzenesulfonamide* **30** were prepared from the commercially available 4-amino-indole or known 6-amino-indole [27] and **8** and subsequent hydrolysis. 6-Bromo-4-amino-indole **27,** the building block for sulfonamide **28**, was accessible from dinitrotoluic acid **24** in three steps. The photo-Hunsdiecker reaction [28] provided the 4-bromo-2,6-dinitrotoluene **25** [29]. Subsequent Batcho-Leimgruber synthesis with *N*,*N*-dimethylformamid-dimethylacetal and reductive cyclisation of the enamine **26** afforded **27** in 51% yield *(figure 5).*

3. Pharmacology

The affinity of the compounds for 5-HT_6 human receptors was measured using [^{3}H]-LSD. The compounds behaved as competitive antagonists causing a parallel shift in the dose response curve to 5-HT and had no effect on the basal levels of cAMP, suggesting that they are antagonists at the 5-HT_6 receptor. For ligands that displayed pK_i values >7, log *D* was determined as an indication for brain penetration. Finally, compounds with log *D*>1.0 were tested for their ef-

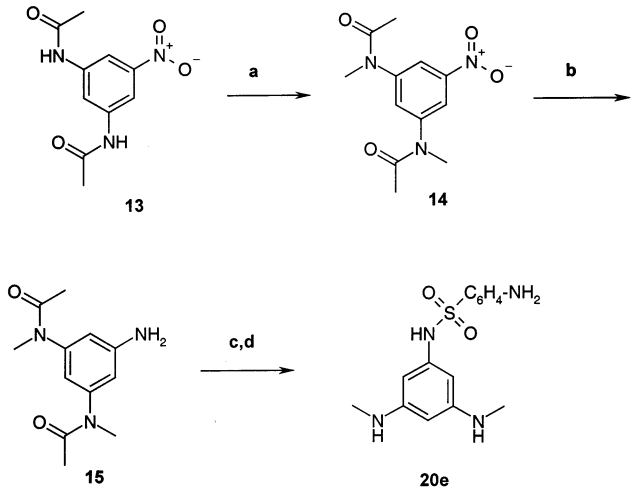

Figure 3. (a) NaH, Mel, THF; (b) H_2, Pd/C EtOH; (c) **8**, pyridine, 60°C; (d) 1 N NaOH, 1 N HCl.

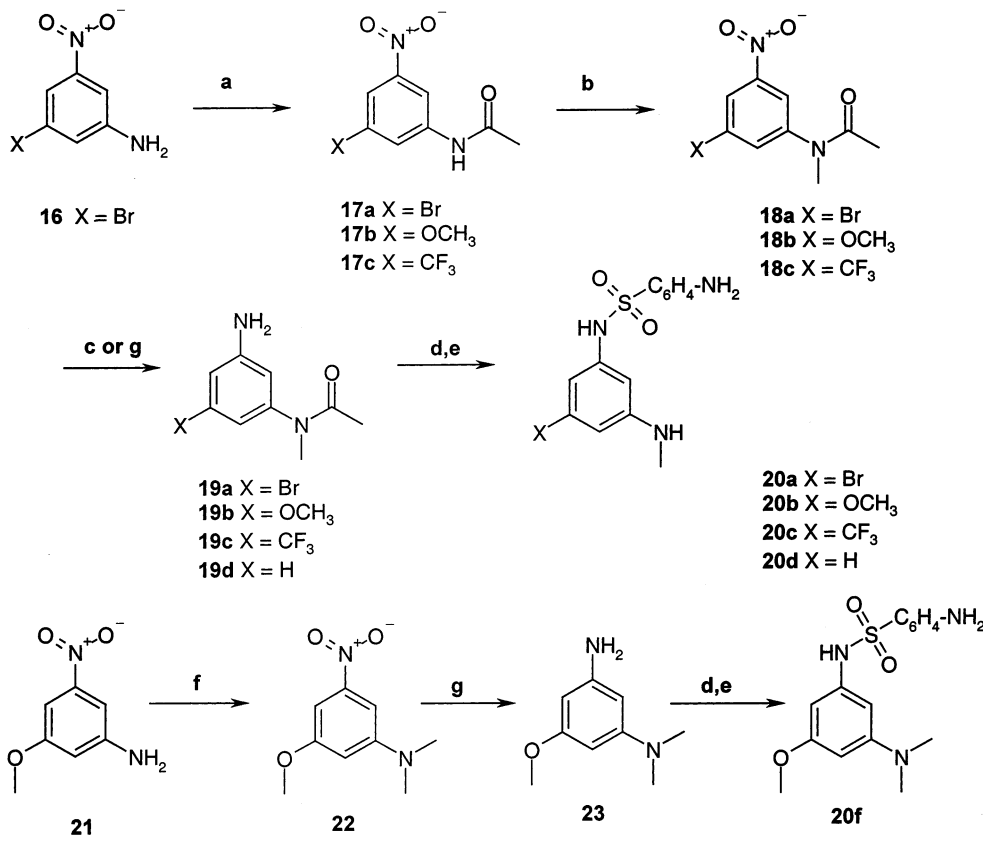

Figure 4. (a) Ac_2O, pyridine; (b) NaH, Mel, THF; (c) $SnCl_2$, AcOH; (d) **8**, pyridine–acetone, room temperature; (e) 1 N NaOH, 1 N HCl; (f) CH_2O, $NaCNBH_3$, AcOH; (g) Pd/C, H_2, MeOH.

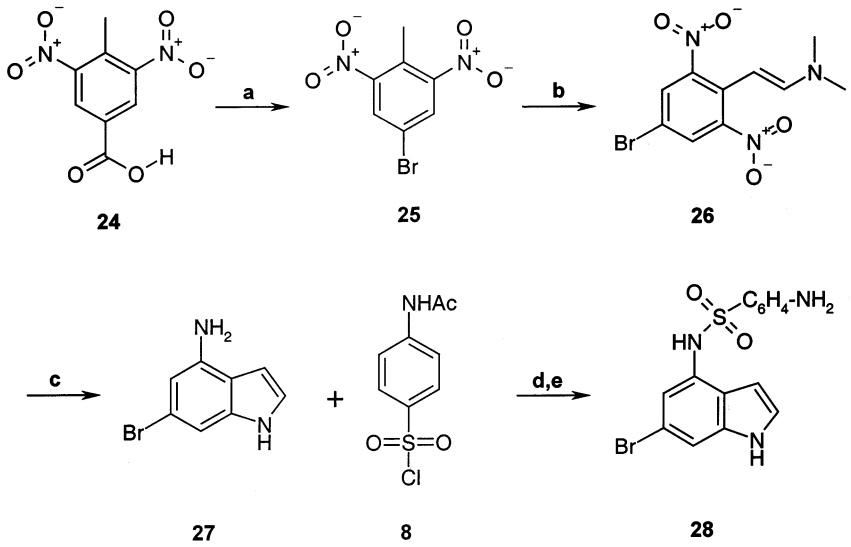

Figure 5. (a) Br_2, HgO red, hν, CCl_4; (b) $(Me)_2NCH(OMe)_2$, DMF; (c) $TiCl_3$, HCl 10%; (d) pyridine, 60°C; (e) 1 N NaOH, 1 N HCl.

Table I. Binding affinity and log *D* values for the pyrimidine derivatives **6**.

	R_1	R_2	R_3	R_4	pK_i (5-HT_6)	log *D* [a]
a	CH_3	H	CH_3	H	7.3	0.16
b	CH_3	H	C_2H_5	H	7.2	0.06
c	CH_3	H	*i*-C_3H_7	H	6.2	nd
d	CH_3	H	CH_3	CH_3	6.8	nd
e	CH_3	H	–$(CH_2)_3$–		6.7	nd
f	CH_3	H	–$(CH_2)_4$–		5.8	nd
g	C_2H_5	H	CH_3	H	6.7	nd
h	C_2H_5	H	C_2H_5	H	7.1	0.63
i	C_2H_5	H	*i*-C_3H_7	H	6.3	nd
j	C_2H_5	H	CH_3	CH_3	6.2	nd
k	C_2H_5	H	–$(CH_2)_3$–		6.7	nd
l	*i*-C_3H_7	H	C_2H_5	H	6.7	nd
m	CH_3	CH_3	CH_3	H	7.3	nd
n	CH_3	CH_3	C_2H_5	H	7.4	0.22
o [32]	CH_3	CH_3	CH_3	CH_3	6.6	nd
p	CH_3	CH_3	–$(CH_2)_3$–		6.8	nd
q	–$(CH_2)_3$–		CH_3	H	7.4	nd
r	–$(CH_2)_4$–		CH_3	H	7.1	2.44
s	H	H	CH_3	H	6.0	nd
t	H	H	C_2H_5	H	5.9	nd

[a] Octanol–20 mM phosphate buffer + 5%/vol DMSO at pH 7.4.

fectiveness in reversing a scopolamine-induced passive avoidance retention deficit in rats.

4. Results

SAR studies within the pyrimidine series **6** (*table I*) showed that methyl and ethyl groups at the amino substituent (mono and disubstitution) as well as small rings such as azetidine and pyrrolidine in position 2 gave compounds with affinity similar to the lead structure **6a**. Larger groups (>C3) and no substitution at the amino group in this position, as well as a secondary amino group at position 6 led to reduced affinity. Omitting the 4-amino functionality of **6a** or replacing it by other substituents like halogens or alkyl groups caused a loss in potency (results not shown). An increased binding displayed the analogous pyridine derivatives **11a**,**b** and **12a**–**c** (*table II*). The bromo substituted compound **11a** and **11b** [22, 23] showed a somewhat reduced affinity at the 5-HT_6 receptor, but increased lipophilicity (**11a**). The 2,6-diamino substituted pyridines **12a** and **12b** exhibited increased affinity for the 5-HT_6 receptor. Their log *D* values, albeit, were rather low.

Replacement of the heterocyclic nucleus with a phenyl ring (*table III*) produced high affinity ligands in the case of bromo-amino (**20a**), trifluoromethyl-amino (**20c**) and methoxy-amino (**20b, 20f**) substitution whereas diamino (**20e**) and monoamino (**20d**)

Table II. Binding affinity and log *D* values for the pyridine derivatives **11** and **12**.

	R_1	R_2	pK_i (5-HT_6)	log *D* [a]
11a	$NHCH_3$	Br	7.3	1.78
11b	NHC_2H_5	Br	6.8	nd
12a	$NHCH_3$	$NHCH_3$	7.8	0.03
12b	$NHCH_3$	$N(CH_3)_2$	7.9	<-1.5
12c	$N(CH_3)_2$	$N(CH_3)_2$	6.7	nd

[a] For footnotes refer to *table I*.

Table III. Binding affinity and log *D* values for the benzene derivatives **20**.

	R_1	R_2	pK_i (5-HT_6)	log *D* [a]
a	Br	$NHCH_3$	7.7	1.7
b	OCH_3	$NHCH_3$	7.4	0.36
c	CF_3	$NHCH_3$	7.5	2.0
d	H	$NHCH_3$	6.2	nd
e	$NHCH_3$	$NHCH_3$	6.7	nd
f	OCH_3	$NH(CH_3)_2$	7.8	1.22

[a] For footnotes refer to *table I*.

Table IV. Binding affinity and log *D* values for the indole derivatives.

	pK_i (5-HT$_6$)	log *D* [a]
28	7.3	1.77
29	7.2	0.41
30	5.9	nd

[a] For footnotes refer to *table I*.

Table V. Passive avoidance results and log *D* values of selected compounds.

cpd	MED [a] po (mg kg^{-1})	log *D*	pK_i (5-HT$_6$)
6r	3	2.4	7.1
11a	10	1.8	7.3
20a	10	1.7	7.7
20c	3	2.0	7.5
20f	10	1.2	7.8
28	10	1.8	7.3

[a] Minimal effective dose to reduce the scopolamine-induced retention deficit based on analysis with a two-tailed Chi square test with a *p*-value <0.05 accepted as statistically significant.

substitution was less favorable. Incorporation of the amino nitrogen of this series of ligands into a 4-sulfamoylsubstituted indole led to antagonists with pK_i values >7 (**28**, **29**). The 6-substituted indole derivative **30**, however, displayed virtually no affinity (*table IV*).

After ip administration **6a** produced a behavioural syndrome in rats consisting of yawning, stretching and chewing, which could be antagonised by atropine and scopolamine, suggesting a modulation of cholinergic transmission. Therefore, some of our 5-HT$_6$ antagonists were studied in an animal model where a cholinergic deficit produced by scopolamine underlies a cognitive dysfunction, the passive avoidance task [30]. Post-training scopolamine administration produces a robust retention deficit, which can be reversed by compounds enhancing cholinergic function, e.g. acetylcholinesterase inhibitors. Oral doses were tested at half-log intervals to determine a minimal effective dose (MED) for the selected compounds, as shown in *table V*. For comparison, the MED values for the reference acetylcholinesterase inhibitors donepezil, rivastigmine and metrifonate evaluated under the same experimental conditions were 3, 0.3 and 10 mg kg^{-1}, respectively. In addition, none of the compounds from this program showed an activity for muscarinic and nicotinic receptors up to 10 μM concentration in a CEREP screen.

In accordance with the assumption that compounds with a log *D* between 2 and 3.5 are most favorably suited for brain penetration [31] we found for **6r** and **20c** (log $D>2$) MEDs of 3 mg kg^{-1} whereas compounds with $1<\log D<2$ (**11a, 20a, 20f** and **28**) exhibited a MED of 10 mg kg^{-1}. Despite comparable or even superior potency in vitro this might reflect a more suitable physico-chemical profile for in vivo activity of the first ones.

The replacement of the sulfonamide functionality by a sulfon group led to high affinity ligands, which will be reported in due course.

5. Experimental protocols

5.1. Chemistry

5.1.1. General

Melting points were determined in capillary tubes (Büchi 530 apparatus) and are uncorrected. Column chromatography was carried out using silica gel (230–400 mesh, Merck) and 0.3–1.0 bar pressure. Spectra were recorded with the following instruments: IR (cm^{-1}): Nicolet-7199-FT-IR. ^{1}H-NMR (δ values in ppm relative to internal TMS, coupling constants *J* in Hz): Bruker AC-250 (250 MHz). MS: MS9 updated with a Finnigan MAT data system SS 200. Elemental analyses (C, H, N) for novel compounds were within 0.4% of the theoretical values.

5.1.1.1. 4-Amino-N-(2,6-bis-methylamino-pyrimidin-4-yl)-benzenesulfonamide **6a**

4-Amino-*N*-(2,6-dimethoxy-pyrimidin-4-yl)-benzenesulfonamide **1** (5.0 g,16 mmol) was dissolved in 60 mL methylamine in ethanol (33%) (60 mL) and stirred in an autoclave at 150°C for 30 h. The mixture was cooled, freed completely from solvent, triturated in 70 mL of methanol for 2 h and suction filtered. 4.2 g (84%) **6** were obtained as gray crystals; m.p. 303–305°C.

5.1.1.2. 4-Amino-N-(2,6-bis-ethylamino-pyrimidin-4-yl)-benzenesulfonamide **6h**

Using the same procedure as for the synthesis of **6a**, **6h** was obtained from 4-amino-*N*-(2,6-dimethoxy-pyrimidin-4-yl)-benzenesulfonamide (1.50 g, 4.83 mmol) and ethylamine (193 mmol, 0.79 g, 40%) as pale beige crystals, m.p. 245–250°C, MS (ISP): 335.2 $[M-H]^+$, which

were converted into a hydrochloride (1:1.5), m.p. 197–208°C. Anal. ($C_{14}H_{20}N_6O_2S$ 1:1.5 HCl) C, H, N, Cl, S.

5.1.2. General procedure for the preparation of N-(2-NR$_1$R$_2$-6-chloro-pyrimidine-4-yl)-4-amino-benzenesulfonamide ***5***

A mixture of 2-*N*R$_1$R$_2$-4,6-dichloropyrimidine (6 mmol) **2** and *N*-(4-sulfamoyl-phenyl)-acetamide potassium salt (3.03 g, 12 mmol) **3** was stirred in 1-methyl-2-pyrrolidone (10 mL) at 140°C for 8 h. After evaporation of the solvent, the residue was partitioned between ethyl acetate and water. The water phase was washed once with ethyl acetate and then acidified with 1 N HCl. The crystals of **4** were filtered off under suction, washed with water and directly used for the next step.

N-[4-(2-*N*R$_1$R$_2$-6-Chloropyrimidin-4-ylsulfamoyl)-phenyl]-acetamide **4** (2.6 mmol) was dissolved in 1 N NaOH (31 mL) and boiled at reflux for 3 h. The mixture was extracted twice with ethyl acetate. The aqueous phase was acidified with 3 N HCl and the precipitate of **5** was filtered off under suction.

5.1.2.1. 4-Amino-N-(6-chloro-2-methylamino-pyrimidin-4-yl)-benzenesulfonamide ***5a***

White crystals (89%); m.p.: >110°C; ^{1}H-NMR (DMSO): 11.15 (s, br, 1H), 7.60 (s, br, 2H), 7.35 (s, br, 1H), 6.57 (d, $J = 8.75$ Hz, 2H), 6.08 (s, 2H), 6.05 (s, 1H), 2.70 (d, $J = 4.75$ Hz, 3H); MS (ISN): m/e 312 $[M-H]^-$.

5.1.2.2. 4-Amino-N-(6-chloro-2-ethylamino-pyrimidin-4-yl)-benzenesulfonamide ***5b***

White crystals (90%); m.p.: 172–173°C; ^{1}H-NMR (DMSO): 11.10 (br, 1H), 7.63 (d, 8.75, 2H), 6.58 (d, $J = 8.75$ Hz, 2H), 6.11 (s, 2H), 6.05 (s, 1H), 3.21 (m, $J = 6.75$ Hz, 2H), 1.05 (t, $J = 6.75$ Hz, 3H), MS (ISP): m/e 328 $[M+H]^+$.

5.1.2.3. 4-Amino-N-(6-chloro-2-isopropylamino-pyrimidin-4-yl)-benzenesulfonamide ***5c***

White crystals (55%); m.p.: 94–95°C; ^{1}H-NMR (DMSO): 11.00 (s, 1H); 7.60 (br, 2H), 7.30 (br, 1H), 6.58 (d, $J = 8.75$ Hz, 2H), 6.10 (s, 2H), 6.00 (s, 1H), 3.85 (m, 1H), 1.07 (d, 6.5 Hz, 6H); MS (ISP): m/e 342 $[M+H]^+$.

5.1.2.4. 4-Amino-N-(2-azetidin-1-yl-6-chloro-pyrimidin-4-yl)-benzenesulfonamide ***5d***

Beige crystals (60%); m.p.: >203°C (dec.), ^{1}H-NMR (DMSO): 11.20 (br, s, 1H); 7.46 (d, $J = 8.75$ Hz, 2H), 6.52 (d, $J = 8.75$ Hz, 2H), 5.97 (s, 1H), 5.75 (br, s, 2H), 3.89 (t, $J = 7.5$ Hz, 4H), 2.21 (p, $J = 7.5$ Hz, 2H); MS (ISP): m/e 340 $[M+H]^+$.

5.1.2.5. 4-Amino-N-(6-chloro-2-pyrrolidin-1-yl-pyrimidin-4-yl)-benzenesulfonamide ***5e***

Beige crystals (58%); m.p.: 229–230°C; ^{1}H-NMR (DMSO): 11.25 (br, s, 1H); 7.56 (d, $J = 9$ Hz, 2H); 6.59 (d, $J = 9$ Hz, 2H); 6.10 (s, 2H); 6.04 (s, 1H); 3.33 (br, s, 4H); 1.87 (br, s, 4H); MS (ISN) m/e 352 $(M-H)^-$.

5.1.2.6. 4-Amino-N-(2-amino-6-chloro-pyrimidine-4-yl)-benzenesulfonamide ***5f***

Beige crystals (86%); m.p.: >265°C (dec.); ^{1}H-NMR (DMSO): 11.00 (br, s, 1H); 7.61 (d, $J = 9$ Hz, 2H), 7.00 (br, s, 2H), 6.58 (d, $J = 9$ Hz, 2H), 6.08 (s, 3H); MS (ISP) m/e 300 $[M+H]^+$.

5.1.3. General procedure for the preparation of N-(2-NR$_1$R$_2$-6-NR$_3$R$_4$-pyrimidin-4-yl)-4-amino-benzenesulfonamides ***6b–6n, 6p–6t***

N-(2-NR$_1$R$_2$-6-Chloropyrimidin-4-yl)-4-amino-benzenesulfonamide (1.76 mmol) **5a–g** was dissolved in an ethanolic amine solution (8 M, 176 mmol) and stirred in an autoclave at 130°C for 16 h. The suspension was filtered, the precipitate was dissolved in ethanol and treated with charcoal, filtered and the solvent removed. The residue was suspended in ethanol and filtered.

5.1.3.1. 4-Amino-N-(6-ethylamino-2-methylamino-pyrimidin-4-yl)-benzenesulfonamide ***6b***

From **5a** with ethylamine: white crystals (56%); m.p.: 252°C (dec.); ^{1}H-NMR (DMSO): 10.60 (br, s, 1H), 7.41 (d, $J = 8.75$ Hz, 2H), 7.25 (br, s, 1H), 6.52 (d, $J = 8.75$ Hz, 2H), 6.40 br, s, 1H), 5.67 (s, 2H), 5.25 (s, 1H), 3.20 (s, br, 2H), 2.72 (d, $J = 4.5$ Hz, 3H), 1.05 (t, $J = 6.75$ Hz, 3H); MS (ISN): m/e 321 $(M-H)^-$. Anal. ($C_{13}H_{18}N_6O_2S$) C, H, N, S.

5.1.3.2. 4-Amino-N-(6-isopropylamino-2-methylamino-pyrimidin-4-yl)-benzenesulfonamide ***6c***

From **5a** with isopropylamine: beige crystals (36%); m.p. 240°C (dec.); ^{1}H-NMR (DMSO): 10.60 (br, 1H); 7.40 (d, $J = 8.75$ Hz, 2H), 7.25 (br, s, 1H), 6.52 (d, $J = 8.75$ Hz, 2H), 6.40 (br, s, 1H), 5.70 br, s, 2H), 5.30 (s, 1H), 4.10 (br, 1H), 2.71 (d, $J = 4.5$ Hz, 3H), 1.07 (d, $J = 6.5$ Hz, 6H); MS (ISP): m/e 337 $[M+H]^+$. Anal. ($C_{14}H_{20}N_6O_2S$) C, H, N, S. Found: 24.33. Calc.: 24.98.

5.1.3.3. 4-Amino-N-(6-dimethylamino-2-methylamino-pyrimidin-4-yl)-benzene-sulfonamide ***6d***

From **5a** with dimethylamine: beige crystals (78%); m.p.: >300°C; ^{1}H-NMR (DMSO): 10.70 (br, s, 1H); 7.43 (d, $J = 8.75$ Hz, 2H), 6.52 (d, $J = 8.75$ Hz, 2H), 6.40 (br, s, 1H), 5.72 (s, 2H), 5.33 (s, 1H), 2.93 (s, 6H), 2.73 (d, $J = 4.5$ Hz, 3H); MS (ISP): m/e 323 $[M+H]^+$. Anal. ($C_{13}H_{18}N_6O_2S$) C, H, N, S.

5.1.3.4. 4-Amino-N-(6-azetidin-1-yl-2-methylamino-pyrimidin-4-yl)-benzenesulfonamide ***6e***

From **5a** with azetidine: white crystals (72%); m.p.: 295–296°C; ^{1}H-NMR (DMSO): 10.50 (br, s, 1H); 7.42 (d, $J = 8.75$ Hz, 2H), 6.53 (d, $J = 8.75$ Hz, 2H), 5.74 (s, 2H), 5.04 (s, 1H), 3.88 (t, $J = 7.5$ Hz, 4H), 2.70 (d, $J = 5.0$ Hz, 3H), 2.28 (qi, $J = 7.5$ Hz, 2H); MS (ISP): m/e 335 $[M+H]^+$. Anal. ($C_{14}H_{18}N_6O_2S$) C, H, N, S.

5.1.3.5. 4-Amino-N-(2-methylamino-6-pyrrolidin-1-yl-pyrimidin-4-yl)-benzenesulfonamide ***6f***

From **5a** and pyrrolidine: beige crystals (63%); m.p.: >300°C; ^{1}H-NMR (DMSO): 10.75 (br, s, 1H), 7.43 (d, $J = 8.75$ Hz, 2H), 6.52 (d, $J = 8.75$ Hz, 3H), 5.70 (s, 2H), 5.21 (s, 1H), 3.0–3.5 (m, 4H), 2.73 (d, $J = 4.5$ Hz, 3H), 1.86 (br, s, 4H); MS (ISN): m/e 347 ($M-H)^-$. Anal. ($C_{15}H_{20}N_6O_2S$) C, H, N, S.

5.1.3.6. 4-Amino-N-(2-ethylamino-6-methylamino-pyrimidin-4-yl)-benzenesulfonamide ***6g***

From **5b** with methylamin: beige crystals (54%); m.p.: 261–263°C; ^{1}H-NMR (DMSO): 10.50 (br, s, 1H); 7.41 (d, $J = 8.75$ Hz, 2H), 7.25 (br, s, 1H), 6.52 (d, $J = 8.75$ Hz, 3H), 5.70 (br, s, 2H), 5.30 (br, s, 1H), 3.23 (m, $J = 6.75$ Hz, 2H), 2.68 (br, s, 3H), 1.06 (t, $J = 6.75$ Hz, 3H); MS (ISP): m/e 323 $[M+H]^+$.

5.1.3.7. 4-Amino-N-(6-ethylamino-2-isopropylamino-pyrimidin-4-yl)-benzenesulfonamide ***6i***

From **5b** with isopropylamine: white crystals (43%); m.p.: 266–267°C; ^{1}H-NMR (DMSO): 10.25 (s, 1H), 7.40 (d, $J = 8.75$ Hz, 2H), 7.25 (br, s, 1H), 6.51 (d, $J = 8.75$ Hz, 2H), 6.35 (br, s, 1H), 5.67 (s, 2H), 5.25 (s, 1H), 3.95 (sext, $J = 6.5$ Hz, 1H), 3.2 (br, s, 2H), 1.11 (d, $J = 6.5$ Hz, 6H), 1.05 (t, $J = 7.25$ Hz, 3H); MS (ISN): m/e 349 $[M-H]^-$. Anal. ($C_{15}H_{22}N_6O_2S$) C, H, N, S.

5.1.3.8. 4-Amino-N-(6-dimethylamino-2-ethylamino-pyrimidin-4-yl)-benzenesulfonamide ***6j***

From **5b** with dimethylamine: white crystals (83%); m.p.: 301–302°C; ^{1}H-NMR (DMSO): 10.50 (br, s, 1H); 7.43 (d, $J = 8.75$ Hz, 2H), 6.53 (d, $J = 8.75$ Hz, 3H), 5.72 (s, 2H), 5.32 (s, 1H), 3.23 (qd, $J = 4.75$ Hz, 4.75, 2H), 2.97 (s, 6H), 1.06 (t, $J = 4.75$ Hz, 3H); MS (ISP): m/e 337 $[M+H]^+$. Anal. ($C_{14}H_{20}N_6O_2S$) C, H, N, S.

5.1.3.9. 4-Amino-N-(6-azetidin-1-yl-2-ethylamino-pyrimidin-4-yl)-benzenesulfonamide ***6k***

From **5b** and azetidine: white crystals (78%); m.p.: 292–293°C; ^{1}H-NMR (DMSO): 10.50 (br, s, 1H), 7.42 (d, $J = 8.75$ Hz, 2H), 6.53 (d, $J = 8.75$ Hz, 3H), 5.73 (s, 2H), 5.03 (s, 1H), 3.88 (t, $J = 7.5$ Hz, 4H), 3.20 (qd, $J = 7.0$, 7.0 Hz, 2H), 2.26 (p, $J = 7.5$ Hz, 2H), 1.04 (t, $J = 7.0$ Hz, 3H), MS (ISP): m/e 349 $[M+H]^+$. Anal. ($C_{15}H_{20}N_6O_2S$) C, H, N, S.

5.1.3.10. 4-Amino-N-(6-ethylamino-2-isopropylamino-pyrimidin-4-yl)-benzenesulfonamide ***6l***

From **5c** and ethylamine: white crystals (43%); m.p.: 266–267°C; ^{1}H-NMR (DMSO): 10.25 (br, s, 1H), 7.40 (d, $J = 8.75$ Hz, 2H), 7.25 (br, s, 1H), 6.51 (d, $J = 8.75$ Hz, 2H), 6.35 (br, s, 1H), 5.67 (s, 2H), 5.25 (s, 1H), 3.95 (sext, $J = 6.5$ Hz, 1H), 3.20 (q, $J = 7.25$ Hz, 2H), 1.11 (d, $J = 6.5$ Hz, 6H), 1.05 (t, $J = 7.25$ Hz, 3H): MS (ISN): m/e 349 $[M-H]^-$. Anal. ($C_{15}H_{22}N_6O_2S$) C, H, N, S.

5.1.3.11. 4-Amino-N-(2-dimethylamino-6-methylamino-pyrimidin-4-yl)-benzenesulfonamide ***6m***

From **5g** [27] and methylamine: beige crystals (21%); m.p.: 253–254°C; ^{1}H-NMR (DMSO): 10.20 (br, s, 1H); 7.48 (d, $J = 8.75$ Hz, 2H), 6.50 (br, s, 1H), 6.54 (d, $J = 8.75$ Hz, 2H), 5.90 (s, 2H), 5.34 (s, 1H), 2.98 (s, 6H), 2.68 (s, 3H); MS (ISP): m/e 323 $[M+H]^+$. Anal. ($C_{13}H_{18}N_6O_2S$) C, H, N.

5.1.3.12. 4-Amino-N-(2-dimethylamino-6-ethylamino-pyrimidin-4-yl)-benzenesulfonamide ***6n***

From **5g** and ethylamine: beige crystals (40%); m.p.: 237–238°C; ^{1}H-NMR (DMSO): 10.20 (br, s, 1H), 7.48 (d, $J = 8.75$ Hz, 2H), 6.90 (br, s, 1H), 6.55 (d, $J = 8.75$ Hz, 2H), 5.90 (br, s, 2H); 5.35 (s, 1H); 3.16 (m, 2H); 2.96 (s, 6H); 1.05 (t, $J = 7.25$ Hz, 3H); MS (ISP): m/e 337 $[M+H]^+$. Anal. ($C_{14}H_{20}N_6O_2S$) C, H, N, S.

5.1.3.13. 4-Amino-N-(6-azetidin-1-yl-2-dimethylamino-pyrimidin-4-yl)-benzenesulfonamide ***6p***

From **5g** and azetidine: beige crystals (61%); m.p.: 239–240°C; ^{1}H-NMR (DMSO): 10.25 (br, s, 1H); 7.48 (d, $J = 8.75$ Hz, 2H), 6.55 (d, $J = 8.75$ Hz, 2H), 5.94 (s, 2H), 5.11 (s, 1H); 3.85 (t, $J = 7.25$ Hz, 4H); 2.94 (s, 6H), 2.25 (p, $J = 7.25$ Hz, 2H); MS (ISP): m/e 349 $[M+H]^+$. Anal. ($C_{15}H_{20}N_6O_2S$) C, H, N, S.

5.1.3.14. 4-Amino-N-(2-azetidin-1-yl-6-methylamino -pyrimidin-4-yl)-benzenesulfonamide ***6q***

From **5d** and methylamine: light beige crystals (27%); m.p.: >260°C (dec.); ^{1}H-NMR (DMSO): 10.70 (br, s, 1H), 7.46 (d, $J = 8.75$ Hz, 2H), 7.10 (br, s, 1H); 6.53 (d, $J = 8.75$ Hz, 2H), 5.78 (br, s, 2H), 5.36 (br, s, 1H), 3.94 (t, $J = 7.5$ Hz, 4H); 2.65 (s, 3H), 2.20 (p, $J = 7.5$ Hz, 2H); MS (ISP): m/e 335 $[M+H]^+$. Anal. ($C_{14}H_{18}N_6O_2S$) C, H, N, S.

5.1.3.15. 4-Amino-N-(6-methylamino-2-pyrrolidin-1-yl-pyrimidin-4-yl)-benzenesulfonamide ***6r***

From **5e** and methylamine: beige crystals (30%); m.p. 299–301°C. ^{1}H-NMR (DMSO) 10.3 (b, 1H), 7.46 (d, 2H, $J = 8.5$ Hz), 6.92 (b, 1H), 6.53 (d, 2H, $J = 8.6$ Hz), 5.79 (bs, 2H), 5.33 (s, 1H), 3.37 (m, 4H), 2.68 (s, 3H), 1.83 (s, 4H). MS (ISP) m/e 349.3 $[M+H^+]$. Anal. ($C_{15}H_{20}N_6O_2S$) C, H, N.

5.1.3.16. 4-Amino-N-(2-amino-6-methylamino-pyrimidin-4-yl)-benzenesulfonamide ***6s***

From **5f** and methylamine: beige crystals (10%); m.p.: 285°C (dec.); ^{1}H-NMR (DMSO): 10.60 (s, 1H); 7.40 (br, s, 1H); 7.39 (d, $J = 8.75$ Hz, 2H); 6.50 (br, s, 2H); 6.51 (d, $J = 8{,}75$ Hz, 2H); 5.64 (s, 2H), 5.30 (br, s, 1H), 2.68 (br, s, 3H): MS (ISP) m/e 295 $[M+H^+]$. Anal. ($C_{11}H_{14}N_6O_2S$) C, H, N, S.

5.1.3.17. 4-Amino-N-(2-amino-6-ethylamino-pyrimidin-4-yl)-benzenesulfonamide ***6t***

From **5f** and ethylamine: beige crystals (53%); m.p. 272–274°C; ^{1}H-NMR (DMSO): 10.60 (s, br, 1H), 7.39 (d, $J = 8.75$ Hz, 2H), 7.25 (br, s, 1H), 6.51 (d, $J = 8.75$ Hz, 2H), 6.45 (br, s, 2H), 5.63 (s, 2H), 5.30 (br, s, 1H), 3.25 (br, s, 3H); 1.03 (t, $J = 7.25$ Hz, 3H); MS (ISP) m/e 309 $[M+H^+]$. Anal. ($C_{12}H_{16}N_6O_2S$) C, H, N, S.

5.1.4. Synthesis of N-(pyridin-4-yl)-4-amino-benzenesulphonamides

5.1.4.1. N-[4-(2,6-Dibromo-pyridin-4-ylsulfamoyl)-phenyl]-acetamide ***9***

A solution of 4-amino-2,6-dibromo-pyridine (1.24 g, 5 mmol) **7** and 4-acetaminobenzenesulfochloride **8** (1.30 g, 5.5 mol) in pyridine (25 mL) was stirred at 60°C for 16 h. The solvent was removed and the residue was taken up in 1 N HCl (50 mL) and extracted twice with ethyl acetate (50 mL). The organic phase was washed with brine, dried (Na_2SO_4) and evaporated. The residue was purified by flash-chromatography (SiO_2, ethyl acetate–hexane 4:1) to yield 1.77 g (79%) of **9** as yellow crystals, m.p.: >260°C (dec.); ^{1}H-NMR (DMSO): 11.70 (br, s, 1H), 10.42 (s, 1H), 7.82 (m, 4H), 7.20 (s, 2H), 2.08 (s, 3H); MS (ISN): m/e 448 $[M-H^-]$. Anal. ($C_{13}H_{11}Br_2N_3O_3S$) C, H, N, Br, S.

5.1.4.2. 4-Amino-N-(2,6-dibromo-pyridin-4-yl)-benzenesulfonamide ***10***

A solution of *N*-[4-(2,6-dibromo-pyridin-4-ylsulfamoyl)-phenyl]-acetamide **9** (1.25 g, 3.5 mmol) in 1 N NaOH (35 mL) was boiled at reflux for 2 h. After cooling the mixture was adjusted to pH 6 with 2 N HCl and the precipitate was filtered off, washed with water and dried. Chromatography (SiO_2, ethyl acetate–hexane 1:2 → 1:1) yielded 1.22 g (86%) **10** as beige crystals, m.p.: 220–222°C; ^{1}H-NMR (DMSO): 11.30 (br, s, 1H); 7.51 (d, $J = 8.75$ Hz, 2H), 7.17 (s, 1H), 6.62 (d, $J = 8.75$ Hz, 2H), 6.24 (s, 2H); MS (EI): m/e 252 [M^+, 100], 173, 171. Anal. ($C_{11}H_9Br_2N_3O_2S$) C, H, N, Br, S.

5.1.4.3. 4-Amino-N-(2-bromo-6-methylamino-pyridin-4-yl)-benzenesulfonamide ***11a***

4-Amino-*N*-(2,6-dibromo-pyridin-4-yl)-benzenesulfonamide **10** (0.81 g, 2 mmol) was stirred in a 8 M solution of methylamine in ethanol (35 mL) in an autoclave at 130°C for 44 h. The reaction mixture was evaporated and the residue was purified by flash-chromatography (SiO_2, ethyl acetate–hexane 1:2) to yield 0.56 g (80%) **11a** as white crystals, m.p.: 173–174°C (dec.); ^{1}H-NMR (DMSO): 10.40 (s, 1H), 7.46 (d, $J = 8.75$ Hz, 2H); 6.86 (d, $J = 4.75$ Hz, 1H), 6.59 (d, $J = 8.75$ Hz, 2H), 6.31 (s, 1H), 6.12 (m, 3H), 2.63 (d, $J = 4.75$ Hz, 3H), 6.12 (m, 3H); MS (ISN): m/e 357, 355 $[M-H^-]$. Anal. ($C_{12}H_{13}BrN_4O_2S$) C, H, N, Br, S.

5.1.4.4. 4-Amino-N-(2-bromo-6-ethylamino-pyridin-4-yl)-benzenesulfonamide ***11b***

4-Amino-*N*-(2,6-dibromo-pyridin-4-yl)-benzenesulfonamide 10 (1.0 g, 2.46 mmol) was stirred in a 4 M solution of ethylamine in dioxane (20 mL) in an autoclave at 135°C for 20 h. The reaction mixture was evaporated and the residue was purified by flash-chromatography (SiO_2, ethyl acetate–hexane) to yield 0.285 g (31%) **11b** as beige crystals, m.p.: >133°C (dec.); ^{1}H-NMR (DMSO): 10.40 (s, 1H); 7.46 (d, $J = 8.75$ Hz, 2H), 6.85 (s, br, 1H), 6.59 (d, $J = 8.75$ Hz, 2H), 6.28 (s, 1H), 6.12 (s, 3H); 3.1 (qd, $J = 7.5$, 7.5 Hz, 2H), 1.05 (t, $J = 7.5$ Hz, 3H); MS (ISN): m/e 371, 369 $[M-H^-]$. Anal. ($C_{13}H_{15}BrN_4O_2S$) C, H, N, Br, S.

5.1.4.5. 4-Amino-N-(2,6-bis-methylamino-pyridin-4-yl)-benzenesulfonamide ***12a***

4-Amino-*N*-(2,6-dibromo-pyridin-4-yl)-benzenesulfonamide **10** (0.90 g, 2 mmol) was stirred in a 8 M solution of methylamine in ethanol (35 mL) in an autoclave at 160°C for 16 h. The reaction mixture was evaporated and the residue purified by flash-chromatography (SiO_2, ethyl acetate–hexane 1:1) to yield 0.34 g (55%) of a brown oil, which was converted into the hydrochloride **12a** (HCl 1:2) as off-white crystals; m.p.: 189–192°C (dec.), ^{1}H-NMR (DMSO): 12.12 (s, 1H), 10.94 (s, 1H), 7.80 (br, s, 2H), 7.53 (d, $J = 8.75$ Hz, 2H), 7.30–6.50 (br, 3H); 6.64 (d, $J = 8.75$ Hz, 2H); 5.60 (s, 2H), 2.76 (s, 6H); MS (ISN): m/e 306 [M−H$^-$]. Anal. ($C_{13}H_{17}N_5O_2S$.1:2 HCl) C, H, N, Cl, S.

5.1.4.6. 4-Amino-N-(2,6-bis-dimethylamino-pyridin-4-yl)-benzenesulfonamide ***12b***

4-Amino-*N*-(2,6-dibromo-pyridin-4-yl)-benzenesulfonamide **10** (0.90 g, 2 mmol) was stirred in a 5.6 M solution of dimethylamine in ethanol (35 mL) in an autoclave at 160°C for 16 h. The reaction mixture was evaporated and the residue purified by flash-chromatography (SiO_2, ethyl acetate–hexane 1:1) to yield 0.54 g (81%) **33** as beige crystals, m.p. 157–160°C (dec.), ^{1}H-NMR (DMSO): 9.88 (s, 1H), 7.46 (d, $J = 8.75$ Hz, 2H), 6.55 (d, $J = 8.75$ Hz, 2H), 6.00 (s, 2H), 5.37 (s, 2H), 2.86 (s, 12H). Anal. (for hydrochloride) ($C_{15}H_{21}N_5O_2S$.1:2 HCl) C, H, N, Cl, S.

5.1.4.7. 4-Amino-N-(2-dimethylamino-6-methylamino-pyridin-4-yl)-benzenesulfonamide ***12c***

4-Amino-*N*-(2-bromo-6-methylamino-pyridin-4-yl)-benzenesulfonamide **11a** (0.71 g, 2 mmol) was stirred in a 5.6 M solution of dimethylamine in ethanol (35 mL) in an autoclave at 160°C for 16 h. The reaction mixture was evaporated and the residue purified by flash-chromatography (SiO_2, ethyl acetate–hexane 1:1) to yield 0. 424 g (66%) **34** as beige crystals; m.p.: 98°C (dec.); ^{1}H-NMR (DMSO): 9.80 (s, 1H); 7.44 (d, $J = 8.75$ Hz, 2H); 6.55 (d, $J = 8.75$ Hz, 2H); 5.98 (s, 2H); 5.92 (q, $J = 4.75$ Hz, 1H), 5.48 (s, 2H), 2.84 (s, 6H) 2.62 (d, $J = 4.75$ Hz, 3H), 2.84 (s, 6H), MS (ISP): $m/e = 322$ ($C_{14}H_{20}N_5O_2S^+$).

5.1.5. General procedure for the preparation of 4-amino-N-(R_1,R_2-phenyl)-benzenesulfonamide ***20a–f***

A solution of 2.6 mmol of the aniline and 4-acetaminobenzenesulfochlorid **8** (0.86 g, 3.7 mmol) in acetone (10 mL) and pyridine (0.5 mL) was stirred for 1 h. The solvent was removed and the residue suspended in CH_2Cl_2 (10 mL) and 1 N HCl (10 mL). The precipitate was filtered off, washed with H_2O and dried in vacuo. The solid was dissolved in 2 N NaOH (30 mL) and heated at 100°C for 2 h. After cooling to room temperature the pH was adjusted to 5 with 25% HCl. The mixture was extracted three times with 20 mL CH_2Cl_2 (20 mL), the combined organic layers were washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash-chromatography on silica gel to give the sulfonamides **20a–f** (*table III*).

5.1.5.1. 4-Amino-N-(3-methylamino-phenyl)-benzenesulfonamide ***20d***

From *N*-(3-amino-phenyl)-*N*-methyl-acetamide **19d** [33] and **8**: yellowish solid (97%); m.p.: 134–135°C; ^{1}H-NMR (DMSO): 9.55 (s, 1H), 7.38 (d, $J = 8.5$ Hz, 2H), 6.85 (t, $J = 7.5$ Hz, 1H), 6.52 (d, $J = 8.5$ Hz, 2H), 6.29 (s, 1H), 6.24 (d, $J = 7.5$ Hz, 1H), 6.12 (d, $J = 7.5$ Hz, 1H), 5.93 (s, 2H), 5.60 (q, $J = 5$ Hz, 1H), 2.56 (d, $J = 5$ Hz, 3H); MS (EI): m/e 277 [M$^+$], 213 (100). Anal. ($C_{13}H_{15}N_3O_2S$) C, H, N, S.

5.1.5.2. 4-Amino-N-(3,5-bis-methylamino-phenyl)-benzenesulfonamide hydro-chloride ***20e***

From *N*-[3-(acetyl-methyl-amino)-5-amino-phenyl]-*N*-methyl-acetamide **15** and **8**, followed by treatment with HCl in ethanol: pink colored amorphous solid (40%); ^{1}H-NMR (DMSO): 10.05 (s, 1H); 7.49 (d, $J = 8.5$ Hz, 2H), 8.0–6.5 (br, 6.5H), 6.63 (d, $J = 8.5$ Hz, 2H); 6.39 (s, 2H), 6.27 (s, 1H), 2.67 (s, 6H); MS (ISN): m/e 305 [M−H$^-$]. Anal. ($C_{14}H_{18}N_4O_2SHCl_{2.6}$) C, H, N, Cl, S. Found: 13.36. Calc.: 13.84.

5.1.5.3. 4-Amino-N-(3-bromo-5-methylamino-phenyl)-benzenesulfonamide ***20a***

From *N*-(3-Amino-5-bromo-phenyl)-*N*-methyl-acetamide **19a** and **8**; colorless foam (85%); ^{1}H-NMR ($CDCl_3$): 7.58 (d, $J = 8.5$ Hz, 2H), 6.61 (d, $J = 8.5$ Hz, 2H), 6.48 (s, 1H), 6.46–6.40 (m, 2H), 6.30 (dd, $J = 2$ and 2 Hz), 4.11 (s, br, 2H), 3.78 (q, br, $J = 5$ Hz, 1H), 2.74 (d, $J = 5$ Hz, 3H); MS (ISP):m/e 358, 356 [M+H$^+$]; Anal. ($C_{13}H_{14}BrN_3O_2S$) C, H, N, Br, S.

5.1.5.4. 4-Amino-N-(3-methoxy-5-methylamino-phenyl)-benzenesulfonamide ***20b***

From 4-Amino-*N*-(3-methoxy-5-methylamino-phenyl)-benzenesulfonamide **51** and **8**; colorless foam (65%). ^{1}H-NMR (DMSO): 9.54 (s, 1H), 7.38 (d, $J = 8.5$ Hz, 2H), 6.52 (d, $J = 8.5$ Hz, 2H), 5.94 (s, 1H), 5.86 (dd,

$J = 2$ and 2 Hz, 1H), 5.68 (dd, $J = 2$ and 2 Hz, 1H), 5.58 (q, $J = 5$ Hz, 1H), 3.57 (s, 3H), 2.54 (d, $J = 5$ Hz, 3H); MS (EI): m/e 307 [M^+], 243 (100). Anal. (C_{14} $H_{17}N_3O_3S$) C, H, N, S.

5.1.5.5. 4-Amino-N-(3-dimethylamino-5-methoxy-phenyl)-benzenesulfonamide ***20f***

From 4 - Amino - *N* - (3 - dimethylamino - 5 - methoxy-phenyl)-benzenesulfonamide **23** and **8**. beige crystals (39%); m.p.: 161.0–161.5°C; ^{1}H-NMR (DMSO): 9.82 (s, 1H); 7.41 (d, $J = 8.5$ Hz, 2H), 6.53 (d, $J = 8.5$ Hz, 2H), 6.03 (dd, $J = 2$ and 2 Hz, 1H), 6.00 (dd, $J = 2$ and 2 Hz, 1H); 5.96 (s, br, 2H), 5.83 (dd, $J = 2$ and 2 Hz, 1H), 3.61 (s, 3H), 2.78 (s, 6H); MS (EI): m/e 321 [M^+], 257 (100). Anal. (C_{15} $H_{19}N_3O_3S$) C, H, N, S.

5.1.5.6. 4-Amino-N-(3-methylamino-5-trifluoromethyl-phenyl)-benzenesulfonamide ***20c***

From 4-Amino-*N*-(3-methylamino-5-trifluoromethyl-phenyl)-benzenesulfonamide **10c** and **8**. light yellow crystals (72%); m.p.: 125°C; ^{1}H-NMR ($CDCl_3$): 7.58 (d, $J = 8.5$ Hz, 2H); 6.67 (s, 1H), 6.60 (d, $J = 8.5$ Hz, 2H), 6.51 (s, br, 2H), 6.49 (s, br, 1H), 4.11 (s, 2H), 3.97 (q, br, $J = 5$ Hz, 1H), 2.79 (d, $J = 5$ Hz, 3H); MS (ISP): m/e 346.2 [$M+H^+$]. Anal. ($C_{14}H_{14}F_3N_2O_2S$) C, H, F, N, S.

5.1.6. Preparation of the anilins ***15, 19a–19c*** *and* ***23***

5.1.6.1. N-[3-(Acetyl-methyl-amino)-5-nitro-phenyl]-N-methyl-acetamide ***14***

N-(3-acetylamino-5-nitro-phenyl)-acetamide 13 (0.24 g, 1 mmol) was suspended in THF (20 mL), treated with NaH (0.092 g, 2.3 mmol) and DMF (10 mL) and stirred at room temperature for 15 h. Methyl iodide (0.29 mL, 4.6 mmol) was added and the mixture was stirred for 48 h. After evaporation of the solvent, the residue was taken up in water (100 mL) and extracted four times with ethyl acetate (80 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), filtered and evaporated. The residue was purified by flash-chromatography (SiO_2, CH_2Cl_2–MeOH 97:3) to give 0.2 g (75%) **14** as a brown oil. ^{1}H-NMR ($CDCl_3$): 8.02 (s, 2H); 7.54 (s, 1H), 3.38 (s, 6H), 2.10 (s, br, 6H); MS (EI): m/e 265 [M^+], 223 (100), 181, 135.

5.1.6.2. N-[3-(Acetyl-methyl-amino)-5-amino-phenyl]-N-methyl-acetamide ***15***

N-[3-(acetyl-methyl-amino)-5-nitro-phenyl]-*N*-methyl-acetamide **14** (0.19 g, 0.72 mmol) was dissolved in ethanol (15 mL), treated with Pd/C (10%) (19 mg) and hydrogenated at room temperature for 2 h. The catalyst was filtered off, the solvent was distilled off and the residue was purified by flash-chromatography (SiO_2, ethyl acetate) to give 0.16 g (94%) **15** as white crystals; m.p.: 179–181°C; ^{1}H-NMR ($CDCl_3$): 6.48 (s, 2H); 6.40 (s, 1H), 3.90 (s, br, 2H), 3.24 (s, 6H), 1.94 (s, 6H); MS (EI): m/e 235 [M^+, 100], 193, 150.

5.1.6.3. N-(3-Bromo-5-nitro-phenyl)-acetamide ***17a***

Acetic anhydride (1.6 mL, 16.7 mmol) was added to a solution of 3-bromo-5-nitro-phenylamine **16** (1.5 g, 6.9 mmol) in pyridine (15 mL). The reaction mixture was stirred for 12 h and than poured into water (100 mL). After stirring for 15 min, the precipitate was collected by filtration to yield after drying in vacuo at 50°C 1.6 g (90%) **17a** as a beige solid. ^{1}H-NMR (DMSO): 10.55 (s, br, 1H); 8.47 (dd, $J = 2$ and 2 Hz, 1H), 8.20 (dd, $J = 2$ and 2 Hz, 1H), 8.03 (dd, $J = 2$ and 2 Hz, 1H), 2.10 (s, 3H); MS (EI): m/e 260 [M^+], 43 (100).

5.1.6.4. N-(3-Bromo-5-nitro-phenyl)-N-methyl-acetamide ***18a***

To a solution of *N*-(3-bromo-5-nitro-phenyl)-acetamide **17a** (1.60 g, 6.2 mmol) in *N*,*N*-dimethyl-formamide (30 mL), NaH (55–65% Dispersion in oil) (0.296 g, 6.8 mmol) was added. After 1 h methyliodide (1.15 mL, 3.0 mmol) was added and the solution stirred for 16 h at room temperature. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (20 mL) and washed with H_2O (15 mL). The aqueous layer was washed twice with CH_2Cl_2 (20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash-chromatography (SiO_2, CH_2Cl_2–methanol 45:1) to give 1.5 g (88%) **47** as a brown oil. ^{1}H-NMR ($CDCl_3$): 8.32 (dd, $J = 2$ and 2 Hz, 1H); 8.05 (dd, $J = 2$ and 2 Hz, 1H), 7.74 (dd, $J = 2$ and 2 Hz, 1H), 3.34 (s, 3H), 2.05 (s, br, 3H); MS (EI): m/e 274, 272 [M^+], 232, 230 (100).

5.1.6.5. N-(3-Amino-5-bromo-phenyl)-N-methyl-acetamide ***19a***

18a (1.50 g, 5.5 mmol) in ethanol (25 mL) and HCl conc. (10 mL) $SnCl_2.2H_2O$ (5.0 g, 22 mmol) were added to a solution of 1.50 g (5.5 mmol) *N*-(3-bromo-5-nitro-phenyl)-*N*-methyl-acetamide. The reaction mixture was stirred for 24 h and then poured into crushed ice. The pH of the solution was adjusted to 10 with 4 N NaOH-solution. The aqueous mixture was extracted twice with ethyl acetate (20 mL) and the combined organic layers were washed with brine, dried ($MgSO_4$), filtered and

concentrated in vacuo. The residue was purified by flash-chromatography on silica gel (CH_2Cl_2–MeOH 19:1) to give 1.00 g (74%) **19a** as a beige solid. ^{1}H-NMR ($CDCl_3$): 6.79 (dd, $J = 2$ and 2 Hz, 1H); 6.71 (dd, $J = 2$ and 2 Hz, 1H), 6.40 (dd, $J = 2$ and 2 Hz, 1H), 3.86 (s, br, 2H), 3.20 (s, 3H), 1.92 (s, br, 3H); MS (EI): *m*/*e* 244, 242 [M^+], 202, 200 (100).

5.1.6.6. N-(3-Methoxy-5-nitro-phenyl)-N-methyl-acetamide ***18b***

Obtained from **17b** as a beige solid (90%) as described for **18a**. ^{1}H-NMR ($CDCl_3$): 7.65–7.72 (m, 2H); 7.08 (s, br, 1H), 3.92 (s, 3H), 3.30 (s, 3H), 1.98 (s, br, 3H); MS (EI): *m*/*e* 224 [M^+], 182 (100).

5.1.6.7. N-(3-Amino-5-methoxy-phenyl)-N-methyl-acetamide ***19b***

Obtained from **18b** as a beige oil (65%) as described for **15**. ^{1}H-NMR ($CDCl_3$): 6.19 (dd, $J = 2$ and 2 Hz, 1H); 6.14–6.08 (m, 2H), 3.80 (s, br, 2H), 3.26 (s, 3H), 3.21 (s, 3H), 1.93 (s, br, 3H); MS (EI): *m*/*e* 194 [M^+].

5.1.6.8. N-(3-Methoxy-5-nitro-phenyl)-dimethyl-amine ***22***

Paraformaldehyde (1.8 g, 59.3 mmol) was added to a solution of 1.00 g (5.9 mmol) of 3-methoxy-5-nitro-phenylamine **21** (1.00 g, 5.9 mmol) in acetic acid (40 mL, 1.8 g, 59.3 mmol), followed by 1.8 g (28.8 mmol) $NaCNBH_3$ (1.8 g, 28.8 mmol) at 10°C. After stirring for 16 h at room temperature the solution was poured into ice/water (100 mL) and the pH adjusted to 10 with conc. NaOH. The solution was extracted three times with CH_2Cl_2 (150 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash-chromatography (SiO_2, CH_2Cl_2–hexanes 4:1) to give 0.85 g (73%) **53** as an orange solid. ^{1}H-NMR ($CDCl_3$): 7.19 (dd, 2.1 and 2.1, 1H); 7.09 (dd, $J = 2.1$ and 2.1 Hz, 1H), 6.45 (dd, $J = 2.1$ and 2.1 Hz, 1H), 3.85 (s, 3H), 3.01 (s, 6H); MS: (EI) *m*/*e* 196 [M^+].

5.1.6.9. 5-Methoxy-N,N-dimethyl-benzene-1,3-diamine ***23***

Pd on charcoal (10%) (100 mg) was added to a solution of (3-methoxy-5-nitro-phenyl)-dimethyl-amine **22** (0.84 g, 4.3 mmol) in methanol (25 mL) and the resulting suspension was hydrogenated for 16 h. After filtration and evaporation of the solvent, 0.68 g (95%) of **54** was obtained as a beige oil. ^{1}H-NMR ($CDCl_3$): 5.76 (dd, $J = 2$ and 2 Hz, 1H); 5.20 (d, $J = 2$ Hz, 2H), 3.75 (s, 3H), 3.59 (s, br, 2H), 2.89 (s, 6H); MS (ISP): *m*/*e* 166 [M^+].

5.1.6.10. N-Methyl-N-(3-nitro-5-trifluoromethyl-phenyl)-acetamide ***18c***

Obtained from **23** as a red oil (88%) according to the procedure for **18a**. ^{1}H-NMR ($CDCl_3$): 8.47 (s, br, 1H), 8.32 (s, br, 1H), 7.82 (s, br, 1H), 3.41 (s, 3H), 2.12 (s, br, 3H); MS (EI): *m*/*e* 262 [M^+], 220 (100).

5.1.6.11. N-(3-Amino-5-trifluoromethyl-phenyl)-N-methyl-acetamide ***19c***

Obtained from **18c** as a beige solid (85%) according to the procedure for **15**. ^{1}H-NMR ($CDCl_3$): 6.85 (s, br, 1H); 6.80 (s, br, 1H), 6.63 (s, br, 1H), 4.01 (s, br, 2H), 3.24 (s, 3H), 1.92 (s, br, 3H); MS (ISP): *m*/*e* 232 [M^+].

5.1.6.12. 5-Bromo-2-methyl-1,3-dinitro-benzene ***25***

Bromine (15.45 mL, 300 mmol) was slowly added to a suspension of 3,5-dinitro-4-methyl benzoic acid **24** (45.2 g, 20 mmol) and HgO red (65.0 g, 300 mol) in CCl_4 (800 mL), which had been heated to 65°C by means of a 800 W lamp. The reaction mixture was stirred at this temperature for another 2.5 h, then cooled to ambient temperature and filtered. The filtrate was washed with sat. bicarbonate solution and the organic phase was dried (Na_2SO_4) and evaporated. The residue (35 g) was treated with ammonia (100 mL) in methanol at 80°C for 1 h. After evaporation water (200 mL) and 2N HCl (80 mL) were added and the aqueous solution was extracted twice with ethyl acetate (150 mL). The combined organic phases were washed with brine, dried (Na_2SO_4) and evaporated to yield 27.13 g (52%) **25** as orange colored crystals; m.p.: 88–89°C; ^{1}H-NMR ($CDCl_3$): 8.06 (s, 2H), 2.45 (s, 3H); MS (EI): *m*/*e* 262, 260 [M^+], 254, 243 (100), 89.

5.1.6.13. 6-Bromo-1H-indol-4-ylamine ***27***

5-Bromo-2-methyl-1,3-dinitro-benzene **25** (1.3 g, 5 mmol) and dimethyl formamid dimethylacetal (0.95 mL, 5.5 mmol) were dissolved in DMF (40 mL) and heated to reflux for 16 h. Upon evaporation one obtained crude (E)-[2-(4-bromo-2,6-dinitro-phenyl)-vinyl]-dimethyl-amine **26** (1.3 g), which was treated with $TiCl_3$ (34 mL (15% in 10% HCl)) for 0.5 h. The reaction mixture was poured onto 2 N NaOH (100 mL) and extracted three times with ethyl acetate (80 mL). The combined organic phase was dried (Na_2SO_4), filtered and evaporated. The residue was purified by flash-chromatography (SiO_2, hexane–ethyl acetate 4:1) to yield 0.24 g (51%) **27** as an

off-white solid; m.p.: 128°C; ^{1}H-NMR ($CDCl_3$): 8.10 (s, br, 1H), 7.07 (m, 1H), 7.0 (m, 1H), 6.54 (d, $J = 1.5$ Hz, 1H), 6.42 (m, 1H), 3.97 (s, br, 2H); MS (EI): *m/e* 212, 210 [M^+, 100], 131, 104. Anal. ($C_8H_7BrN_2$) C, H, N.

5.1.6.14. 4-Amino-N-(6-bromo-1H-indol-4-yl)-benzenesulfonamide ***28***

Obtained as an off-white solid (49%) from **27** and **8** as described for **10**; m.p.: 229°C; ^{1}H-NMR ($CDCl_3$): 11.19 (s, 1H), 9.91 (s, 1H), 7.40 (d, $J = 8.7$ Hz, 2H), 7.22 (s, 2H), 6.99 (s, 1H), 6.71 (s, 1H) 6.49 (d, $J = 8.7$ Hz, 2H), 5.95 (s, 2H); MS (ISP): *m/e* 368, 366 [$M+H]^+$. Anal. ($C_{14}H_{12}Br\ N_3O_2S$) C, H, N.

5.1.6.15. 4-Amino-N-(1H-indol-4-yl)-benzenesulfonamide ***29***

Obtained as a white foam (66%) from 4-amino-indole (Fluka) and **8** as described for **10**; ^{1}H-NMR (DMSO): 11.00 (br, s, 1H), 9.6 (br, s, 1H), 7.39 (d, $J = 9$ Hz, 2H), 7.20 (t, 1H), 7.08 (d, 1H), 6.88 (m, 2H), 6.68 (s, 1H), 6.46 (d, $J = 9$ Hz, 2H), 5.86 (s, 2H); MS (ISP): *m/e* 288 $[M+H]^+$. Anal. ($C_{14}H_{13}N_3O_2S$) C, H, N.

5.1.6.16. 4-Amino-N-(1H-indol-6-yl)-benzenesulfonamide ***30***

Obtained as beige crystals (55%) from 6-amino-indole [22] and **8** as described for **10**; m.p.: 186–188°C (dec.); ^{1}H-NMR (DMSO): 10.94 (br, s, 1H), 9.52 (s, 1H), 7.32 (d, $J = 9$ Hz, 3H), 7.22 (s, 1H), 7.14 (s, 1H), 6.73 (d, $J = 8.5$ Hz, 1H); 6.47 (d, $J = 9$ Hz, 2H), 6.29 (s, 1H), 5.88 (br, s, 2H); MS (ISP): *m/e* 288 $[M+H]^+$. Anal. ($C_{14}H_{13}N_3O_2S$) C, H, N.

5.2. Log D determination

Log *D* values were determined as partition coefficients between water–octanol mixtures starting from DMSO solutions dispensed in aqueous buffer (TAPSO, pH 7.4) at 50 mM concentration. These solutions were incubated by shaking for 2 h with 1-octanol and were kept standing overnight to reach equilibrium. After separation of the layers the concentration of the aqueous phase was determined by UV-absorption (OD measurement) and the log *D* value was calculated from the measured and the reference OD.

5.3. Receptor binding assays

The affnity at the human 5-HT_6 receptor was measured on membranes obtained from Hela cells stably expressing the human 5-HT_6 receptor. Hela cells were grown in Dulbecco's modified Eagle's medium (DMEM)+10% foetal bovine serum (FBS) containing penicillin (100 iu mL^{-1}) and streptomycin (100 mg mL^{-1}) in a humidified atmosphere (5% CO_2). The cells were detached with phosphate buffered saline (PBS) containing 1 mM EDTA, washed with PBS by two centrifugations (10 min, 500 g) and the resulting pellet was resuspended in 50 mM, ice-cold Tris–HCl (pH 7.4) containing 10 mM $MgCl_2$ and 0.5 mM EDTA by use of a polytron homogeniser (15 s at maximal speed), at a concentration corresponding to 4×10^7 cells per mL and aliquots were stored at −80°C. 5-HT_6 receptor binding assays were performed with [^{3}H]-lysergic acid diethylamide ([^{3}H]-LSD); specific activity 86 Ci $mmol^{-1}$, Amersham). Membranes corresponding to 4×10^5 cells per assay tube were used for the binding assay, resuspended in an assay buffer consisting of Tris–HCl 50 mM, pargyline 10^{-5} M, $MgCl_2$ 5 mM and ascorbic acid 0.1%, pH 7.4. For estimations of the expression levels and the affinity of [^{3}H]-LSD for the receptor binding sites, saturation experiments were performed with eight concentrations of [^{3}H]-LSD (0.163± 20 nM). Competition curves were constructed with seven concentrations of the displacing agents (one data point per log unit of concentration: 10^{-10}–10^{-4} M). Binding assays consisted of 100 mL of the membrane preparation expressing the 5-HT_6 receptor, 50 mL of [^{3}H]-LSD and 50 mL of a displacing drug or assay buffer. Non-specific binding was measured in the presence of 10^{-5} M 5-HT. Incubations were carried out for 1 h at 37°C and reactions were stopped by rapid filtration through Whatmann GF/B filters by use of a Filtermate 196 (Packard Canberra). The filters were washed with 362 mL Tris–HCl (50 mM, pH 7.4) and the radioactivity retained on the filters was measured by scintillation spectroscopy in 50 mL of scintillation fluid. All experiments were performed in triplicate and repeated three times. The dissociation constants for [^{3}H]-LSD binding to human 5-HT_6 receptors, IC50 values, K_i values and Hill coefficients were calculated by use of EBDA and LIGAND [34, 35].

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