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Discovery of 5-HT$_6$ receptor ligands based on virtual HTS

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Abstract—Based on a pharmacophore alignment on a 5-HT$_6$ ligand applying 4SCan® technology, a new lead series was identified and further structurally investigated. K_is down to 8 nM were achieved.

The human 5-HT$_6$ receptor was first reported in 1996 and is now one of at least fourteen 5-HT receptors, which are grouped into seven families 5-HT$_1$ through 5-HT$_7$. Except for 5-HT$_3$, which is a ligand-gated ion channel, all subtypes represent GPCRs. The 5-HT$_6$ receptor is almost exclusively expressed in the CNS, it is positively coupled to adenylate cyclase and appears to regulate glutaminergic and cholinergic neuronal activity. For its pharmacological function, an involvement in schizophrenia, cognition, memory and learning, appetite control, convulsive disorder, affective state, and seizure is discussed. A few representatives of 5-HT$_6$ ligands have already entered clinical trials for the therapeutic indications anxiety and cognitive impairment associated with schizophrenia and Alzheimer's disease and displayed first encouraging results.[1]

As crystal structure data was not available for this receptor, a usually preferable in silico docking approach could not be applied to the discovery of new structural types of 5-HT$_6$ ligands. Based on a highly potent agonist E-6837 (Fig. 1), which was generated at Esteve by focused syntheses of designed molecules,[2,3] a pharmacophore alignment on this compound was performed using 4SCan®[4] with a virtual library of around 3.3 Mio commercially available compounds. A set of top 2000 compounds with the highest alignment score was further cross evaluated—likewise by alignment—against other known 5-HT$_6$ receptor ligands (e.g., SB-271046, SB-357134, Ro 04-6790; K_is = 1.3, 3.2 and 50 nM, respectively),[5] to give additional criteria for a final selection of 235 compounds, which was submitted for biological testing in a binding assay on the 5-HT$_6$ receptor.[6] This initial screen directly led to the identification of three structurally closely related compounds **1**–**3** with K_i values down to 61 nM (Fig. 1). Nitroarene **1** was identified to be a partial agonist,[7] for which a receptor profile (72 targets) was screened at a compound concentration of 10 μM, including adrenoceptors, dopaminergic,

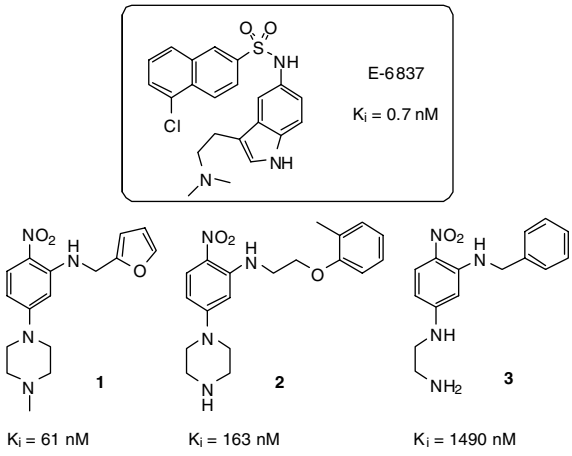

Figure 1. Template for a pharmacophore alignment and hit structures **1**–**3** from biological testing.

Keywords: Serotonin receptor; 5-HT$_6$; GPCR; Molecular modeling; vHTS.
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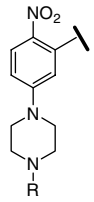
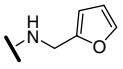
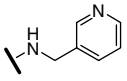
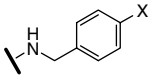
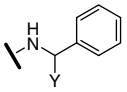
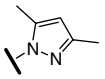
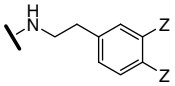
histaminergic, and other serotonergic receptors as well as the serotonin transporter (SERT). Inhibition above 90% was observed only for 5-HT_{2B}, 5-HT_{2C}, histamine H_1 and dopamine D_3 receptors, and SERT, whereas inhibitions of 5-HT_3, 5-HT_4, and 5-HT_{5a} were below 40%, and ranged from 61% to 77% for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_7 at a 10 μM concentration.

Several 2,4-diaminated nitroarenes are commercially available due to their simple preparation involving S_NAr strategies, thus allowing for an easy validation of the hit class established by compounds **1**–**3** and for gaining first ideas on a SAR. Further 60 nitroarenes were selected by a 2D similarity search for this purpose, ordered, and tested (for a selection, cf. Tables 1 and 2). However, a synthetic route had to be established in parallel for the attachment of two different amines to an aromatic ring when envisaging a replacement of the nitro head group by other functionalities like carboxylic acids, nitriles, and arylethers and striving for a free choice of amination positions within the arene (Scheme 1). In general, a selection of head groups and the 2-amino substituent focused on attaining a broad structural diversity, resulting in a structural set appropriate for the establishment of a computational CoMFA model for further enhancement of affinity toward the 5-HT_6 receptor. The second amine (to be located in the 4- or 5-position) was defined to be *N*-methylpiperazine for almost all molecules to be synthesized as present in hit molecule **1**. This portion was supposed to be robust, easily introducible by Pd chemistry, and did not require additional protective group chemistry. For a realization of this strategy, a twofold transition metal catalyzed aromatic amination sequence was established. A detailed methodical evaluation of this approach using palladium and copper chemistry with a discussion of advantages and disadvantages over S_NAr reactions can be deduced from reference.[8] Appropriately halogenated arenes required as starting materials were not commercially available for all desired head groups in place and had to be synthesized according to standard procedures, involving aniline oxidation to the corresponding nitroarenes using perborates (in **4** and **5**, Scheme 1),[9] etherification (in **5**) as well as esterification (in **5** and **6**). Substrates obtained by these steps were next submitted to a twofold aromatic amination sequence, as were commercially available benzonitrile **8**, nitroarene **9**, and anisole **10** (Fig. 2).[8]

Final functional group transformations proved to be highly intriguing for nitroarenes like **7** (X = NO_2), the nitro group of which was scheduled to be reduced and acetylated or sulfonylated. A regioselective acylation within a 1,2-diaminoarene prepared from, for example, **11** by nitro reduction (Scheme 2) was not expected to succeed, but had to be realized eventually due to the failure of a Boc protection of the secondary amine function in *ortho* position to the nitro group prior to said reduction (e.g., Boc_2O, CH_2Cl_2, cat. DMAP, rt, 6 h), which was caused by the strong electron withdrawing character of the latter. Nitro reduction was performed using Pd/C and hydrogen to give the corresponding aniline, which was next acetylated with acetic anhydride to give a mixture of mono- and diacetylated products (Scheme 2).

Table 1. 4-Piperazinylnitroarenes

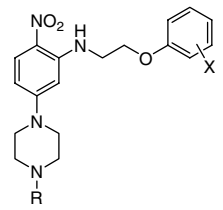


Entry	2-Amino substituent	R = Me K_i [nM] (inhib. @ 10 μM)	R = H K_i [nM] (inhib. @ 10 μM)
1		61[a]	25[a]
2		1267	1512
			
3	X = H	— (74%)	28[b]
4	X = CI	59	8[b]
5	X = F	172[c]	n.d.
			
6	Y = Me	12[a]	— (13%)
7	Y = Ph	4489	n.d.
8		250	482
			
9	Z = H	119	33[a]
10	Z = OMe	— (24%)	— (59%)

n.d., not determined.
[a] Partial agonist.
[b] Full agonist.
[c] All compounds commercially available except for this one.

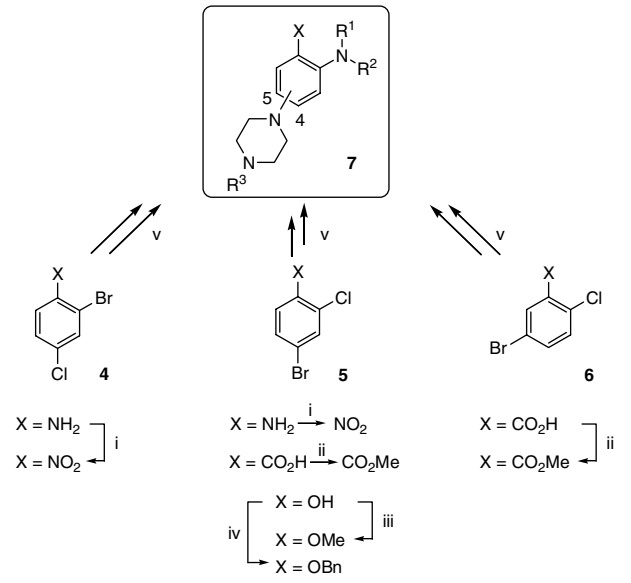
Separation of these products could only be achieved partly by preparative LCMS, positions of acetyl groups within the resulting pure compounds **12** were assigned by NMR investigations. N-acetylated 2-phenoxyaniline and 2-pyrazolylaniline (Fig. 3) were synthesized accord-

Table 2. 2-(2-Aryloxyethylamino)nitroarenes



Entry	X =	R = Me K_i [nM] (inhib. @ 10 μM)	R = H K_i [nM]
11	H	n.d.	114
12	2-OMe	19[a]	
13	3-OMe	— (22%)	
14	4-OMe	245	
15	2-Me	26[a]	163
16	3-Me	— (10%)	
17	4-tBu	80	
18	3-Me, 4-Me	— (67%)	
19	4-CI	— (21%)	290

n.d., not determined; all compounds commercially available.
[a] Partial agonist.



Scheme 1. Reagents and conditions: (i) HOAc, $NaBO_3 \cdot H_2O$, 60 °C, 3 h, 48%; (ii) MeOH, cat. H_2SO_4, reflux, 12 h, 95%; (iii) dimethyl sulfate, K_2CO_3, acetone, reflux, 5 h, 98%; (iv) BnBr, K_2CO_3, acetone, reflux, 5 h, 93%; (v) [Pd] or [Cu] catalyzed amination reactions, cf. Ref. 8; NR^1R^2 = different amines (cf. Tables); R^3 = Me, Bu, Ph, 2-pyridyl.

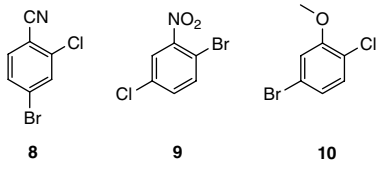
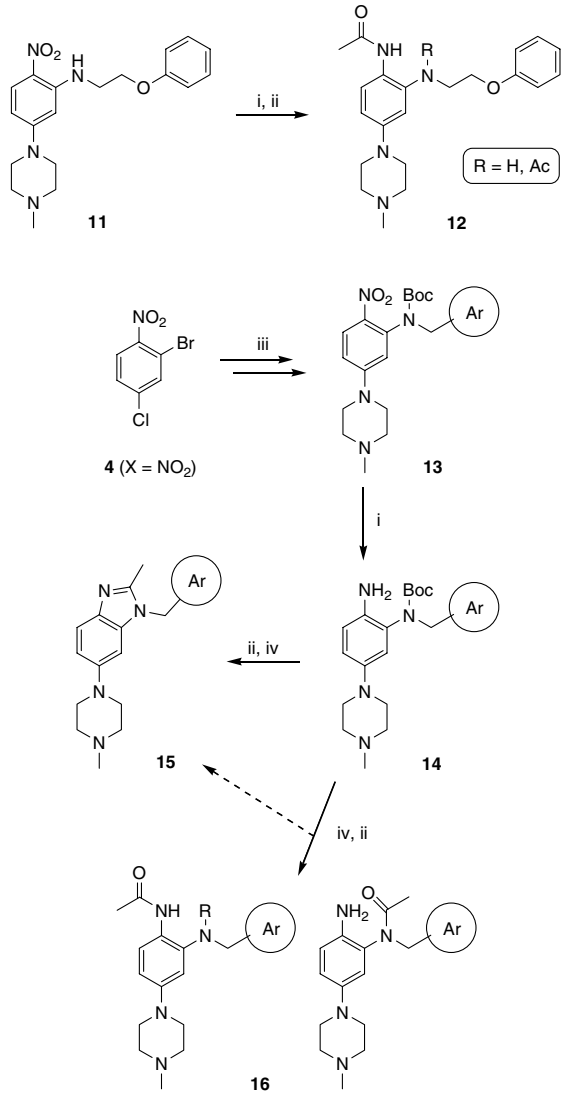


Figure 2. Building blocks for a twofold aromatic amination sequence.

ingly to give each one single product. For *ortho*-(arylmethylamino)nitroarenes, the nitro group could not be reduced selectively in the presence of the *N*-(arylmethyl) unit under various reduction conditions. As mentioned above, an *N*-Boc protecting group was not easily introduced, so that the synthetic strategy for these compounds had to be altered starting with the first transition metal catalyzed reaction: *N*-Boc-benzylamine or *N*-Boc-furfurylamine was directly coupled to the corresponding 2-bromo-nitroarene **4** (X = NO_2), and upon second amination,[8] reduction of the nitro group in **13** succeeded without removal of the benzyl or furfuryl group. In order to obtain a clean acetylation of the aniline group of **14**, the synthetic pathway had to follow N-acetylation prior to the Boc-removal step. Unfortunately, standard conditions for Boc-removal led to a quantitative intramolecular condensation within the N-acetylated intermediate to give benzimidazole **15**, which was isolated and tested for Ar = furyl, but proved to be inactive on the 5-HT_6 receptor. Thus, Boc-removal had to be realized prior to N-acetylation, which, in this case, resulted only in the formation of minor amounts of **15**, but gave rise to a product mixture of mono- and



Scheme 2. Reagents and conditions: (i) H_2, Pd/C, THF or diisopropylether, rt, 2–20 h; (ii) Ac_2O, THF, rt, 1 h; (iii) [Pd] or [Cu] catalyzed amination reactions, cf. Ref. 8; (iv) 4.0 M HCl/dioxane, rt, 1 h.

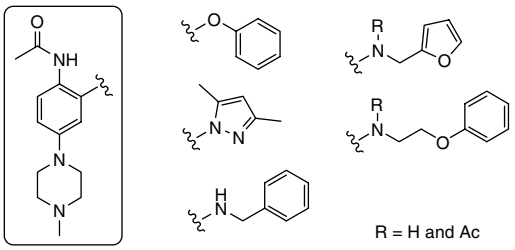


Figure 3. Inactive *N*-acetyl compounds.

diacetylated products **16**, not all components of which could be isolated. N-Sulfonylation (preparation of compounds **18** and **19**, Fig. 4) followed the same protocol, using mesylchloride or phenylsulfonylchloride instead of acetic anhydride (THF, rt, 8 h). Carboxylic acids (Table 4, entry 28; Table 5, entry 34) were prepared from the corresponding methyl esters by saponification (LiOH, H_2O/dioxane, rt, 20 h).

SAR analysis was performed varying head groups, the 2-amino substituent with great structural diversity, the position of the 2nd amine (4- vs 5-amination), and the structure of the latter within a narrow scaffold. A clear SAR, however, was not apparent for all of these variations.

From a series of nitroarenes, biological data were acquired for each pair of 2-amino compounds with either an unsubstituted or a N-methylated piperazine unit (Tables 1 and 2), the comparison of which disclosed that N-methylation at this position might be rather detrimental to affinity. This, however, was not a general tendency as for some compounds, the discrepancy in affinity was not found to be dramatic if there was any at all (Table 1, entries 1, 2, and 8). For the 1-phenethylamino substituted compound of entry 6 (Table 1), the N-methylated compound even displayed an excellent K_i of 12 nM as compared to a virtual inactivity of the corresponding N-unsubstituted derivative (similar but less emphasized for entry 15, Table 2). As to the aromatic portion of the 2-(arylmethylamino) substituent within the R = Me series, unsubstituted phenyl and 3-pyridyl displayed only moderate affinity (an inhibition of 75% at 10 μM usually correlated into a K_i around 1–2 μM), which was clearly disadvantageous compared to 2-furfuryl (entry 1 vs 2 and 3). Here, *para*-halogenation of the phenyl ring resulted in a boost in affinity, with chlorine being favorable over fluorine (entries 4 and 5 vs 3), as

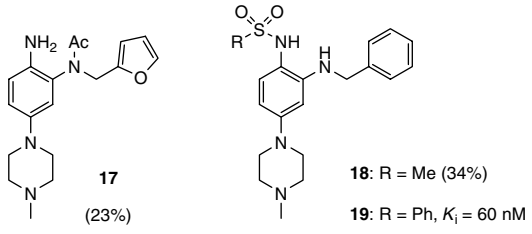


Figure 4. Aniline and sulfonamides; inhibition at 10 μM given in parentheses.

was likewise observed for the incorporation of an α-methyl within the benzylamine unit (entry 6). Exchange of this methyl by a sterically more demanding phenyl resulted in a drop in affinity (entry 7 vs 6). Extension of the carbon chain toward phenethylamine also resulted in a good affinity with a K_i of 119 nM (entry 9 vs 3). Interestingly, these correlations could not be transferred to the R = H series in Table 1, where the benzylamino derivative was equipotent as furfurylamino- and 2-phenethylaminoarenes (entry 3 vs 1 and 9). The 1-phenethylamino derivative of entry 6, however, displayed virtually no activity. It can be assumed, that the additional methylation at the piperazine nitrogen resulted in a small but distinct variation of orientation of the molecule within the receptor pocket, which led to a slightly different positioning of the benzyl unit, which seems to be favorable within the R = H series, but is now not allowing for additional steric encumbrance in the α position of the benzylamine.

Among the 2-benzylaminonitroarenes, further evaluation of possible N-substituents (not shown) for the 4-piperazinyl group revealed *N*-(2-hydroxyethyl), *N*-(2-pyridyl), and *N*-acetyl to lead to rather inactive compounds. *N*-(*n*-Butyl) and *N*-phenyl displayed at least an inhibition of around 55% at 10 μM (as compared to 74% for the *N*-Me derivative, Table 1, entry 3). *N*-Benzyl derivatization resulted in an excellent K_i of 34 nM and thus represents a promising substitution pattern for further synthetic efforts. Other piperazine derivatives were tested within the 2-furfurylaminonitroarene series (not shown), for which N-attachment of differently substituted benzoyl groups or mesyl and phenylsulfonyl units gave rise to no affinity again. Likewise inactive were compounds with the piperazine being replaced by a piperidine or morpholine (which were still N-attached to the nitroarene). From this collection of data it can be concluded that an additional basic amine nitrogen is required for attaining good binding properties, as compounds without this nitrogen or with a nitrogen with reduced basicity due to, for example, acylation displayed no significant affinities. Furthermore, a rigidified diamine framework proved to be essential, as compound **3** with its 2-aminoethylamine displayed a decrease in activity by a factor of 50 as compared to the piperazinyl derivative of entry 3 (Table 1, R = H).

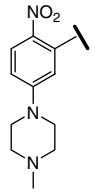
Within the 2-aryloxyethylamino series (Table 2), only two examples are present with a pair of data for R = H and R = Me, not allowing for a conclusion concerning its influence on 5-HT_6 affinity (entries 15 and 19). A 2-substitution of the aryloxy moiety seemed to be best, *meta*-substitution rather lead to poor affinities (entries 12 and 15 vs 13 and 16, respectively). In general, a 3-substituent, either alone or in combination with a 4-substituent, did not yield decent affinities (cf. Table 2, entries 13, 16, 18 and Table 1, entry 10—even though on a different scaffold). Substitution in *para* position was tolerated, with the only exception being a 4-chloro substituent within the R = Me series (entries 14 and 17 vs 19). Again, a huge discrepancy was attained here for the latter for the R = H series, in which 4-chlorophenoxy substitution resulted in a K_i of 290 nM.

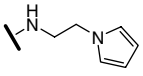
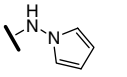
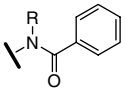
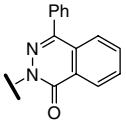
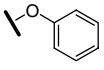
Varying the general framework for the 2-substituent of the nitroarenes, direct attachment of a pyrazole (Table 1, entry 8) or an *N*-aminopyrrole (Table 3, entry 21) was tolerated and resulted in moderate K_is of 250–480 nM. Incorporation of 2-(*N*-pyrrolyl)ethylamine as compared to 2-phenethylamine was clearly unfavorable (Table 3, entry 20 vs Table 1, entry 9). Attachment of amide functions as in entries 22 through 25 proved to be non-desirable as well, with only a sterically most demanding 4-phenylphthalazinone as amine substituent giving rise to a micromolar affinity. Obtained only as a side product in one of the palladium catalyzed amination reactions,[8] the diaryl ether of entry 26 was found to be among the most potent 5-HT_6 ligands, which represents a highly intriguing scaffold for further investigations.

An exchange of the nitro head group for a carboxylic ester, acid or alkoxy groups (Table 4), which seemed desirable as nitroarenes do not belong to the most favorable pharmacophores, resulted in rather mediocre affinities for the 5-HT_6 receptor with only three compounds displaying a K_i around 450 nM, each with a different 2-amino group: for the ester, a 2-phenethylamino residue proved to be superior (entry 27), anisole derivatives were most active with a 2-benzylamino group (entry 30), and benzyloxy derivatives showed such an affinity only for 2-furfurylamino substitution (entry 31). However, the benzyloxy series displayed the broadest structural window with regard to affinities, which differed only by a factor of 3 for all three amino groups tested (between 402 and 1284 nM), which was only matched by the benzonitriles (entry 29), showing one of the best potencies for non-nitroarenes with a K_i of 108 nM. A similar benzonitrile derivative with a 2-phenoxyethylamino substitution (not shown) gave rise to a K_i of 380 nM.

Acetylamino or sulfonylamino head groups resulted mostly in inactive compounds (Figs. 3 and 4 and Table 5, entry 36), with the only exception being phenylsulfonamide **19** with an excellent K_i of 60 nM, as compared to almost no affinity for the corresponding mesylamide **18**. When changing the regiochemistry by moving the

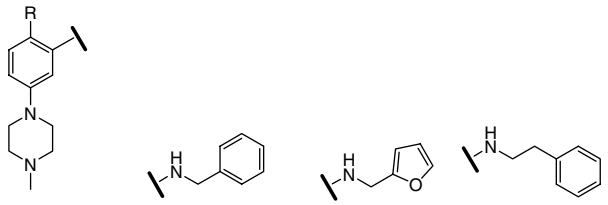
Table 3. 4-Piperazinylnitroarenes



Entry	2-Amino substituent	K_i [nM] (inhib. @ 10 μM)
20		— (78%)
21		481
		
22	R = H	Inactive
23	R = Me	— (49%)
24		— (24%)
25		1221[a]
26		27[b]

[a] Compound commercially available.
[b] Partial agonist.

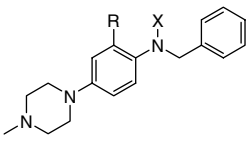
Table 4. Substitution of the nitro head group



Entry	R =	K_i [nM] (inhib. @ 10 μM)	K_i [nM] (inhib. @ 10 μM)	K_i [nM] (inhib. @ 10 μM)
3/1/9	NO_2	— (74%)	61	119
27	CO_2Me	— (72%)	— (31%)	462
28	CO_2H	— (49%)	Inactive	Inactive
29	CN	108[a]	1365	n.d.
30	OMe	409	— (53%)	1013
31	OBn	928	402	1284

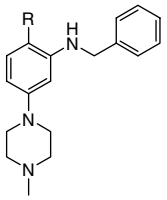
n.d., not determined.
[a] Partial agonist.

Table 5. Substitution of the nitro head group and change in substitution pattern



Entry	R =	X =	K_i [nM] (inhib. @ 10 μM)
32	NO_2	H	— (64%)
33	CO_2Me	H	1888
34	CO_2H	H	— (37%)
35	OMe	H	— (49%)
36	NHAc	Ac	Inactive
37	NH_2	Ac	164

Table 6. Affinity data on other receptors



Receptor	R = CN K_i [nM] (inhib. @ 1 μM)	R = $PhSO_2NH$ (**19**) —(inhib. @ 1 μM)
5-HT_{2A}	47	— (61%)
5-HT_{2B}	— (68%)	— (63%)
5-HT_{2C}	— (72%)	— (14%)
5-HT_7	— (52%)	— (6%)
H_1	— (65%)	— (2%)

N-methylpiperazine from the 4- into the 5-position (Table 5), affinities were quite low for all head groups tested, except for the aniline of entry 37 with an K_i of 164 nM, which, however, possesses a certain potential for displaying mutagenic effects in vivo.

As already observed for nitroarene **1** (Fig. 1 and Table 1, entry 1, R = Me), most other compounds tested for functionality were found to be partial agonists at the 5-HT_6 receptor as well (marked in Tables 1–4). In contrast, the benzylamino-nitroarenes of Table 1, entries 3 and 4, R = H displayed full agonism. Further investigations are currently being made to explore potential correlations of the magnitude of the agonistic effect with substitution patterns within this structural class of 5-HT_6 ligands.

For sulfonamide **19** and the 2-benzylaminobenzonitrile of entry 29 being identified as new lead structures for further structural evaluations (K_is around or below 100 nM), some selectivity data was acquired (Table 6). Both compounds displayed no selectivity issues at a compound concentration of 1 μM at 5-HT_{2B}, 5-HT_{2C}, 5-HT_7, and H_1-receptors, which was also true at 5-HT_{2A} for compound **19**. The benzonitrile showed good affinity toward the latter (K_i below 100 nM), selectivity of which has hence to be addressed in further synthetic endeavors.

In summary, a highly potent class of 5-HT_6 ligands was identified using the 4SCan® in silico technology, with activities down to a K_i of 8 nM for nitroarenes and 108 and 60 nM for a benzonitrile and a phenylsulfonamide, respectively. Some SAR trends have been identified, a conclusive and elaborated analysis should better be performed by computational methods (CoMFA model), though, which will be published in due course. Highly intriguing variants within this structural class to be further explored would be diarylethers like the one in Table 3, entry 26, with different substitution patterns at the aryloxy substituent combined with other head groups like nitrile, phenylsulfonamide, or benzyloxy, which are still under investigation.

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