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2-Aminoimidazole-based antagonists of the 5-HT_6 receptor – a new concept in aminergic GPCR ligand design

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Abstract

A new strategy in the design of aminergic GPCR ligands is proposed – the use of heterocyclic basic moieties in place of the evergreen piperazine or alicyclic and aliphatic amines. This hypothesis has been tested using a benchmark series of 5-HT_6R antagonists obtained by coupling variously substituted 2-aminoimidazole moieties to the well established 1-benzenesulfonyl-1*H*-indoles, which served as the ligands cores. The crystallographic studies revealed that upon protonation, the 2-aminoimidazole fragment triggers a resonance driven conformational change leading to a form of higher affinity. This molecular switch may be responsible for the observed differences in 5-HT_6R activity of the studied chemotypes with different amine-like fragments. Considering the multiple functionalization sites of the embedded guanidine fragment, diverse libraries were constructed, and the relationships between the structure and activity, metabolic stability, and solubility were established. Compounds from the *N*-(1*H*-imidazol-2-yl)acylamide chemotype (**10a**–**z**) exhibited high affinity for 5-HT_6R and very high selectivity over 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 and D_2 receptors (negligible binding), which was attributed to their very weak basicity. The lead compound in the series 4-methyl-5-[1-(naphthalene-1-sulfonyl)-1*H*-indol-3-yl]-1*H*-imidazol-2-amine (**9i**) was shown to reverse the cognitive impairment caused by the administration of scopolamine in rats indicating procognitive potential.

Keywords

2-aminoimidazole; serotonin; 5-HT_6 antagonist; non-basic; GPCR; Alzheimer's disease.

Introduction

Until now, the chemical space of ligands of aminergic G protein-coupled receptors (GPCR) has been expanding mainly by the employment of novel bioisosteric building blocks, resulting in a large diversity of core scaffolds, aromatic systems, linkers, hydrogen bond donors and acceptors. This is in sharp contrast to the very narrow pool of amine-like groups that have been used to replace the aminoalkyl chains of endogenous neurotransmitters and classical ligands. The commonly used bioisosteres include piperazine,[1] piperidine,[2] tetrahydropyridine,[3] morpholine, pyrrolidine[4] and azetidine. More sophisticated saturated heterocyclic and cycloalkyl structures include spirocyclic amines[5,6] and bicyclic amines.[7] Interestingly, hardly any attempts have been made to employ aromatic basic groups in the design of aminergic GPCR ligands. In our previous paper, we characterized a series of 5-(1*H*-indol-3-yl)-1-alkylimidazoles which were potent and selective 5-HT_7 receptor agonists.[8] This discovery supported the idea of introducing aromatic basic moieties into serotonin receptor ligands.

2-Aminoimidazole (2-AI) remains an underexplored basic scaffold in the GPCR ligand field, and it has been found to be a common molecular framework of numerous marine alkaloids and synthetic antibacterial (anti-biofilm) agents. The serotonergic activity of marine alkaloids containing the 2-AI motif was revealed in 1984, well before the discovery and cloning of several subtypes of serotonin receptors (Figure 1).[9,10]

Affinity of some 2-AI alkaloids for other GPCR's was also reported in the following years.[11–14]

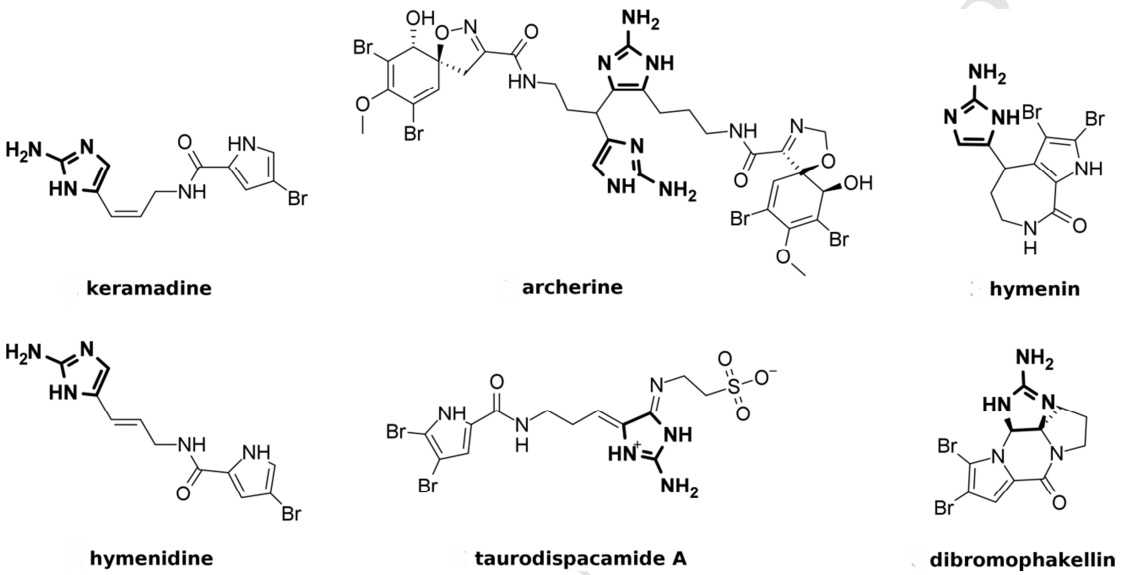


Figure 1. Marine alkaloids exhibiting aminergic GPCR activity. The compounds keramadine[9] and hymenidine,[10] at 15 μM, reversed the contractile effect of 1 μM serotonin on an isolated rabbit aorta, while the contraction caused by KCl or noradrenaline was not affected. The compounds archerine[11] and taurodispacamide A[12] at high concentrations blocked the effects of histamine in a screening assay conducted on the guinea pig ileum. The compound hymenin[13] acted as a competitive antagonist of α-adrenoreceptors in vascular smooth muscles in a screening assay. Dibromophakellin showed agonistic activity against α_{2B} adrenoreceptor with an EC_{50} of 4.2 μM.[14]

Contrary to their well-recognized anti-biofilm properties, synthetic 2-aminoimidazole derivatives have not been shown to interact with aminergic GPCRs, or with other monoamine binding CNS-related targets. The widespread occurrence of 2-AI in natural bioactive compounds and the previous use of this fragment in medicinal chemistry suggest that 2-AI might serve as a guanidine mimic that can be chemically modified to have the desired basicity (Figure 2).[15]

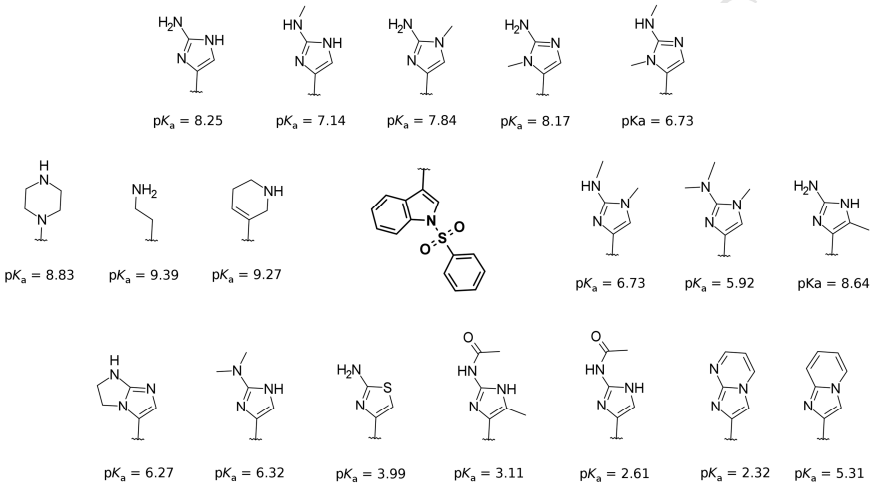


Figure 2. Comparison of the calculated pK_a of aminoimidazoles, 2-aminothiazole and other cyclic amidines vs. the classical 5-HT$_6$R ligand amine groups. The calculations were made for structures containing the standardized 5-HT$_6$R ligand core: 1-benzenesulfonyl-1*H*-indole. A comparison of the basicity of various amidines can be found in a review by Sullivan et. al.[15]

5-HT$_6$ is a G$_s$ coupled serotonin receptor that is involved in regulating cholinergic transmission.[16,17] This receptor has long been a hope for Alzheimer's disease patients

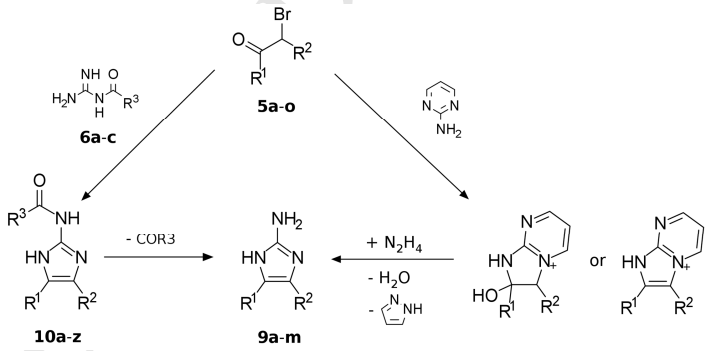
since its antagonists were shown to significantly relieve the disease symptoms in animal models, possibly by restoring the physiological acetylcholine levels in the brain.[18] Numerous structurally diverse antagonists were reported, a majority of which contributed to the arylsulfonyl chemotypes.[19–22] The most advanced of several clinical candidates, Idalopirdine (LuAE58054) did not show convincing activity in phase III clinical trial.[23] The development of Intepirdine (SB-742457) and Cerlapirdine (WAY-262,531) have been halted. An 1-arylsulfonyl-1*H*-indole derivative SUVN-502 was shown safe and well-tolerated during phase I clinical trial.[1,24] Among the clinical leads, AVN-211 holds a remarkable place as an antagonist developed for treating schizophrenia, and it was confirmed to relieve positive symptoms while producing procognitive effects.[25,26]

The primary objective of this work was to validate the usefulness of 2-AI as a basic fragment in aminergic GPCR ligands. Once this was achieved by employing 2-AI as a bioisostere of the classical piperazine/alkylamino fragment in a series of potent and selective 5-HT_6R antagonists, the next goal was to tune the ADME properties of the series to find suitable lead compounds. Differently functionalized 2-aminoimidazoles were used to obtain compounds based on a 2-aminoimidazole scaffold while pursuing the optimal solubility, activity and metabolic stability.

Results

Chemistry

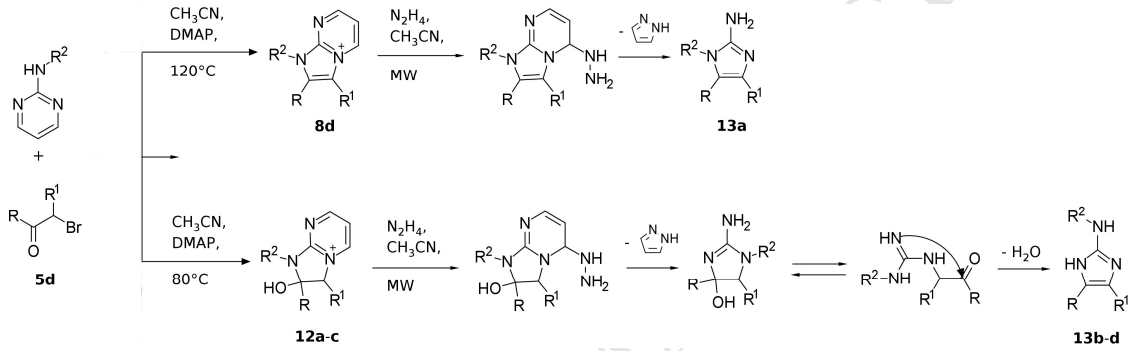
5-Aryl-2-aminoimidazoles can be synthesized using several different protocols, although only two were found practical for the compounds that we designed and were tolerant of broad scope of reagents applied in the present study. Both synthetic methods rely on the condensation of bromoketones with reagents carrying the guanidine synthon. The first method involves the formation of 2-hydroxy-1*H*,2*H*,3*H*-4λ^5-imidazo[1,2-*a*]pyrimidin-4-ylium salts from 2-aminopyrimidine derivatives and haloketones.[27–29] Strict control of the reaction conditions leads to either an alcohol or an aromatized product (Scheme 1). The nucleophilic addition of hydrazine and the subsequent cleavage of the pyrimidinium ring, which results in the formation of pyrazole lead to substituted or unsubstituted 2-aminoimidazole derivatives.



Scheme 1. Two approaches for synthesizing 5-aryl-2-aminoimidazoles. Both pathways use bromoketones as starting materials.

The cleavage of an aromatized salt yields a simple 1,4,5-substituted 2-aminoimidazole product (**13a**), whereas the cleavage of a non-aromatic salt leads to a rearrangement that

resembles the Dimroth mechanism, giving rise to 2,4,5-substituted 2-aminoimidazole (**13b-d**, Scheme 2).[27] The acylated and unsubstituted 2-AI's could be concisely prepared from acylguanidines, via condensation with bromoketones and subsequent deprotection with acid. Acylguanidines can be prepared from guanidine free base and esters.[30]



Scheme 2. Approaches to alkyl-substituted 2-aminoimidazole synthesis. The order of aromatization and hydrazine addition is crucial to the structure of the product. A Dimroth-type rearrangement may be responsible for the alkyl group migration.[27]

The initial experiments involved the condensation of unprotected indol-3-yl based bromoketones with amidine derivatives that did not yield the desired products.Condensation of unprotected 2-bromo-1-(1*H*-indol-3-yl) propan-1-one with acetylguanidine did not yield the desired 2-aminoimidazole derivative, instead the formation of a product M^+/Z=412 was observed which could potentially be explained by the dimerization of the substrate and condensation with acetylguanidine.

Thus, the indoles were first protected with the appropriate arylsulfonyl groups

giving rise to compounds **1a**–**h**; the 3 position was next functionalized via Friedel-Crafts acylation that employed acid anhydrides and aluminum chloride, yielding **2a**–**l** (Scheme 3). The final compounds that were based on 4- and 6-imidazole-substituted 1-arylsulfonylindole scaffolds (**9l**, **9m** and **10x**–**z**) were synthesized starting from the appropriate indolenitriles (Scheme 3, procedure 3). The nitriles were treated with alkyllithiums (Grignard reagents could potentially be used instead but were found less suitable in reagent screening) followed by hydrolysis in dilute hydrochloric acid.[31] The resulting 4- and 6-acylindoles **3a**–**c** were protected with an arylsulfonyl group via phase transfer catalyzed on water reaction with arylsulfonyl chlorides yielding **4a**–**c**. A mild bromination using $CuBr_2$ and ethyl acetate-chloroform mixture was found to be a very convenient way to produce the desired bromoketones **5a**–**o** in high yields, and it has a very simple work-up.[32] It was found that only the acetylindole derivatives (**2a**–**c** and **4a**) were prone to overbromination, whereas the α-substituted ketones could be reacted with an excess of $CuBr_2$, which resulted in a 100% conversion. 1-[1-(Benzenesulfonyl)-2-methyl-1*H*-indol-3-yl]-2-bromopropan-1-one **5p**, which could not be synthesized by direct bromination was obtained via Friedel-Crafts acylation of 1-benzenesulfonyl-2-methyl-1*H*-indole (**1i**) with bromopropionyl bromide (Scheme 3).

The acylguanidines **6a**–**c** were obtained from the appropriate ethyl esters and freshly prepared guanidine free base. The alkyl substituted 2-aminoimidazoles were synthesized using a modified microwave-assisted method.[28] The starting

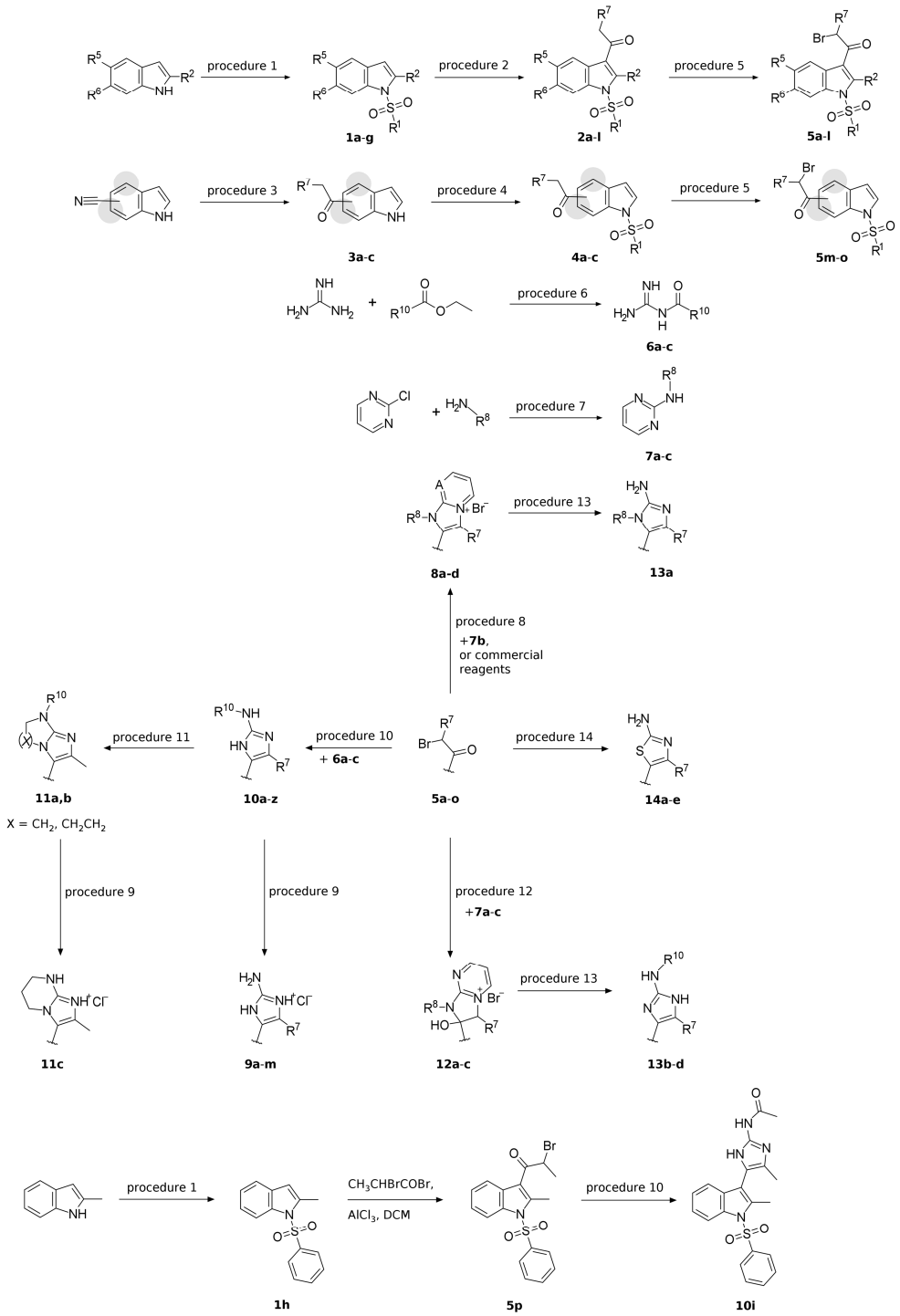
N-alkylpyrimidin-2-amines **7a**–**c** were prepared from 2-chloropyrimidine and the appropriate amines (for low nucleophilicity amines, such as 2,2-difluoroethylamine long reaction times were required).[33]

The condensation of bromoketones with *N*-alkylpyrimidin-2-amines was conducted in pressure tubes immersed in an oil bath and led to **8a**, **8c**, **8d** and **12a**–**c**. Screening experiments revealed that acetonitrile was the solvent of choice, and that the highest temperature at which the product did not aromatize was 80°C. Compound **8b** was synthesized in a similar manner, starting from 2-aminopyridine.

2-Acetamidoimidazoles **10a**–**z** were conveniently prepared via the condensation of bromoketones with a large excess of acylguanidines **6a**–**c** in warm DMF.[34] The products were often easily precipitated by the addition of water, which solubilized the unreacted acylguanidine while the byproducts were simply triturated out with ethyl acetate or acetone. Compounds **10a**, **10b**, **10d**, **10f**–**h**, **10n**, **10o and 10y** were converted into hydrochloride salt. The unprotected 2-aminoimidazoles **9a**–**m** were prepared from their acetylated counterparts (**10a**–**c**, **10e**–**g**, **10j**, **10m**, **10p**, **10s**, **10u** and **10y**), by hydrolysis in boiling dilute hydrochloric acid. The resulting hydrochlorides **9a**–**m** could not be converted back to the free bases, as a chemical reaction occurs at pH>9. This finding was in contrast to the more chemically stable 2-acetamidoimidazoles **10a**–**z**, which remain intact in highly basic solutions.

1*H*,2*H*,3*H*-Imidazo[1,2-*a*][1,3]diazole **11a** and **11c** and

5*H*,6*H*,7*H*,8*H*-imidazo[1,2-*a*]pyrimidine **11b** were synthesized via cyclization from 2-acetamidoimidazoles and 1,2-dibromoethane or 1,3-dibromopropane, respectively (Scheme 3, procedure 11). Both possible isomers were detected by LC-MS and the peak integral ratio was 1:5 however, only the major peak was isolated, and its structure was determined via 2D NMR. The deprotection of **11a** with hydrochloric acid yielded **11c**. 2-Aminothiazoles **14a**–**e** were synthesized from thiourea and bromoketones (Scheme 3, procedure 12).



Scheme 3. Synthetic figure for the final 2-aminoimidazole and 2-aminothiazole compounds. The final compounds, **8a–c**, **9a–m**, **10a–z**, **11a–c**, **13a–d** and **14a–e**, were

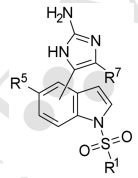
synthesized from common substrates, i.e., bromoketones via condensation with embedded amidines. Reagents and conditions: Procedure 1: 1. NaH, THF, 2. arylsulfonyl chloride; procedure 2: $AlCl_3$, DCM, acid anhydride; procedure 3: 1. Alkyllithium, THF, -78°C 2. conc. HCl, reflux; procedure 4: NaOH, TBAB, arylsulfonyl chloride, H_2O, toluene; procedure 5: AcOEt and $CHCl_3$ (1:1), $CuBr_2$, reflux; procedure 6: EtOH, r.t.; procedure 7: EtOH, Et_3N, MW; procedure 8: 2-aminopyridine or 2-aminopyrimidine, MeCN, 120°C; procedure 9: conc. HCl, reflux; procedure 10: acylguanidine, DMF; procedure 11: Cs_2CO_3, DMF, $BrCH_2CH_2Br$ or $BrCH_2CH_2CH_2Br$, 60°C; procedure 12: MeCN, DMAP, 80°C; procedure 13: MeCN, $N_2H_4xH_2O$, MW: 10 minutes, 100°C, 200W; procedure 14: thiourea, MeCN, 40°C.

Structure-affinity and structure-ADME relationships

The pilot compounds **9a** (R7 = H, Table 1) and **9b** (R7 = Me), which were structurally the simplest within the series showed high 5-HT_6R affinity (K_i = 10 nM and 14 nM, respectively) and moderate selectivity over 5-HT_{1A}, 5-HT_{2A} and D_2 receptors, **9b** being more selective over 5-HT_{1A}R and D_2R. Extending the alkyl chain at the 5th position of the imidazole to an ethyl decreased the potency even further (**9c**, R7 = Et, K_i = 29 nM). The incorporation of a methoxy group at the 5th position of the indole nucleus resulted in a significant enhancement in binding affinity; compounds **9d** (K_i = 2 nM) and **9e** (K_i = 3 nM) were the most potent derivatives within the series.[35] The change from benzenesulfonyl group to 3-fluorobenzenesulfonyl (**9d** and **9e** vs. **9g** and **9h**),

1-naphthylsulfonyl (**9i**, **9j**) and 2-naphthylsulfonyl (**9k**) resulted in lowered affinity. The shift of the 2-aminoimidazole moiety to the 4 position of the indole diminished the affinity roughly threefold (**9a** vs **9l)**. A major enhancement of 5-HT_6R binding was observed with the shift of the 2-aminoimidazole to the 6 position of the indole in compound **9m** (K_i = 4 nM), but this was accompanied by a sharp drop in solubility (which was in the 1–10 mg/mL range for **9m**).

Table 1. Structure and binding data of compounds **9a**–**m**. The amine-like group within the chemotype is 1*H*-imidazol-2-amine (2-aminoimidazole).



ID	R1	R5	R7	IM *	K_i [nM]				
					5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7	D_2
9a	Ph	H	H	3	126±17	891±114	10±2	2455±377	87±7
9b	Ph	H	Me	3	N.A.	100±13	14±3	9120±842	794±81
9c	Ph	H	Et	3	N.D.	107±9	29±4	3467±403	1585±186
9d	Ph	OMe	H	3	N.A.	794±155	2±1	1148±178	1202±173
9e	Ph	OMe	Me	3	N.A.	676±82	3±1	7079±947	N.D.
9f	Ph	OMe	Et	3	N.A.	661±97	19±2	N.A.	1622±99
9g	3-F-Ph	OMe	H	3	N.D.	N.D.	9±2	N.D.	7079±802

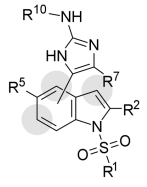
9h	3-F-Ph	OMe	Me	3	N.A.	1122±215	9±1	N.A.	N.D.
9i	1-Naph	H	Me	3	N.A.	490±52	8±2	2512±303	1175±144
9j	1-Naph	OMe	Me	3	N.A.	1660±201	10±3	1096±91	1318±162
9k	2-Naph	OMe	Me	3	N.D.	1047±173	9±2	3715±411	2344±427
9l	Ph	H	H	4	N.D.	2291±359	34±4	N.A.	3981±511
9m	Ph	H	Me	6	N.A.	1175±93	4±1	776±63	708±65

*IM – position at the indole being the 2-aminoimidazole attachement point. N.D. – not determined N.A. – not active, K_i > 10000 nM

The intermediate **10a** (Table 2), which was used to obtain compound **9a** (procedures 10 and 9 from Scheme 4), exhibited considerable affinity for the 5-HT_6 receptor (K_i = 79 nM), despite its very low basicity (calculated pK_a = 3.11). Compound **10a** not only was considered promising (because it had less hydrogen bond donors and higher lipophilicity), but its lower basicity contributed to a very high selectivity over the related targets at the cost of low solubility. For most of the 1-(arylsulfonyl)-1*H*-indole cores, triplets of analogs namely an unsubstituted 2-aminoimidazole, 2-acetamidoimidazole and 2-propanamidoimidazole (the order complies with decreasing *in vitro* potency for most entries) were synthesized. There were no sharp SAR trends among compounds **10b**–**10i**; **10e** had the highest affinity (K_i = 10 nM), and **10b**, **10f** and **10g** exhibited very high metabolic stability (Table 7). In general, compounds **9a**–**m** showed fine selectivity, and the acylated chemotype (compounds **10a**–**z**) provided ultimate selectivity i.e. did not bind to targets other than 5-HT_6R, which could potentially be attributed to their very low basicity.

A comparison of the highly basic naphthylsulfonyl-indole-based compounds **9i**, **9j** and **9k** (9 nM ≤ K_i ≤ 10 nM), and their low-basicity counterparts: **10p**, **10r**, **10s**, **10t** and **10u** (10 nM ≤ K_i ≤ 15 nM and 23 nM, respectively) clearly showed that the activity of the naphthyl derivatives was not greatly affected by the 5-methoxy substituent on indole or by the switch from 1-naphthyl to 2-naphthyl. The only compound that did not follow this pattern was **10w** (K_i = 455 nM), which might be beyond the capacity limits of the receptor binding pocket. The substitution at the 3 position of the phenylsulphonyl moiety with a fluorine atom was investigated in the series **10n**, **10o** (vs **9e**, **9g**), and **9g**, **9h** (vs **9d**, **9e**) and neither produced any significant effect on the activity in the case of low-basicity compounds nor enhanced the metabolic stability, while **9g** and **9h** (K_i = 8 nM and 9 nM, respectively) were found approximately three-fold less potent than **9d** and **9e**. The depth of the pocket housing the acyl group in the low-basicity compounds was determined by the synthesis of **10j** (K_i = 3 nM). The chemical space was expanded with **10y** and **10z**, the indol-6-yl-based compounds (K_i = 15 nM and 17 nM, respectively).

Table 2. Structure and binding data of the compounds **10a**–**z**. The amine-like group within the chemotype is *N*-(1*H*-imidazol-2-yl)acylamide (2-acylamidoimidazole).



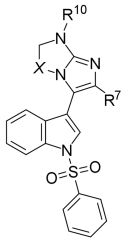
ID	R1	R2	R5	R7	R10	IM*	pK_i [M]				
							5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	5-HT$_7$	D$_2$
10a	Ph	H	H	H	Ac	3	N.A.	N.D.	79±12	N.A.	N.A.
10b	Ph	H	H	Me	Ac	3	N.A.	N.A.	25±4	N.A.	N.A.
10c	Ph	H	H	Et	Ac	3	N.D.	N.A.	263±31	N.A.	N.A.
10d	Ph	H	OMe	H	Ac	3	N.A.	N.D.	18±3	N.A.	N.A.
10e	Ph	H	OMe	Me	Ac	3	N.A.	N.A.	10±2	N.A.	N.A.
10f	Ph	H	OMe	Et	Ac	3	N.A.	N.A.	31±5	N.A.	N.A.
10g	Ph	H	OMe	Me	CH_3CH_2CO	3	N.D.	N.D.	14±2	N.A.	N.A.
10h	Ph	H	OMe	Et	CH_3CH_2CO	3	N.D.	N.D.	16±3	N.D.	N.A.
10i	Ph	Me	H	Me	Ac	3	N.A.	N.D.	83±5	N.A.	N.A.
10j	Ph	H	OMe	Me	$PhCH_2CO$	3	N.D.	N.A.	3±1	N.A.	N.A.
10k	Ph	Me	F	H	Ac	3	N.D.	N.A.	759±92	N.D.	N.A.
10l	Ph	Me	F	H	CH_3CH_2CO	3	N.A.	7943±1752	107±8	8511±743	N.D.
10m	3-F-Ph	H	OMe	H	Ac	3	N.D.	N.A.	50±7	N.A.	N.A.
10n	3-F-Ph	H	OMe	Me	Ac	3	N.A.	N.A.	14±3	N.A.	N.A.
10o	3-F-Ph	H	OMe	Me	CH_3CH_2CO	3	N.A.	N.A.	9±2	N.A.	N.A.
10p	1-Naph	H	H	Me	Ac	3	N.D.	N.D.	13±1	N.D.	N.D.
10r	1-Naph	H	H	Me	CH_3CH_2CO	3	N.D.	N.A.	15±4	N.A.	N.A.
10s	1-Naph	H	OMe	Me	Ac	3	N.D.	N.A.	10±2	N.A.	N.A.
10t	1-Naph	H	OMe	Me	CH_3CH_2CO	3	N.D.	N.D.	10±1	N.D.	N.D.
10u	2-Naph	H	OMe	Me	Ac	3	N.D.	N.A.	23±3	N.A.	N.A.

10w	2-Naph	H	OMe	Me	CH_3CH_2CO	3	N.D.	N.A.	455±29	N.A.	N.A.
10x	Ph	H	H	Me	CH_3CH_2CO	4	N.A.	N.A.	501±73	N.D.	N.D.
10y	Ph	H	H	Me	Ac	6	N.D.	N.A.	15±3	N.D.	5248± 314
10z	Ph	H	H	Me	CH_3CH_2CO	6	N.D.	7413±1 477	17±4	N.D.	3631± 562

*IM – position at the indole being the *N*-(1*H*-imidazol-2-yl)acetamide attachement point. N.D. – not determined N.A. – not active, K_i > 10000 nM

Bicyclic 2-acetamidoimidazoles **11a** (K_i = 585 nM Table 3) and **11b** (K_i = 280 nM) exhibited low affinity and extremely low water solubility. Compound **11a** was insoluble in most organic solvents and crystallized readily from warm DMSO. This scaffold was investigated in an effort to optimize the ADME and receptor affinity but was discontinued because no improvement in affinity was observed while the solubility decreased steeply.

Table 3. Structure and binding data for compounds **11a**–**e**. The amine-like group within the chemotype is 1*H*,2*H*,3*H*-imidazo[1,2-*a*][1,3]diazole in **11a** and **11c** 5*H*,6*H*,7*H*,8*H*-imidazo[1,2-*a*]pyrimidine in **11b**.



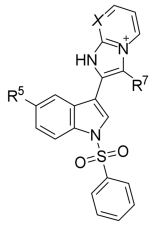
ID	R7	R10	X	pK_i [M]

				5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	5-HT$_7$	D$_2$
11a	Me	Ac	CH_2	N.A.	N.A.	585±157	N.A.	N.A.
11b	Me	Ac	CH_2CH_2	N.A.	N.A.	280±61	N.A.	N.A.
11c[*]	Me	H	CH_2	N.A.	5129±891	93±18	N.A.	1230±243

[*] Compound **11c** was obtained and tested as the hydrochloride. N.A. – not active, K_i > 10000 nM

Compound **8a** (structurally similar to **11a**–**c**) (Table 4) which served as an intermediate in the synthesis of **9a** exhibited considerable binding affinity for 5-HT$_6$R (K_i = 50 nM) and high selectivity over the related targets. This rather serendipitous discovery encouraged us to explore the receptor affinity of 1,3-substituted 1*H*-4λ^5-imidazo[1,2-*a*]pyrimidin-4-ylium salts. Although they had better solubility than did **11a**–**c**, the **8a** derivatives, **8b** (78 nM) and **8c** (250 nM), did not exhibit any improvement in binding affinity, **8a** did not have good chemical properties (e.g., highly susceptible to nucleophiles) and was later found to be metabolically unstable (Table 7) thus, the chemotype was not extended any further.

Table 4. Structure and binding data for compounds **8a**–**c**. The amine-like group within the chemotype is imidazo[1,2-*a*]pyrimidine of **8a** and **8c** and imidazo[1,2-*a*]pyridine in **8b**.

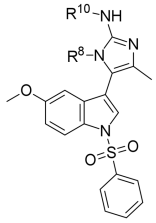


ID	R5	R7	X	pK_i [M]				
				5-HT$_{1A}$	**5-HT$_{2A}$**	**5-HT$_6$**	**5-HT$_7$**	**D$_2$**
8a	H	H	N	N.A.	N.A.	50±6	N.A.	N.D.
8b	OMe	Me	C	N.D.	N.A.	78±9	N.A.	3162±528
8c	OMe	Me	N	N.A.	N.A.	250±17	N.A.	N.A.

N.D. – not determined, N.A. – not active, K_i > 10000 nM

Several *N*-alkylated derivatives of 2-aminoimidazole were also synthesized, including the fluorinated compounds **13a** (K_i = 157 nM) and **13b** (K_i = 21 nM, Table 5). The isomers **13a** and **b** displayed highly different activities and compound **13b** with the exocyclic nitrogen substituted was more potent. Compound **13c** was found to be significantly less potent than its non-basic counterpart **10j**.

Table 5. Structure and binding data for compounds **13a**–**d**. The amine-like group within the chemotype is a *N*-alkyl-*1H*-imidazol-2-amine in **13a** and 1-substituted *1H*-imidazol-2-amine in **13b**–**d**.

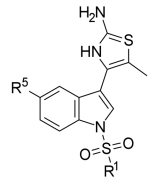


ID	R8	R10	pK_i [M]				
			5-HT$_{1A}$	**5-HT$_{2A}$**	**5-HT$_6$**	**5-HT$_7$**	**D$_2$**
13a	CH_2CHF_2	H	N.A.	3236±725	157±41	N.A.	3802±586
13b	H	CH_2CHF_2	N.A.	1230±187	21±4	N.A.	3981±819
13c	H	EtPh	N.A.	1905±423	83±6	N.A.	2138±414
13d	H	Bn	N.A.	N.D.	21±3	2455±319	1175±101

N.D. – not determined

2-Aminothiazoles **14a**–**e** (Table 6) were found to be active, as the switch to this amine-like moiety neither decreased the 5-HT$_6$ receptor binding nor yielded any hit compounds. The most potent thiazole **14c** had a K_i = 44 nM.

Table 6. Structure and binding data for compounds **14a**–**e**. The amine-like group in this chemotype is 1,3-thiazol-2-amine (2-aminothiazole).



ID	R1	R5	pK_i [M]

			5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_6$	5-HT$_7$	D$_2$
14a	Ph	OMe	N.D.	2455±612	105±19	N.A.	N.A.
14b	2-Naph	OMe	N.D.	N.D.	65±5	N.D.	N.D.
14c	1-Naph	OMe	N.D.	N.D.	44±3	N.D.	N.D.
14d	1-Naph	H	N.A.	N.A.	69±8	N.A.	N.A.
14e	Ph	H	N.A.	N.D.	100±13	N.A.	5129±1158

N.D. – not determined

The metabolic stability, which was a key factor used to establish the lead compounds was assessed in an *in vitro* experiment using rat liver microsomes. Compounds **10b**, **10f**, **10g** were found to be very stable (Table 7). Most of the tested 2-acylamidoimidazoles exhibited pronounced stability, when compared to compounds **8a**, **9i** and **9m**. Only compounds **8a**, **10a** and **9m** were found to have lower stability than the benchmark compound Idalopirdine (LuAE58054).

Table 7. Metabolic stability: clearance (rat liver microsomes) and half-lives of selected compounds.

ID	Clearance [µL/min/mg]	$t_{1/2}$ [min]
8a	230.3	6
9i	60.3	23
9m	104	13
10a	>460	<3

10b	7.3	190
10d	43.0	32
10f	10.8	129
10g	17.1	81
10h	43.4	32
10n	53.5	26
10o	35.4	39
13b	39.0	36
Idalopirdine	99.3	N.C.
Intepirdine	6.6	N.C.
Donepezil	17.6	79

N.C. – not calculated

Physicochemical descriptors (i.e., ClogP, pK_a and TPSA) were calculated using ChemAxon Instant JChem and the water solubility range was determined experimentally for compounds **9b**, **9d**, **9i**, **10b**, **10y** and **13b** (Table 8). The selected compounds were screened for several anti-targets including adrenergic, dopamine, histamine, muscarinic and serotonin receptors and the level of binding to the *h*ERG potassium channel was measured (Table 8). The low binding of all of the tested compounds to *h*ERG suggests a low risk of cardiac side effects mediated by this channel. Compounds **9b** and **9d** showed marginal binding, and **10b**,and **9i** did not exhibit any functional activity at 5-HT_{2B}R. The functional assays clearly demonstrated the ability of compounds **10b**, **10y** and **9i** to block the 5-HT_6R at low concentration. The K_b constants were calculated based on the

observed inhibition of cAMP production (induced by 5-CT) at different concentrations of the tested compound. The values obtained were consistent with the results of the binding experiments.

Table 8. Calculated descriptors, solubility ranges and anti-target profiles of the selected compounds.

		9b	**9d**	**9i**	**10b***	**10y***	**13b**
CLogP		2.44	2.15	3.96	2.50	2.50	3.58
TPSA		93.77	103.0	93.77	96.85	96.85	89.01
M.W.		352.41	368.41	402.47	394.45	394.45	446.47
pK_a		8.41	8.60	8.64	2.90	3.10	7.47
Solubility		high	high	medium	low	very low	medium
5-HT$_6$R K_b [nM]		N.D.	N.D.	4.8	1.3	3.1	13
alpha$_{1A}$ (% inh. at 10^{-6} M)**		12.2	33.1	10.7	-1.5	-4.2	4.0
H_1 (h)	(% inh. at 10^{-6} M)**	N.D.	N.D.	70.1	-5.9	-1.9	1.0
	(%agonist at 10^{-5} M)	N.D.	N.D.	-1.3	N.D.	N.D.	N.D.
	(% antagonist at 10^{-5} M)	N.D.	N.D.	25.7	N.D.	N.D.	N.D.
M_1 (h) (% inh. at 10^{-6} M)**		3.8	6.7	-0.3	-13.4	-10.7	-4.0
M_5 (h) (% inh. at 10^{-6} M)**		7.6	5.6	N.D.	N.D.	N.D.	N.D.
5-HT$_{2C}$ (h)	(% inh. at 10^{-6} M)**	71.6	82.0	52.7	22.4	N.D.	28.0
	(% agonist at 10^{-5} M)	N.D.	N.D.	21.3	N.D.	N.D.	N.D.
	(% antagonist at 10^{-5} M)	N.D.	N.D.	50.3	N.D.	N.D.	N.D.

hERG (h) (% inh. 10^{-6} M)**	N.D.	N.D.	25.7	2.4	N.D.	23.0
Function 5-HT_{2B}R (h) (% inh. 10^{-6} M)**	N.D.	N.D.	-2.8	-1.9	N.D.	N.D.
5-HT_{2B} (h) (% inh. at 10^{-6} M)**	4.5	1.7	N.D.	N.D.	N.D.	N.D.

*Compound was tested as hydrochloride.

**Experiments were performed at Eurofins Cerep. Water solubility ranges: high >10 mg/mL; medium 1–10 mg/mL; low 0.1–1 mg/mL; very low <0.1mg/mL. Physicochemical descriptors: partition coefficient (ClogP), topological surface area (TPSA), molecular weight (M.W.) and acidity dissociation constant (pK_a) were calculated using ChemAxon software.

Lead compound selection

The non-basic chemotypes (i.e., **10**, **11** and **14**) were omitted from further development due to insufficient water solubility. The highest water solubility was observed for compounds from chemotypes **8** and **9**. Compound **9b** showed mediocre blood brain barrier permeability in mice (unpublished data). Compound **9i** was thus chosen as the lead compound based on the calculated physicochemical descriptors (i.e., higher lipophilicity than **9b**), *in vitro* activity, solubility data and metabolic stability assay results.

The cytotoxicity of compound **9i** was assessed in the HepG2 cell line by measuring both cell membrane damage and cell viability. The compound was mildly cytotoxic (i.e., it had an IC_{50} = 15.7 µM in the PrestoBlue assay) and damaged cell membranes at a

similar concentration level (i.e., 18.4 µM for Toxi Light assay). Moreover, **9i** was found to be non-mutagenic in a mini AMES test up to 10 µM and inhibited CYP3A4 and CYP2D6 at a concentration of 1.7 µM and 6.4 µM, respectively. The compound was non-toxic in female rats (LD_{50} > 2000 mg/kg) with no apparent adverse effects observed at the highest dose.

In vivo pharmacology

The procognitive properties of compound **9i** were determined; it had the potential to reverse the scopolamine-induced novel object recognition (NOR) impairment, which serves as a model of Alzheimer's disease cholinergic system failure. There were no significant differences in the time spent exploring two identical objects in the familiarization phase in any group (data not shown).

One hour after the inter trial interval (ITI), vehicle-treated, but not scopolamine-treated rats spent significantly more time exploring a novel object than they spent exploring a familiar one. Thus, the administration of scopolamine (1.25 mg/kg) abolished the ability to discriminate novel and familiar objects in a recognition (T2) trial. This deficit was reduced by administering compound **9i** (at 1 but not 0.3 mg/kg) and donepezil (at 1 but not 0.3 mg/kg).

It has been concluded that the lack of efficacy of the current Alzheimer's disease treatments may be overcome with the use of combined therapy. One of the latest

approaches involves the administration of a 5-HT_6R antagonist together with an acetylcholinesterase inhibitor.[36] Thus, the efficacy of **9i** as a part of an 5-HT_6R antagonist-acetylcholinesterase inhibitor cocktail was tested. Co-administration of inactive doses of compound **9i** (0.3 mg/kg) with donepezil (0.3 mg/kg), facilitated cognitive performance. Because the analyses of exploration time during the recognition trial and the discrimination index (DI) yielded the same results, only the DI data are presented (Figure 3). The DI data were analyzed by a one-way ANOVA followed by a Newman–Keul's post hoc test.

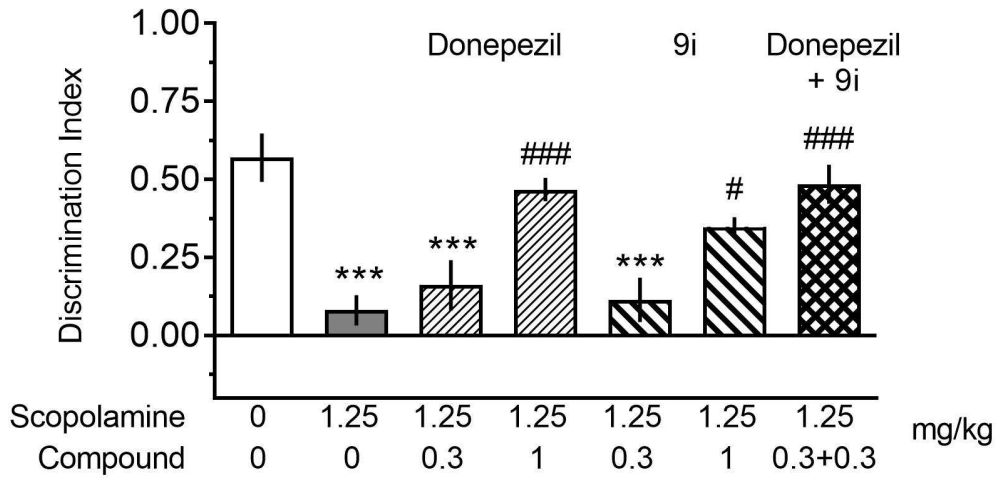


Figure 3. Effects of **9i**, donepezil and their combined administration on scopolamine-induced cognitive impairment in rats. Animals were treated with the experimental compounds and scopolamine at 120 and 30 min before the learning trial, and were tested 1 hour later in the recognition trial. The data are expressed as the mean ± standard error of the mean of the discrimination index (DI). N = 9–10 animals per group.

Symbols: ***p < 0.001, a significant reduction in the DI compared with the vehicle-treated group; # p < 0.05, ### p < 0.001, a significant increase in the DI compared with the scopolamine-treated group. Note that one rat from the scopolamine + **9i** (1 mg/kg) group was excluded to fulfill the D'Agostino & Pearson and Shapiro-Wilk normality tests criteria (Prism 7 software).

Determination of the basicity of compounds **9i** and **10b**

In an acidic solution, 2-aminoimidazole could be expected to undergo a two stage protonation:

2-AI $+H^+ =$ **2-AIH**$^+$ $\quad p\boldsymbol{K}_1$

2-AIH$^+ + H^+ =$ **[2-AIH$_2$]**$^{2+}$ $\quad p\boldsymbol{K}_2$

Based on the presented equilibria (protonation/deprotonation) two dissociations constants should be expected: pK_1 for NH_2 group and pK_2 for N(1) in imidazole ring system. The experimental data reports only one base centre with pK_a = 8.46 for 2-aminoimidazole[37] and pK_a = 7.18 for 2-aminobenzimidazole suggesting the equivalence of N(1) and (N3) nitrogen atoms in imidazoline ring system (Supporting Information figure S1). Due to a very high stability of the 2-aminoimidazolium cation, the second protonation is not observed in aquous solutions and requires the action of superacids.[38] The difference between pK_a of imidazole and the suggested imidazoline moiety (ΔpK_a =1.46) for 2-aminoimidazole vs imidazole and (ΔpK_a =1.73) for

2-aminobenzimidazole vs benzimidazole, let us postulate imidazoline tautomeric forms for both 2-aminoimidazole and benzimidazole derivatives.

Results from the titration curve of **9i** (Figure 4) confirm that its acid-base properties are similar to imidazole and 2-aminobenzimidazole (Supporting Information figure S1). Contrary to **9i**, compound **10b** seemed to be resistant to protonation which could be explained as a result of acetylation of the amine group at (2-NH_2) position resulting in formation of an intramolecular hydrogen bond leading to very low solubility. The relative order of the pK_a values was as follows:

$\mathbf{pK_{a\ 10b}} < pK_{a\ benzimidazole} < \mathbf{pK_{a\ 9i}} < pK_{a\ imidazole} < pK_{a\ 2\text{-}aminobenzimidazole} < pK_{a\ 2\text{-}aminoimidazole}$

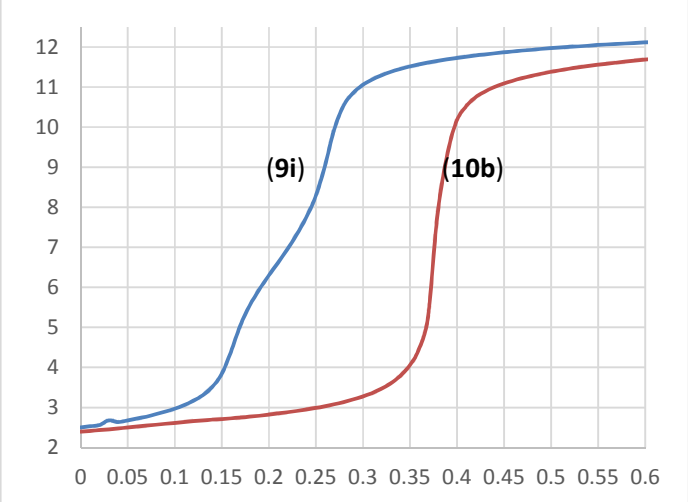
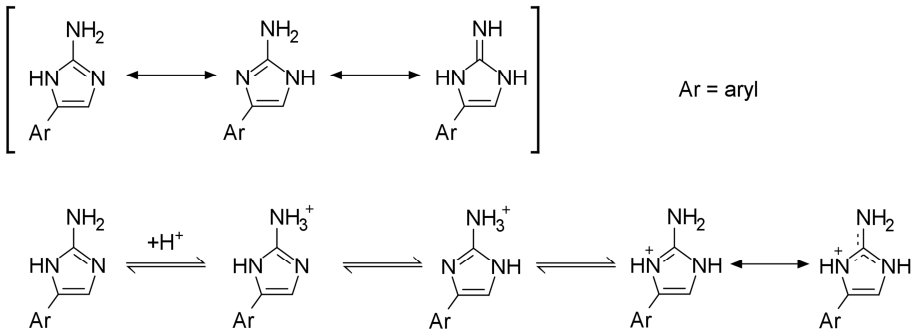


Figure 4. Titration curve pH = f(V_{KOH}) of **9i** (0.0025 M) +HCl (0.004 M), V_0 = 4 mL and **10b** (0.0044 M) + HCl (0.008 M), V_0 = 4.5 mL as titrand D in tertiary solvent system H_2O/DMSO/Polysorbate 80 with 0.098 M KOH as titrant T.

We have also considered the influence of tautomeric equilibria on the pK_a values of the

analysed compounds. Benzimidazoles are predominantly basic compounds having the ability to form salts with acids, however benzimidazole (pK_a = 5.35) is a considerably weaker base than imidazole (pK_a = 7.0). Similarly, a 1-ethyl-5-(indole-3-yl)-imidazole derivative AGH-192 (pK_a = 5.48), which was described in our recent paper, was found to be a weaker base than imidazole.[39] This difference in the basic strength is a reflection of the conjugation between the imidazole and aromatic ring systems. Conjugation increases the number of contributing states in the resonance sense, thus enhancing the chemical stability of the molecule. The possible tautomeric forms for 2-aminoimidazole and their protonated forms are presented in Scheme 4.



Scheme 4. Resonance structures of 5-aryl-2-aminoimidazole and the tautomeric equilibrium of its protonated form.

Crystal structure and binding mode analysis

Two crystal structures of 2-aminoimidazole derivatives were determined – the chosen lead **9i**, representing the set of basic compounds and **10b** as an exemplary non-basic compound. Both molecules exhibit similar L-shape geometry, which is a characteristic

feature of 5-HT_6R ligands.[40] Analysis of the single molecule geometry in the crystal structure suggested different molecular properties with a plausible impact on the interaction mode. Compound **9i** is partially protonated under physiological conditions, so it was crystalized as the hydrochloride salt. The positive charge is located on the guanidine fragment. As a consequence, there is a small electron withdrawing-effect in the direction of the 2-aminoimidazole that affects the π-electron distribution of the indole ring. The observed effect is combined with the sulfur atom moving closer to the indole mean plane (6.5° and 5.3° for **9i** and **10b**, respectively) (Figure 5A, molecule in magenta) and the six-membered ring becoming an attractive acceptor of the weak hydrogen C-H...π interactions (mainly in the C5-C6-C7 region). On the contrary, the acyl substituent at the exocyclic amine group of the 2-AI, lowers the basicity and this non-protonated form exhibits an electron-donating effect (deduced from the increased interior angle on the atom of the 2-aminoimidazole ring directly bound to C3 of the indole (details are in the Supporting Information)), leading to a more attractive acceptor of the C-H...π interaction located on the five-membered pyrrole ring and a significant deviation of the sulfur atom from the indole's mean plane (~ 17.3°) (Figure 5A, molecule in green). [The detailed crystal structure analysis is presented in the Supporting Information File.]

To further investigate observations based on the crystal structure analysis, the potential binding mode of compounds **9i** (protonated) and **10b** (not protonated) was determined

via molecular docking (Figure 5). Both molecules have relatively similar geometries and comparable orientations of the 5-HT_6R binding site as reported previously. The postulated interaction with D3.32 is conserved to form two and one strong N-H...O bonds, for **9i** and **10b**, respectively (for the former, one of the interactions is charge assisted). The arylsulfonyl fragment of both molecules occupied a hydrophobic cavity formed by residues of the transmembrane helices 3–5. The sulfonyl group was in the proximity of N6.55, allowing an additional stabilizing interaction, as postulated in the literature.[40,41] The minimal shift of the indole ring observed for ligand **9i** allows for possible C-H...π interactions (T-shape) with F6.51, F6.52 and F7.35, with the acceptor localized in the C5-C6-C7 region (discussed/observed in the crystal structure). These weak interactions have an additional stabilizing effect which could explain the higher binding affinity for the 5-HT_6R.

To find the typical interactions formed by the 2-aminoimidazole moiety within the protein binding site, the PDB was searched, which provided 6 structures of protein-ligand complexes (resolution limit up to 2.5 Å). In most of the analyzed crystal data, the 2-aminoimidazole ring formed strong hydrogen bonds with electronegative acceptors (carbonyl oxygen atoms in most cases); in non-protonated form, it also served as an acceptor of the weak C-H...π interactions that were observed in the small molecular crystal structure packing and in the predicted binding mode. Only in two structures (3MFW and 3MFV – the same metalloprotein with different ligands) was the π...π

interaction observed between the mentioned aromatic ring and two histidine side chains. However, the 2-aminoimidazole ring is less prone to form π...π interactions than C-H...π interactions.

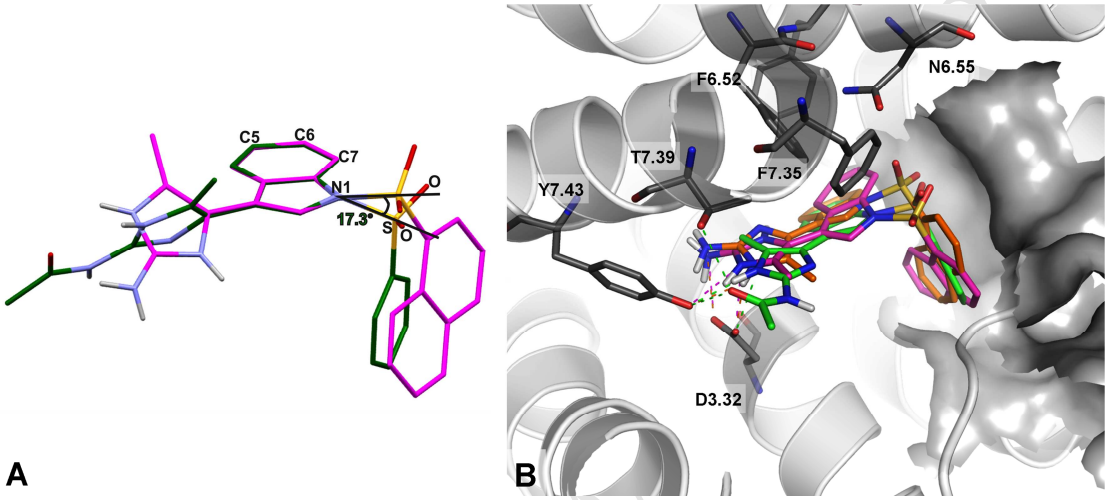


Figure 5. A – superposition of the molecular conformations observed in the crystal structure, showing a nearly co-planar orientation of the sulfur atom with respect to the indole ring for compound **9i** (magenta) and the angular deviation of the sulfur atom from the mean plane of indole observed in the conformation of molecule **10b** (green); B – the superposition of compounds **9i** in protonated (magenta) and unprotonated (orange) forms and **10b** (green) docked to the 5-HT_6R homology model built on the β2 adrenergic template.

Conclusions

Aromatic basic groups such as imidazoles or thiazoles have been almost absent in the medicinal chemistry space of aminergic receptor ligands, and there are only a few

examples of these structures being used to design other GPCR ligands. To date, only limited attempts have been made to escape the chemical space of classical amine groups, i.e., primary, secondary and tertiary amines.

The incorporation of 2-aminoimidazole as a bioisostere of the classical amine groups of the 5-HT_6 receptor ligands yielded highly active compounds with various physicochemical profiles. Several chemotypes were investigated in order to characterize the possible substitution patterns of 2-aminoimidazole.

In water solution, lead compound **9b** could exist as a monobasic compound with pK_a = 6.74, while *N*-acetylation in **10b** reduced the salt formation capability in aqueous media, solubility, (N1-N3) tautomerization and drastically lowered pK_a value. Lowering the basicity below the level necessary for the compounds to exist as a protonated form at physiological pH only slightly affected the 5-HT_6R affinity, but ultimately yielded selective derivatives. The analysis of the crystal structures of compounds **9i** and **10b** combined with the results of docking experiments to 5-HT_6R homology models revealed a plausible binding mode that is consistent with the SAR. The crystallographic study revealed that **9i**, upon protonation undergoes a conformational change and electron density transfer which can both significantly reinforce the binding.

The chosen lead compound **9i** was shown to be an orally active procognitive agent. It reversed the cognitive impairment caused by scopolamine in rats after *p.o.* administration both alone (at 1 mg/kg) and in combination with an inactive dose of

donepezil (both at 0.3 mg/kg).

A thorough pharmacodynamic and pharmacological characterization of different core structures that are more drug-like than the prototypical 1-benzenesulfonyl-1*H*-indole core structures conjugated to different 2AIs could potentially yield 5-HT_6R ligands with even higher blood-brain barrier permeation, a better ADMET profile and better water solubility. Our recent work on indole-imidazole agonists of the 5-HT_7R indicate that indeed imidazole can be successfully used as a replacement for the classically used amine groups in drug-like serotonin receptor ligands.[39,42]

It is likely that the incorporation of 2-amidoimidazole based moieties described in this study can be used to obtain new ligands of aminergic receptors as potential therapeutics or molecular probes. The multiple functionalization sites of 2-AI can successfully be used to tailor the ADME properties to meet specific requirements. We can expect that many other molecules based on 2-aminoimidazole and different cores are yet to be developed as novel ligands of other GPCR's.

Experimental

Chemicals. All organic reagents were purchased from Sigma-Aldrich, Apollo Scientific, or Combi-Blocks and were used without purification. Solvents and inorganic reagents were acquired from the company Chempur. Reaction progress was monitored by TLC on

Merck Silica Gel 60 F 254 on aluminium plates. Column chromatography was performed on Merck Silica Gel 60 (0.063–0.200 mm; 70–230 mesh ASTM) and aluminum oxide (activated, neutral, Brockmann I).

Analytical Methods. UPLC/MS analysis was performed on Waters TQD spectrometer combined with UPLC Acquity H-Class with PDA eLambda detector. Waters Acquity UPLC BEH C18 1.7 μm 2.1x50 mm chromatographic column was used, at 40°C, 0.300 mL/min flow rate and 1.0 μL injection volume (the samples were dissolved in LC-MS grade acetonitrile, typically at a concentration of 0.1–1 mg/mL prior to injection). All mass spectra were recorded under electrospray ionization in positive mode (ESI+) and chromatograms were recorded with UV detection in the range of 190-300 nm. The gradient conditions used were: 80% phase A (water + 0.1% formic acid) and 20% phase B (acetonitrile + 0.1% formic acid) to 100% phase B (acetonitrile + 0.1% formic acid) at 3.0 minutes, kept till 3.5 minutes, then to initial conditions until 4.0 minutes and kept for additional 2.0 minutes. Total time of analysis – 6.0 minutes.

^{1}H, ^{13}C NMR and 2D NMR spectra were recorded on Bruker Avance III HD 400 NMR and Bruker AVANCE 500 MHz spectrometers. All samples were dissolved in DMSO-d_6 with TMS as the internal standard. The spectral data of the compounds refer to their free bases except for compounds **9a-m** which were sampled as hydrochlorides.

HRMS spectra of representative compounds were measured on a Bruker Daltonics ultrafleXtreme (MALDI-TOF/TOF) apparatus and calibrated to an internal standard.

All presented compounds were of at least 95% purity as determined by UPLC (UV, 190-300 nm). The spectral data for all compounds is included in the Supporting Information.

Software. Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 17.24.0, 2017, ChemAxon. JChem Base was used for structure searching and chemical database access and management, JChem 18.3.0, 2018, ChemAxon. (www.chemaxon.com) Mendeley was used for citation, Mendeley 19.1.2, 2018, Mendeley Ltd. (www.mendeley.com)

General procedures for the final compounds

General procedures 1-7 for all the intermediates and the full characterization of the intermediates and final compounds can be found in Supporting Information.

Synthesis and characterization of pyrimidinium salts: 8a–d

A mixture of 2-aminopyridine (94 mg, 1 mmol) or 2-aminopirymidine (95 mg, 1 mmol) and 1-[1-(benzenesulfonyl)-1*H*-indol-3-yl]-2-bromoketone (1.02 mmol) was dissolved in acetonitrile and held at 120°C. The progress of the reaction was controlled with TLC (approx. 96 hours). The product was purified by trituration with acetone or column

chromatography using neutral alumina eluted with $CHCl_3$.

General procedure 9 for the synthesis of 2-amino-5-[1-(benzenesulfonyl)-1*H*-indol-X-yl]-1*H*-imidazol-3-ium chlorides: 9a–m and 11c

N-{5-[1-(benzenesulfonyl)-1*H*-indol-3-yl]-1*H*-imidazol-2-yl}acetamide (1 mmol) was placed in a round-bottom flask, then 30 mL MeOH was added followed by 0.5 mL concentrated hydrochloric acid. The mixture was refluxed until all the substrate was converted (approximately 2-3 h, monitored with LC-MS), and evaporated to dryness. The product could be triturated with acetone if necessary.

General procedure 10 for the synthesis of *N*-{5-[1-(benzenesulfonyl)-1*H*-indol-3-yl]-1*H*-imidazol-2-yl}acylamides: 10a–z

The intermediates 1-arylsulfonyl-X-(2-bromoacyl)indole (10 mmol) and acylguanidine (70 mmol) were dissolved in 40 mL DMF. The mixture was stirred at 40°C for 96 h, diluted with 100 mL water and vacuum filtered. The resulting solid was dried, triturated with ethyl acetate or acetone, suction filtered and dried. If it was not possible to purify the product by trituration, flash chromatography using a 4- to 5-cm bed of neutral alumina eluted with $CHCl_3$:hexane mixtures was employed, followed by trituration with ethyl acetate or acetone. Hydrochloride salts were prepared as follows: 3 milimoles of compound **10** were suspended in isopropyl alcohol (20 ml). A solution of hydrogen

chloride in isopropyl alcohol (3.3 mmol, of 20% solution, 0.6 g). After complete dissolution of the free base, an amount of diethyl ether sufficient to produce a slight turbidity was added. The deposited solid was filtered and vacuum dried.

General procedure 11 for the synthesis of compounds: 11a–b

N-{5-[1-(benzenesulfonyl)-1*H*-indol-3-yl]-1*H*-imidazol-2-yl}acetamide (0.5 mmol) was dissolved in 6 mL DMF. Then the cesium carbonate (815 mg, 2.5 mmol) was added. Dibromoethane or dibromopropane (1.5 mmol) was added dropwise and the mixture was held at 60°C and monitored by TLC until 100% conversion was achieved. The excess dibromoethane or dibromopropane was evaporated. Water was added to the mixture, and the precipitate was suction filtered and rinsed with 100 mL of water and 30 mL of acetone.

General procedure 13 for cleavage of pyrimidinium salts with hydrazine: 13a–d

Compounds 3-[1-(benzenesulfonyl)-5-methoxy-1*H*-indol-3-yl]-1-alkyl-2-methyl-1*H*-4λ5-imidazo[1,2-*a*]pyrimidin-4-ylium bromide or 3-[1-(benzenesulfonyl)-5-methoxy-1*H*-indol-3-yl]-1-alkyl-3-hydroxy-2-methyl-1*H*,2*H*,3*H*-4λ^5-imidazo[1,2-*a*]pyrimidin-4-ylium bromide (1 mmol) were separately placed in a microwave reactor, and 5 mL acetonitrile and 80% solution of hydrazine monohydrate (440 µM, 7 mmol) were added. The mixture was heated using microwave irradiation for

10 minutes at 100°C and 200 W. Then the mixture was placed in a round-bottomed flask, 20 mL toluene was added and solvents were evaporated to dryness. Pyrazole was washed out with hot distilled water twice, and the mixture was dried under a vacuum. The crude product was triturated with hexane:acetone mixture to yield the pure product.

General procedure 14 for the synthesis of 4-[1-(arylsulfonyl)-1*H*-indol-3-yl]-1,3-thiazol-2-amines: 14a-e

1-arylsulfonyl-X-(2-bromoacyl)indole (1 mmol) and thiourea (5 mmol) were dissolved in 15 mL acetonitrile and held at 40°C for 96 h. Typically, the products were suction filtered at this point. If the precipitation did not occur, the mixture was evaporated to dryness, brought to a boil with 15 mL H_2O, cooled to room temperature and vacuum filtered. The product could be purified by trituration with ethyl acetate or acetone.

***In Vitro* Pharmacology**

Cell Culture. HEK293 cells that stably expressed the human serotonin 5-HT_{1A}R, 5-HT_6 and 5-HT_{7b}R or the dopamine D_{2L}R (obtained using Lipofectamine 2000, Invitrogen) or CHO-K1 cells with a plasmid containing the sequence coding for the human serotonin 5-HT_{2A} receptor (Perkin Elmer) were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were grown in Dulbecco's Modified Eagle's Medium containing 10% dialyzed fetal bovine serum and 500 μg/mL G418 sulfate. For membranes preparations, the cells were subcultured into 150 cm^2 cell culture flasks, grown to 90% confluence,

washed twice with phosphate buffered saline (PBS) prewarmed to 37 °C, pelleted by centrifugation (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol, and stored at −80°C.

5-HT_{1A}/5-HT_{2A}/5-HT_6/5-HT_7/D_2 Radioligand Binding Assays. The membrane preparation and general assay procedures for the cloned receptors were adjusted to a 96-microwell format, as described in our previous papers.[43,44] The cell pellets were thawed and homogenized in 10 volumes of assay buffer using an Ultra Turrax tissue homogenizer, and were centrifuged twice at 35,000 g for 15 min at 4 °C and were incubated for 15 min at 37 °C between centrifugation rounds. The composition of the assay buffers was as follows: for 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate; for 5-HT_{2A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$ and 0.1% ascorbate; for 5-HT_6R: 50 mM Tris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$, and for 5-HT_{7b}R: 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate; for dopamine D_{2L}R: 50 mM Tris–HCl, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 0.1% ascorbate.

All assays were incubated in a total volume of 200 μL in 96-well microtiter plates for 1 h at 37°C, except for 5-HT_{1A}R and 5-HT_{2A}R, which were incubated at room temperature and 27°C, respectively. The equilibration process was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester and the radioactivity that was retained on the filters was quantified using a Microbeta plate reader (PerkinElmer, USA).

For the displacement studies, the assay samples contained the following as radioligands (PerkinElmer, USA): 1.5 nM [^{3}H]-8-OH-DPAT (135.2 Ci/mmol) for 5-HT$_{1A}$R; 2 nM [^{3}H]-ketanserin (53.4 Ci/mmol) for 5-HT$_{2A}$R; 2 nM [^{3}H]-LSD (83.6 Ci/mmol) for 5-HT$_6$R, 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT$_7$R or [^{3}H]-Raclopride (74.4 Ci/mmol). Non-specific binding was defined using 10 μM of 5-HT in 5-HT$_{1A}$R and 5-HT$_7$R binding experiments, whereas 20 μM of mianserin, 10 μM of methiothepine or 1 μM of (+)-butaclamol was used in the 5-HT$_{2A}$R, 5-HT$_6$R and D$_{2L}$R assays, respectively. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated using the Cheng-Prusoff equation[45] and the results were expressed as the means of at least two independent experiments.

Affinity of the reference drugs: 5-HT$_{1A}$R, Buspirone – pK_i = 7.49 M (K_i = 32.2 ± 2.9 nM); 5-HT$_{2A}$R and 5-HT$_6$R, Olanzapine – pK_i = 8.25 M (K_i = 5.6 ± 1.1 nM) and pK_i = 8.05 M (K_i = 8.8 ± 1.3 nM), respectively; 5-HT$_7$R, Clozapine – pK_i = 7.32 M (K_i = 48.4 ± 5.6 nM); D$_2$R, Ziprasidone – pK_i = 9.07 M (K_i = 0.9 ± 0.2 nM).

Functional cAMP Assay Protocol. The antagonistic properties of the compounds at the 5-HT$_6$R were evaluated, as their ability to inhibit cAMP production induced by the agonist 5-CT (100 nM) in HEK293 cells overexpressing 5 HT$_6$R. Each compound was tested in triplicate at 8 concentrations (10^{-11} − 10^{-4}M). Cells (expressing 5-HT$_6$R) were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were grown in Dulbecco's modified Eagle's medium containing 10% dialyzed fetal bovine serum and

500 mg/mL G418 sulfate. For the functional experiments, the cells were grown in 75 cm^2 flasks to 90% confluence, washed twice with prewarmed (37°C) PBS and then collected in (0.48 mM) EDTA by centrifugation (5 min, 160 x g). The supernatant was aspirated, and the cell pellet was resuspended in stimulation buffer (1 × HBSS, 5 mM HEPES,0.5 mM IBMX, 0.1% BSA). Total cAMP was measured using a LANCE cAMP detection kit (PerkinElmer), according to the manufacturer instructions. To quantify cAMP, the cells (5 μL) were incubated with various compounds (5 μL) for 30 min at room temperature, in a 384-well white opaque microtiter plate. After incubation, the reaction was stopped and cells were lysed by the addition of a 10 μL working solution (*i.e.*, 5 μL Eu-cAMP and 5 μL ULight-anti-cAMP). The assay plate was incubated for 1 h at room temperature. The time-resolved fluorescence resonance energy transfer (TR-FRET) was detected by an Infinite M1000 Pro (Tecan) using instrument settings from the LANCE cAMPdetection kit manual. The K_b values were calculated from the Cheng−Prusoff equation[45] specific for the analysis of functional inhibition curves: $K_b = IC_{50}/(1 + A/EC_{50})$, where A is the agonist concentration, IC_{50} is the antagonist concentration producing a 50% reduction in the response to the agonist, and EC_{50} is the agonist concentration, which causes a half of the maximal response. The results were expressed as the means of at least two independent experiments.

Metabolic stability. The tested compounds were incubated in triplicate with a rat liver microsomal fraction at 37°C in the presence of metabolic phase I cofactors (NADP,

G6P,G6P dehydrogenase, $MgCl_2$) necessary for metabolic transformations. The concentration of a non-metabolized test compound in the reaction mixture was measured using HPLC-LC/MS at 4 time points: 0, 20, 40 and 60 min of incubation. The AUC of the compounds at those time points were compared with AUC at point 0 to obtain % loss of parental compound. The data obtained were used to calculate the intrinsic clearance (Cl(int)) and the half-life (T½). The metabolic activity of the microsomes was assessed by measuring the respective stabilities of two standards of low and high metabolic stability, verapamil and donepezil.

Cytotoxicity. The human hepatocellular carcinoma cells (HepG2) were cultured using standard procedures (protocol from ATCC). Briefly, cells were cultured in Dulbecco's Modified Eagle's Medium – high glucose, (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies), with added 100 IU/mL penicillin (Sigma Aldrich) and 100 µg/mL streptomycin (Sigma Aldrich), incubated at 37°C, 5% CO_2. For the test of compounds with the HepG2 cells line, hepatocytes were seeded on 96-well culture plate (Falcon) at a density of 2x104 cells per well in fresh medium. Cells were grown for 24 hours in the incubator (37°C, 5% CO_2) before performing experiments. After addition of test compounds, cell were incubated for further 24 hour. Four concentration of test compound were teste: 1 µM, 10 µM, 50 µM and 100 µM. PrestoBlue reagent (Invitrogen) to assess cell viability and ToxiLight bioassay (Lonza) to determine the cell membrane damages were used according to the manufacturer's

protocol. The flurescence and luminescence signal was measured by POLARstar Omega, plate reader (BMG Labtech).

CYP inhibition. The luminescent CYP3A4 P450-Glo™ and CYP2D6 P450-Glo™ assays and protocols were provided by Promega (Madison, WI, USA). The stock solutions (10 mM) of ketoconazole (KE), quinidine (QD) and examined compounds were performed in DMSO. The 4X concentrated dilutions were prepared before the assays (0.04-100 μM). The enzymatic reactions were conducted in white polystyrene, flat-bottom Nunc™ MicroWell™ 96-Well Microplates (Thermo Scientific, Waltham, MA USA). The CYPs, proluciferin and examined compounds (25 μL/well) were preincubated first for 5 min and next the NADPH Regeneration System was added (25 μL) to start the reaction. The final concentrations of KE and examined compounds were in range from 0.01 μM to 25 μM. The final concentrations of QD were from 0.001 to 10 μM. The control reactions for measure the 100% of CYPs activity and minus-CYP negative control reactions for measure background luminescence were also prepared. The microplate were incubated in room temperature for 30 min (CYP3A4) or 45 min (CYP2D6). Finally, LDR was added (50 μl/well) and after 20 min of incubation in room temperature the luminescence signal was measured with a microplate reader (EnSpire) in luminescence mode. The signal produced by CYPs without the presence of compounds was considered as a 100% of CYP activity. The IC_{50} values were calculated using GraphPad Prism 5 Software.

Mini AMES test. Ames microplate fluctuation protocol (MPF) assay was performed with Salmonella typhimurium strain TA100, enabling the detection of base-pair substitution. Bacterial strain as well as exposure and indicator medium were obtained from Xenometrix AG (Allschwil, Switzerland). The mutagenic potential of tested structures was evaluated by incubation of bacteria with the test compound at a concentration of 1 µM and 10 µM for 90 min (37°C) in exposure medium, containing limited amount of histidine. After addition of indicator medium each well of the 24-well plate was aliquoted into 48 wells of a 384-well plate.

The occurrence of reversion events to histidine prototrophy was observed as a growth of bacteria in the indicator medium without histidine after 72 h of incubation in 37°C temperature. Bacterial growth in 384-well plates was visualized by color change of medium from violet to yellow due to addition of pH indicator dye. The absorbance was measured with a microplate reader (EnSpire) at 420 nm. The reference mutagen NQNO (0.5 µM) was used as positive control in performed experiments. The medium control baseline (MCB) was calculated, as derived from the mean number of revertants in the medium control plus one standard deviation. Data points with fold increase $\geqslant$ 2.0 and binomial B-value $\geqslant$ 0.99 as mutagenic alert.

***In vivo* pharmacology**

NOR procedure. The procedure was based on the earlier study of Popik et al.,[46] 1-aminocyclopropanecarboxylic acid (ACPC) produces procognitive but not

antipsychotic-like effects in rats.

The experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Animal Experiments, Institute of Pharmacology.

Male Sprague–Dawley rats (Charles River, Germany) weighing ~250 g at the arrival were housed in the standard laboratory cages, under standard colony A/C controlled conditions: room temperature 21±2°C, humidity (40–50%), 12-hr light/dark cycle (lights on: 06:00) with *ad libitum* access to food and water. Rats were allowed to acclimatize for at least 7 days before the start of the experimental procedure. During this week animals were handled for at least 3 times. Behavioral testing was carried out during the light phase of the light/dark cycle.

At least 1 h before the start of the experiment, rats were transferred to the experimental room for acclimation. Rats were tested in a dimly lit (25 lx) “open field” apparatus made of a dull gray plastic (66×56×30 cm). After each measurement, the floor was cleaned and dried.

Procedure consisted of habituation to the arena (without any objects) for 5 min, 24 hours before the test and test session comprised of two trials separated by an inter trial interval (ITI). For scopolamine-induced memory impairment paradigm, 1 hour ITI was chosen. During the first trial (familiarization, T1) two identical objects (A1 and A2) were

presented in opposite corners, approximately 10 cm from the walls of the open field. In the second trial (recognition, T2) one of the objects was replaced by a novel one (A=familiar and B=novel). Both trials lasted 3 min and animals were returned to their home cage after T1. The objects used were the glass beakers filled with the gravel and the plastic bottles filled with the sand. The heights of the objects were comparable (~12 cm) and the objects were heavy enough not to be displaced by the animals. The sequence of presentations and the location of the objects was randomly assigned to each rat. The animals explored the objects by looking, licking, sniffing or touching the object while sniffing, but not when leaning against, standing or sitting on the object. Any rat spending less than 5 s exploring the two objects within 3 min of T1 or T2 was eliminated from the study. Exploration time of the objects and the distance traveled were measured using the Any-maze® video tracking system. Based on exploration time (E) of two objects during T2, discrimination index (DI) was calculated according to the formula: DI = (EB–EA)/(EA+AB).

Scopolamine, used to attenuate learning, was administered at the dose of 1.25 mg/kg (i.p.) 30 before familiarization phase (T1). The **9i** and donepezil compounds were administered p.o. 120 min before familiarization phase (T1).

Toxicity class determination. The study of compound **9i** was performed according to the OECD Guideline for Testing of Chemicals No. 425: Acute Oral Toxicity – Up-and-Down Procedure. The detailed procedure can be found in Supporting

Information.

Potentiometric titration:

The potentiometric microtitrations were performed in thermostated 10 mL cell using a CerkoLab microtitration unit, fitted with pH electrode (Hydromet ERH-13-6). The electrode was calibrated with the use of buffer solutions: pH = 4.00, pH = 7.00 and pH = 10.00. Titrant T(0.098 M KOH) was standardised according to the general analytical procedure and protected from carbon dioxide. Double distilled water of conductivity approximately 0,18 μS/cm was used for the preparation of all aqueous solutions. Other solvents and reagents : DMSO, HPLC grade, 99.5% and Polysorbate 80 were supplied by Sigma Aldrich. The composition of the titrand (D) solution was as follows: 0.0025M.

Solubilization procedure for aminoimidazole derivatives: Appropriate amount of compounds **9i**xHCl was dissolved in 1 mL of 0.1 M HCl, 1 mL DMSO and finally 1% solution of Polysorbate 80 was added to prepare 25 mL of stock solution. The concentration of Polysorbate 80 was above CMC (critical micelle concentration) of surfactant with HLB = 15. The elaborated solubilization system could be recommended for low soluble aminoimidazole derivatives.

Analytical Procedure: Volume V0 = 4 mL of titrand D was titrated with 0,098 M of titrant T by using CerkoLab System, equipped with a 5 mL syringe pump. Titrant (T) was added to titrand (D) in increments of 0,005 ml, with a pause of 10 s. The pKa values were calculated from the experimental data points {(Vj,pHj) / j=1,…..,N} according to

the Kostrowicki and Liwo algorithm.[47,48] The determinated pK_a values are in agreement with pKa reported for standard compounds in water.[15]

Procedure validation can be found in Supporting Information.

Molecular modeling

To study the molecular mechanism of action for synthesized library of compounds with the 5-HT_6R, a previously generated[49] and tested homology models built on the β2 adrenergic receptor crystal structure as a template, was used.

The 3-dimensional structures of the ligands were prepared using LigPrep,[50] and the appropriate ionization states at pH = 7.4±1.0 were assigned using Epik.[51] One low energy ring conformation per ligand was generated. The Protein Preparation Wizard was used to assign the bond orders, appropriate amino acid ionization states and to check for steric clashes. The receptor grid was generated (OPLS3 force field)[52] by centring the grid box with a size of 14 Å on Asp3.32. Automated docking was performed using Glide at the XP level with the flexible docking option turned on.[53]

Supporting Information Available: Characterization details for intermediate products and final compounds, toxicological data for compound **9i**, crystal structure determination of compound **9i** and **10b**, ^{1}H NMR, ^{13}C NMR and LC-MS spectra (PDF).

Molecular formula strings (CSV)

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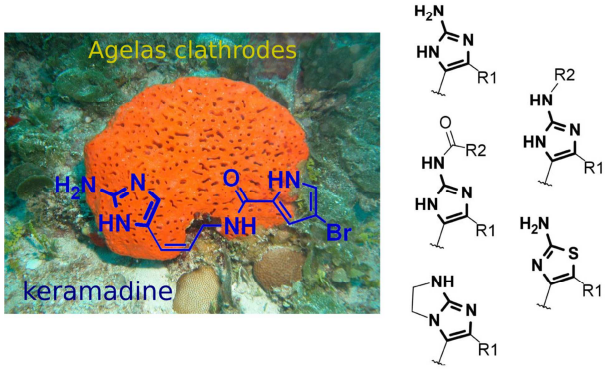
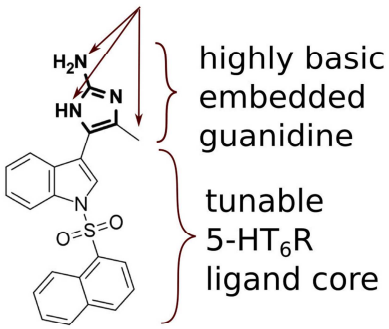
Artwork

Novel serotonin receptor chemotypes inspired by marine sponge alkaloids

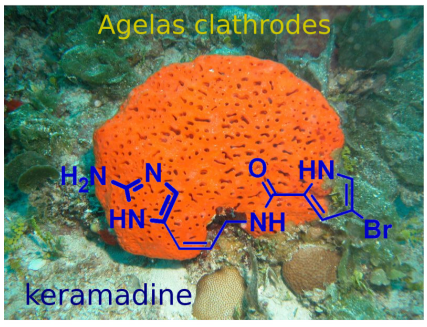


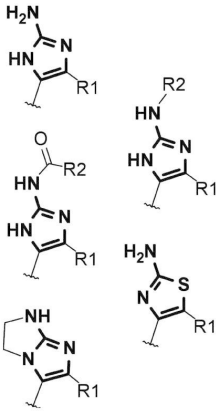
Multiple functionalization sites:



AHN-208 (**9i**, K_b = 5 nM): a selective 5-HT_6R antagonist

Novel serotonin receptor chemotypes inspired by marine sponge alkaloids





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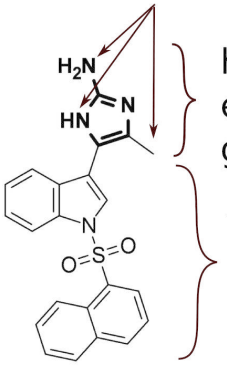

AHN-208 (**9i**, K_b = 5 nM): a selective 5-HT_6R antagonist

- The first synthetic aminergic GPCR ligands built around the 2-aminoimidazole fragment,
- Several differently functionalized 2-aminoimidazole based fragments tested,
- Potent and very selective 5-HT_6R antagonists revealed,
- A significant conformational change occurs upon protonation of the amidine fragment,
- Compound **9i** (AHN-208) exhibited procognitive properties in NOR assay.