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Identification of fused bicyclic heterocycles as potent and selective 5-HT$_{2A}$ receptor antagonists for the treatment of insomnia

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ABSTRACT

A series of fused bicyclic heterocycles was identified as potent and selective 5-HT$_{2A}$ receptor antagonists. Optimization of the series resulted in compounds that had improved PK properties, favorable CNS partitioning, good pharmacokinetic properties, and significant improvements on deep sleep (delta power) and sleep consolidation.

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Serotonin (5-HT) involvement in the regulation of sleep and wakefulness has been well documented.[1] 5-HT actions in the CNS are mediated by multiple receptor subtypes that are classified into seven subfamilies.[2] Of particular interest has been the 5-HT$_2$ subfamily, which includes three subtypes: 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$. Among these three receptors, only 5-HT$_{2A}$ and 5-HT$_{2C}$ are distributed in areas of the CNS that are implicated in the regulation of sleep and waking.[3] Recent preclinical and clinical evidence suggest that 5-HT$_{2A}$ antagonism might be effective in the treatment of sleep maintenance insomnia.[4] Indeed, several selective 5-HT$_{2A}$ inverse agonists have undergone clinical evaluation for the treatment of insomnia including eplivanserin, volinanserin, pruvanserin and nelotanserin.[5]

We recently reported the synthesis and in vivo evaluation of phenethylpiperazine amides based on pyrazole acids. Our initial hit **1** had poor pharmacokinetic properties in rat that we ascribed to its low microsomal stability ($T_{1/2}$ = 14 min); as a result, it was not very potent in vivo. Compound **1** also showed significant hERG channel blockade which could be a potential safety concern. Optimization of the pyrazole portion and replacing the phenethylpiperazine linker with phenethanone piperazine linker resulted in **2** and **3** (Fig. 1). These two analogs had improved pharmacokinetic properties and acceptable in vitro safety profiles and were highly efficacious in improving deep sleep and sleep consolidation in the rat.[6]

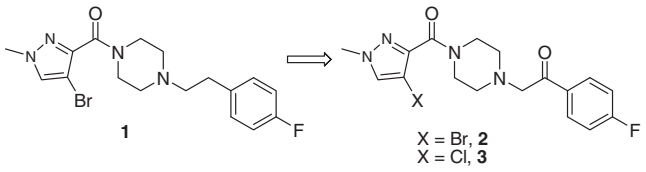

Figure 1. From hit **1** to lead **2** and **3**.

Despite its poor metabolic stability **1** was still attractive as a chemical starting point as a result of its very favorable CNS penetration. Samples taken during an experiment to measure attenuation of DOI-induced hypolocomotion in rat were used to determine a brain:plasma ratio of >2. Thus as an alternative approach to the optimization of this lead we decided to keep the phenethylpiperazine linker and explore other aromatic acids to replace the bromo-pyrazole acid of **1**. Our goal was to identify compounds that had improved metabolic stability that therefore may have better exposure and duration of action in vivo while maintaining the favorable CNS partitioning. Herein we describe the discovery and optimization of a series of fused bicyclic heterocycles using this approach that was able to provide the in vivo efficacy comparable to **2** or **3**.

From previous SAR, it was known that 2,4-difluoro substitution of the phenyl ring provided a modest but significant increase in 5-HT$_{2A}$ binding affinity versus the 4-fluoro substitution of the phenyl

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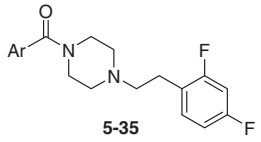
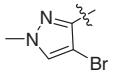
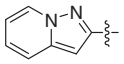
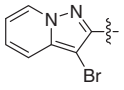
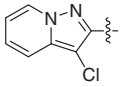
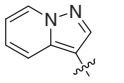
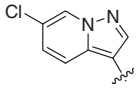
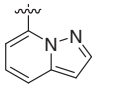
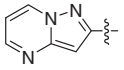
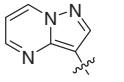
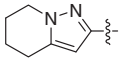
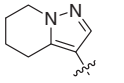
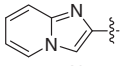
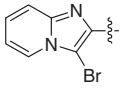
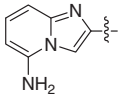
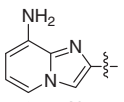
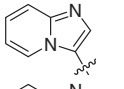
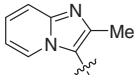
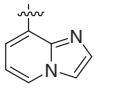
Table 1
Binding affinities of 5,6-fused heteroaromatic acid derivatives at human 5-HT_{2A} and 5-HT_{2C} receptors



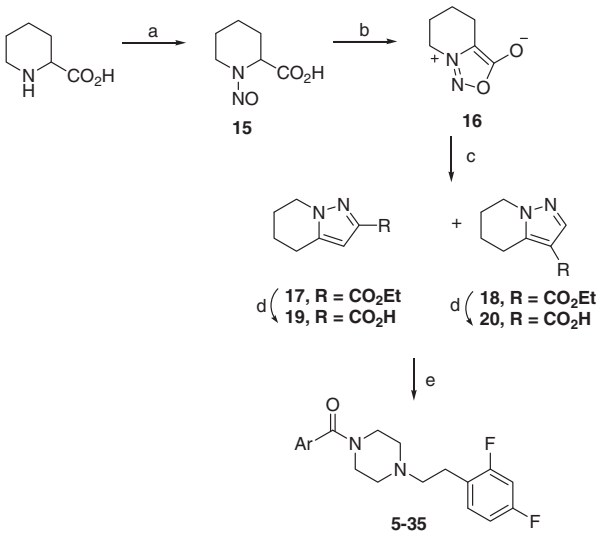
5-35

Compd	Ar	5-HT_{2A} K_i[a] (nM)	5-HT_{2C} K_i[a] (nM)	Selectivity[b]
4		1.1	2820	2563
5		5.7	467	82
6		0.8	496	620
7		0.6	394	656
8		1.8	246	136
9		5.9	575	97
10		0.4	194	485
11		5.6	1360	242
12		3.8	910	240
13		2.6	717	275
14		18	2877	160
29		4.3	596	138
30		1.9	581	305
31		1.4	152	108
32		2.7	190	70
33		1.7	739	434
34		3.5	997	284
35		1.6	524	327

[a] K_i values are the mean of at least three experiments performed in triplicate, determined from ten concentrations, all K_i values were calculated from IC_{50} values using the method of Cheng and Prusoff[10] with standard deviation <0.4 log units.
[b] h5-HT_{2C}/h-5HT_{2A}.

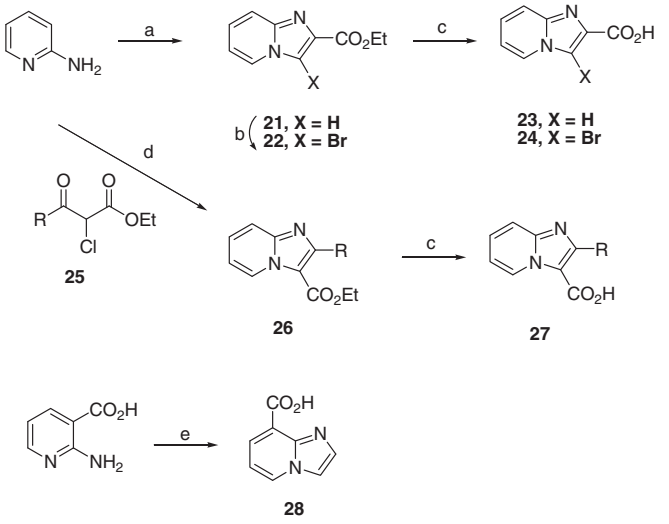
ring with the phenethylpiperizine linker, so this group was kept constant while we made an extensive exploration of 5,6-fused bicyclic heteroaromatic groups in place of the bromo-pyrazole of **1**.

We began with pyrazolo[1,5-*a*]pyridines in which the pyrazole was fused with a phenyl group. Three different acid positions were explored using commercially available, pyrazolo[1,5-*a*]pyridine 2-acid, 3-acid and 7-acids. In vitro competitive binding assays with radiolabeled 2,5-dimethoxy-4-iodoamphetamine (^{125}I-DOI) were used to determine K_i values for all compounds against recombinant h5-HT_{2A} and h5-HT_{2C} receptors (Table 1). The direct monocyclic analog **4** was included as comparison. The selectivity ratio for the 5-HT_{2A} receptor versus the 5-HT_{2C} receptor was calculated based on K_i values. As can be seen, the 5-HT_{2A} receptor binding affinity of these phenyl-fused pyrazoles was comparable to that of monocyclic pyrazole analog **4**. The 7-acid derivative **10** was slightly more potent than the 2-acid (**5**) or 3-acid (**8**) derivatives. As observed previously, introduction of halogen to the pyrazole ring led to increased affinity for the 5-HT_{2A} receptor, this was confirmed by **6** and **7** whose binding affinity was improved by 7–8-fold compared to **5**. Interestingly, introduction of halogen to the pyridine ring, such as **9**, had little effect on the 5-HT_{2A} receptor binding affinity. Adding another nitrogen to the pyridine ring resulted in pyrazolopyrimidine core, these compounds (**11** and **12**) retained 5-HT_{2A} receptor binding affinity and had slightly lower affinity for the 5-HT_{2C} receptor. Compared to the monocyclic pyrazole analog **4**, these phenyl-fused-pyrazole derivatives were less selective for the 5-HT_{2A} receptor versus the 5-HT_{2C} receptor. To examine if this reduced selectivity was due to the extra phenyl group fused with the pyrazole ring, cyclohexyl-fused pyrazoles with an acid at both 2- and 3-positions were prepared for incorporation into test compounds. As outlined in Scheme 1, esters **17** and **18** were isolated according to literature methods.[7] *DL*-pipecolic acid was nitrosated using $NaNO_2$ in glacial acetic acid to give acid **15**, and treatment with trifluoroacetic anhydride provided the sydnone **16**. Cycloaddition with ethyl propiolate yielded both regioisomers, with the major isomer having the ester group at the 2-position. Base hydrolysis of the separated esters afforded acids **19** and **20**. As shown in Table 1, the 2-acid derivative **13** had higher 5-HT_{2A} binding affinity than the 3-acid derivative **14**. However, the pyrazoles fused with the cyclohexyl group did not have much improvement with respect to 5-HT_{2A} receptor selectivity compared to pyrazoles fused with the phenyl group.



5-35

Scheme 1. Synthesis of fused pyrazole acids. Reagents and conditions: (a) $NaNO_2$, CH_3CO_2H; (b) $(CF_3CO)_2O$; (c) ethyl propiolate, xylene, reflux; (d) NaOH, EtOH; (e) 2,4-difluorophenethyl piperazine, HATU, TEA, THF.



Scheme 2. Synthesis of imidazopyridine acids. Reagents and conditions: (a) ethyl bromopyruvate, CH_3CN; (b) Br_2, CH_3CO_2H; (c) NaOH, EtOH; (d) CH_3CN, 80 °C; (e) bromoacetaldehyde dimethyl acetal, CH_3CN.

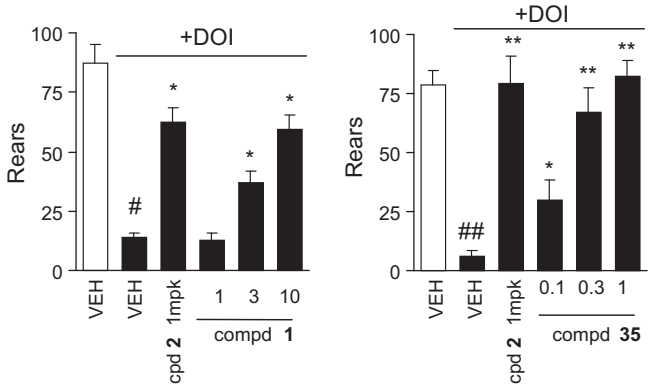
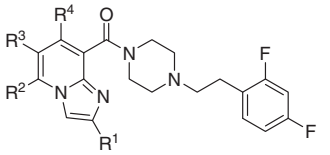


Figure 2. Attenuation of DOI-induced hypolocomotion in rat. DOI (1 mg/kg) administered 10 min prior to locomotor testing induced a decrease in locomotor activity which was reversed by 5-HT_{2A} antagonists: compound **1**, **2**, and **35**. $^{\#\#}P$ <0.01 versus VEH/VEH, $^{**}P$ <0.01 versus VEH/DOI, $^{*}P$ <0.05 versus VEH/DOI.

Moving the position of nitrogen in the pyrazolo[1,5-*a*]pyridine provided imidazo[1,2-*a*]pyridine scaffolds.[8] Again three different acid positions (2-, 3- and 8-acids), were explored. Synthesis of the acids was outlined in Scheme 2. Condensation of 2-aminopyridine with ethyl bromopyruvate afforded ethyl imidazo[1,2-*a*]pyridine 2-carboxylate **21** and bromination using bromine in acetic acid introduced a bromo group at the 3-position (**22**). Saponification furnished the imidazo[1,2-*a*]pyridine 2-acids **23** and **24**.

Table 3
Binding affinities of imidazo[1,2-*a*]pyridine 8-acid derivatives at human 5-HT_{2A} and 5-HT_{2C} receptors



Compd	R^1	R^2	R^3	R^4	5-HT_{2A} K_i[a] (nM)	5-HT_{2C} K_i[a] (nM)
36	Br	H	H	H	1.7	682
37	Cl	H	H	H	1.5	391
38	CN	H	H	H	2.8	6,900
39	H	H	Br	H	5.0	474
40	H	Me	H	Me	3.6	153

[a] K_i values are the mean of at least three experiments performed in triplicate, determined from ten concentrations, all K_i values were calculated from IC_{50} values using the method of Cheng and Prusoff[9] with standard deviation <0.4 log units.

Similarly, condensation of 2-aminopyridine with a 2-chloro-β-ketoester derivative **25** provided ethyl imidazo[1,2-*a*]pyridine 3-carboxylate **26** which was then hydrolyzed to the acid **27**. Imidazo[1,2-*a*]pyridine 8-acid **28** was prepared from 2-aminonicotinic acid and bromoacetaldehyde dimethyl acetal in one step. The SAR of these imidazo[1,2-*a*]pyridine acids (**29**–**35**) was similar to that of pyrazolo[1,5-*a*]pyridine acids (Table 1). They retained binding affinity at the 5-HT_{2A} receptor across different acid positions but with somewhat reduced selectivity at the 5-HT_{2A} receptor compared to the monocyclic analog **4**. One further observation was that substitution of a hydrophilic amino group on the pyridine ring (**31** and **32**) further decreased selectivity at the 5-HT_{2A} receptor.

In contrast to the SAR of phenyl substitutions and piperazine ethyl linker modifications, which could change 5-HT_{2A} binding affinity significantly with only subtle changes,[6] activity was retained across a wide range of heteroaromatic acid derivatives. The in vitro profiles of these heteroaromatic acid derivatives were similar with respect to both their 5-HT_{2A} receptor binding affinity and selectivity; therefore in vivo potency and CNS partitioning were used as criteria to differentiate the compounds. Compounds were evaluated for their ability to attenuate DOI-induced hypolocomotion in rats. DOI is a potent 5-$HT_{2A/C}$ receptor agonist that crosses the blood–brain barrier. Administration of DOI in rats produces a decrease in total locomotor activity, including a suppression of rearing. This inhibition can be reversed by co-administration of a centrally acting 5-HT_{2A} antagonist. Thus compounds were evaluated in a rat DOI screen at a single dose of 3 mg/kg (in expectation of their low exposure similar to that of **1**) and their efficacies were compared to 1 mg/kg of **2**. One striking early result was **35**, at the screening dose of 3 mg/kg it was equipotent with 1 mg/kg of **2**, so a dose–response at lower doses was conducted. As shown in Figure 2, compound **35** had ED_{50} of 0.3 mg/kg, making it as potent as **2**, and it had good CNS partitioning with a

Table 2
Parmacokinetic parameters of **1** and **35** after oral dose in rat, brain and plasma concentration from DOI reversal studies in rat[a]

Compd	C_{max} (ng/mL)	AUC_{last} (h ng/mL)	$T_{1/2}$ (h)	IV CL_{total} (L/h/kg)	Vss (L/kg)	F[d] (%)
1	48[b]	91[b]	1.4	9.3	8.1	9
35	943[c]	440[c]	0.5	3.5	1.7	47
	Dose (mg/kg)	Brain[e] (ng/mL)	Plasma[e] (ng/mL)	B/P[f]		
1	3	16.8	8.3	2.02		
35	0.3	14.5	11	1.32		

[a] Values represent the mean of $n = 3$ animals.
[b] Values from 10 mg/kg dose.
[c] Values from 3 mg/kg dose.
[d] % F calculated relative to a 2 mg/kg iv dose.
[e] Samples taken at the 0.75 h time point.
[f] Brain to plasma ratio.

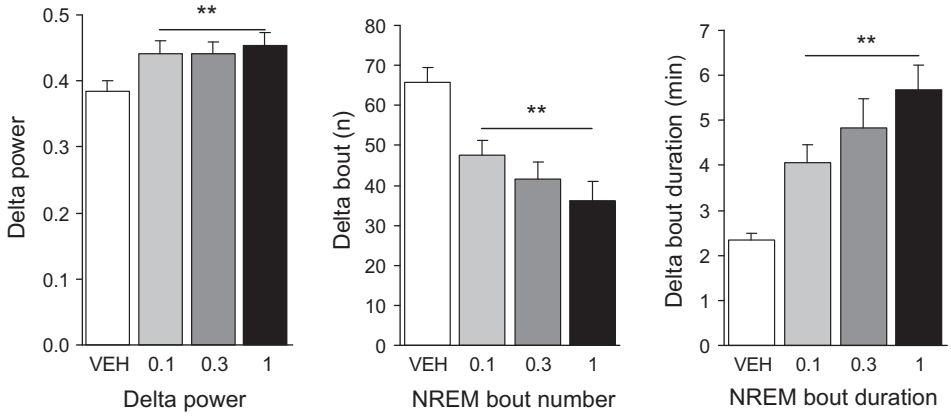


Figure 3. Rat sleep study of compound **35**. Data represent the sum total of sleep measures recorded 5 h after compound administration. It induces a statistically significant increase in delta power (left figure), and a dose-dependent statistically significant increase in sleep consolidation, as indicated by the concomitant decrease in NREM bout number (middle figure) and increase in NREM bout duration (right figure). $^{*}P$ <0.05; $^{**}P$ <0.01 versus vehicle treated controls.

brain to plasma ratio of 1.32 at 0.3 mg/kg (Table 2). Compound **35** was thus 10 times more potent than the initial starting point **1** in the DOI reversal assay. Further assessment of pharmacokinetics in rat revealed this improvement was most likely the result of the good oral bioavailability of **35** (47%), which had a much higher exposure level than **1** (AUC 440 h ng/mL) (Table 2). Interestingly, this greater bioavailability was observed despite a lack of improvement in microsomal stability compared to **1** ($T_{1/2}$ for **35** = 16 min in rat liver microsomes). Given the excellent efficacy of **35** in the in vivo DIO reversal assay, the imidazo[1,2-*a*]pyridine 8-acid core was further examined to introduce substitutions to both the imidazole and pyridine ring portions (Table 3). Of note is **38**, substituted with a nitrile group at the 3-position, it was the most highly 5-HT_{2A} selective compound among all the 5,6-fused bicyclic heterocycles prepared. However, none of these substituted analogs were more potent in the in vivo DIO reversal assay than **35**.[9] As a result **35** was further evaluated in a sleep study at doses of 0.1, 0.3 and 1 mg/kg PO (Fig. 3). In this study, compound was administered orally 6 h after lights on in the middle of the rat's inactive period (subjective night). Both delta power, a measure of deep sleep, and sleep consolidation were analyzed. **35** significantly increased delta power 1 h after dosing at 0.1 mg/kg compared to vehicle treated controls, and the effect lasted 3–4 h following dosing. In addition, **35** significantly and dose-dependently increased sleep consolidation, as indicated by the decrease in non-rapid eye movement (NREM) bout number and increase in NREM bout duration.

As a result of its excellent activity in the DOI reversal assay and good CNS penetration, **35** was further profiled. Selectivity screening against a panel of 70 human GPCRs, ion channels and transporters (CEREP) showed it had no binding of >50% of control at 10 μM, on any target tested including dopamine receptors D1-5, α1-adrenergic and α2-adrenergic receptors. CYP inhibition assays using human liver microsomes revealed **35** was not an inhibitor of any major CYP isoforms (1A2, 2C9, 2C19, 2D6 and 3A4; IC_{50} >40 μM). However, in the patch clamp in a full concentration-response assay, **35** inhibited hERG channel function with an IC_{50} of 1.9 μM. From these data, we calculated that there likely still remained a significant margin between the hERG inhibition and the plasma concentration required for efficacy. The latter was estimated as 11 ng/mL based on the 45 min time point (close to the T_{max}) at the fully efficacious dose of 0.3 mg/kg in rat. This concentration equated to approximately 33 nM. With the plasma protein binding in human measured at 67.4%, this would give a free fraction of ~11 nM in vivo. Hence we adjudged there would likely be a satisfactory margin (approximately 175-fold) between this calculated free fraction concentration and the IC_{50} from the hERG study for this compound. However, we decided that further evaluation would be prudent at this stage and so a cardiovascular assessment in a telemeterized dog model was conducted, wherein no adverse effects were observed on hemodynamic or ECG measures at doses up to 30 mg/kg.

The identification of **35** with potent in vivo antagonist activity demonstrated that improved exposure could be achieved with a phenethylpiperazine linker if appropriate acid group cores were identified and this compound was selected for further development.

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9. Synthesis of 35: To a mixture of 2-aminonicotinic acid (0.69 g, 5.00 mmol) in CH3CN (20 mL) was added bromoacetaldehyde dimethyl acetal (0.59 mL, 5.00 mmol). The resulting slurry was heated to 150 °C under microwave irradiation for 2 h. The resulting precipitate was filtered off and washed with CH3CN and hexane to afford *H*-imidazo[1,2-*a*]pyridine-8-carboxylic acid (0.924 g) as a grey solid. Exact mass calculated calcd for $C_8H_6N_2O_2$: 162.04; Found: LC–MS m/z = 163.1($M+H^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.58–7.69 (m, 1H), 8.15 (d, J = 2.27 Hz, 1H), 8.50 (dd, J = 7.45, 1.14 Hz, 1H), 8.56 (d, J = 2.02 Hz, 1H), 9.20 (dd, J = 6.69, 1.14 Hz, 1H). To a solution of *H*-imidazo[1,2-*a*]pyridine-8-carboxylic acid (36.6 mg, 226 μmol), 1-(2,4-difluorophenethyl)piperazine dihydrochloride (45.0 mg, 150 μmol), and triethylamine (210 μl, 1504 μmol) in DMF (0.75 mL) was added 1-propylphosphonic acid anhydride solution (50 wt % in ethyl acetate, 183 μL, 0.301 mmol). The mixture was stirred for 2 h, quenched with water and purified by HPLC to give 35 (33.0 mg) as a white solid. Exact mass calculated for $C_{20}H_{20}F_2N_4O$: 370.16; Found: LC–MS m/z = 371.4 ($M+H^+$). ^{1}H NMR (400 MHz, chloroform-d_6) δ ppm 2.45–2.53 (m, 2H), 2.57–2.64 (m, 2H), 2.64–2.71 (m, 2H), 2.74–2.84 (m, 2H), 3.29–3.43 (m, 2H), 3.85–3.98 (m, 2H), 6.69–6.92 (m, 3H), 7.10–7.21 (m, 1H), 7.23–7.28 (m, 1H), 7.64 (d, J = 1.26 Hz, 1H), 7.69 (d, J = 1.26 Hz, 1H), 8.18 (dd, J = 6.82, 1.26 Hz, 1H).
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