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Synthesis and pharmacological evaluation of aminopyrimidine series of 5-HT$_{1A}$ partial agonists

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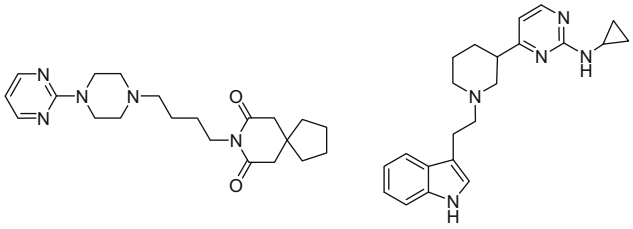
ABSTRACT

Aminopyrimidine **2** (4-(1-(2-(1*H*-indol-3-yl)ethyl)piperidin-3-yl)-*N*-cyclopropylpyrimidin-2-amine) emerged from a high throughput screen as a novel 5-HT$_{1A}$ agonist. This compound showed moderate potency for 5-HT$_{1A}$ in binding and functional assays, as well as moderate metabolic stability. Implementation of a strategy for improving metabolic stability by lowering the lipophilicity (*c*Log*D*) led to identification of methyl ether **31** (4-(1-(2-(1*H*-indol-3-yl)ethyl)piperidin-3-yl)-*N*-(2-methoxyethyl)pyrimidin-2-amine) as a substantially improved compound within the series.

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter that is known to play a role in regulating numerous physiological functions, including thermoregulation, vasoconstriction, sexual behavior, appetite, and sleep. Additionally, alterations in 5-HT-mediated mechanisms are associated with a range of neuropsychiatric conditions, such as anxiety, depression, and bipolar disorder. Among the various 5-HT receptors, the 5-HT$_{1A}$ subtype has been most extensively studied.[1,2] 5-HT$_{1A}$ agonists and partial agonists have demonstrated clinical effectiveness in the treatment of anxiety[3] and depression.[4,5] Recent preclinical and clinical studies also suggest that 5-HT$_{1A}$ agonists may be useful in treating dementia,[6] Parkinson's disease,[7] pain associated with spinal cord injuries,[8] ischemia,[9] and ADHD.[10] To date, buspirone (**1**, Fig. 1) is the only 5-HT$_{1A}$ agonist marketed in the US. Its market potential has been limited by side effects, a short half-life (2–3 h) that necessitates three-times a day (tid) dosing,[11] and formation of a metabolite, 2-(piperazin-1-yl)pyrimidine, which is a potent α_2-adrenergic receptor antagonist.[12] As part of an effort to identify novel 5-HT$_{1A}$ agonists, compound **2** (Fig. 1) emerged as a lead compound following a high throughput screen of the Pfizer compound collection. Whereas the activity of the 3-(2-(piperidin-1-yl) ethyl) indole moiety at 5-HT receptors is known,[13] compound **2** represents a novel template against this receptor class. This compound showed moderate potency for 5-HT$_{1A}$ in binding and functional assays, as well as moderate stability in an in vitro human liver microsome (HLM) assay. Lead optimization efforts around this compound focused on improving 5-HT$_{1A}$ binding potency, functional activity, and metabolic stability.

The synthesis of novel analogs of **2** was accomplished using the versatile chloropyrimidine intermediate **8** (Scheme 1). The synthesis of **8** began with piperidine 3-carboxylic acid (**3**), which was protected as the *tert*-butyl carbamate **4**. Subsequent Weinreb amide formation and methyl Grignard addition afforded ketone **5**, which was further elaborated to vinylogous amide **6** using Bredereck's reagent. Cyclocondensation with guanidine provided 2-aminopyrimidine **7**, and diazotization in the presence of $CuCl_2$ yielded 2-chloropyrimidine **8**. Although the conversion of **7**–**8** proceeded in modest yield, **8** was readily accessible on 100 g scale for use in medicinal chemistry studies.

Chloropyrimidine **8**, which presents potential points of diversification at both the 2-pyrimidyl position and at the piperidine nitrogen, served as a useful template for parallel synthesis of a ser-



1
5-HT$_{1A}$ Ki = 15 nM

2
5-HT$_{1A}$ Ki = 75 nM

Figure 1. Buspirone and 5-HT$_{1A}$ agonist **2**.

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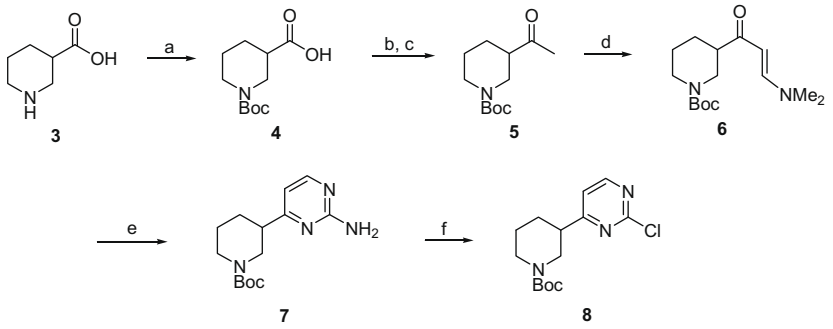


Scheme 1. Reagents and conditions: (a) Boc_2O (1.2 equiv), NaOH (2.1 equiv), dioxane/water, 3 °C—rt (88%); (b) HN(Me)(OMe) (1.2 equiv), EDCI (1.2 equiv), Et_3N (3.0 equiv), HOBt (0.2 equiv), CH_2Cl_2, 3 °C—rt (92%); (c) MeMgCl (2.0 equiv), MTBE, −10 °C—rt; (d) *tert*-butoxy-bis(dimethyamino)-methane, reflux; (e) NaOMe (2.0 equiv), MeOH, guanidine (1.5 equiv), 3 °C—rt (87%, three steps); (f) isoamyl nitrite (1.3 equiv), $CuCl_2$ (1.2 equiv), CH_3CN, 65 °C (20%).

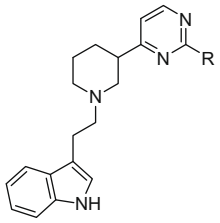
ies of 2-alkylaminopyrimidines (**11**–**28**). Treatment of **8** at 65 °C with a variety of primary amines (see Tables 1–3) afforded aminopyrimidines **9** (see Scheme 2). Liberation of the piperidine amine functionality using 4 N HCl followed by N-alkylation of primary alkyl bromides[14] under microwave heating conditions provided the array of diverse 2-alkylaminopyrimidines (**11**–**28**).[15].

One aspect of the SAR studies on lead compound **2** focused on the effect of minor modifications of the alkylamino group at the 2-pyrimidyl position. Our preliminary studies showed that higher 5-HT_{1A} binding affinity and intrinsic activity could be achieved by replacement of the cyclopropyl group with slightly longer or bulkier alkyl groups (Table 1). However, these improvements in 5-HT_{1A} binding and functional activities were accompanied by a significant decrease in HLM stability. Although racemates were used for the majority of our early SAR work in this series, the effect of the stereocenter at the 3-piperidyl position was examined using single enantiomers **15** and **16**, which were resolved using chiral HPLC. Our study of **15** and **16** shows no significant difference in 5-HT_{1A} binding affinities between the two enantiomers, but the (−)-enantiomer, **15**, was shown to be a weaker partial agonist than the (+)-enantiomer, **16**.

In optimizing lead compound **2**, we also investigated modification of the 3-ethylindole portion of the molecule. Recognizing the potential metabolic soft spots presented by the indole motif, a blocking approach toward improving HLM stability was undertaken. Compounds **17**–**21** were prepared in order to study the effect of blocking the C2, C5, C6, and C7 positions of the indole nucleus (Table 2). Although 5-HT_{1A} binding potency improved somewhat with the addition of fluoro or chloro substituents at the C5 and C7 positions of the indole (Table 2, compounds **17**–**21**), the HLM stability was generally reduced in these compounds compared to lead compound **2** (compounds **17**–**19; 21**). Likewise, replacement of the indole system with alternate fused bicyclic heterocycles or carbocycles, including benzodioxane, benzothiophene, or indane systems (compounds **22**–**24**) led to a modest improvement in 5-HT_{1A} binding affinity and a significant decrease in HLM stability. Analysis of **2** using the MetaSite software[19] for prediction of metabolic soft spots suggested that the observed cytochrome P450-mediated metabolism in this series has a high probability of occurring at the position on the ethyl tether adjacent to the piperidine nitrogen. Compound **25** was prepared to test whether increased steric bulk at this position would interfere with metabolic degradation. However, this change had little effect on either 5-HT_{1A} binding or HLM stability. In vitro metabolite identification studies in this series showed that one major metabolite resulted from oxidation of the pseudobenzylic position on the ethylene tether. Ketone **26** was prepared in order to test the effect of blocking this site. Unfortunately, this modification led to complete loss of 5-HT_{1A} binding potency and also did not improve HLM stability.

Due to the failure of our blocking strategy to produce metabolically stable analogs, compounds **27** and **28** were prepared to test the hypothesis that polar, monocyclic heterocycles might serve as suitable substitutes for the indole. These compounds did indeed show significant improvement in HLM stability; however, 5-HT_{1A} binding affinity was dramatically reduced. Not surprisingly, in our studies of compounds **2**–**28** (Tables 1 and 2), a strong correlation between *c*Log*D*[20] and HLM stability was observed. Whereas modification of the size of the alkyl groups on the alkyl amino substituent was well tolerated by 5-HT_{1A} (Table 1), replacement of the

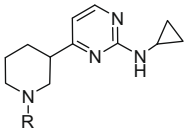
Table 1
Binding, functional activity, and microsomal stability of 2-alkylamino pyrimidines

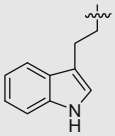
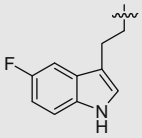
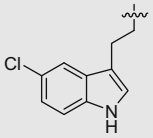
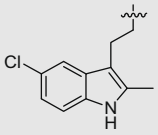
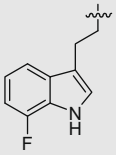
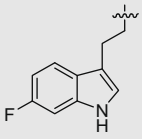
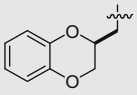
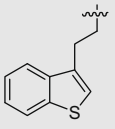
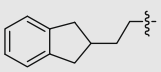
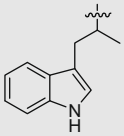
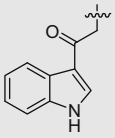


Compound	R	Ent	5-HT_{1A} K_i (nM)[a]	FLIPR IA (%)[b]	FLIPR EC_{50} (nM)	HLM Clint[c] (mL/min/kg)
1	—	—	15	72	41	148
2		±	75.3	38	138	35
11		±	21.3	82	147	94
12		±	7.4	88	140	134
13		±	32	—	—	135
14		±	5.2	—	—	138
15	*n*-PrNH	—	11.2	50	302	81
16	*n*-PrNH	+	16.3	90	78	178

[a] Serotonin$_{1A}$ scintillation proximity assay (SPA) binding assay; IC_{50} values are the mean of at least two experiments carried out in duplicate.[16]
[b] FLIPR assay for agonism at serontonin$_{1A}$ receptors; EC50 and IA values are the mean of at least two experiments carried out in duplicate.[17]
[c] Human Liver Microsome Incubation assay.[18]

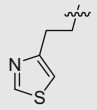
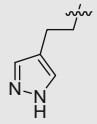
Table 2
Binding, functional activity, and microsomal stability of modified heterocyclic analogs



Compound	R	$5HT_{1A}$ K_i[a] (nM)	FLIPR IA (%)	FLIPR EC_{50}[b] (nM)	*c*Log*D*[c]	HLM Clint[d] (mL/min/kg)
2		75.3	38	138	1.7	35
17		23.6	28	169	2.1	40
18		10.4	–	–	2.8	49
19		26.6	–	–	3.1	135
20		35.1	29	216	2.1	28
21		64.5	–	–	2.0	65
22		37.8	–	–	2.5	60
23		38.5	–	–	4.2	143
24		42.5	10	290	3.1	106
25		40.2	–	–	1.6	60
26		3420	–	–	3.1	36

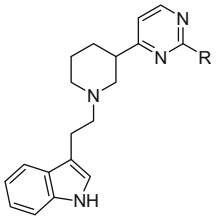
(*continued on next page*)

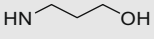
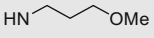
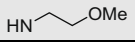
Table 2 (*continued*)

Compound	R	5HT$_{1A}$ K_i[a] (nM)	FLIPR IA (%)	FLIPR EC$_{50}$[b] (nM)	*c*Log*D*[c]	HLM Clint[d] (mL/min/kg)
27		780	–	–	0.8	15
28		4450	–	–	0.3	<7.6

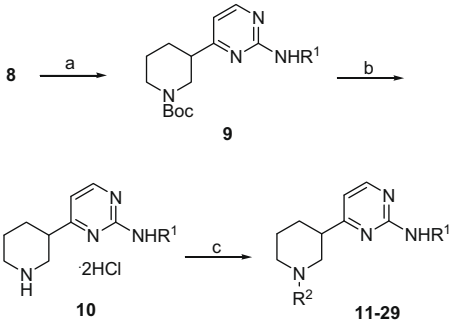
[a] IC$_{50}$ values are the mean of at least two experiments carried out in duplicate.
[b] EC$_{50}$ and IA values are the mean of at least two experiments carried out in duplicate.
[c] Calculated log*D* value (pH 7.4).
[d] Human Liver Microsome Incubation assay.[16]

Table 3
Log*D* modification via alkyl amino side chain modification



Compound	R	5HT$_{1A}$ K_i[a,b] (nM)	FLIPR IA (%)	FLIPR EC$_{50}$[c] (nM)	*c*Log*D*	HLM Clint[d] (mL/min/kg)
2		75.3	38	138	1.7	35
29		175	91	51.4	0.8	12.3
30		78.3	91	65	1.4	34.4
31		66.3	94	40.7	1.2	9.6

[a] Calculated Log*D* value (pH 7.4).
[b] IC$_{50}$ values are the mean of at least two experiments carried out in duplicate.
[c] EC$_{50}$ and IA values are the mean of at least two experiments carried out in duplicate.
[d] Human Liver Microsome Incubation assay.[16]



Scheme 2. Reagents and conditions: (a) R^1NH_2, 65 °C; (b) 4 N HCl in dioxane, rt; (c) R^2Br, CH_3CN/H_2O, Et_3N or NaOH, 150 °C (microwave), 45 min.

indole core with smaller, less lipophilic heterocycles was not well tolerated (Table 2, compounds **27**–**28**). These findings suggested that modulating *c*Log*D* by modification of the alkyl amino group might be a fruitful approach toward improving HLM stability while maintaining 5-HT$_{1A}$ binding affinity and functional activity.

To test the effect of adding polar groups to the alkyl amino terminus, compounds **29**–**31** were prepared (Table 3). As expected, reduction of Log*D* by the addition of a terminal hydroxyl group improved HLM stability (**29**); however, 5-HT$_{1A}$ binding affinity was reduced somewhat by this modification. Capping of the alkyl amino group with a methyl ether proved to be a better solution to the problem. Although no improvement in metabolic stability was observed with compound **30**, 5-HT$_{1A}$ binding was not adversely affected by this modification, and functional activity was significantly improved over that of the simple alkyl amino derivatives. Ultimately, the best balance of potency and stability was achieved with methyl ether **31**. As predicted, the reduction of *c*Log*D* in compound **31** relative to lead compound **2** afforded a significant improvement in HLM stability. Furthermore, this modification did not affect 5-HT$_{1A}$ binding potency and led to improved 5-HT$_{1A}$ functional activity, and the compound showed good in vivo brain exposure in rat.[21]

Identification of aminopyrimidine **2** as a lead compound represented an important first step toward discovery of a novel 5-HT$_{1A}$ agonist with a superior pharmacokinetic profile to buspirone. As part of our lead optimization efforts, several strategies toward improving the pharmacological and pharmacokinetic profile for aminopyrimidine **2** were investigated. The initial approach of blocking potential metabolic soft spots was ineffective. One plausible explanation for this finding is that as single positions were blocked on the relatively lipophilic analogs, a compensatory increase in the rate of P450-mediated formation of alternate

metabolites resulted in no net improvement in clearance.[22] A more effective strategy utilized lowering the lipophilicity (*c*Log*D*) by addition of a methyl ether at the alkyl amino terminus of the lead molecule. Using this approach, methyl ether **31** emerged as a substantially improved compound within the series. Additional studies required for further advancement of **31** will include study of the single enantiomers of the compound in both in vitro and in vivo pharmacological and pharmacokinetic studies.

Acknowledgments

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14. (a) Alkyl bromides were prepared from the corresponding alcohols using CBr_4 (1.5 equiv) and PPh_3 (1.5 equiv) in CH_2Cl_2, rt, 16 h; Alcohols that were not commercially available were prepared according to the following method (b) Campos, K. R.; Woo, J. C. S.; Lee, S.; Tillyer, R. D. *Org. Lett.* **2004**, *6*, 79.
15. Compounds **12** and **25** were prepared as diasteromeric mixtures from racemic starting materials.
16. Assayed according to published protocol: Graham, J. M.; Coughenour, L. L.; Barr, B. M.; Rock, D. L.; Nikam, S. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 489.
17. Standard FLIPR techniques and CHO cells transfected with human 5-HT_{1A} receptor and Gα16 were used. Cells were plated on 384-well plates ($\sim$25,000 cells/well). Growth media was removed and cells were loaded at 37 °C with 25 μl/well DMEM containing 4.6 μM Fluo-4 dye and 2.5 mM probenecid. After 1 h, cells were washed using assay buffer containing a final ionic composition (mM) of: 1.26 $CaCl_2$, 0.81 Mg_2SO_4, 5.55 glucose, 10.0 HEPES, 5.37 KCl, 0.44 KH_2PO_4, 0.34 Na_2HPO_4, 136.89 NaCl, 2.5 probenecid. Plates were equilibrated for 30 min post-loading in a 37 °C, CO_2-gassed incubator. A 5 min incubation period at rt was initiated within FLIPR with a 20 μl addition of (3×) drug-containing buffer or 20 μl addition of a (2×) buffer solution containing serotonin. Fluorescence was monitored at 5 s intervals for 315 s. Data was analyzed as peak height or maximum fluorescent counts above basal.
18. Assay method adapted from published protocols: (a) Riley, R. J.; McGinnity, D. F.; Austin, R. P. *Drug Metab. Dispos.* **2005**, *33*, 1304; (b) Obach, R. S. *Drug Metab. Dispos.* **1999**, *27*, 1350.
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20. Calculated Log*D* values (*c*Log*D*) using ACD/Labs 9.03.
21. Compound **31** dosed in rat ($n = 4$) 10 mg/kg (SC); mean concentrations (ng/mL) 1 h post dose; plasma: 798.3 ± 224.3; brain: 1013.7 ± 80.4.
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