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Solubilized phenyl-pyrazole ureas as potent, selective 5-HT_{2A} inverse-agonists and their application as antiplatelet agents

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ABSTRACT

Potent 5-HT_{2A} inverse-agonists containing phenyl-pyrazole ureas with an amino side chain were identified. Optimization of this series resulted in selective compounds that proved effective in modulating 5HT-induced amplification of ADP-stimulated human platelet aggregation.

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The 5-HT_{2A} receptor is one of at least 15 different serotonin (5-HT) receptor subtypes, several of which regulate important behavioral responses.^{1,2} Inverse-agonists of the 5-HT_{2A} receptor are known to improve sleep maintenance^{3,4} and to alleviate negative symptoms in schizophrenia.^{5,6} Several clinical candidates have been developed for these central nervous system (CNS) indications,⁴ including volinanserin (Sanofi-Aventis),⁷ and pimavanserin (Acadia, Fig. 1).^{8,9}

In addition to its CNS activities, the 5-HT_{2A} receptor plays an important role in regulating cardiovascular functions, including platelet aggregation.^{10,11} By itself, serotonin does not significantly alter platelet function, but has been found to amplify aggregation induced by an agonist such as collagen, epinephrine, thrombin or ADP. 5-HT_{2A} inverse-agonists are known to prevent this amplification and reduce the formation of blood clots (thrombogenesis).¹²

Previously, we identified a novel series of phenyl-pyrazole urea-based 5-HT_{2A} inverse-agonists which unlike most known inverse-agonists of the receptor, lack a basic amine function.¹³ While these compounds are potent 5-HT_{2A} inverse-agonists, they possess limited intrinsic solubility. This study focuses on our attempts to mitigate this potential liability by incorporating an amino moiety onto the phenyl-pyrazole urea scaffold. The ability of the resulting

compounds to attenuate serotonin-induced amplification of platelet activation was then evaluated.

We began our investigation by modifying compounds we already had available as part of our 5-HT_{2A} insomnia program.¹³ Using the potent (K_i = 1.5 nM) but insoluble inverse-agonist **1** as a starting point, demethylation with boron tribromide furnished phenol **2** in 45% yield (Scheme 1). Initial attempts at alkylation of **2** using Williamson ether synthesis conditions (DBU, alkyl halide) led to a mixture of products, but utilizing a Mitsunobu protocol¹⁴ afforded **3** in 57% yield.¹⁵

In cultured HEK cells transfected with the constitutively active 5-HT_{2A} receptor, **3** inhibited serotonin-independent inositol phosphate accumulation (IP) with an IC₅₀ of 1.9 nM, demonstrating that it is a functional inverse-agonist.¹⁶ A K_i of 0.28 nM was determined by a competitive binding assay against ¹²⁵I-radiolabeled DOI at the 5-HT_{2A} receptor (Table 1). However, **3** showed only sixfold selectivity at the related 5-HT_{2C} receptor (K_i = 1.6 nM). Further evaluation of **3** in a multi-receptor panel (CEREP) found submicromolar activ-

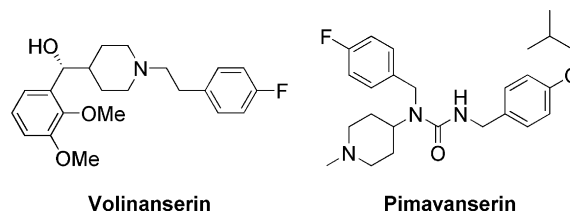
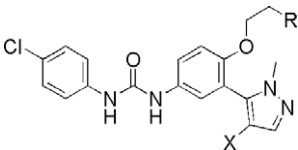


Figure 1. Structures of known 5-HT_{2A} inverse-agonists.

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Table 1
Amino solubilizing group SAR


Compounds	X	R	5-HT _{2A} K _i ^a (nM)	5-HT _{2C} K _i ^a (nM)
3	Br		0.28	1.6
12	Cl		0.26	3.0
13	H		0.15	9.0
14	Br		0.66	21.4
15	Cl		0.43	24.0
16	H		0.41	58.9
17	Br		0.63	12.7
18	Cl		0.61	20.0
19	H		0.19	213
20	H		0.43	8.8
21	H		0.55	24.3
22	H		4.0	151
23	H		0.58	187
24	H		0.22	98.7
25	H		17.6	133
26	H		2.1	102.7
27	H		0.96	216
28	H		0.42	66.2
29	H		0.12	29.6
30	H		0.69	5.4
31	H		0.99	50.7
32	H		0.84	98
33	H		16.2	481

^a Mean of at least three determinations with s.d. < 0.4 log units.

ity against several other GPCRs, including muscarinic M1, dopamine D1 and D5, and μ opiate. Compound **3** also showed 73% inhibition of the hERG channel at 1 μ M, likely preventing its use in any clinical applications. The pharmacokinetic profile of **3** was evaluated in male Sprague-Dawley rats. Compound **3** was dosed at 2.0 mg/kg (iv) or 10.0 mg/kg (po) using 80% PEG400 in PBS (pH 7.4) and had a $t_{1/2}$ of 5.1 h and $F = 19\%$. Compound **3** demonstrated significantly improved solubility compared with **1**, both under neutral conditions (0.2 mg/ml in a pH 7.0 sodium phosphate buffer) and acidic conditions (16 mg/ml in a pH 4.0 sodium acetate buffer).

While the potency of **3** was adequate, a more selective compound, particularly with respect to hERG channel blockade, was needed for possible clinical applications. A new synthetic route was developed in order to more readily access a larger variety of solubilized phenyl-pyrazole ureas (Scheme 2). Starting with **4**,¹⁵ demethylation with aluminum trichloride afforded phenol **5**. A Mitsunobu coupling of **5** with 2-bromoethanol led to **6**. Pyrazole halogenation could be achieved by treating **6** with NCS or NBS to yield **7** or **8**, respectively. Intermediates **6–8** were then reacted with an amine to furnish **9a–9c**. Amide hydrolysis under basic conditions followed by coupling with an isocyanate led to the desired final products **11a–c**.

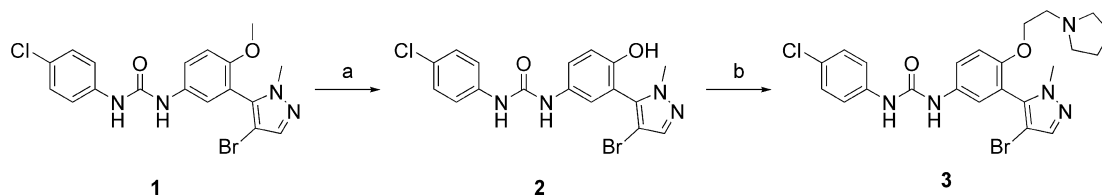
Our initial SAR efforts were directed at determining the necessity of pyrazole halogenation for potency at the 5-HT_{2A} receptor. Pyrrolidine, piperidine, and morpholine analogs were prepared in brominated, chlorinated, and nonhalogenated versions. From examination of the data in Table 1 (compounds **3**, **12–19**), it is clear that pyrazole halogenation (X = Br or Cl) is not required for potency at the 5-HT_{2A} receptor. In addition, removal of the pyrazole halogen generally leads to compounds with increased selectivity for the 5-HT_{2A} receptor compared to the 5-HT_{2C} receptor.

Holding the rest of the structure constant, a set of molecules incorporating a wide range of amino substituents (R in Table 1) were synthesized. Several compounds, including those substituted with piperidine (**16**), morpholine (**19**), and 4-acetylpiperazine (**27**), maintained subnanomolar potency in the competitive 5-HT_{2A} binding assay, but showed significantly improved selectivity over 5-HT_{2C} compared to the pyrrolidine analog **13**.¹⁶

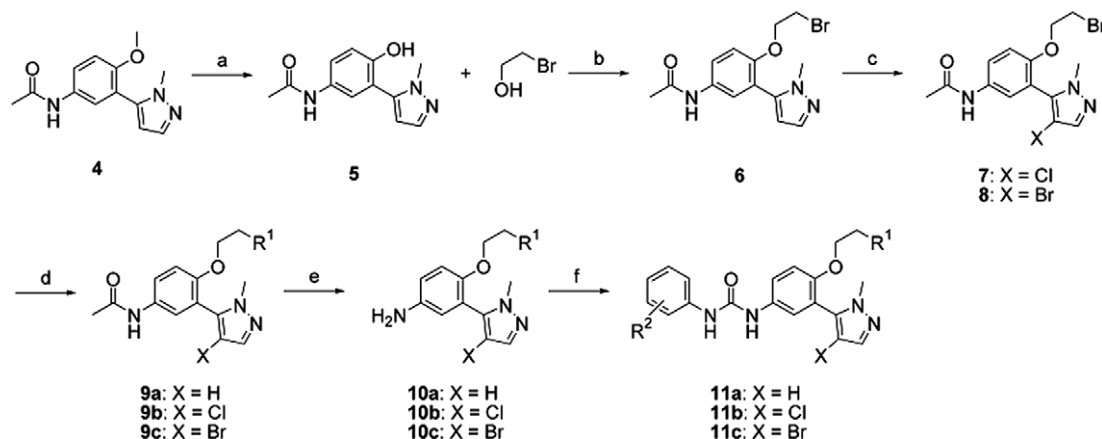
Two analogs bearing a longer three carbon tether (**21** and **22**) were prepared by a Mitsunobu coupling route similar to that shown in Scheme 1. While **22** proved to be significantly less potent and selective than its two carbon chain analog **19**, no such difference was seen between **21** and **20**.

Next, a phenyl urea optimization was undertaken, using piperidine as the amino side chain (Table 2). The results of this study show that a range of electron withdrawing and donating substituents were well tolerated on the phenyl ring of the urea. Compounds bearing substituents on the 3- or 4-positions of the phenyl ring proved to be significantly more potent than those bearing the same substituent at the 2-position. Several compounds (**38**, **39**, and **42**) were found to have 5-HT_{2A} binding affinities of less than 1 nM with selectivities of better than 500-fold for 5-HT_{2A} over 5-HT_{2C}.

Several of these compounds were evaluated for their ability to inhibit serotonin-induced amplification of ADP-stimulated human platelet aggregation. Aggregation was measured turbidometrically at 37 °C. Platelet rich human plasma was pre-incubated with the phenyl-pyrazole urea for 1 min before aggregation was induced by the simultaneous addition of 1 μ M serotonin and 1 μ M ADP. In the absence of an inhibitor, addition of ADP by itself causes approximately 10–20% of maximal aggregation, while addition of both serotonin and ADP causes maximal aggregation. The IC₅₀ is defined as the concentration of inhibitor (the phenyl-pyrazole urea) at which half of the serotonin amplification effect was reversed.¹⁷



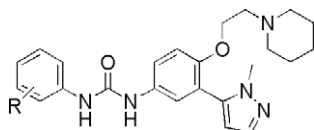
Scheme 1. Reagents and conditions: (a) BBr_3 , 1,2-dichloroethane, 100 °C, 45%; (b) PPh_3 , DIAD, 1-(2-hydroxyethyl)pyrrolidine, THF, rt, 57%.



Scheme 2. Reagents and conditions: (a) AlCl_3 , 1,2-dichloroethane, 0–80 °C, 83%; (b) PPh_3 , DIAD, THF, rt, 82%; (c) for **7**: NCS, MeOH, 150 °C, 47%; for **8**: NBS, DMA, rt, 2 h, 93%; (d) $\text{R}^1\text{-H}$, DMF, DIEA; (e) NaOH in MeOH/ H_2O , 150 °C, 30 min; (f) R^2NCO , CH_2Cl_2 , rt.

Ureas **38**, **39**, and **42** were found to inhibit serotonin-induced amplification of ADP-stimulated human platelet aggregation with IC_{50} 's of 31, 17, and 6 nM, respectively. Table 3 shows inhibition of platelet aggregation data for a range of compounds with 5-HT_{2A} binding affinities of less than 2 nM. In general, the addition of a basic amine moiety led to compounds which showed greater effects on platelet aggregation than related insoluble compounds (for instance, compound **3** had an IC_{50} of 21 nM while compound **1** had an IC_{50} of 296 nM). These compounds are all less potent in the platelet inhibition assay than in the binding assay, but the reduction in potency varies widely from 3.5-fold (compound **49**) to over 500-fold (compound **55**).

Table 2
Phenyl urea SAR

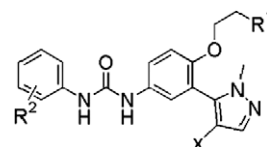


Compounds	R	5-HT _{2A} K_i^a (nM)	5-HT _{2C} K_i^a (nM)
34	H	1.4	1090
35	2-Cl	2.5	223
36	3-Cl	0.33	21.7
16	4-Cl	0.41	58.9
37	2-F	6.1	1150
38	3-F	0.99	760
39	4-F	0.84	466
40	4-Me	1.4	104
41	3-OMe	3.3	403
42	4-OMe	0.54	385
43	3-CF ₃	0.25	2.9
44	4-CF ₃	0.34	18.9
45	3-Acetyl	14.3	59.1
46	2,4-F	3.2	1170
47	2-F, 5-Me	2.1	210
48	3,5-F	0.91	147

^a Mean of at least three determinations with s.d. < 0.4 log units.

Table 3

Inhibition of 5HT-induced amplification of ADP-stimulated human platelet aggregation



Compounds	R ¹	R ²	X	5-HT _{2A} K_i^a (nM)	5-HT _{2C} K_i^a (nM)	Inhibition of platelet aggregation IC_{50} (nM)
49		4-F	H	0.86	53.2	3
50		2,4-F	Br	0.18	12.4	10
51		2,4-F	Cl	0.28	11.0	7
52		4-F	Br	0.36	9.0	21
53		4-OH	Br	1.9	810	17
3		4-Cl	Br	0.28	1.6	21
42		4-OMe	H	0.54	385	6
39		4-F	H	0.84	466	17
38		3-F	H	0.99	760	31
54		2,4-F	Cl	0.15	405	34

Table 3 (continued)

Compounds	R ¹	R ²	X	5-HT _{2A} K _i ^a (nM)	5-HT _{2C} K _i ^a (nM)	Inhibition of platelet aggregation IC ₅₀ (nM)
55		3,5-F	Cl	0.31	37.8	183
56		3-F	H	0.82	1230	89

^a Mean of at least three determinations with s.d. < 0.4 log units.

One of the compounds showing the greatest 5HT_{2A}/5HT_{2C} selectivity, **54**, was evaluated in multi-receptor panel (CEREP) and proved to be significantly more selective than **3**, giving submicromolar activity against only the 5HT_{2A} and 5HT_{2C} receptors. In addition, **54** showed only 7% inhibition of the hERG channel at 1 μ M¹⁸ and was soluble under acidic conditions (1.9 mg/ml in 0.01 N HCl), though not at neutral pH (0.01 mg/ml in a sodium phosphate buffer). The pharmacokinetic profile of **54** was evaluated in rats. Compound **54** had a $t_{1/2}$ of 2.8 h and $F = 11\%$.

In summary, addition of an amino group to the previously identified phenyl-pyrazole urea scaffold led to several compounds with high 5-HT_{2A} potency and good selectivity over the related 5-HT_{2C} receptor. Compounds were identified that were highly effective at inhibiting serotonin-induced amplification of ADP-stimulated human platelet aggregation and which had reduced off-target liabilities.

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- Inhibition of the hERG channel varied based on the substituents in the compounds, but in general molecules containing the less basic morpholino group, like **54** and **56** (29% at 3 μ M), showed less inhibition than those with more basic groups like **32** (90% at 3 μ M) and **50** (64% at 1 μ M).