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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.

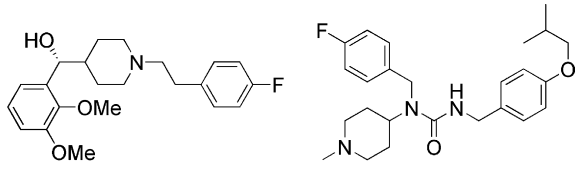
The 5-HT$_{2A}$ receptor is one of at least 15 different serotonin (5-HT) receptor subtypes, several of which regulate important behavioral responses.[1,2] Inverse-agonists of the 5-HT$_{2A}$ receptor are known to improve sleep maintenance[3,4] and to alleviate negative symptoms in schizophrenia.[5,6] 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 protocol[14] 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 IC$_{50}$ 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 ^{125}I-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-



Volinanserin Pimavanserin

Figure 1. Structures of known 5-HT$_{2A}$ inverse-agonists.

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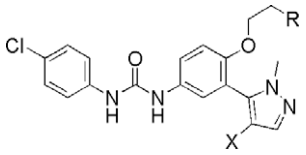
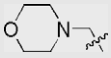
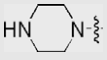
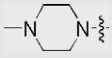
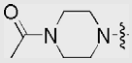
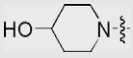
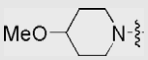
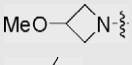
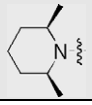
Table 1
Amino solubilizing group SAR



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 μM, 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 μM serotonin and 1 μM 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 IC$_{50}$ is defined as the concentration of inhibitor (the phenyl-pyrazole urea) at which half of the serotonin amplification effect was reversed.[17]