Bioorganic & Medicinal Chemistry Letters 19 (2009) 5486–5489

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Peter I. Dosa *, Sonja Strah-Pleynet, Honnappa Jayakumar, Martin Casper, Marc Decaire, Yifeng Xiong, Juerg Lehmann, Karoline Choi, Katie Elwell, Amy Wong, Robert R. Webb, John W. Adams, Juan Ramirez, Jeremy G. Richman, William Thomsen, Graeme Semple, Bradley R. Teegarden

Arena Pharmaceuticals Inc., 6166 Nancy Ridge Drive, San Diego, CA 92121, United States

ARTICLE INFO

Article history:
Received 12 June 2009
Revised 10 July 2009
Accepted 14 July 2009
Available online 18 July 2009

Keywords:
5-HT$_{2A}$
Antiplatelet
Antithrombosis

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.

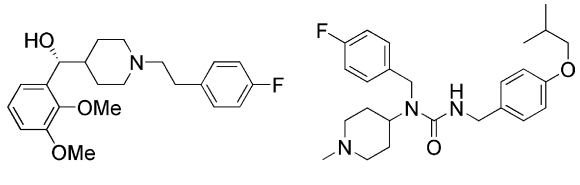
The 5-HT$_{2A}$ receptor is one of at least 15 different serotonin (5-HT) receptor subtypes, several of which regulate important behavioral responses.[1,2] Inverse-agonists of the 5-HT$_{2A}$ receptor are known to improve sleep maintenance[3,4] and to alleviate negative symptoms in schizophrenia.[5,6]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



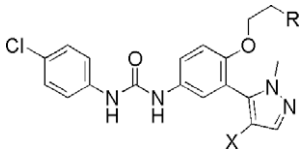
Volinanserin Pimavanserin

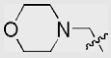
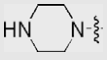
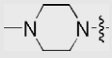
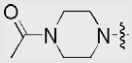
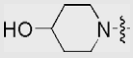
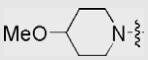
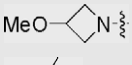
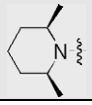
Figure 1. Structures of known 5-HT$_{2A}$ inverse-agonists.

* Corresponding author at present address: Institute for Therapeutics Discovery and Development, Room 455, College of Pharmacy, University of Minnesota, 717 Delaware Street SE, Minneapolis, MN 55414, United States.
E-mail address: pidosa@umn.edu (P.I. Dosa).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.073

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**.

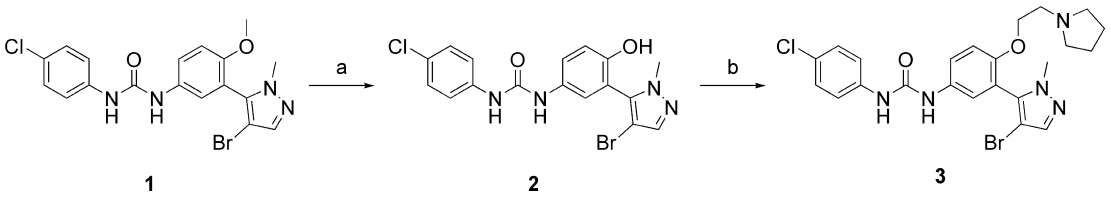
Our initial SAR efforts were directed at determining the necessity of pyrazole halogenation for potency at the 5-HT$_{2A}$ receptor. Pyrrolidine, piperidine, and morpholine analogs were prepared in brominated, chlorinated, and nonhalogenated versions. From examination of the data in Table 1 (compounds **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]

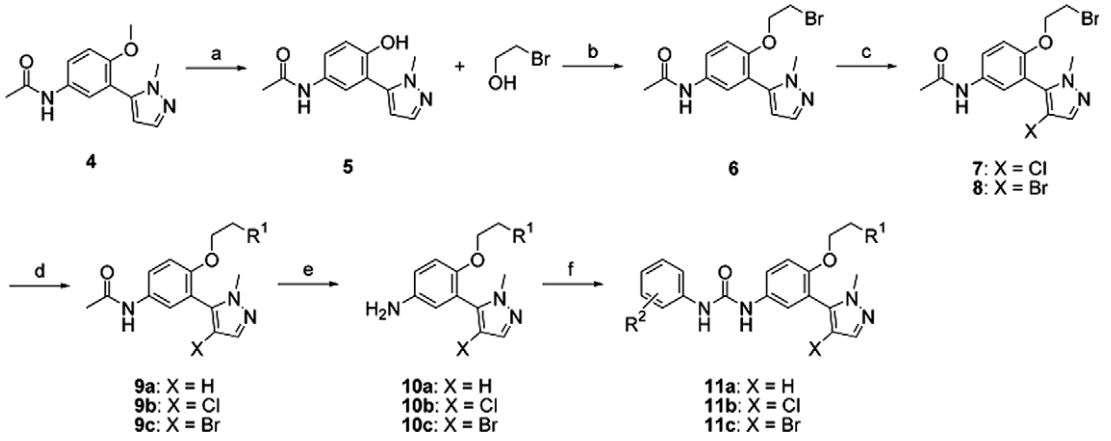
Two analogs bearing a longer three carbon tether (**21** and **22**) were prepared by a Mitsunobu coupling route similar to that shown in Scheme 1. While **22** proved to be significantly less potent and selective than its two carbon chain analog **19**, no such difference was seen between **21** and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



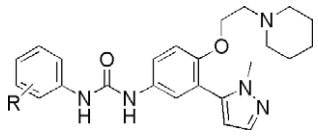
Scheme 1. Reagents and conditions: (a) BBr_3, 1,2-dichloroethane, 100 °C, 45%; (b) PPh_3, DIAD, 1-(2-hydroxyethyl)pyrrolidine, THF, rt, 57%.



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) R^1-H, DMF, DIEA; (e) NaOH in MeOH/H_2O, 150 °C, 30 min; (e) 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**).

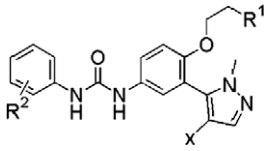
Table 2
Phenyl urea SAR



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 μM[18] 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.

Acknowledgements

We thank Hong Zheng, John Frazer, Rosemarie Angeles and Diane Yuskin for their excellent assistance in running assays and Dan Connelly for useful discussions.

References and notes

1. Hoyer, D.; Hannon, J. P.; Martin, G. R. *Pharmacol., Biochem. Behav.* **2002**, *71*, 533.
2. Sanders-Bush, E.; Fentress, H.; Hazelwood, L. *Mol. Interventions* **2003**, *3*, 319.
3. Sharpley, A. L.; Solomon, R. A.; Fernando, A. I.; da Roza Davis, J. M.; Cowen, P. J. *Psychopharmacology* **1990**, *101*, 568.
4. Teegarden, B. R.; Al-Shamma, H.; Xiong, Y. *Curr. Topics Med. Chem.* **2008**, *8*, 969.
5. Abi-Dargham, A.; Laruelle, M.; Aghajanian, G. K.; Charney, D.; Krystal, J. *J. Neuropsych. Clin. Neurosci.* **1997**, *9*, 1.
6. Di Pietro, N. C.; Seamans, J. K. *Pharmacopsych.* **2007**, *40*, S27.
7. Kehne, J. H.; Baron, B. M.; Carr, A. A.; Chaney, S. F.; Elands, J.; Feldman, D. J.; Frank, R. A.; Van Giersbergen, P. L. M.; McCloskey, T. C.; Johnson, M. P.; McCarty, D. R.; Poirot, M.; Senyah, Y.; Siegel, B. W.; Widmaier, C. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 968.
8. Vanover, K. E.; Weiner, D. M.; Makhay, M.; Veinbergs, I.; Gardell, L. R.; Lameh, J.; Del Tredici, A. L.; Piu, F.; Schiffer, H. H.; Ott, T. R.; Burstein, E. S.; Uldam, A. K.; Thygesen, M. B.; Schlienger, N.; Andersson, C. M.; Son, T. Y.; Harvey, S. C.; Powell, S. B.; Geyer, M. A.; Tolf, B.; Brann, M. R.; Davis, R. E. *J. Pharm. Exp. Ther.* **2006**, *317*, 910.
9. Roberts, C. *Curr. Opin. Invest. Drugs* **2006**, *7*, 653.
10. De Clerck, F. F.; Herman, A. G. *Fed Proc.* **1983**, *42*, 228.
11. Nagatomo, T.; Rashid, M.; Abul Muntasir, H.; Komiyama, T. *Pharmacol. Ther.* **2004**, *104*, 59.
12. De Clerck, F. F.; Janssen, P. A. *J. Hypertens.*, **1990**, S87.
13. Teegarden, B. R.; Li, H.; Jayakumar, H.; Strah-Pleynet, S.; Dosa, P. I.; Selaya, S. D.; Kato, N.; Elwell, K. H.; Davidson, J.; Cheng, K.; Saldana, H.; Frazer, J. M.; Whelan, K.; Foster, J.; Espitia, S.; Webb, R. R; Beeley, N.R.A.; Kilduff, T.S.; Morairty, S.; Thomsen, W.; Al-Shamma, H., submitted for publication.; For an example of another non-basic 5-HT_{2A} antagonist see: Ladduwahetty, T.; Boase, A. L.; Mitchinson, A.; Quin, C.; Patel, S.; Chapman, K.; MacLeod, A. M. *Bioorg. Med. Chem. Lett.* **2006**, 3201.
14. Hughes, D. L. In *Organic Reactions*; Paquette, L. A., Ed.; Wiley: New York, 1992; Vol. 42, pp 335–656.
15. a For experimental details, see: Teegarden, B.; Jayakumar, H.; Li, H.; Strah-Pleynet, S.; Dosa, P. I. *Preparation of diaryl and arylheteroaryl urea derivatives, in particular pyrazolylphenyl ureas, as 5-HT2A serotonin receptor modulators*. WO2005012254, 2005.; b Teegarden, B.; Xiong, Y.; Strah-Pleynet, S.; Jayakumar, H.; Dosa, P. I.; Feichtinger, K.; Casper, M.; Lehmann, J.; Jones, R. M.; Unett, D. J. *Preparation of 3-phenylpyrazole derivatives as modulators of 5-HT2A serotonin receptors for the treatment of related disorders*. WO2006055734, 2006.
16. The solubilities of compound **13** (0.25 mg/ml in sodium phosphate buffer and 2.8 mg/ml in 0.01 N HCl) and compound **19** (0.15 mg/ml and 3.3 mg/ml) were similar to that of compound **3**.
17. Adams, J. W.; Ramirez, J.; Ortuno, D.; Shi, Y.; Thomsen, W.; Richman, J. G.; Morgan, M.; Dosa, P.; Teegarden, B. R.; Al-Shamma, H.; Behan, D. P.; Connolly, D. T. *Eur. J. Pharmacol.* **2008**, *586*, 234.
18. 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 μM), showed less inhibition than those with more basic groups like **32** (90% at 3 μM) and **50** (64% at 1 μM).