Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2089–2092
Bioorganic & Medicinal Chemistry Letters

Synthesis and in vitro activity of 1-(2,3-dichlorophenyl)-*N*-(pyridin-3-ylmethyl)-1*H*-1,2,4-triazol-5-amine and 4-(2,3-dichlorophenyl)-*N*-(pyridin-3-ylmethyl)-4*H*-1,2,4-triazol-3-amine $P2X_7$ antagonists

Alan S. Florjancic,[a,*] Sridhar Peddi,[a] Arturo Perez-Medrano,[a] Biqin Li,[b] Marian T. Namovic,[a] George Grayson,[a] Diana L. Donnelly-Roberts,[a] Michael F. Jarvis[a] and William A. Carroll[a,*]

[a]*Abbott Laboratories, Global Pharmaceutical Research and Development, 100 Abbott Park Road, Abbott Park, IL 60064, USA*
[b]*Department of Medicinal Chemistry, Abbott Bioresearch Center, 381 Plantation Street, Worcester, MA 01605, USA*

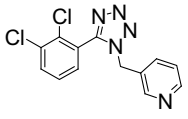
Received 19 December 2007; revised 23 January 2008; accepted 23 January 2008
Available online 30 January 2008

Abstract—A novel series of aminotriazole-based $P2X_7$ antagonists was synthesized, and their structure–activity relationships (SAR) were investigated for activity at both human and rat $P2X_7$ receptors. Most compounds showed greater potency at the human receptor although several analogs were discovered with potent activity ($pIC_{50} \geqslant 7.5$) at both human and rat $P2X_7$.

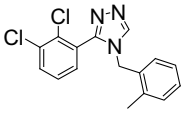
The $P2X_7$ receptor is an ATP-activated ion channel that has received a notable amount of attention in recent years from the academic and pharmaceutical sectors as a potential therapeutic target.[1–5] $P2X_7$ is found on a variety of cell types dealing with inflammation and immune function,[1] such as mast cells, macrophages, and lymphocytes, and the receptor's activation has been found to be one of the most potent stimuli of IL-1β release. Receptor activation also drives the release of a variety pro-inflammatory cytokines such as TNF-α as well as giant cell formation and mast cell degranulation, implicating it in a variety of potential disease states. Although its presence on neurons has been debated,[6,7] $P2X_7$ is expressed in the central nervous system on microglia and astrocytes.[8] On glial cells, activation of $P2X_7$ has been found to mediate the release of glutamate,[9] which is involved in the neurotransmission of painful sensory signals, potentially making $P2X_7$ useful in treating a variety of pain states. Studies with $P2X_7$ KO mice showed a reduction in collagen-induced arthritis severity,[10] and offered protection to the KO animals from inflammatory pain, and partial nerve ligation-induced neuropathic pain.[11]

The potential therapeutic indications of inhibiting $P2X_7$ have led to a recent proliferation in the number and quality of small molecule antagonists in both the patent and scientific literature.[2–5] Agents from several disclosed chemical classes have been found to possess activity in rodent models of inflammatory and neuropathic pain.[5] AZ9056, a $P2X_7$ antagonist of undisclosed structure, is reportedly under clinical investigation for rheumatoid arthritis and inflammatory bowel disease.[12]

Recent reports from our laboratories have described the in vitro and in vivo characterization of substituted aryltetrazoles[13] and aryltriazoles,[14] exemplified, respectively, by **1** and **2**. Both of these $P2X_7$ antagonists



1



2

displayed activity in a rat model of neuropathic pain. Interestingly, although attachment of the phenyl and

Keywords: P2X7; Antagonist; Aminotriazole.
* Corresponding authors. Tel.: +1 847 938 6041; fax: +1 847 935 5466 (W.A.C.); e-mail: william.a.carroll@abbott.com

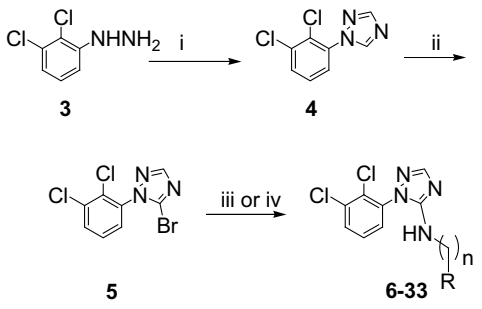
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.095

benzyl groups on adjacent atoms was a requirement for $P2X_7$ activity, changing the positions of the nitrogen atoms in the heterocyclic core with respect to the appended groups had little impact on activity. In an effort to further explore the structure–activity relationships around this pharmacophore, additional variations on the linker between the heterocyclic core and the benzylic appendage were investigated on two regioisomeric triazoles. Surprisingly, it was found that the prototypical methylene linker of **1** and **2** could be replaced with an amino or aminomethyl linker with retention or improvement in potency at both human and rat $P2X_7$ receptors.

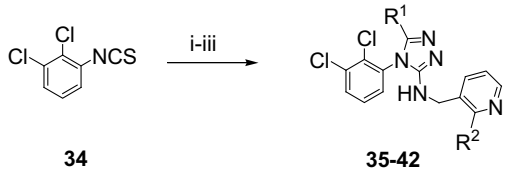
Earlier work in our laboratories on triazole and tetrazole cores established that the 2,3-dichlorophenyl substitution pattern on the left-hand side enhanced the potency toward $P2X_7$. To quickly survey the aminotriazole systems, we opted to hold the left-hand side 2,3-dichlorophenyl moiety constant throughout our SAR studies.

The target 5-amino-1-phenyl-1,2,4-triazoles **6**–**33** were prepared using the synthetic route shown in Scheme 1. 2,3-Dichlorophenylhydrazine[15] was reacted with formamide at elevated temperature to provide the triazole **4** which was in turn brominated under radical conditions to give the key intermediate **5**.[16] Direct reaction of **5** with anilines was unsuccessful under a variety of conditions, however Buchwald–Hartwig coupling conditions gave compounds **6**–**12** in low to moderate yields, all unoptimized. Some intriguing preliminary data led our main area of interest to be centered on compounds which contained an aminomethylene linker. We were able to generate a medium-sized library of these compounds by treating **5** with a variety of commercial and custom benzyl amines neat at 90 °C for 4–12 h to give compounds **13**–**33** in 10–48% yields.

Synthesis of the regioisomeric 3-amino-4-phenyl-1,2,4-triazoles **35**–**42** was accomplished utilizing a one-pot sequence (Scheme 2) starting from commercially available 2,3-dichloroisothiocyanate **34**. Treatment with a benzyl amine for approximately 1h in THF gave the intermediate thiourea, which, by addition of hydrazine in the presence of base and $HgCl_2$ for several hours, gave the corresponding aminoguanidine. The crude compounds were then heated to reflux in the presence of an ortho-



Scheme 1. Reagents and conditions: (i) formamide, 120 °C, 16 h, 72%; (ii) NBS, benzoyl peroxide, CCl_4, reflux, 12 h, 35–42%; (iii) $n = 0$; RNH_2, $Pd_2(dba)_3$, XANTPHOS, NaOtBu, toluene, 100 °C, 12 h; (iv) $n = 1$; RCH_2NH_2 (2–4 equiv), 90 °C, 4–12 h, 10–48%.



Scheme 2. Reagents and conditions: (i) RNH_2, THF, rt, 1 h; (ii) NEt_3, $HgCl_2$, NH_2NH_2, rt, 2–14 h; (iii) $R^1C(OEt)_3$, HCOOH, THF, reflux, 2 h, 20–35%.

formate under acidic conditions to afford ring closure, giving the aminotriazole compounds **35**–**42** in 20–35% yield over three steps.

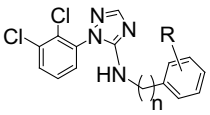
In vitro $P2X_7$ activity was assessed using the recombinant rat and human receptors. Antagonist potencies were determined by measuring the inhibition of Ca^{2+} flux with a fluorometric imaging plate reader (FLIPR) using Fluo-4 as the dye and benzoylbenzoylATP (BzATP) as the agonist.[17]

Previously, extensive SAR studies around the preferred benzyl group of **2** revealed that substitution in the ortho position gave rise to the most potent compounds. With these results as a guide, compounds **6**–**12** were targeted to ascertain if this same trend held true with an amino linker (Table 1). Indeed, both the methyl (**7**), ethyl (**8**), and methoxy (**9**) analogs proved more potent than the unsubstituted compound **6**. As the groups became larger (**10**–**12**), however, the trend reversed leading to analogs of lesser or equal potency to **6**. Relative to compound **2**, with a methylene linker, the analogous 2-methyl compound **7** displayed similar or improved potency at rat and human $P2X_7$. Interestingly, the corresponding analog of **7** wherein the NH linker was replaced with oxygen showed very weak potency.[18]

Lengthening the linker by insertion of a methylene unit gave rise to aminotriazole compounds **13**–**25**. Although the unsubstituted analog **13** displayed significantly improved potency over **6**, the previously observed trend of enhanced potency with *ortho*-substitution was much less prominent here or absent. Compounds **14**, **17**, **20** and **21**, for example, possess essentially equivalent potency to **13**. The 2,3-disubstituted analogs **23** and **24** similarly did not improve upon **13**. Only compound **22** shows an appreciable potency increase, which manifests itself most noticeably at $rP2X_7$. Surprisingly, the much bulkier pyridin-3-yloxy compound **25** demonstrated similar potency to the analogs with smaller groups in the *ortho* position. In contrast to the decreasing potency with larger groups seen with compounds **7**–**12**, activity seems to be independent of size with the extra atom in the linker. This result gives some indication that with the increased length of the linker, there is more tolerance for varying the substitution at this position. Regarding substitution in the *meta* and *para* positions, **15**, **16** and **18**, **19** were significantly less potent which is consistent with previous observations.[14]

On the right-hand side of the pharmacophore, replacement of phenyl with pyridine resulted in a loss of

Table 1. 5-Amino-1-(2,3,-dichlorophenyl)-1,2,4-triazole regioisomer: N-phenyl and N-benzyl SAR



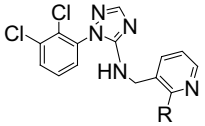
Compound	*n*	R	hP2X$_7$pIC$_{50}$[a]	rP2X$_7$pIC$_{50}$[a]
1	—	—	6.91	6.51
2	—	—	7.11	6.68
6	**0**	H	6.44	5.41
7	0	2-Me	7.75	6.62
8	0	2-Et	7.34	6.54
9	0	2-OMe	6.99	5.69
10	0	2-OEt	5.73	4.96
11	0	2-OCF$_3$	6.21	5.26
12	0	2-Morpholin-4-yl	5.47	5.31
13	1	H	7.32	6.68
14	1	2-Me	7.68	6.70
15	1	3-Me	6.81	5.99
16	1	4-Me	6.32	5.15
17	1	2-OMe	7.24	6.15
18	1	3-OMe	6.54	5.86
19	1	4-OMe	5.79	4.98
20	1	2-OEt	7.34	6.31
21	1	2-SMe	7.31	6.54
22	1	2-SO$_2$Me	7.70	7.40
23	1	2,3-Di-OMe	7.51	6.87
24	1	2,3-Di-Me	7.17	6.20
25	1	2-(Pyridin-3-yloxy)	7.33	6.80

[a] Values are means of 2–4 experiments. Standard deviations ranged from 0.01 to 0.30 with an average of 0.14. Compounds tested at the recombinant human and rat P2X$_7$ receptors as described.[17]

potency (**13** vs **26** and **27**). Incorporation of a substituent in the 2-position of the 3-pyridyl group (**28**–**33**), however, returned potency to the level observed with a substituted phenyl. Compound **32**, for example, is similarly potent to **25** at rP2X$_7$ and 3-fold more potent at hP2X$_7$. This group of structural analogs (**28**–**33**) further underscores the range of tolerated substitutions in the ortho position in this series, with amine (**29**–**31**), arylether (**25** and **32**), and aryl (**33**) groups all showing potent activity. Although many compounds in this series display about 10-fold greater potency at human over rat P2X$_7$, the azetidine and pyrrolidine analogs **29** and **30** show comparatively small species difference (2- to 3-fold).

A smaller group of regioisomeric aminotriazoles **35**–**42** was also evaluated for activity at human and rat P2X$_7$ (Table 3). This core afforded a synthetic handle which allowed for limited probing of chemical space on the aminotriazole at a position which was previously inaccessible (R^1 group). In general, similar SAR trends were observed in this regioisomeric series in that ortho substitution (R^2) substantially increased potency (~10-fold), there was a high degree of tolerance for substitution at R^2 and compounds displayed similar potency at hP2X$_7$ to those in Table 2 (cf. **28**, **30**, **32**, **33** vs **36**–**38**, and **40**). Although the compounds in Table 3 generally showed greater potency for human over rat, the species differences were less marked here for most analogs. For example, compounds **37**, **39**, and **40** showed

Table 2. 5-Amino-1-(2,3-dichlorophenyl)-1,2,4-triazole regioisomer: N-pyridylmethyl SAR



Compound	R	hP2X$_7$ pIC$_{50}$[b]	rP2X$_7$ pIC$_{50}$[b]
26	H	6.93	5.88
27	H[a]	<5	<5
28	Me	7.93	6.53
29	Azetidin-1-yl	8.04	7.59
30	Pyrrolidin-1-yl	7.90	7.55
31	Morpholin-4-yl	7.55	6.67
32	Pyridin-3-yloxy	7.86	6.89
33	Thiophen-3-yl	7.82	6.86

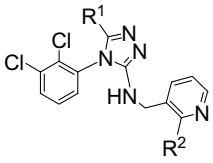
[a] 4-Pyridyl.

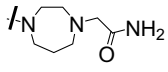
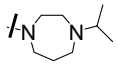
[b] Values are means of 2–4 experiments. Standard deviations ranged from 0.01 to 0.20 with an average of 0.11. Compounds tested at the recombinant human and rat P2X$_7$ receptors as described.[17]

no appreciable species differences and compound **38** was only 3-fold less potent at rat whereas its counterpart **33** was 10-fold less potent at rP2X$_7$ (Table 2). The presence of a methyl group at R^1 (cf. **38** and **39**), had little effect on rP2X$_7$ potency compared to 3-fold loss of activity at hP2X$_7$. With the aim of exploring potentially water-solubilizing functionality, compounds **41** and **42** were synthesized.[19] Both analogs maintained moderate to potent activity at hP2X$_7$ demonstrating that additional hydrogen bond donating and accepting groups may be installed in this position.

Overall, the wide variety of different functional groups tolerated in the ortho (Tables 1–3) position suggests that these moieties may not engage in specific receptor inter-

Table 3. 3-Amino-4-(2,3-dichlorophenyl)-1,2,4-triazole regioisomer: N-pyridylmethyl SAR



Compound	R^1	R^2	hP2X$_7$pIC$_{50}$[a]	rP2X$_7$ pIC$_{50}$[a]
35	H	H	6.45	5.95
36	H	Me	7.54	6.75
37	H	Pyrrolidin-1-yl	7.83	7.92
38	H	Thiophen-3-yl	7.96	7.44
39	Me	Thiophen-3-yl	7.40	7.34
40	H	Pyridin-3-yloxy	7.51	7.45
41	H		7.49	NT
42	H		7.17	NT

[a] Values are means of 2–3 experiments. NT, not tested. Standard deviations ranged from 0.02 to 0.20 with an average of 0.12. Compounds tested at the recombinant human and rat P2X$_7$ receptors as described.[17]

actions at $hP2X_7$ beyond that afforded by relatively simple substituents such as methyl and methoxy. At $rP2X_7$, however, the two regioisomeric aminotriazoles appear to diverge somewhat with regard to the preferred substitutions in this part of the pharmacophore. A narrower range of substituents in the 5-amino-1-phenyl-1,2,4-triazole regioisomer displayed enhanced $rP2X_7$ potency compared to the 3-amino-4-phenyl-1,2,4-triazole regioisomer. Whereas in the former case amino substitution (e.g., **29** and **30**) met this criteria, in the latter case somewhat greater flexibility was observed with amino (**37**), aryl (**38**) and arylether (**40**) groups all having enhanced $rP2X_7$ activity. The sulfone **22** also showed enhanced $rP2X_7$ potency, however this substitution was not investigated in both regioisomers. A generally more balanced activity across species is clearly a desirable feature of this subgroup of compounds that is shared by few other $P2X_7$ antagonists.[14,20]

In summary, a new series of potent and selective[21] aminotriazole $P2X_7$ antagonists has been discovered. These studies have provided us with a wider understanding of the overall $P2X_7$ pharmacophore by expanding the diversity of linkers as well as substitutions on the right hand aromatic group. The aminotriazole analogs described herein were found to possess enhanced potency relative to earlier $P2X_7$ triazoles. Additionally, structural features were uncovered that confer potent activity at both the human and rat $P2X_7$ receptors.

References and notes

1. DiVirgilio, F.; Vishwanath, V.; Ferrari, D. *Handb. Exp. Pharmacol.* **2001**, *151*, 355.
2. Baraldi, P. G.; DiVirgilio, F.; Romagnoli, R. *Curr. Top. Med. Chem.* **2004**, *4*, 1707.
3. Romagnoli, R.; Baraldi, P. G.; DiVirgilio, F. *Expert Opin. Ther. Patents* **2005**, *15*, 271.
4. Gunosewoyo, H.; Coster, M. J.; Kassiou, M. *Curr. Med. Chem.* **2007**, *14*, 1505.
5. Donnelly-Roberts, D. L.; Jarvis, M. F. *Br. J. Pharmacol.* **2007**, *151*, 571.
6. Sim, J. A.; Young, M. T.; Sung, H.-Y.; North, R. A.; Surprenant, A. *J. Neurosci.* **2004**, *24*, 6307.
7. Anderson, C. M.; Nedergaard, M. *Trends Neurosci.* **2006**, *29*, 257.
8. Collo, G.; Neidhart, S.; Kawashima, E.; Kosco-Vibois, M.; North, R. A.; Buell, G. *Neuropharmacology* **1997**, *36*, : 1277.
9. Papp, L.; Vizi, E. S.; Sperlágh, B. *Neuroreport* **2004**, *15*, 2387.
10. Labasi, J. M.; Petrushova, N.; Donovan, C.; McCurdy, S.; Lira, P.; Payette, M. M.; Brissette, W.; Wicks, J. R.; Audoly, L.; Gabel, C. A. *J. Immunol.* **2002**, *168*, 6436.
11. Chessell, I. P.; Hatcher, J. P.; Bountra, C.; Michel, A. D.; Hughes, J. P.; Green, P.; Egerton, J.; Murfin, M.; Richardson, J.; Peck, W. L.; Grahames, C. B. A.; Casula, M. A.; Yiangou, Y.; Birch, R.; Anand, P.; Buell, G. N. *Pain* **2005**, *114*, 386.
12. Adis Data Information (2007). http://pipeline.dialog.com/abb_lab/adrd.htm.
13. Nelson, D. W.; Gregg, R. J.; Kort, M. E.; Perez-Medrano, A.; Voight, E. A.; Wang, Y.; Grayson, G.; Namovic, M. T.; Donnelly-Roberts, D. L.; Niforatos, W.; Honore, P.; Jarvis, M. F.; Faltynek, C. R.; Carroll, W. A. *J. Med. Chem.* **2006**, *49*, 3659.
14. Carroll, W. A.; Kalvin, D. M.; Perez Medrano, A.; Florjancic, A. S.; Wang, Y.; Donnelly-Roberts, D. L.; Namovic, M. T.; Grayson, G.; Honore, P.; Jarvis, M. F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4044.
15. Nagarajan, K.; Talwalker, P. K.; Kulkarni, C. L.; Venkateswarlu, A.; Prabhu, S. S.; Nayak, G. V. *Ind. J. Chem. Sect. B* **1984**, *12*, 1243.
16. X-Ray crystal analysis verified that the bromination occurred exclusively at the 5-position of the triazole ring.
17. Honore, P. M.; Donnelly-Roberts, D.; Namovic, M.; Hsieh, G.; Zhu, C.; Mikusa, J.; Hernandez, G.; Zhong, C.; Gauvin, D.; Chandran, P.; Harris, R.; Perez-Medrano, A.; Carroll, W.; Marsh, K.; Sullivan, J.; Faltynek, C.; Jarvis, M. F. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1376.
18. For 1-(2,3-dichlorophenyl)-5-(*o*-tolyloxy)-1*H*-1,2,4-triazole: $hP2X_7$ $pIC_{50} = 5.8$; $rP2X_7$ $pIC_{50} = 5.6$.
19. Compounds **41** and **42** were prepared via the corresponding Boc-protected homopiperazine intermediate that was deprotected and alkylated with the appropriate alkylating agent.
20. Furber, M.; Alcaraz, L.; Bent, J. E.; Beyerbach, A.; Bowers, K.; Braddock, M.; Caffrey, M. V.; Cladingboel, D.; Collington, J.; Donald, D. K.; Fagura, M.; Ince, F.; Kinchin, E. C.; Laurent, C.; Lawson, M.; Luker, T. J.; Mortimore, M. M. P.; Pimm, A. D.; Riley, R. J.; Roberts, N.; Robertson, M.; Theaker, J.; Thorne, P. V.; Weaver, R.; Webborn, P.; Willis, P. *J. Med. Chem.* **2007**, *50*, 5882.
21. Compounds **22**–**23**, **25**, **29**, **30**, and **33** were inactive at $P2X_1$, $P2X_2$, $P2X_{2/3}$, $P2X_4$, $P2Y_1$, and $P2Y_2$ receptors at 10 μM.