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Synthesis and in vitro activity of *N*-benzyl-1-(2,3-dichlorophenyl)-1*H*-tetrazol-5-amine $P2X_7$ antagonists

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ABSTRACT

Synthesis and biological evaluation of a novel class of substituted *N*-benzyl-1-(2,3-dichlorophenyl)-1*H*-tetrazol-5-amine derivatives resulted in the identification of potent $P2X_7$ antagonists. These compounds were assayed for activity at both the human and rat $P2X_7$ receptors. On the benzyl moiety, a variety of functional groups were tolerated, including both electron-withdrawing and electron-donating substituents. *Ortho*-substitution on the benzyl group provided the greatest potency. The *ortho*-substituted analogs showed approximately 2.5-fold greater potency at human compared to rat $P2X_7$ receptors. Compounds **12** and **38** displayed $hP2X_7$ pIC_{50}s >7.8 with less than 2-fold difference in potency at the $rP2X_7$.

The $P2X_7$ receptor is a purinergic ion channel specifically activated by endogenous adenosine-5′-triphosphate (ATP).[1] The $P2X_7$ receptor is expressed on numerous cell types, including macrophages, epidermal Langerhan's cells, osteoclasts, and microglial cells.[2–7] Activation of the $P2X_7$ receptor on cells of the immune system leads to the liberation of proinflammatory interleukin-1β (IL-1β),[8] giant cell formation, degranulation, and L-selectin shedding. $P2X_7$ antagonists have been proposed to be beneficial in various disease states and conditions, such as osteoarthritis, rheumatoid arthritis, psoriasis and other chronic inflammatory diseases, in which activation of the $P2X_7$ receptor may induce the release of IL-1β.[9] $P2X_7$ knockout mice showed a reduced inflammatory response in an experimental arthritis model, demonstrating a role of the $P2X_7$ receptor in inflammation.[10]

Furthermore, $P2X_7$ knockout mice showed a reduction in inflammatory thermal hyperalgesia and nerve injury-induced mechanical allodynia, as compared to matched wild type mice,[11] suggesting a role for $P2X_7$ in pain transmission. The $P2X_7$ receptor on glial cells, regulates the release of glutamate,[12] a neurotransmitter involved in pain transmission.[13] Hence, targeting $P2X_7$ receptors on glial cells under pathological conditions may culminate in the development of $P2X_7$ antagonists that have therapeutic utility in the treatment of various pain states.[14]

The foregoing results have generated interest into the development of selective $P2X_7$ antagonists and a steady increase in the \literature describing new $P2X_7$ antagonists.[15–18] From our own laboratories, a series of substituted aryltetrazoles,[19] aryltriazoles[20] and benzyltriazolamines,[21] exemplified by **1, 2, 3**, and **4**, respectively, (Fig. 1) were found to be potent in vitro $P2X_7$ antagonists. Moreover, **1** and **2** were characterized in vivo and displayed activity in a rat model of neuropathic pain.[19,20] In this Letter, we describe the preparation and in vitro biological activity of a novel series of *N*-benzyl-1-(2,3-dichlorophenyl)-1*H*-tetrazol-5-amine derivatives.

The 5-aminotetrazoles described in this Letter were synthesized by a known methodology[22] that was modified to a two-step one

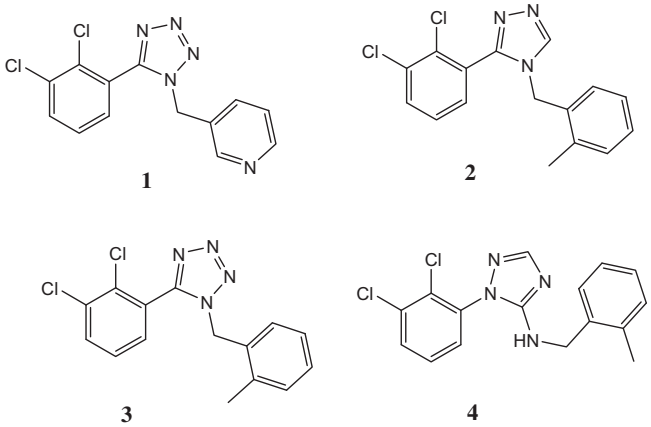

Figure 1. Structures of $P2X_7$ antagonists previously described.

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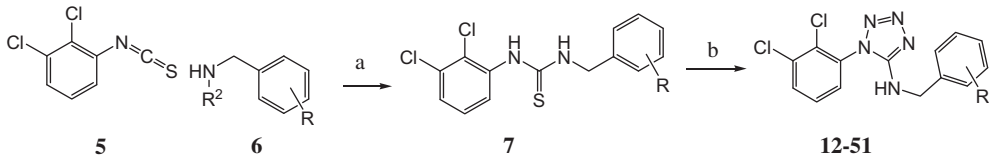
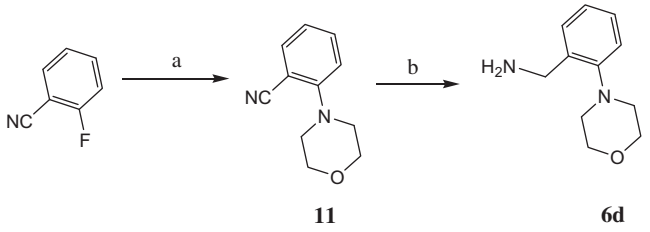


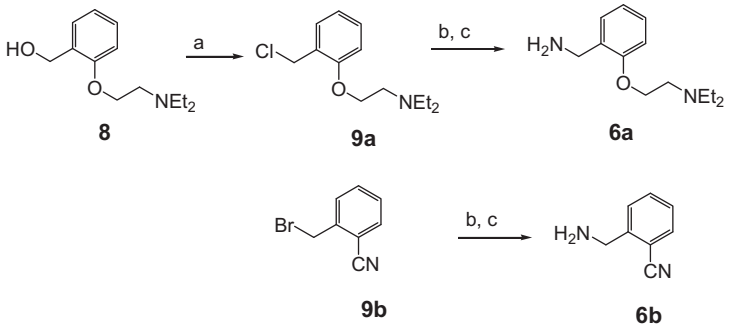
Scheme 1. Reagents and conditions: (a) THF, 22 °C; (b) NaN_3, $Hg(OAc)_2$ or $HgCl_2$, Et_3N, THF.

pot reaction as depicted in Scheme 1. Reaction of 2,3-dichlorophenylisothiocyanate **5** and benzylamines **6** in THF afforded the corresponding thioureas **7**. Subsequent treatment of **7** in the same pot with sodium azide in the presence of mercuric acetate or mercuric chloride and triethylamine provided aminotetrazoles **12–51**.[23] The benzylamine (**6a**), a precursor of aminotetrazole **24**, was synthesized from the corresponding alcohol **8**[24] as described in Scheme 2. Reaction of alcohol **8** with thionyl chloride afforded the chloride **9a**. Treatment of **9a** with sodium azide in acetone followed by catalytic hydrogenation with Pd/C of the corresponding azide intermediate provided the benzylamine **6a**. The amine **6b** was prepared from the benzyl bromide **9b** following the same sequence of reactions as described from benzyl chloride **9a** to amine **6a**. The benzylamine **6c** was prepared according to Scheme 3. Carboxylic acid **10** was reduced with borane to give the benzyl alcohol **8b** which was treated with carbon tetrabromide and triphenylphosphine to afford the benzyl bromide **9c**. Subsequent reaction of bromide **9c** with sodium azide followed by reduction with lithium aluminum hydride provided benzylamine **6c**. The benzylamine **6d**, a precursor of aminotetrazole **25**, was synthesized as shown in Scheme 4. 2-Fluorobenzonitrile was treated with morpholine under microwave conditions to give the benzonitrile intermediate **11**. Nitrile **11** was subjected to catalytic hydrogenation using Raney-Ni in ammonia/methanol to give the benzylamine **6d**.

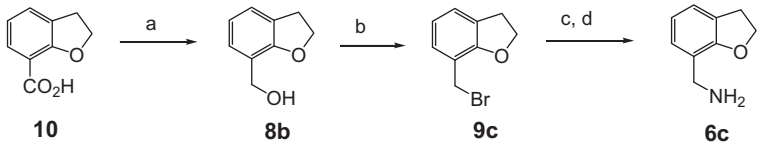
The aminotetrazole analogs were assayed for in vitro $P2X_7$ activity as antagonists at recombinant human and rat receptors. Inhibition of Ca^{2+} was measured with a fluorometric imaging plate reader (FLIPR), using Fluo-4 as the calcium-sensing dye and benzoylbenzoic ATP (BzATP) as the agonist.[25,26] In vitro data for compounds **1–4**, and **12–45** is presented in Table 1. Our initial SAR study evaluated modifications and substitution on the benzyl ring. In general, compounds **12–45** were less potent at the rat than the human $P2X_7$ receptor in a range that varied from 2- to 16-fold. Investigation of the *ortho*-substitution on the benzyl group displayed several analogs that possessed essentially equivalent potency to the unsubstituted analog **15**. Compounds **12**, **13**, **16**, **18**, and **21**, for example, were equipotent to **15** at both the human and rat $P2X_7$ receptors. Moreover, analogs **12**, **13, 16**, and **18** showed only 2- to 4-fold species difference between the human and rat $P2X_7$ receptors. Interestingly, analog **17** with the 2-methylphenyl group directly attached to the nitrogen and the 2-methylbenzyl analog **16** exhibited equivalent potency to each other at both the human and rat $P2X_7$ receptors. In contrast, N-methylation on the nitrogen of the benzylamino group was detrimental to $P2X_7$ activity.[27] Compounds with basic groups on the *ortho*-position such as **23, 24**, and **25** showed comparable activity to other *ortho*-substituted analogs such as the 2-chloro compound **29.** Compounds **23–25** also showed a narrow species difference of 2- to 4-fold. Also noteworthy with compounds **24** and **25** is the



Scheme 4. Reagents and conditions: (a) morpholine, THF, microwave, 110 °C, (b) Raney-Ni, H_2, NH_3/MeOH, 22 °C.

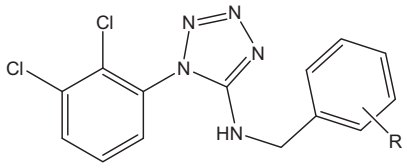


Scheme 2. Reagents and conditions: (a) SO_2Cl_2, 0 °C; (b) NaN_3, acetone, reflux; (c) Pd/C, H_2, MeOH.



Scheme 3. Reagents and conditions: (a) BH_3, THF, 22 °C; (b) CBr_4, PPh_3, CH_2Cl_2, 22 °C; (c) NaN_3, DMF, 22 °C; (d) $LiAlH_4$, THF, 0 °C.

Table 1
SAR of *N*-benzyl-1-(2,3-dichlorophenyl)-1*H*-tetrazol-5-amines **12–45**



Compd	R	hP2X$_7$ pIC_{50}[a]	rP2X$_7$ pIC_{50}[a]
1		6.91	6.51
2		7.11	6.68
3		7.75	7.16
4		7.68	6.70
12	2-$MeSO_2$	7.94	7.67
13	2-MeO	7.86	7.25
14	3-MeO	7.39	6.76
15	H	7.84	7.26
16	2-Me	7.78	7.30
17[b]		7.78	7.37
18	2-F	7.74	7.12
19	3-F	7.66	7.25
20	4-F	7.40	6.90
21	2-CN	7.74	6.99
22	2-MeS	7.62	7.36
23	2-$(Me)_2N$	7.62	6.99
24	2-$(Et)_2NCH_2CH_2O$	7.56	7.16
25	2-Morpholin-4-yl	7.55	7.34
26	2-(CHF_2O)	7.50	7.14
27	2-EtO	7.26	6.97
28	2-Me_2CHO	6.96	6.94
29	2-Cl	7.50	6.98
30	3-Cl	6.92	6.33
31	4-Cl	6.59	6.03
32	2-CF_3	7.13	6.92
33	3-CF_3	6.63	5.99
34	4-CF_3	6.36	5.15
35	2-OCF_3	7.13	6.73
36	3-OCF_3	6.25	6.02
37	4-OCF_3	5.72	4.80
38	2,3-diMeO	7.83	7.73
39	2,3-(OCH_2O)	7.83	6.82
40	2,3-(OCH_2CH_2)	7.70	6.66
41	2,3-diMe	7.55	6.61
42	2,3-diF	7.26	6.64
43	2,5-diF	7.52	6.85
44	2-F-5-Cl	7.15	6.28
45	2-Cl-3, 6-diF	7.06	6.10

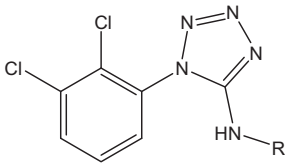
[a] Values are the mean of 2–5 experiments.
[b] Phenyl ring is directly attached to the nitrogen.

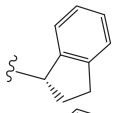
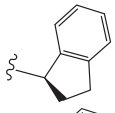
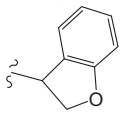
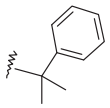
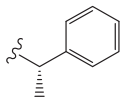
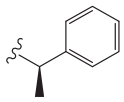
tolerance for the larger diethylaminoethyl and morpholinyl groups at the *ortho*-position.[28] Increasing the size of the 2-alkylether substitution, in contrast, produced a decrease in potency at both the human and rat receptors. Ethers **26–28** were 2-, 4- and 8-fold, respectively, less potent than the 2-methoxy analog **13**.

Comparison of the human P2X$_7$ activity of the three mono fluoro substituted analogs, **18–20**, revealed that the 2 and 3-fluoro analogs were equipotent, but the 4-substituted analog **20** was 2-fold less potent than analogs **18** and **19**. However, the 3-methoxy and 3-chloro analogs **14** and **30** were 3- and 4-fold less potent than the 2-methoxy (**13**) and 2-chloro analogs (**29**). The 4-chloro analog **31** was 8-fold less active than the 2-chloro analog **29**. A parallel effect was observed with the trifluoromethyl and trifluoromethoxy substitutions. The 3-substituted analogs **33** and **36** were 3- and 7-fold less potent than the 2-susbtituted analogs **32** and **35,** and the 4-substituted analogs **34** and **37** were 6- and 25-fold less potent than the 2-substituted analogs **32** and **35**.

The 2,3-disubstituted analogs **38–40** showed comparable potency to the 2-methoxy ether **13** at the human receptor. Although

Table 2
SAR of *N*-benzyl-1-(2,3-dichlorophenyl)-1*H*-tetrazol-5-amines **46–51**



Compd	R	hP2X$_7$ pIC_{50}[a]	rP2X$_7$ pIC_{50}[a]
46		7.80	7.23
47		7.53	6.54
48		7.70	6.70
49		7.57	6.84
50		7.41	6.10
51		7.68	6.08

[a] Values are the mean of 2–5 experiments.

compounds **39** and **40** showed a 10-fold species difference between the human and rat P2X$_7$ receptors, compound **38** displayed no significant difference in potency across species. A slight decrease of 2- and 4-fold in potency at the human P2X$_7$ receptors was observed with the 2,3-dimethyl, 2,5-difluoro and 2,3-difluoro substituted analogs **41, 43**, and **42**. Trisubstitution on the phenyl ring led to a less active analog **45**.

Substitution on the benzylic carbon was also investigated. Shown in Table 2 are the in vitro data for compounds **46–51**, which have either ring formation between the *ortho*-position and the benzylic position or simple methyl substitution on the benzylic carbon. All of the compounds in Table 2 possessed comparable activity at the human P2X$_7$ receptor. Activity at the rat receptor, however, was more sensitive both to stereochemistry and the presence or absence of ring formation with the *ortho*-position. Examination of the effect of the stereochemistry in this series showed that the (*R*) enantiomer **46** was 2-fold more potent than its antipode **47** at the human P2X$_7$ receptor but 5-fold more potent at the rat receptor. A more dramatic loss of rat activity was seen with the non-ring compounds **50** and **51**; these were 20- and 40-fold less active, respectively, at rP2X$_7$. Compound **48**[29] shows that insertion of oxygen into the indane ring was well tolerated at the human receptor, but this compound also showed 10-fold lower activity at rP2X$_7$. A slight decrease of 2- and 3-fold in potency at the human and rat P2X$_7$ receptors was observed with the *gem*-dimethyl analog **49**, compared with the unsubstituted analog **15**.

Compared to the benzylaminotriazole compound **4**, the corresponding benzylaminotetrazole **16** showed superior potency at the rat P2X$_7$ receptor. Additionally, the difference in potency across

species was attenuated for **16** compared to **4**; **16** showed only a 3-fold difference, whereas triazole **4** displayed 10-fold lower activity at rP2X$_7$. This greater potency for the tetrazole compared to the triazole in the benzylamino variation parallels the greater potency for tetrazole versus triazole seen previously with the directly attached benzyl group,[20] and shown here in the comparison of compound **2** with compound **3**. The in vitro potencies of the three *ortho*-methylphenyl-substituted tetrazoles **3**, **16**, and **17** show no significant differences at either rat or human receptors. From a synthetic chemistry perspective, however, the method to prepare the benzylaminotetrazoles was more amenable to analog generation. This allowed a more expansive exploration of the SAR trends on the right-hand aromatic group in this series compared with compounds like **1** and **3**.[19]

In summary, we have discovered a novel series of *N*-benzyl-1-(2,3-dichlorophenyl)-1*H*-tetrazol-5-amine derivatives that are potent P2X$_7$ receptor antagonists. This structural variation shows improved rat potency over the corresponding triazole core (e.g., **4**), but similar P2X$_7$ potency compared to the earlier tetrazole **3**. Convenient synthetic methodology allowed extensive SAR studies to be conducted on the right-hand side of the molecule. This work revealed the preferred substitution patterns for the *N*-benzylamine moiety: 2-substituted and 2,3-disubstituted phenyl groups produced potent P2X$_7$ receptor antagonists. Analogs **12** and **38** are particularly interesting because of their high potency and narrow species difference. These studies also revealed opportunities to substitute in the *ortho* -position of the right-hand phenyl with much larger and potentially water-solubilizing groups (e.g., **24** and **25**). The results of studies with more diverse substitution in the *ortho*-position will be reported in due course.

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23. Representative procedure for the preparation of aminotetrazoles **12–51**. A solution of amine **6** (0.74 mmol) in anhydrous THF (10 mL) was treated drop wise with isothiocyanate **5** (0.74 mmol). The solution was stirred at room temperature for 6 h and then mercuric chloride or mercuric acetate (0.74 mmol) was added followed by sodium azide (2.21 mmol, 3.0 equiv). The reaction was stirred at room temperature for 8 h. The black mixture was filtered through a pad of Celite, washed with THF and concentrated under reduced pressure. The residue was purified by reversed phase HPLC on a Waters Nova-Pak® HR C18 6um 60 Å Prep-Pak® cartridge column (40 mm × 100 mm) using a gradient of 0–70% acetonitrile:10 mM ammonium acetate over 8 min (10 min run time) at a flow rate of 70 mL/min) to yield the aminotetrazoles **12–51**.
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27. The compound *N*-benzyl-1-(2,3-dichlorophenyl)-*N*-methyl-1*H*-tetrazol-5-amine had a hP2X$_7$ pIC_{50} = 5.25 and rP2X$_7$ pIC_{50} = 5.44.
28. The results of studies into diverse groups having greater size or basic character in the *ortho*-position will be the subject of a future publication.
29. Compound **48** also inhibited IL-1β release in THP-1 cells with pIC_{50} = 8.6. For a description of the assay and the activity of a more well-characterized analog see Ref. 30. For a more extensive correlation between FLIPR activity and IL-1β release see Ref. 19.
30. Honore, P.; Donnelly-Roberts, D.; Namovic, M.; Zhong, C.; Wade, C.; Chandran, P.; Zhu, C.; Carroll, W.; Perez-Medrano, A.; Iwakura, Y.; Jarvis, M. F. *Behav. Brain Res.* **2009**, *204*, 77.