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Novel and potent 3-(2,3-dichlorophenyl)-4-(benzyl)-4*H*-1,2,4-triazole $P2X_7$ antagonists

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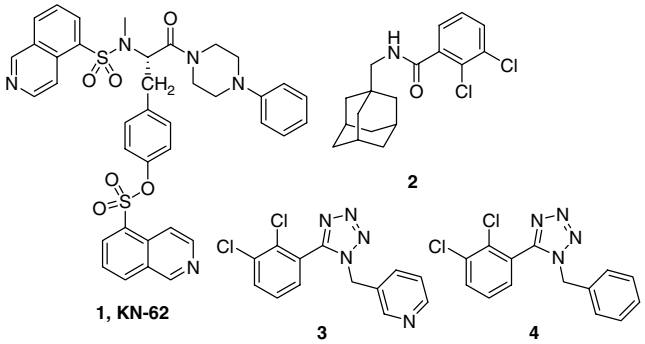
Abstract—Structure–activity relationship (SAR) studies were conducted around early tetrazole-based leads **3** and **4**. Replacements for the tetrazole core were investigated and the pendant benzyl substitution was reoptimized with a triazole isostere. Triazole-based $P2X_7$ antagonists were identified with similar potency to the lead compound **4** but with improved physiochemical properties. Compound **12** was active in a rat model of neuropathic pain.

The P2X receptors ($P2X_1$–$P2X_7$) comprise a family of ligand-gated ion channels that are activated by ATP.[1] A functional ion channel forms through the hetero- or homomeric assembly of P2X subunits. Each subunit, in turn, consists of a single polypeptide chain with two membrane spanning regions and both amino and carboxy terimini being intracellular. Of the various P2X receptors, the $P2X_7$ receptor is unique in that it does not form heteromeric complexes with other P2X receptor subunits.[2] Brief (millisecond) activation of the $P2X_7$ receptor with ATP results in a non-selective cation (Na^+, Ca^{2+}, K^+) flux, whereas longer exposure (>30 min) to ATP gives rise to cytolytic pore formation.[3]

The $P2X_7$ receptor is located primarily on cells of the immune system[4] such as macrophages, monocytes, mast cells, and lymphocytes as well as on glial cells (astrocytes and microglia) in the central nervous system. The presence of the $P2X_7$ receptor on neurons, however, has yet to be definitively established.[5] Activation of the $P2X_7$ receptor on cells of immunological origin causes a release of pro-inflammatory cytokines,[4] including IL-1β, a process that may play an important role in the development and progression of inflammatory, neurodegenerative[6–9] or chronic pain[10,11] conditions in either the peripheral or central compartments. Recent studies with $P2X_7$ knockout (KO) mice support a prominent role for $P2X_7$ in inflammation and pain. These KO mice showed a reduced incidence and severity of arthritic symptoms compared to wild-type mice.[12] Additionally, $P2X_7$ KO mice were protected from the development of both adjuvant-induced inflammatory pain and partial nerve ligation induced neuropathic pain.[13] Blockade of $P2X_7$ with a small molecule antagonist, thus, could provide a novel therapeutic approach to the treatment of pain as well as neurodegenerative and inflammatory disorders.



Structure–activity relationship (SAR) studies of $P2X_7$ selective ligands have appeared in the literature for several chemical series.[14–19] KN-62 (**1**), a tyrosine-based non-competitive $P2X_7$ antagonist, was the first small molecule inhibitor to be identified.[20] Recently, a more

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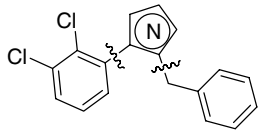
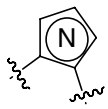
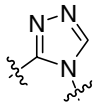
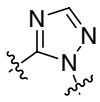
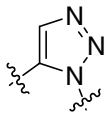
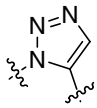
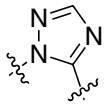
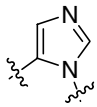
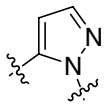
'druglike' series of adamantylbenzamides, exemplified by **2**, have been described that possess potent activity to competitively block $P2X_7$ receptors.[18] As described in a recent report, we have found that 1-benzyl-5-(2,3-dichlorophenyl)-tetrazoles are potent $P2X_7$ antagonists with compound **3** showing activity in a rat model of neuropathic pain.[21a] The studies described herein extend the SAR investigations around **3** by examining heterocyclic replacements for the tetrazole core (see Table 1) and subsequent reoptimization of the pendant nitrogen substituent (Tables 2 and 3).

Scheme 1 illustrates the straightforward 3-step method utilized to prepare the triazole **5** as well as the examples

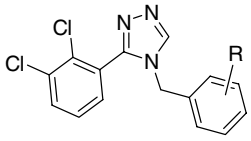
Table 1.



Compound		hP2X$_7$ pIC$_{50}$[a]
4		7.40
5		6.28
6		6.52
7		6.22
8		6.37
9		6.68
10		5.74
11		5.22
2		7.19

Values are means of at least three experiments.
[a] Compounds tested at the recombinant hP2X$_7$ receptor as described.[21]

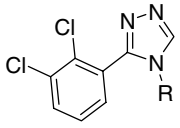
Table 2.

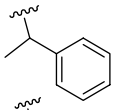
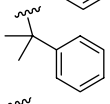
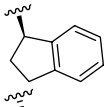
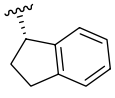


Compound	R	hP2X$_7$ pIC$_{50}$[a]
5	H	6.28
12	2-Me	7.11
13	2-OMe	6.72
14	2-CF_3	6.88
15	2-CHF_2	6.93
16	2-SMe	7.31
17	2-SMe[b]	7.57
18	2-SO_2Me[b]	6.84
19	2,3-Benzo	7.23
20	2-Me, 5-F	7.32
21	2-CF_3,5-F	7.31
22	2-F, 5-CF_3	6.04
23	3-OMe	6.67
24	3-CF_3	5.89
25	4-CF_3	5.05
26	2,3-DiMeO	6.88
27	2,3-DiCl	6.72
28	2,4-DiCl	6.48
29	2,5-DiCl	6.86

Values are means of at least three experiments.
[a] Compounds tested at the recombinant hP2X$_7$ receptor as described.[21]
[b] 2-Cl,3-CF_3 substitution on left hand phenyl rather than 2,3-dichlorophenyl.

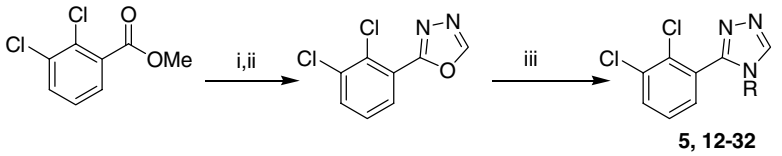
Table 3.



Compound		hP2X$_7$ pIC$_{50}$[a]
30		5.98
31		4.88
32		7.72
33		7.30

Values are means of at least three experiments.
[a] Compounds tested at the recombinant hP2X$_7$ receptor as described.[21]

in Tables 2 and 3. Conversion of methyl 2,3-dichlorobenzoate to the oxadiazole intermediate in two steps proceeded in high yield. Transformation of the oxadiazole to the triazole by reaction with amines proved to be a reaction that could be run in parallel, allowing for the rapid SAR exploration of the R group off the triazole nitrogen. Although the yields for this



Scheme 1. Reagents and conditions: (i) NH_2NH_2, EtOH; (ii) $HC(OEt)_3$, *p*-TsOH, toluene, Δ, 90% yield for two steps; (iii) RNH_2, toluene, Δ.

latter transformation usually did not exceed 50%,[22] the desired product was easily separated from any by-products by either reverse phase HPLC or normal phase column chromatography.[23]

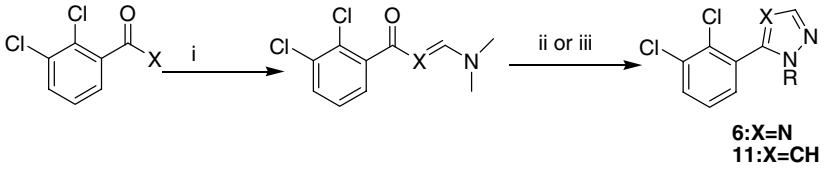
The triazole and pyrazole analogs **6** and **11** were synthesized using the same general method (Scheme 2) wherein either the starting acetophenone or benzamide was reacted first with DMF-dimethylacetal, followed by condensation with benzylhydrazine under buffered conditions.[24]

The 1,2,3-triazole **7** was prepared in three steps (Scheme 3) from 1,2-dichloro-3-iodobenzene via Sonogashira coupling with trimethylsilylacetylene followed by cleavage of the TMS group and 1,3-dipolar cycloaddition of the primary acetylene with benzylazide. This latter reaction provided the desired 1,5-regioisomer in low yield (10%) due to the preferred formation of the 1,4-regioisomer (~20%). Reaction (not shown) of 1-azido-2,3-dichlorobenzene with 3-phenyl-1-propyne in toluene for 18 h at 100 °C provided **8** in low yield (20%), again due to the preferred formation of the 1,4-regioisomer in 66% yield.

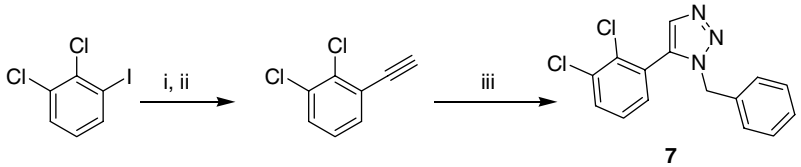
The triazole **9** was prepared in a straightforward fashion (Scheme 4) by condensing 2,3-dichlorophenylhydrazine with *N*-formylphenylacetamide in glacial acetic acid at reflux.

Finally, the imidazole isostere **10** was synthesized by benzylation of 4-bromoimidazole[25] and subsequent microwave-facilitated Suzuki coupling (Scheme 5). In the case of the benzylation, the 1,4 regioisomer again predominated (59%). The Suzuki reaction and product purification were not optimized, accounting for the low yield.[26]

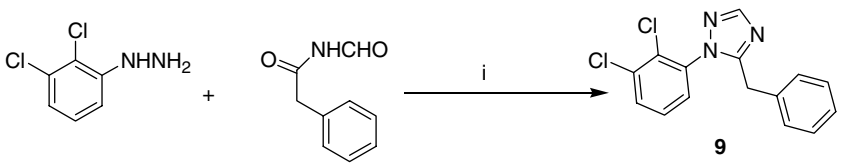
With 2,3-dichlorophenyl and benzyl substitutions held constant (Table 1), heterocyclic replacements for the tetrazole moiety were evaluated. There was observed a progressive loss of $P2X_7$ potency in the order tetrazole > triazole > imidazole > pyrazole, with the various triazole regioisomers **5**–**9** demonstrating similar $P2X_7$ potency regardless of connectivity (C versus N) to the phenyl and benzyl groups. In view of this latter result, it would appear that the position of the nitrogens in the central ring does not strongly influence activity. Rather, a stronger relationship seems to exist between the overall electron density of the core heterocycle and $P2X_7$ potency. The most electron deficient ring (tetrazole) demonstrates the greatest potency, whereas the more electron rich imidazole and pyrazole rings are



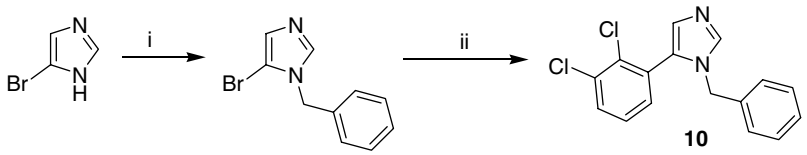
Scheme 2. Reagents and conditions: (i) DMF dimethylacetal, Δ. X=CH_3 yield = 90%; X=NH_2 yield = 44%; (ii) X=CH: $BnNH_2NH_2$·HCl, NaOAc, H_2O/MeOH (1:10), 90 °C; yield = 46%; (iii) X=N: $BnNH_2NH_2$·HCl, NaOAc, 70% AcOH/dioxane (1:1), 90 °C, yield = 41%.



Scheme 3. Reagents and conditons: (i) HC≡CTMS, $PdCl_2(PPh_3)_2$, CuI, Et_3N, Δ; (ii) K_2CO_3, MeOH; 70% yield for two steps; (iii) BnN_3, EtOH, 80 °C, 10% yield.



Scheme 4. Reagent and condition: (i) AcOH, reflux, 28% yield.



Scheme 5. Reagents and conditon: (i) BnCl, TBAB, KOtBu, 21% yield; (ii) 2,3-dichlorophenylboronic acid, $Pd(PPh_3)_2Cl_2$, DME/EtOH/H_2O (7:3:2), microwave, 10 min, 6% yield.

Table 4.

Compound	THP-1 IL-1β pIC_{50}[a]	$rP2X_7$ pIC_{50}[a]
12	6.43	6.68
20	6.62	6.26
32	7.12	7.11
4	5.60	7.12
2	NT	6.09

Values are means of at least three experiments.
[a] Compounds tested at the recombinant $rP2X_7$ receptor and for IL-1β release as described.[21]

the weakest and the various triazoles are intermediate in terms of both potency and electron density. From these, the triazole **5** was selected for further study to determine if optimization of the *N*-benzyl substitution could result in analogs that recapture the potency of **4**. Using parallel synthesis techniques, a library of approximately 60 analogs was prepared around **5** utilizing a variety of benzylamines (selected analogs shown in Table 2). Briefly, *ortho*-substitution tended to increase $P2X_7$ potency as for analogs **12**–**18**, whereas *para*-substitution decreased potency (**25**) and *meta*-substitution produced mixed effects (**23**, **24**). In the *ortho*-position the sulfone **18** was approximately fivefold less potent than the corresponding sulfide **17**. From the small set of *ortho*-substituted examples, it is clear that a variety of electron donating and withdrawing groups are tolerated although the best activity is observed with the electronically more neutral substitutions (**12**, **16**). There was typically a significant degree of tolerance for substitution in the meta- and *para*-positions as long as an *ortho*-substituent was present (**19**–**21**, **26**–**29**). However the reduced activity for compound **22** is consistent with the *meta*-CF_3 substituted **24** also being weaker. A number of phenethylamine analogs were also generated in this fashion, however, none of these showed any appreciable $P2X_7$ activity (data not shown). Changing the substitution on the left hand phenyl from 2,3-dichloro to 2-chloro-3-trifluoromethyl provided comparable potency (**16** vs **17**). Further exploration of the left-hand side substitution was not undertaken.

Substitution on the benzylic carbon was also investigated. Interestingly, methyl (**30**) and dimethyl (**31**) progressively decreased $P2X_7$ potency, however, tying back the methyl to form an indane provided the most potent analog from this series (**32**).[27] In this case, the (R) enantiomer **32** was 2–3 times more potent than the (S) enantiomer **33**.

As shown in Table 4, several compounds were evaluated for their ability to inhibit IL-1β release from human THP-1 cells. All three of these compounds blocked IL-1β release, although with less potency than that observed to inhibit Ca^{2+} flux. In addition, the compounds in Table 4 were tested at the $rP2X_7$ receptor where they also generally showed reduced potency, a potential confounding factor when conducting in vivo efficacy studies in rats.

Compound **12** was further evaluated in vivo for its antinociceptive activity in the rat Chung model[28] of neuropathic pain. When dosed intraperitoneally, compound **12** produced a dose-dependent reversal of mechanical allodynia at the 30 min timepoint with an ED_{50} of 125 μmol/kg and a maximum efficacy of 68%. Consistent with this activity, **12** demonstrated good bioavailability (ip) in the rat (62%) and a half-life (1.7 h) commensurate with the duration of the behavioral assessment.[29]

One of the limitations of earlier tetrazole analogs lacking the pyridine moiety on the right side of the pharmacophore was that of low solubility precluding the routine in vivo evaluation of many analogs.[21a] Triazole analogs like **12**, however, possess a nitrogen basic enough to permit salt formation[27] and sufficient solubility to permit pharmacokinetic and efficacy studies in vivo. The improved ADME properties of the triazole isostere allowed for exhaustive exploration of the benzyl group SAR without the restriction that pyridine be present on the right side of the molecule. These studies thus compliment our earlier work on the left side of the pharmacophore[21a] and allowed for the diversification of $P2X_7$ chemical space and the identification of novel, selective[30] analogs with potency comparable to available reference compounds. These studies have also provided additional tool compounds with which to probe the potential of $P2X_7$ antagonists in the treatment of inflammatory and neurodegenerative disorders.

References and notes

1. Girdler, G.; Khakh, B. S. *Curr. Biol.* **2004**, *14*, R6.
2. North, R. A. *Physiol. Rev.* **2002**, *82*, 1013.
3. Morelli, A.; Ferrari, D.; Bolognesi, G.; Rizzuto, R.; DiVirgilio, F. *Drug Dev. Res.* **2001**, *52*, 571.
4. DiVirgilio, F.; Vishwanath, V.; Ferrari, D. *Handb. Exp. Pharmacol.* **2001**, *151*, 355.
5. Sim, J. A.; Young, M. T.; Sung, H.-Y.; North, R. A.; Surprenant, A. *J. Neurosci.* **2004**, *24*, 6307; Andersen, C. M.; Nedergaard, M. *Trends Neurosci.* **2006**, *29*, 257.
6. LeFeuvre, R. A.; Brough, D.; Touzani, O.; Rothwell, N. J. *J. Cereb. Blood Flow Metab.* **2003**, *23*, 381.
7. LeFeuvre, R.; Brough, D.; Rothwell, N. *Eur. J. Pharmacol.* **2002**, *447*, 261.
8. Rampe, D.; Wang, L.; Ringheim, G. E. *J. Neuroimmunol.* **2004**, *147*, 56.

9. Wang, X.; Arcuino, G.; Takano, T.; Lin, J.; Peng, W. G.; Wan, P.; Li, P.; Xu, Q.; Liu, Q. S.; Goldman, S. A.; Nedergaard, M. *Nat. Med.* **2004**, *10*, 821.
10. Raghavendra, V.; DeLeo, J. A. *Adv. Mol. Cell Biol.* **2004**, *31*, 951.
11. Milligan, E. D.; Maier, S. F.; Watkins, L. R. *Sem. Pain Med.* **2003**, *1*, 171.
12. 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.
13. 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.
14. Baraldi, P. G.; Romagnoli, R.; Tabrizi, M. A.; Falzoni, S.; DiVirgilio, F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 681.
15. Ravi, R. G.; Kertesy, S. B.; Dubyak, G. R.; Jacobsen, K. A. *Drug Dev. Res.* **2001**, *54*, 75.
16. Chen, W.; Ravi, R. G.; Kertesy, S. B.; Dubyak, G. R.; Jacobsen, K. A. *Bioconjugate Chem.* **2002**, *13*, 1100.
17. Baraldi, P. G.; del Carmen Nunez, M.; Morelli, A.; Falzoni, S.; DiVirgilio, F.; Romagnoli, R. *J. Med. Chem.* **2003**, *46*, 1318.
18. Baxter, A.; Bent, J.; Bowers, K.; Braddock, M.; Brough, S.; Fagura, M.; Lawson, M.; McInally, T.; Mortimore, M.; Robertson, M.; Weaver, R.; Webborn, P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4047.
19. Alcaraz, L.; Baxter, A.; Bent, J.; Bowers, K.; Braddock, M.; Cladingboel, D.; Donald, D.; Fagura, M.; Furber, M.; Laurent, C.; Lawson, M.; Mortimore, M.; McCormick, M.; Roberts, N.; Robertson, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4043.
20. Gargett, C. E.; Wiley, J. S. *Br. J. Pharmacol.* **1997**, *120*, 1483.
21. (a) 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; (b) Honore, P.; Donnelly-Roberts, D.; Namovic, M. T.; Hsieh, G.; Zhu, C. Z.; Mikusa, J. P.; Hernandez, G.; Zhong, C.; Gauvin, D. M.; Chandran, P.; Harris, R.; Perez Medrano, A.; Carroll, W.; Marsh, K.; Sullivan, J. P.; Faltynek, C. R.; Jarvis, M. F. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1376.
22. A typical experimental procedure goes as follows: 2-(2,3-dichloro-phenyl)-[1,3,4]oxadiazole (2.28 g, 10.6 mmol) and 2-methylbenzylamine (1.4 mL, 1.1 equiv) were heated in toluene (3 mL) for 2 days in a sealed tube at 100 °C. The reaction mixture was directly purified by silica gel flash chromatography eluting with 3% MeOH/EtOAC to provide 1.64 g (49%) of compound **12** as a tan solid.
23. Reverse phase HPLC conditions: waters symmetry C8 column (40 mm × 100 mm, 7 μm particle size) using a gradient of 10–100% acetonitrile: 0.1% aqueous TFA over 12 min (15 min run time) at a flow rate of 70 mL/min. Silica gel flash column chromatography conditions for compound **12**: 3% MeOH/EtOAc.
24. The 1,5-pyrazole regioisomer **11** was the main product in the reaction although the 1,3-regioisomer was also formed as a minor product (6%). The structural assignment was confirmed by NMR studies showing NOE between the benzylic protons and the *ortho*-proton of the 2,3-dichlorophenyl group for **11** whereas this NOE was absent for the minor 1,3-regioisomer. The regiochemistry for the triazole **6** was assigned by analogy to Lin, Y.-i.; Lang, S. A.; Lovell, M. F.; Perkinson, N. A. *J. Org. Chem.* **1979**, *44*, 4160.
25. Diez-Barra, E.; Hoz, A. de la; Sanchez-Migallon, A.; Tejeda, J. *Synth. Commun.* **1993**, *23*, 1783.
26. Compound **10** was purified using the HPLC conditions described above in footnote 23. The analogous Suzuki reaction using 5-bromo-1-(2-methyl-benzyl)-1*H*-imidazole rather than 5-bromo-1-benzyl-1*H*-imidazole provided the product in improved yield (25%) using silica gel chromatography (70:29:1 EtOAc:Hex:Et_3N) to purify the product.
27. Attempts to form the HCl salt of *N*-indanyl analogs like **32** resulted in some decomposition, presumably due to an E1-type process. By contrast, compound **12** was stable to such treatment and the HCl salt was crystallized from Et_2O/EtOH.
28. Kim, S. H.; Chung, J. M. *Pain* **1992**, *50*, 355.
29. Compound **12** was administered at a dose of 5 μmol/kg for the pharmacokinetics study. The following additional pharmacokinetic parameters were measured for compound **12**. Oral bioavailability = 15%; clearance (iv) = 3.1 L/h/kg; V_β = 1.9 L/kg; T_{max} (ip) = 0.33 h; C_{max} (ip) = 0.21 μg/mL.
30. Compound **12** was profiled across a range of ion channels and receptors at Cerep and was found to interact significantly only with the peripheral benzodiazepine site (86% @ 10 μM). Compound **12** was also inactive at $P2X_1$, $P2X_2$, $P2X_{2/3}$, $P2X_4$, $P2Y_1$, and $P2Y_2$ receptors at 10 μM.