Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 435–438
Bioorganic & Medicinal Chemistry Letters

Synthesis and SAR of novel 4,5-diarylimidazolines as potent $P2X_7$ receptor antagonists

Gregory H. Merriman,* Liang Ma, Patrick Shum, Daniel McGarry, Frank Volz, Jeffrey S. Sabol, Alexandre Gross, Zhicheng Zhao, David Rampe, Lin Wang, Friederike Wirtz-Brugger, Bruce A. Harris and Douglas Macdonald

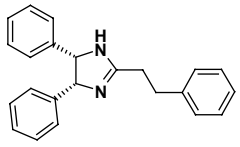
Aventis Inc., 1041 Route 202-206, Bridgewater, New Jersey, NJ 08807-0800, USA

Received 9 August 2004; accepted 20 October 2004

Abstract—A series of 4,5-diarylimidazoline libraries were prepared using high-throughput solid-phase and microwave techniques. The compounds were evaluated as $P2X_7$ antagonists and their SAR is described.

1. Introduction

The $P2X_7$ receptor has received considerable attention over the past few years as a therapeutic target.[1,2] The $P2X_7$ receptor is a ligand-gated ion channel that is present on a variety of cell types, such as, mast cells, macrophages and lymphocytes, all thought to be involved in inflammation and autoimmune processes.[3] Activation of the $P2X_7$ receptor by adenosine triphosphate (ATP) leads to release of the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), as well as giant cell formation, including macrophages and microglial cells and also leads to mast cell degranulation.[4,5] As such, it is anticipated that a $P2X_7$ antagonist will show anti-inflammatory activity and may be of therapeutic use for diseases with this component such as rheumatoid arthritis, Alzheimer's disease and stroke.



1 $P2X_7$ IC_{50} = 0.08 μM

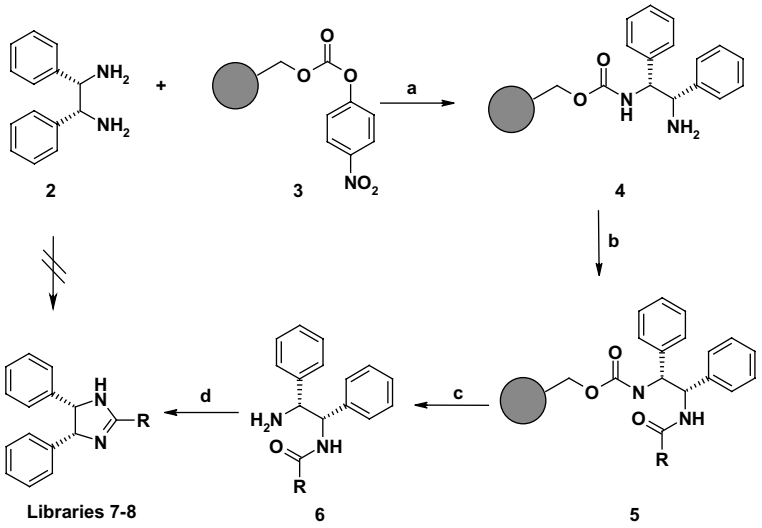
As a result of a high-throughput screening effort we identified **1** as a potent new $P2X_7$ antagonist lead. Here we describe our efforts to rapidly explore the SAR of a series of diarylimidazoline analogs of **1** using a high-throughput medicinal chemistry approach.

2. Chemistry

The design and synthesis of a focused 4,5-diaryl-imidazoline library based on **1** employed a combination of solid phase and microwave techniques. Initially we envisioned a simple condensation of a 1,2-diaryl-1,2-ethanediamine scaffold **2** with a series of carboxylic acid building blocks to afford the desired cyclic imidazoline targets **7** directly (Scheme 1). Unfortunately attempts to prepare the imidazolines in one step under a variety of dehydrative conditions failed to give the desired targets, usually affording a complex mixture of compounds that was difficult to separate. As an alternate approach we explored the stepwise formation of target imidazolines using solid-phase and microwave techniques highlighted in Scheme 1. Thus, coupling of the ethanediamine scaffold **2** with *p*-nitrophenyl-carbonate resin **3** afforded the carbamate bound amine **4** in quantitative yield.[6] A series of carboxylic acids, chosen to explore the chain length, electronic and steric requirements of the pendent aromatic ring, were then coupled using a standard amide coupling with EDC in dimethyl-formamide to afford **5** in quantitative yield.[7] Attempts to cleave and cyclize (**5** → **7**) in one pot using TFA or other

* Corresponding author. Fax: +1 908 231 4774; e-mail: gregory.merriman@aventis.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.052



Scheme 1. Reagents and conditions: (a) CH_2Cl_2, rt; (b) RCO_2H, EDC, DMF; (c) 50% TFA/CH_2Cl_2; (d) TMS-polyphosphate, CH_2Cl_2, microwave 140 °C, 8 min.

strong organic acids with heat failed to give **7**, affording only the uncyclized amine cleavage product **6**. Since **6** could be isolated in high yield and purity we focused on cyclizing **6** → **7** off resin. Our initial attempts to cyclize amides **6** using polymer bound Burgess reagent failed to afford **7** under a variety of conditions. We then turned our attention to the use of trimethylsilyl polyphosphate (TMS-PP), a reagent previously shown to be effective in the preparation of 1,4,5,6-tetrahydropyrimidines.[8] Thus, treatment of **6** with TMS-PP in dichloromethane under microwave irradiation at 140 °C (~12–15 bar) afforded the desired imidazolines in good overall yield.[9] Two focused libraries **7** and **8** were prepared using the aforementioned route to explore the SAR.

3. Results

Target compound $P2X_7$ IC_{50}s were assessed using a cellular (U373 cells stably expressing human $P2X_7$) YO-PRO-1 dye uptake fluorescence assay.[10,11] To assess the appropriate chain length, branching and configurational constraints of the alkyl-aromatic R group in the 2-position of the imidazoline, a small group of compounds (**7a**–**k**) was prepared (Table 1). In general, activity was maintained in compounds with chain lengths 1–4, with a drop in potency once the 3 carbon chain is reached (**7i**). Compounds with aryl moieties directly attached to the imidazoline scaffold were inactive as exemplified by **7h**. Branching with small alkyl groups on the chain was also well tolerated within the series. Alkyl-branched phenethyl substituted compounds **7a**–**e** all maintained activity, however, **7f**, a conformationally restricted *trans*-cyclopropane analog, was inactive at the concentration tested.

The most potent compounds in the group were methyl and *gem*-dimethyl substituted **7a** and **7b**, respectively.

Benzyl substituted analogs also maintained activity as shown with compound **7g**. Notably, there is no apparent enantiomeric preference for the branched substitutent, as both enantiomers of **7g** were prepared and were found to be equipotent.[12]

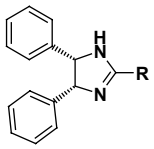
It was anticipated that substitution of the pendent aryl ring would serve to maintain or improve potency as well as potentially offer compounds that would have reduced metabolic liability. Based, in part, on the aforementioned $P2X_7$ antagonists (**7**) and on the relative availability of requisite carboxylic acid building blocks, additional focused libraries of phenethyl and benzyl analogs with substitution on the aryl ring (**8**) were prepared and the activities of a representative group are highlighted in Table 2.

Electron withdrawing and small neutral groups were generally well-tolerated on both the phenethyl ($n = 2$) and benzyl ($n = 1$) analogs. However, electron-donating groups were much less active as exemplified by **8k**,**l**,**m** and **y**. Substitution of the *ortho* and *meta* aryl positions of the phenethyl group gave analogs that tended to be more potent in general than the corresponding *para*-substituted analogs (**8c**,**f**,**h**,**j**). On the other hand *ortho*-substituted benzyl analogs (**8n**,**r**,**s**,**w**) were less potent than corresponding *meta*- and *para*-substituted analogs.

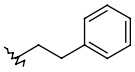
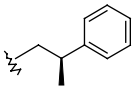
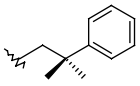
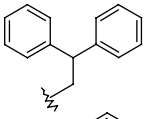
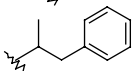
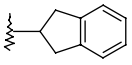
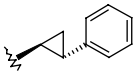
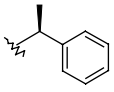
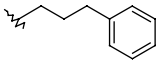
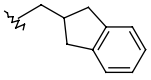
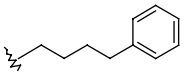
4. Conclusion

In conclusion we have prepared and evaluated a new series of imidazolines as $P2X_7$ antagonists. The chemistry used to prepare this series included a combined solid- and solution-phase approach that was quite general and amenable to library synthesis. The key step in the synthesis was a microwave cyclization using TMS-PP to afford the desired imidazoline targets.

Table 1.



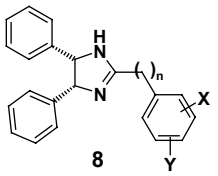
7

Entry	R	$P2X_7IC_{50}$ (μM)[a]
1		0.08
7a		0.03
7b		0.01
7c		0.32
7d		0.57
7e		0.05
7f		>1.00[b]
7g		0.11
7h		3.80
7i		0.54
7j		0.24
7k		0.61

[a] Values are means of experiments performed in duplicate, one or two times.

[b] Indicates the highest concentration tested resulting in no appreciable inhibition.

Table 2.



8

Entry	*n*	X	Y	$P2X_7$ IC_{50} (μM)[a]
1	2	H	H	0.08
8a	2	2-F	H	0.16
8b	2	3-F	H	0.04
8c	2	4-F	H	0.22
8d	2	2-Cl	H	0.07
8e	2	3-Cl	H	0.12
8f	2	4-Cl	H	>1.00[b]
8g	2	3-F	4-F	0.12
8h	2	3-Cl	4-Cl	1.40
8i	2	2-Me	H	0.07
8j	2	4-Me	H	2.19
8k	2	2-OMe	H	0.83
8l	2	4-OMe	H	>3.00[b]
8m	2	3-OMe	4-OMe	>3.00[b]
8n	1	2-F	H	0.58
8o	1	3-F	H	0.08
8p	1	4-F	H	0.08
8q	1	3-F	4-F	0.03
8r	1	2-F	3-F	0.93
8s	1	2-Cl	H	0.16
8t	1	3-Cl	H	0.03
8u	1	4-Cl	H	0.27
8v	1	4-CF_3	H	0.40
8w	1	2-Me	H	0.32
8x	1	4-Me	H	0.08
8y	1	2-OMe	3-OMe	>3.00[b]

[a] Values are means of experiments performed in duplicate; one, two or three times.

[b] Indicates the highest concentration tested resulting in no appreciable inhibition.

The initial investigation of SAR to explore the appropriate chain length, branching and aryl substitution at the 2-position of the imidazoline were described. Several new potent $P2X_7$ antagonists were found offering a variety of substitution patterns that will be used to help further optimize this series.

We are currently preparing additional libraries using the aforementioned chemistry to investigate the SAR of other areas of the scaffold to modulate the physical chemical characteristics and pharmacokinetic profiles of compounds in this series.

References and notes

1. (a) Di Virgilio, F. *Immunol. Today* **1995**, 524; (b) Suprenant, A.; Rassendren, F.; Kawashima, E.; North, R. A.; Buell, G. *Science* **1996**, *272*, 5262, 735.
2. Several groups have recently reported on $P2X_7$ antagonists: (a) 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*(22), 4047; (b) Alcaraz, L.; Baxter, A.; Bent, J.; Braddock, M.; Cladingboel, D.; Donald, D.; Fagura, M.; Laurent, C.; Lawson, M.; Mortimore, M.; McCormick, M.; Roberts, N.; Robertson, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*(22), 4043; (c) Baraldi, P. G.; Nunez, M. C.; Morelli, A.; Falzoni, S.; Di Virgilio, F.; Romagnoli, R. *J. Med. Chem.* **2003**, *46*, 1318; (d) Baraldi, P. G.; Romagnoli, R.; Tabrizi, M. A.; Falzoni, S.; Di Virgilio, F. *Bioorg. Med. Chem. Lett.* **2000**, 681; (e) Ravi, R. G.; Kertsey, S. B.; Dubyak, G. R.; Jacobson, K. A. *Drug Dev. Res.* **2001**, *54*(2), 75; Chessell, I. P.; Michel, A. D.; Humphrey, P. P. A. *Brit. J. Pharmacol.* **1998**, *124*(6), 1314.
3. (a) Collo, G.; Neidhart, E.; Kawashima, E.; Kosco-Vilbois, M.; North, R. A.; Buell, G. *Neuropharmacology* **1997**, *36*(9), 1277; (b) Di Virgilio, F.; Sanz, J. M.; Chiozzi, P.; Falzoni, S. *Progr. Brain Res.* **1999**, *120*, 355.

4. Perregaux, D. G.; McNiff, P.; Laliberte, R.; Conklyn, M.; Gabel, A. G. *J. Immunol.* **2000**, *165*, 4615.
5. Solle, M.; Labasi, J.; Perregaux, D. G.; Stam, E.; Petrushova, N.; Koller, B. H.; Griffiths, R. J.; Gabel, C. A. *J. Bio. Chem.* **2001**, *276*, 1, 125.
6. *Resin preparation*: To a suspension of 10.0 g (13.2 mmol) of polystyrene-4-nitrophenylcarbonate resin (commercially available Advanced ChemTech 1.32 mmol/g loading) in 150 mL of dichloromethane was added 8.41 g (39.6 mmol) of 1,2-diphenylethylenediamine. The mixture was shaken at room temperature for 12 h then filtered and washed with 4×500 mL of dichloromethane and dried in a vacuum oven. Loading was quantitative by UV determination (*p*-nitrophenol λ 320 nm).
7. Rich, D. H.; Singh, J. In *The Peptides: Analysis, Synthesis, Biology*; Academic: New York, 1979; Vol. 1, p 241.
8. Perillo, I. A.; Garcia, M. B.; Niemevz, F.; Orelli, L. R. *J. Heterocycl. Chem.* **1999**, *36*, 105.
9. *General example of EDC coupling and TMS-PP Microwave cyclization*: *4,5-Diphenyl-2-(2-p-tolyl-ethyl)-4,5-dihydro-1H-imidazole* **8i**. To 300 mg (0.40 mmol) of the aforementioned resin suspended in 6 mL of DMF was added 0.15 g (0.78 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) followed by 0.13 g (0.78 mmol) of 3-*p*-tolyl-propionic acid. The mixture was shaken for 12 h and the resin was filtered and washed sequentially with 3×10 mL of DMF, 3×10 mL of methanol and 3×10 mL of dichloromethane. The resin was then treated with 4 mL of 50% trifluoroacetic acid in dichloromethane and stirred for 2 h. The resin was filtered and washed with dichloromethane. The filtrate was concentrated in vacuo to give 135 mg (100%) of the amine as a yellow oil.
A solution of the aforementioned residue in 2 mL of dichloromethane was added 1 mL of TMS-PP in dichloromethane solution (25 g TMS-PP/100 mL). The resulting solution was warmed in the microwave (Smith Synthesizer™—Personal Chemistry) at 140 °C for 8 min final pressure ~15 bar. The work-up was performed using the automated Allex™ liquid–liquid extraction station as follows: The sample was washed with 2×3 mL of 2 N NaOH (aq) and 2×3 mL of brine and then dried over Na_2SO_4. The organic phase was concentrated in vacuo then purified using preparative reverse phase HPLC to afford 66 mg (41%) of the imidazoline **8i**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H, CH_3), 2.82 (t, 2H, CH_2Ph), 3.15 (t, 2H, $CH_2C{=}N$), 5.25 (s, 2H, N–CH–Ph), 6.8 (m, 4H, ArH), 7.0 (m, 6H, ArH), 7.15 (d, 2H, ArH), 7.25 (d, 2H, ArH); LC/MS M^{+1} 342.
10. *$P2X_7$ receptor-mediated dye uptake assay*: Bz-ATP (2′- & 3′-O-(4-benzoylbenzoyl)-adenosine-5′-triphosphate (Sigma Chemical, St. Louis, MO)) activation of the $P2X_7$ receptor was measured using YO-PRO-1 iodide (491/509) dye (Molecular Probes, Eugene, OR) uptake in U373 cells stably expressing the human $P2X_7$ receptor with a method modified from the procedure described by Virginio, C.; MacKenzie, A.; North, R. A.; Surprenant, A. *J. Physiol.* **1999**, *519.2*, 335. This dye uptake assay was used to screen for $P2X_7$ receptor antagonists by measuring inhibition of dye uptake. Briefly, cells were plated and grown overnight on collagen coated 96-well plates at a density of 35,000 cells/well. The following day, culture media was replaced with Mg^{2+} and Ca^{2+}-free Hank's balanced salt solution in the presence of increasing concentrations of test compounds in duplicate and the cells were then incubated for 20 min at 37 °C. Next, 5 mM YO-PRO-1 iodide dye and 300 mM Bz-ATP were added to the cells sequentially and then incubated for 1.5 h at 37 °C. Dye uptake through $P2X_7$ was measured by fluorescence YO-PRO-1 nucleic acid staining using a CytoFluor Series 4000 fluorescence plate reader (PerSeptive Biosystems, Framingham, MA) with an excitation filter of 485/20 and an emission filter of 530/25. Fluorescence in the presence of the test compounds was compared to that in the absence of test substances (control) and IC_{50}s were calculated using XLfit Version 2.0.9 (ID Business Solutions, Ltd, Surrey, UK) and nonlinear sigmoidal curve fit.
11. ATP-stimulated YO-PRO uptake in U373MG cells was abolished by oxidized ATP see: Rampe, D.; Wang, L.; Ringheim, G. E. *J. Neuroimmunol.* **2004**, *147*, 56.
12. Only one enantiomer of **7a** (that shown in Table 1) was prepared in this set of compounds, however, given that **7b** is also quite potent it is anticipated that the opposite enantiomer of **7a** would also be equipotent. Compound **7d** was tested only as a racemic mixture.