Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4819–4823
Bioorganic & Medicinal Chemistry Letters

Novel pyrazinone inhibitors of mast cell tryptase: synthesis and SAR evaluation

Corey R. Hopkins,[a,*] Kent Neuenschwander,[a] Anthony Scotese,[a] Sharon Jackson,[a] Thaddeus Nieduzak,[a] Henry Pauls,[a] Guyan Liang,[b] Keith Sides,[c] Dona Cramer,[c] Jennifer Cairns,[c] Sebastien Maignan[d] and Magali Mathieu[d]

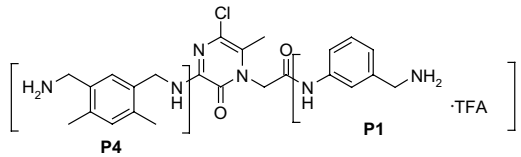
[a]*Department of Medicinal Chemistry, Drug Innovation and Approval, Aventis Pharmaceuticals, Route 202-206, Bridgewater, NJ 08807, USA*
[b]*Department of Medicinal Chemistry/Molecular Modeling, Drug Innovation and Approval, Aventis Pharmaceuticals, Route 202-206, Bridgewater, NJ 08807, USA*
[c]*Department of Molecular Validation and Cell Biology, Drug Innovation and Approval, Aventis Pharmaceuticals, Route 202-206, Bridgewater, NJ 08807, USA*
[d]*Department of Structural Biology, Aventis Pharma, Vitry 94403, France*

Received 7 June 2004; revised 22 July 2004; accepted 23 July 2004
Available online 13 August 2004

Abstract—In this manuscript, the synthesis and SAR evaluation of a novel pyrazinone class of tryptase inhibitors is described. Chemical optimization of the P1 and P4 groups led to the identification of **7p** (K_i = 93 nM) as a potent inhibitor of mast cell tryptase.

Asthma is a disease of ever increasing proportions, one that is expected to nearly double over the next decade.[1] Even though asthma has been afflicting the world population for many years, there still does not exist a general therapy for this indication. The most common therapy includes the use of bronchodilators and anti-inflammatory agents and there has been only one new major therapy approved over the past few years, Singulair®.[2] However, even with this new class of therapy, a great number of asthmatics still rely on a combination therapy of inhaled drugs. Thus, discovery of a novel medication for asthma, preferably an orally acting drug, is the major concentration of a number of research departments.

One such possible target that has been receiving attention from a number of laboratories is tryptase inhibition due to the fact that tryptase has been directly linked to the pathology of asthma.[3] It is a trypsin-like serine-class protease that is found almost exclusively in mast cells.[4] Tryptase is stored in mast cell granules as a stable, heparin-bound tetramer[5] and is released upon mast cell stimulation. Once released, tryptase has been shown to cleave and degrade numerous substrates all of which play important roles as bronchodilators or vasodilators by causing smooth muscle relaxation.[4,6] In addition to these effects, tryptase is also a known mitogen of both dog tracheal[7] and human[8] smooth muscle cells as well as human lung and dermal fibroblasts.[9] These effects are known as airway remodeling[10] and thus tryptase inhibitors would be a novel therapy for this condition. There have been a number of reports of small molecule inhibitors of tryptase,[11] and this report details efforts from our laboratories to discover a novel inhibitor of tryptase.

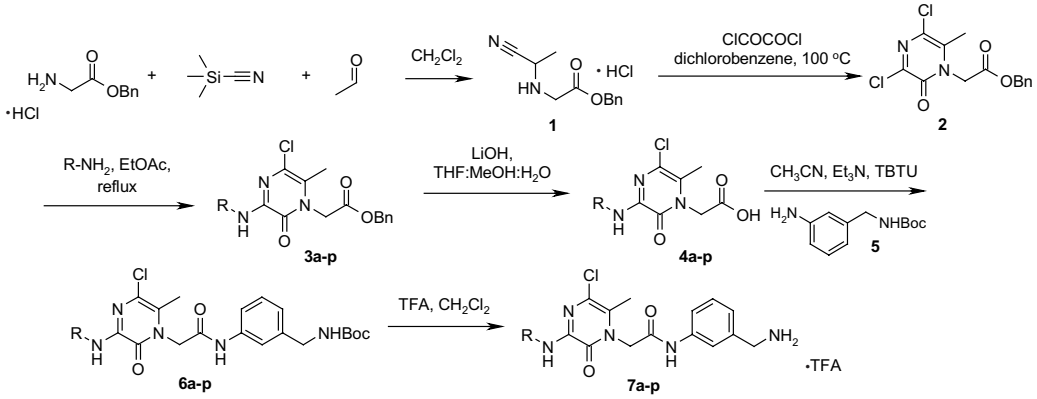


7p
Ki (tryptase) = 93 nM

Pyrazinone compounds of similar structure to **7** are reported serine protease inhibitors.[12] It was at this

Keywords: Mast cell tryptase; SAR; Pyrazinone.
* Corresponding author. Tel./fax: +1-908-231-3351; e-mail: corey.hopkins@aventis.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.07.051



Scheme 1. Synthesis of the pyrazinones **7**.

juncture that we started our program looking at these compounds as possible mast cell tryptase inhibitors. The synthesis of the pyrazinone core structure **2** is outlined in Scheme 1.[13] Glycine benzyl ester hydrochloride, trimethylsilylcyanide, and acetaldehyde were reacted together under modified Strecker conditions to yield the nitrile amino ester **1**. Alternatively, the ethyl ester of **1** could be prepared by a literature method.[14] Next, the pyrazinone **2** was formed by cyclization of **1** with oxalyl chloride in dichlorobenzene[15] at 100 °C. The dichloropyrazinone **2** was treated with a variety of amines in refluxing ethyl acetate to give the amino pyrazinones **3c**–**p** after chloride displacement.[12a] Chloride displacement with anilines could be accomplished by heating the ethyl ester of **2** with neat aniline in the microwave (variable 300 W max, 12 min, 120 °C). Saponification (LiOH, THF/MeOH/H_2O) to acids **4a**–**p** followed by coupling with aniline **5** (CH_3CN, Et_3N, TBTU)[16] delivered the amides **6a**–**p**. The final targets **7** were realized, after Boc deprotection (TFA, CH_2Cl_2), in the salt form.

Compound **7c** was our starting point for the pyrazinone series (Table 1). The compound showed moderate activity (1.5 μM) and we were interested in improving this activity by making changes to the P4 group. The first changes included the incorporation of hydrophobic portions on to the amine; however, these compounds did not improve the activity (**7c**–**7f**). Substituted anilines (**7a**,**b**) exhibited a marked decrease in activity. It was

Table 1. Tryptase inhibition of P4 group modifications for compounds **7a**–**p**[17]

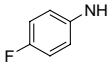
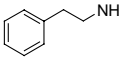
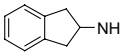
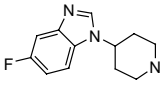
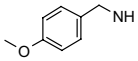
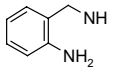
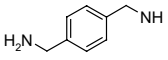
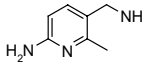
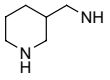
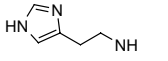
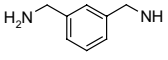
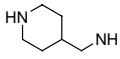
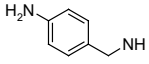
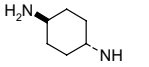
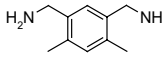
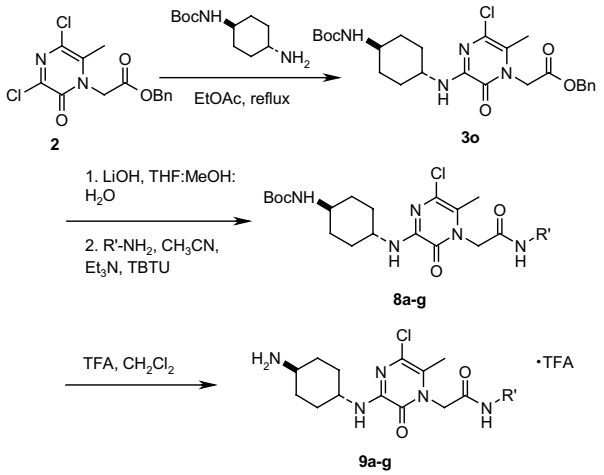
Compd	R-NH	Tryptase K_i, nM[a]
7a		NA[b]
7b		NA[c]
7c		1550
7d		970
7e		1238

Table 1 (*continued*)

Compd	R-NH	Tryptase K_i, nM[a]
7f		1550
7g		875
7h		615
7i		580
7j		518
7k		426
7l		390
7m		290
7n		245
7o		138
7p		93
Babim[d]		140

[a] Values are means of three experiments.
[b] 24% inhibition @ 5 μM.
[c] 11% inhibition @ 5 μM.
[d] Reference compound.[18]



Scheme 2. Synthesis of **9a**–**g**, P1 replacements.

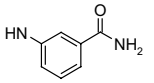
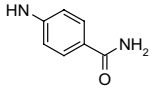
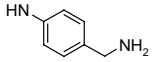
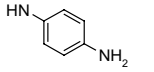
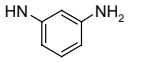
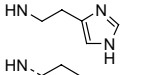
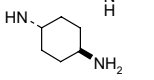
not until more polar groups were investigated that the tryptase activity reached more acceptable levels. To this end, the aryl amines and benzyl amines (**7g**–**7i**) showed improvement in activity to sub-micromolar levels. The P4 site also tolerated non-aromatic substituents. A sterically bulky group delivered affinity for tryptase at a more interesting level (**7p**, K_i = 93 nM). It is of note that this represents a 10-fold increase in tryptase activity by modification of the P4 group.

Concurrent with our efforts at improving the P4 group we pursued optimization of the P1 group on the *trans*-1,4-cyclohexylamine P4 derivative **7m**, which was the best compound at the time and due to the ease of synthesis (Scheme 2). Thus, the mono-Boc protected *trans*-1,4-cyclohexylamine was added to the dichloropyrazinone **2** in refluxing EtOAc to yield **3o**. Next, the acid was saponified (LiOH, THF/MeOH/H_2O) followed by amide formation by coupling with the appropriate amine (CH_3CN, Et_3N, TBTU) to give the intermediates **8a**–**g**. Finally, the targets **9a**–**g** were realized after Boc deprotection (TFA, CH_2Cl_2).

The results from the primary assay for compounds **9a**–**g** can be seen in Table 2. The benzyl amine replacements for **3m** all gave inactive compounds. It is interesting to note that even the *para* substituted derivative **9c**, was inactive. These results suggest that the *meta* substituted benzyl amine is the favored P1 group.

These results are supported by X-ray structure analysis for inhibitor **7o** (Fig. 1) obtained using similar conditions as Bode and co-workers.[5] The analysis shows a number of key interactions for this compound. Namely, the *meta*-benzylamine (P1 group) interacts with both the Asp189 and the carbonyl of Gly219. This explains why the P1 replacements did not show the same activity as **7o**. When the benzyl amine is replaced with either an amide (**9a**,**9b**), or aniline (**9d**,**9e**), these groups do not have the same length nor geometry to retain this interaction. Also, when the benzyl amine is moved to the *para* position (**9c**) this too does not have the required geometry to retain the interaction and hence activity.

Table 2. Tryptase inhibition of P1 group modifications for compounds **9a**–**g**

Compd	R′-NH	Tryptase K_i, μM
9a		18% @ 5 μM
9b		31% @ 5 μM
9c		16% @ 5 μM
9d		29% @ 5 μM
9e		7% @ 5 μM
9f		8% @ 5 μM
9g		2% @ 5 μM

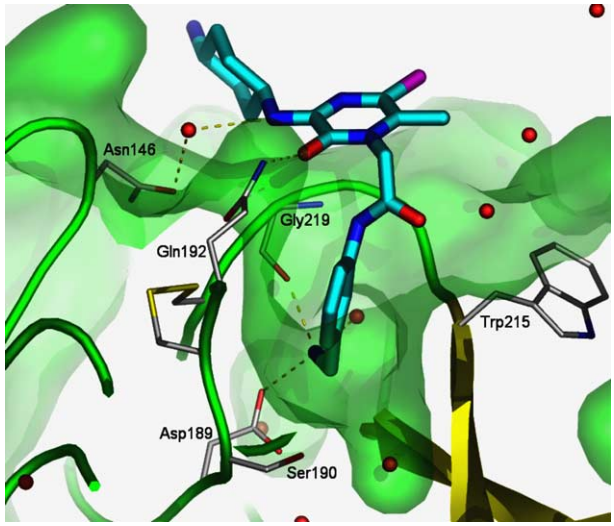


Figure 1. X-ray structure of inhibitor **7o** soaked into β-tryptase. The carbonyl of the pyrazinone ring is engaged in both an external hydrogen bond interaction with Glu192 and an internal hydrogen bond with the amide NH of the benzylamine moiety, which is situated in the active site pocket. The benzyl amine makes an interaction with both Asp189 and the carbonyl of Gly219.

The X-ray structure revealed an alternative binding mode for the P4 group. As shown in Figure 2, instead of binding in the S4 pocket, the 'P4' groups actually stayed in an area close to N146 and Q221, which is not utilized by natural substrates for typical trypsin-like serine proteases. While the S4 pocket in a typical trypsin-like serine protease prefers an aromatic/hydrophobic group, this alternative binding region is more hydrophilic featuring several charged residues; including Q221 directly underneath, N146/D147 on one side, and E217/D60 on another. This alternative binding mode explains the SAR shown in Table 1. As the 'P4' groups become

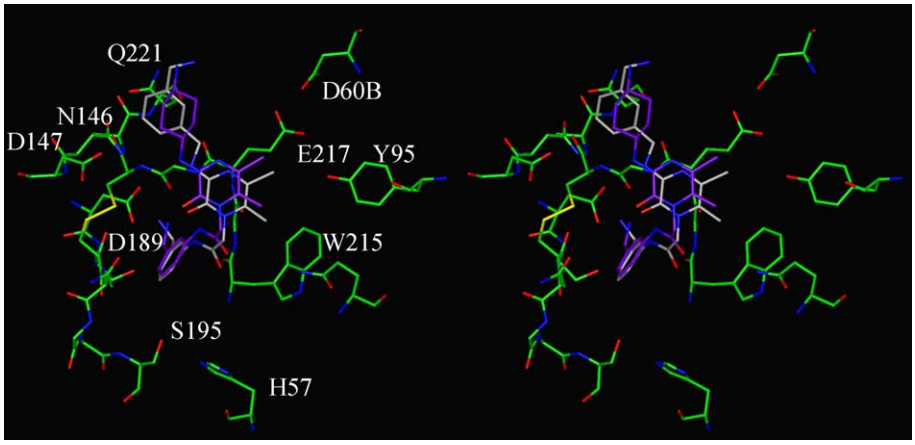


Figure 2. Stereoview of two tryptase inhibitors indicating the alternative binding mode (**7n**, blue; **7o**, grey).

more polar and bulky, the activity increases. Molecular modeling studies indicate that it is rather difficult to achieve tight binding in this surface exposed shallow pocket. As a result, the mobility of these 'P4' groups was high and the H-bonding interactions between inhibitors and enzyme could also be further diluted by water molecules.

In conclusion, our investigation of the SAR at the P4 and P1 chain modifications of the pyrazinone nucleus has identified a potent inhibitor of mast cell tryptase (**7p**, $K_i = 93$ nM). Chemical optimization has shown that bulky, polar substituents for the P4 chain are desired. Also, optimization of the P1 chain has revealed (along with the X-ray structure) that a *meta*-substituted benzylamino amide is the optimal substituent.

References and notes

1. (a) Barnes, P. J.; Chung, K. F.; Page, C. P. *Pharmacol. Rev.* **1998**, *50*, 515–596; (b) Nimmaggadda, S. R.; Evans, R. *Pediatr. Rev.* **1999**, *20*, 111–115.
2. Nayak, A. *Expert Opin. Pharmacother.* **2004**, *5*, 679–686.
3. Molinari, J. F.; Scuri, M.; Moore, W. R.; Clark, J.; Tanaka, R.; Abraham, W. M. *Am. J. Respir. Crit. Care. Med.* **1996**, *154*, 649–653.
4. Brown, J. K.; Jones, C. A.; Rooney, L. A.; Caughey, G. H.; Hall, I. P. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2002**, *282*, L197–L206.
5. Pereira, P. J. B.; Bergner, A.; Macedo-Ribeiro, S.; Huber, R.; Matschiner, G.; Fritz, H.; Sommerhoff, C. P.; Bode, W. *Nature* **1998**, *392*, 306–311. Resolution for reported structures 2.0–2.6 Å.
6. Abraham, W. M. *Am. J. Physiol. Lung Cell Physiol.* **2002**, *282*, L193–L196.
7. Brown, J. K.; Tyler, C. L.; Jones, C. A.; Ruoss, S. J.; Hartmann, T.; Caughey, G. H. *Am. J. Respir. Cell Mol. Biol.* **1995**, *13*, 227–236.
8. Berger, P.; Perng, D. W.; Thabrew, H.; Hollenberg, M. D.; Sanjar, S.; Laurent, G. J.; McAnulty, R. J. *J. Appl. Physiol.* **2001**, *91*, 1372–1379.
9. (a) Akers, I. A.; Parsons, M.; Hill, M. R.; Hollenberg, M. D.; Sanjar, S.; Laurent, G. J.; McAnulty, R. J. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2000**, *278*, L193–L201; (b) Hartmann, T.; Ruoss, S. J.; Raymond, W. W.; Seuwen, K.; Caughey, G. H. *Am. J. Physiol. Lung Cell Mol. Physiol.* **1992**, *262*, L528–L534.
10. Bousquet, J.; Jeffrey, P. K.; Busse, W. W.; Johnson, M.; Vignola, A. M. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, 1720–1721.
11. (a) Clark, J. M.; Moore, W. R.; Tanaka, R. D. *Drugs Future* **1996**, *21*, 811–816; (b) Burgess, L. E. *Drug News Perspect.* **2000**, *13*, 147–156; (c) Sutton, J. C.; Bolton, S. A.; Hartl, K. S.; Huang, M.-H.; Jacobs, G.; Meng, W.; Ogletree, M. L.; Pi, Z.; Schumacher, W. A.; Seiler, S. M.; Slusarchyk, W. A.; Treuner, U.; Zahler, R.; Zhao, G.; Bisacchi, G. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3229–3233; (d) Slusarchyk, W. A.; Bolton, S. A.; Hartl, K. S.; Huang, M.-H.; Jacobs, G.; Meng, W.; Ogletree, M. L.; Pi, Z.; Schumacher, W. A.; Seiler, S. M.; Sutton, J. C.; Treuner, U.; Zahler, R.; Zhao, G.; Bisacchi, G. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3235–3238; (e) Zhao, G.; Bolton, S. A.; Kwon, C.; Hartl, K. S.; Seiler, S. M.; Slusarchyk, W. A.; Sutton, J. C.; Bisacchi, G. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 309–312; (f) Burgess, L. E.; Newhouse, B. J.; Ibrahim, P.; Rizzi, J.; Kashem, M. A.; Hartman, A.; Brandhuber, B. J.; Wright, C. D.; Thomson, D. S.; Vigers, G. P. A.; Koch, K. *Proc. Natl. Acad. Sci.* **1999**, *96*, 8348–8352; (g) Costanzo, M. J.; Yabut, S. C.; Almond, H. R.; Andrade-Gordon, P.; Corcoran, T. W.; de Garavilla, L.; Kauffman, J. A.; Abraham, W. M.; Recacha, R.; Chattopadhyay, D.; Maryanoff, B. E. *J. Med. Chem.* **2003**, *46*, 3865–3876.
12. (a) Burgey, C. S.; Robinson, K. A.; Lyle, T. A.; Sanderson, P. E. J.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Singh, R.; Miller-Stein, C.; White, R. B.; Wong, B.; Lyle, E. A.; Williams, P. D.; Coburn, C. A.; Dorsey, B. D.; Barrow, J. C.; Stranieri, M. T.; Holahan, M. A.; Sitko, G. R.; Cook, J. J.; McMasters, D. R.; McDonough, C. M.; Sanders, W. M.; Wallace, A. A.; Clayton, F. C.; Bohn, D.; Leonard, Y. M.; Detwiler, T. J., Jr.; Lynch, J. J., Jr.; Yan, Y.; Chen, Z.; Kuo, L.; Gardell, S. J.; Shafer, J. A.; Vacca, J. P. *J. Med. Chem.* **2003**, *46*, 461–473; (b) Parlow, J. J.; Case, B. L.; Dice, T. A.; Fenton, R. L.; Hayes, M. J.; Jones, D. E.; Neumann, W. L.; Wood, R. S.; Lachance, R. M.; Girard, T. J.; Nicholson, N. S.; Clare, M.; Stegeman, R. A.; Stevens, A. M.; Stallings, W. C.; Kurumbail, R. G.; South, M. S. *J. Med. Chem.* **2003**, *46*, 4050–4062.
13. All new compounds were characterized by high resolution LCMS and ^{1}H NMR and found to be in agreement with their structures.
14. Araldi, G. L.; Semple, J. E. *PCT Int. Appl.* **2001**, 236.

15. Vekemans, J.; Pollers-Wieers, C.; Hoornaert, G. *J. Heterocycl. Chem.* **1983**, *20*, 919–923.
16. Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 441–445.
17. (a) Tryptase inhibition activity is confirmed using recombinant human βII tryptase expressed in yeast cells. The assay procedure employs a 96 well microplate (Costar 3590) using L-pyroglutamyl-L-prolyl-L-arginine-para-nitroanilide (S2366: Quadratech) as substrate (essentially as described by McEuen et al.[17b]). Assays are performed at room temperature using 0.5 mM substrate ($2 \times K_m$) and the microplate is read on a microplate reader (Beckman Biomek Plate reader) at 405 nm wavelength. Materials and methods for tryptase primary screen (Chromogenic assay): assay buffer—50 mM Tris (pH 8.2), 100 mM NaCl, 0.05% Tween 20, 50 μg/mL heparin; substrate-S2366 (stock solutions of 2.5 mM); purified recombinant βII tryptase enzyme stocks of 310 μg/mL. Protocol (single point determination): add 60 μL of diluted substrate (final concentration of 500 μM in assay buffer) to each well, add compound in duplicates, final concentration of 20 μM, volume 20 μL, add enzyme at a final concentration of 50 ng/mL in a volume of 20 μL. Total volume for each well is 100 μL. Agitate briefly to mix and incubate at room temperature in the dark for 30 min. Read absorbances at 405 nM. Each plate has the following controls—Totals: 60 μL of substrate, 20 μL of buffer (with 0.2% final concentration of DMSO), 20 μL of enzyme. Nonspecific: 60 μL of substrate, 40 μL of buffer (with 0.2% DMSO). Totals: 60 μL of substrate, 20 μL of buffer (no DMSO), 20 μL of enzyme. Nonspecific: 60 μL of substrate, 40 μL of buffer (no DMSO) Protocol (IC_{50} determination). The protocol is essentially the same as above except that the compound is added in duplicates at the following final concentrations: 0.01, 0.03, 0.1, 0.3, 1, 3, 10 μM (all dilutions carried out manually). For every assay, whether single point or IC_{50} determination, a standard compound is used to derive IC_{50} for comparison; (b) McEuen, A. R.; He, S.; Brander, M. L.; Walls, A. F. *Biochem. Pharm.* **1996**, *52*, 331–340.
18. Caughey, G. H.; Raymond, W. W.; Bacci, E.; Lombardy, R. J.; Tidwell, R. R. *J. Pharm. Exp. Exp. Ther.* **1993**, *264*, 676–682.