Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2734–2737
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and biological activity of potent and selective inhibitors of mast cell tryptase

Corey R. Hopkins,[a,*] Mark Czekaj,[a] Steven S. Kaye,[a] Zhongli Gao,[a] James Pribish,[a] Henry Pauls,[a] Guyan Liang,[b] Keith Sides,[c] Dona Cramer,[c] Jennifer Cairns,[c] Yongyi Luo,[d] Heng-Keang Lim,[d] Roy Vaz,[d] Sam Rebello,[d] Sebastian Maignan,[e] Alain Dupuy,[e] Magali Mathieu[e] and Julian Levell[a,*]

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

Received 7 February 2005; revised 31 March 2005; accepted 4 April 2005
Available online 4 May 2005

Abstract—A new series of novel mast cell tryptase inhibitors is reported, which features the use of an indole structure as the hydrophobic substituent on a *m*-benzylaminepiperidine template. The best members of this series display good in vitro activity and excellent selectivity against other serine proteases.

Asthma is a very complex disease that is mediated by a number of factors. The number of cases is expected to double in the next decade[1] and thus there is a huge effort in the pharmaceutical industry to find novel therapies to treat this disease. One target that has garnered the attention of the industry is β-tryptase. It is a serine-like protease that is found almost exclusively in mast cells.[2] Tryptase is stored in intracellular granules as the active form as a heparin bound tetramer and is released upon stimulation of mast cells. Since tryptase has been directly linked to the pathology of asthma,[3] we[4] and others[5] have been working toward finding a small-molecule inhibitor.

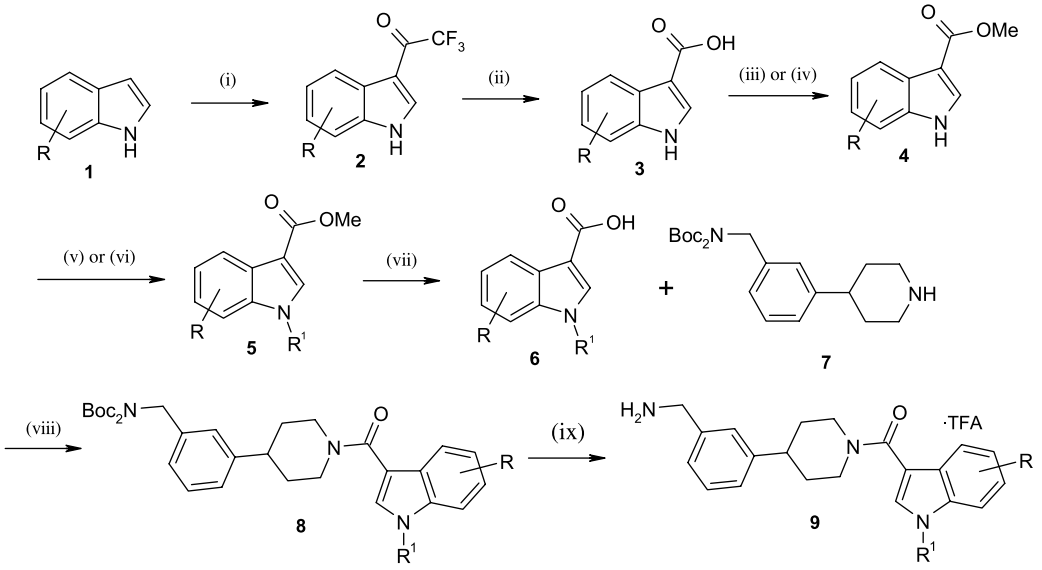
Having established a preferred P1 ligand,[4b] we next explored alternative hydrophobic units to improve potency and hopefully moderate PK and eADMET properties. Based on in-house computer models, we felt that a 3-substituted indole scaffold would lead to desirable inhibitors of mast cell tryptase. Herein we describe the synthesis, SAR, and PK properties of this type of inhibitor.

The synthesis of inhibitors **9** is shown in Scheme 1. The appropriately substituted indole **1** was treated with trifluoroacetic anhydride in DMF to yield the indole-3-trifluoroacetate **2**. The trifluoroacetate was hydrolyzed (20% NaOH (aq)) and the acid was esterified giving **4**. The indole nitrogen was alkylated (NaH, R^1X, and THF or Cs_2CO_3, R^1X, and DMPU) giving **5**, which was then saponified (NaOH (aq), MeOH, and THF) yielding the indole-3-carboxylic acid **6**. Alternatively, the trifluoroacetate **2** could be directly alkylated at the indole nitrogen followed by subsequent hydrolysis to yield acid **6**. This acid was then coupled with the piperidine **7**[4b] (TBTU, DIEA, and DMF) followed by bis-Boc cleavage (TFA and DCM) to realize the desired inhibitors **9** as their TFA salts. Alternative deprotection with HCl/dioxane resulted in the isolation of the HCl salt.

Our first efforts concentrated on making unsubstituted indole compounds. After making several compounds, it was apparent (Table 1) that substitution in the 5- and 7-position (**9b**,**d**, and **e**) of the indole was tolerated while substitution in the 4- and 6-positions gave less active

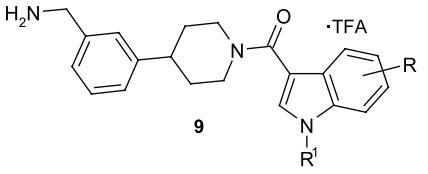
* Corresponding authors. Tel./fax: +1 908 231 3351 (C.R.H.); tel./fax: +1 908 231 4045 (J.L.); e-mail addresses: corey.hopkins@aventis.com; julian.levell@aventis.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.04.002



Scheme 1. Synthesis of indole inhibitors **9**. Reagents and conditions: (i) TFAA, DMF (70–85%); (ii) 20% NaOH (aq) (60–80%); (iii) H_2SO_4, MeOH; (iv) $TMSCHN_2$, Tol, MeOH; (v) NaH, R^1X, THF; (vi) Cs_2CO_3, R^1X, DMPU (50–90%, 2 steps); (vii) NaOH (aq), MeOH, THF (85–95%); (viii) TBTU, DIEA, DMF (90% quant.); (ix) TFA, DCM (50–95%).

Table 1. Tryptase inhibition for indole substituents[6]



Compd	R	R^1	Tryptase K_i, nM	Sol (μM)	CombiPK,[7] F (%)
9a	4-Me	H	>2000	27	ND
9b	5-Me	H	101	40	ND
9c	6-Me	H	>2000	40	ND
9d	7-Me	H	61	26	19
9e	7-F	H	368	40	ND
9f	H	H	290	21	22
9g	H	3-Pyridyl	197	23	ND
9h	H	2-Thiazole	373	13	ND
9i	H	Acetyl	240	32	ND
9j	H	Thiophene-2-carbonyl	246	5.9	ND
9k	H	Benzenesulfonyl	128	7.4	17
9l	H	Methanesulfonyl	162	40	ND
9m	H	Isopropyl	310	36	ND
9n	H	Cyclohexylmethyl	497	29	ND
9o	H	Propyl	185	27	ND
9p	H	Methyl	155	38	ND
9q	H	Cyclopropylmethyl	141	10	ND
9r	H	Ethyl	124	30	11
9s	H	Isobutyl	116	9	ND
9t	H	2-Methoxyethyl	57	30	ND
9u	H	Butyl	49	34	9.3
9v	H	Hexyl	125	30	ND
9w	7-Me	2-Methoxyethyl	32	14	ND
9x	7-Me	Propyl	41	11	ND
9y	7-Me	Ethyl	41	30	26
9z	7-Me	Butyl	68	8.1	33
Babim[8]			140		

compounds (**9a** and **c**). Also, substitution at the 2-position (2-Me) led to a ~10-fold loss of activity (not shown). The 7-Me substituted compound gave very acceptable potency, solubility, and displayed bioavailability that

was a reasonable starting point. Next, our efforts moved toward indoles with varying *N*-substitution (entries **9f–v**). A number of groups were investigated (aryl, alkyl, acyl, and sulfonyl). As can be seen in the table, no substitution (**9f**) as well as aryl, acyl, and sulfonyl groups (**9g–l**) were all inferior to the alkyl substituents. It appears that an sp^3 atom adjacent to the nitrogen is optimal for potency. Also, as the chain length grows, the potency increases (**9t–v**). However, there seems to be an optimal chain length as the potency increases to a point (**9u**) and then starts to diminish as the hexyl (**9v**) substituents potency is less.

Next, we turned our attention to evaluating compounds with variable R^1 while retaining R = 7-Me (entries **9w–z**). There is no significant difference in potency between these four compounds shown, each showing good potency. The combination of the 2-methoxyethyl and butyl *N*-alkyl groups with R = 7-Me substituent did not offer significant improvement in the potency (**9t** vs **w**, 57 nM vs 32 nM) and (**9u** vs **z**, 49 nM vs 68 nM). However, the presence of the R = 7-Me improved the potency with the shorter propyl and ethyl groups (**9o** vs **x**, 185 nM vs 41 nM) and (**9r** vs **y**, 124 nM vs 41 nM). Evaluation of oral bioavailability for selected compounds showed the addition of R = 7-Me afforded improvements in oral bioavailability (**9r** vs **y**, 11% vs 26%) and (**9u** vs **z**, 9.3% vs 33%).

The SAR results are supported by computer modeling analysis for inhibitor **9z** (Fig. 1). 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. The *meta*-benzylamine has been shown in our laboratories to be a key component for potent tryptase activity.[4] Any modification to this group drastically reduces the inhibitory activity. The indole portion fills a hydrophobic void in the enzyme.

Analysis of modifications of the phenyl portion of the indole ring showed two distinct differences. First, substitution at the C-4 position (**9a**) leads to a decrease in activity. This is likely due to the change in the torsion angle of the inhibitor about the amide bond rather than a steric clash with the protein. Second, substitution at the C-6 position (**9c**) also led to a decrease in activity, which may be explained by a steric clash between the two inhibitors in adjacent active sites of the tryptase tetramer. Substitution at either the C-5 or C-7 (**9b**,**d**) are tolerated because they do not either change the torsion angle, or cause steric interactions with the adjacent inhibitor or protein backbone.

Another key interaction is likely that of Tyrosine-95 and the proximal *N*-indole group (Figs. 1 and 2). Thus, due to the constraints of the system, flexible groups on the *N*-indole lead to the most active compounds (**9r–u**).

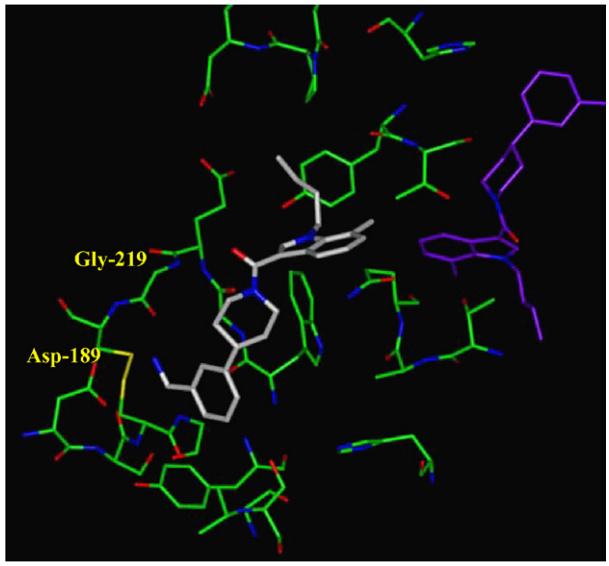


Figure 1. Computer modeled (using InsightII and Discover from Accelrys, and FlexX from BioSolveIT) binding mode of inhibitor **9z** in β-tryptase. The amide carbonyl makes a key hydrogen bond interaction with the nitrogen of Gly219. The benzyl amine inserts into the S1 pocket, forms a salt bridge to Asp189 and hydrogen bonds to oxygens of Gly219 and Ser190. The indole moiety is making good hydrophobic contacts.

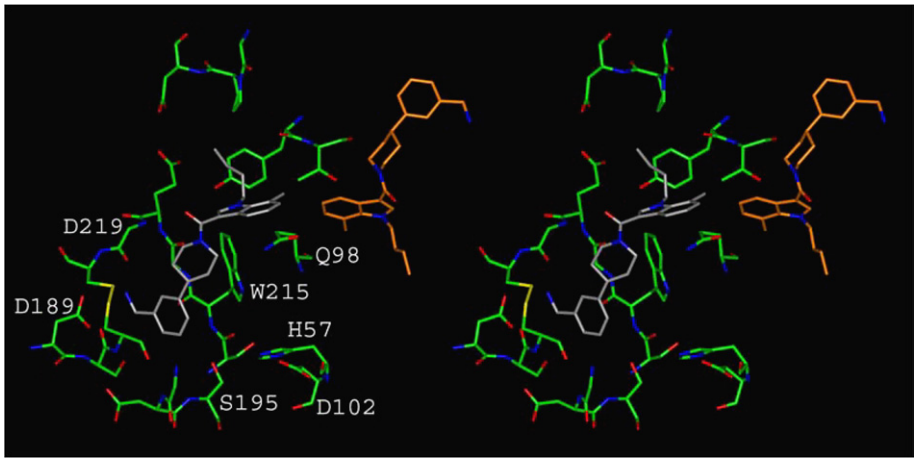
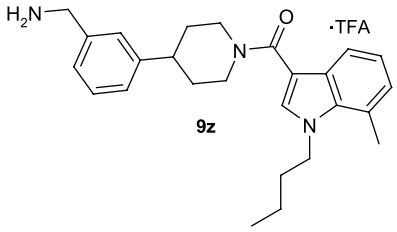


Figure 2. Stereoview of tryptase inhibitor **9z**.

Table 2. Selectivity data for potent tryptase inhibitor, **9z**



9z

Compd	IC_{50} (nM)					
	β-Tryptase	α-Tryptase	Factor Xa	Chymase	Thrombin	Trypsin
9z	68	3694	>10,000	>10,000	>10,000	>10,000

Groups such as acyl, sulfonyl, or branched alkanes are too sterically hindered to rotate away from the Tyrosine-95. In addition, the bottom face of the indole group is blocked and thus inhibitors that do not have an unbranched sp^3 center α (and preferably β) cannot rotate above the indole group. However, as the chain length grows, the activity seems to hit a peak at butyl and starts to decrease with hexyl (**9v**). Combining the two substitutions (7-Me and *N*-alkyl) offered an advantage in potency in the *N*-ethyl and *N*-propyl derivatives (**9r** vs **y**; **9o** vs **x**), but did not offer any substantial advantage using *N*-2-methoxyethyl (**9w**) or the *N*-butyl (**9z**).

Lastly, we turned our attention to selectivity. We wanted to make sure that these compounds would be selective against other, very similar, serine proteases. As can be seen in Table 2, inhibitor **9z** shows excellent selectivity against factor Xa, thrombin, and trypsin (~1000-fold). This compound also shows very good selectivity against other tryptase homologues, for example, α-tryptase (~90-fold selectivity).

In conclusion, we have reported the synthesis and SAR evaluation of a novel class of small-molecule mast cell tryptase inhibitors. The compounds are an extension of our tryptase program and are very potent, orally bioavailable inhibitors. In addition, these compounds have also been shown to be very selective against other similar serine proteases.

References and notes

1. (a) Barnes, P. J.; Chung, K. F.; Page, C. P. *Pharmacol. Rev.* **1998**, *50*, 515; (b) Nimmaggadda, S. R.; Evans, R., III. *Pediatr. Rev.* **1999**, *20*, 111.
2. (a) 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; (b) Cairns, J. A. *Pulm. Pharmacol. Ther.* **2005**, *18*, 55.
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.
4. (a) Hopkins, C. R.; Neuenschwander, K.; Scotese, A.; Jackson, S.; Nieduzak, T.; Pauls, H.; Liang, G.; Sides, K.; Cramer, D.; Cairns, J.; Maignan, S.; Mathieu, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4819; (b) Levell, J.; Astles, P.; Eastwood, P.; Cairns, J.; Houille, O.; Aldous, S.; Merriman, G.; Whiteley, B.; Pribish, J.; Czekaj, M.; Liang, G.; Maignan, S.; Gouilloteau, J.-P.; Dupuy, A.; Davidson, J.; Harrison, T.; Morley, A.; Watson, S.; Fenton, G.; McCarthy, C.; Romano, J.; Mathew, R.; Engers, D.; Sides, K.; Kwong, J.; Tsay, J.; Rebello, S.; Shen, L.; Wang, J.; Luo, Y.; Giardino, O.; Lim, H.-K.; Smith, K.; Pauls, H. *Bioorg. Med. Chem.* **2005**, *13*, 2859.
5. (a) Clark, J. M.; Moore, W. R.; Tanaka, R. D. *Drugs Future* **1996**, *21*, 811; (b) Burgess, L. E. *Drug News Perspect.* **2000**, *13*, 147; (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; (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; (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; (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. U.S.A.* **1999**, *96*, 8348; (g) Costanzo, M. J.; Yabut, S. C.; Almond, H. R., Jr.; 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.
6. For assay details, see Ref. 4.
7. CombiPK was performed in rats using IV and PO doses of 1 and 2 mg/kg, respectively.
8. Reference compound, see: Caughey, G. H.; Raymond, W. W.; Bacci, E.; Lombardy, R. J.; Tidwell, R. R. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 676.