Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4085–4089
Bioorganic & Medicinal Chemistry Letters

Novel, potent, selective, and orally bioavailable human βII-tryptase inhibitors

David Sperandio,* Vincent W.-F. Tai, Julia Lohman, Bernie Hirschbein, Rohan Mendonca, Chang-Sun Lee, Jeffrey R. Spencer, James Janc, Margaret Nguyen, Jerlyn Beltman, Paul Sprengeler, Heleen Scheerens, Tong Lin, Liang Liu, Ashwini Gadre, Alisha Kellogg, Michael J. Green and Mary E. McGrath†

Celera Genomics, 180 Kimball Way, South San Francisco, CA 94080, USA

Received 2 March 2006; revised 28 April 2006; accepted 28 April 2006
Available online 24 May 2006

Abstract—The synthesis of novel [1,2,4]oxadiazoles and their structure–activity relationship (SAR) for the inhibition of tryptase and related serine proteases is presented. Elaboration of the P′-side afforded potent, selective, and orally bioavailable tryptase inhibitors.

Tryptase, a tetrameric serine protease, has been implicated in many of the physiological responses observed in asthma. For example, tryptase activates kininogens, producing the potent bronchoconstrictor bradykinin, and may also amplify its signal by stimulating further mast cell degranulation. Tryptase also processes vasoactive intestinal peptide, down-regulating this bronchodilating agent, and it behaves as a potent mitogen for smooth muscle cells, endothelial cells, and fibroblasts. The mitogenic effects on smooth muscle cells may contribute to the long term airway remodeling, a defining characteristic of chronic asthma that is of therapeutic concern in disease progression.[1–14] Efficacy of the tryptase inhibitor APC-366, in a sheep model of allergic asthma[15] and in clinical trials,[6,12,16] prompted several groups over the last decade to develop additional small molecule inhibitors of tryptase. The successful crystallization of tryptase in 1998 by Pereira et al., revealed a homotetrameric structure that was amenable to structure-guided design.[17] Here we report that P′-side elaboration of an α-ketoheterocycle affords potent and selective tryptase inhibitors.

A screen of our in-house compound collection afforded compound **1** (see Fig. 1) an α-ketoheterocycle with a potency against tryptase of 2.7 μM. Compound **1** is structurally related to RWJ-56423, which entered clinical trials as an inhaled agent for asthma.[18–20] Key interactions of RWJ-56423, which has a potency against tryptase of 10 nM, are a covalent reversible bond between the active site Ser195 and the ketone activated by the benzthiazole moiety, a charge–charge interaction of the guanidine moiety with Asp189 at S1, and several H-bonds of the hydroxyproline moiety at S2.[19]

Most of the interactions at S2 are also likely to be present when RWJ-56423 is bound to trypsin, which explains the potency against trypsin of 8 nM.[18] The S2-sites of tryptase and trypsin are both open, hydrophobic patches on the enzymes which initially did not seem to offer promise for developing tryptase-selective

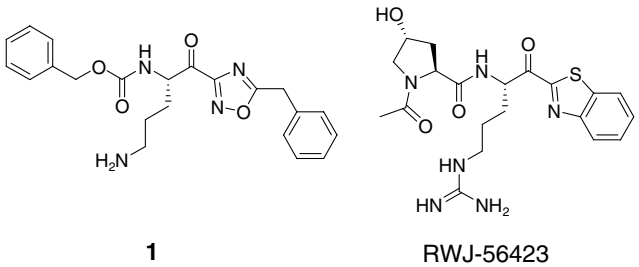


Figure 1. Compound **1** and related α-ketobenzthiazole RWJ-56423.

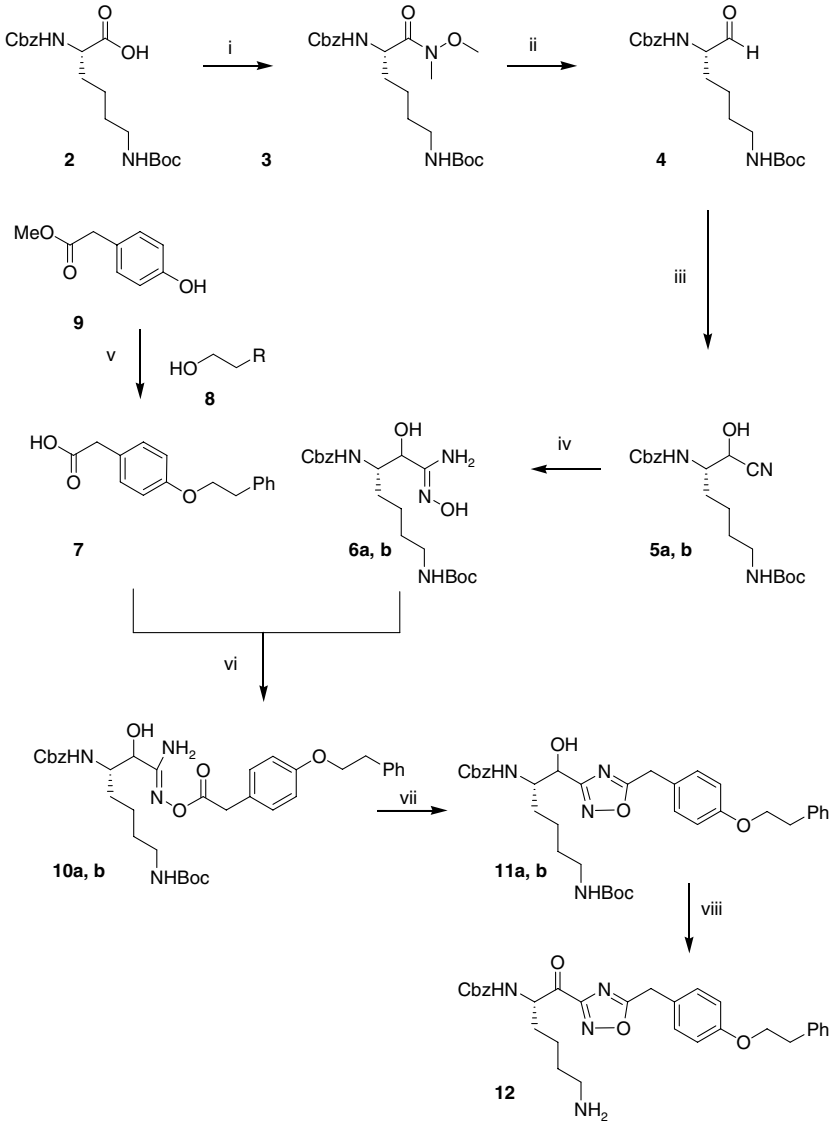
Keywords: α-Keto-[1,2,4]oxadiazoles; Structure–activity relationship; Tryptase inhibitors; Serine proteases; P′-side; Asthma.
* Corresponding author. At present address: Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, USA.Tel.: + 1 650 522 1641; fax: +1 650 522 5899; e-mail: david.sperandio@gilead.com
† Present address: Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, USA.

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2006.04.088

inhibitors. In contrast, the X-ray structure of tryptase shows a hydrophobic pocket on the P′-side of the active site, which is not present in trypsin.[17] This pocket is located about 12–14 Å from Ser195, and was previously explored with chelating inhibitors[8,21] as well as with mechanism-based azetidinone inhibitors.[22–28] Modeling studies of **1** docked into the X-ray structure of tryptase indicate that elaboration of the [1,2,4]oxadiazole of **1** at C-3 with a benzyl moiety affords a vector that points toward the hydrophobic pocket on the P′-side. Based on these observations, we elaborated the P′-side of **1** to improve potency and selectivity.

The synthesis of [1,2,4]oxadiazole containing tryptase-inhibitors is illustrated in Scheme 1. Orthogonally protected *N*-α-benzyloxycarbonyl-*N*-ε-*tert*-butyloxycarbonyl-L-lysine **2** was converted to aldehyde **4** via the Weinreb-amide **3**. Acetone cyanohydrin treatment of **4** afforded the diastereomeric cyanohydrins **5a**,**b** that were converted to *N*-hydroxy-amidines **6a**,**b** in the presence of 50% aqueous hydroxylamine in ethanol at 50 °C. Phenyl ethers **7** were prepared by a Mitsunobu-type coupling reaction of 4-hydroxyphenylacetic acid methyl ester **9** and diversity alcohols **8**, followed by saponification of the ester and acidification. Acylation of the *N*-hydroxy-amidines **6a**,**b** in the presence of phenylacetic acids **9** and carbodiimide followed by thermal intramolecular cyclization (pyridine, 120 °C) afforded the [1,2,4]oxadiazoles **11a**,**b**. Diastereomeric alcohols **11a**,**b** were oxidized using Dess–Martin periodinane in dichloromethane. Final deprotection of the Boc group (TFA–dichloromethane) and conversion to the HCl salt of the *N*-ε-amino group provided the series of inhibitors **12**.

We focused the diversity of the initial set of compounds on the trajectory and length of the linker, and plan to



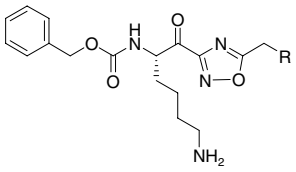
Scheme 1. Reagents and conditions: (i) CH_3NHOCH_3, EDC, HOBt, Hunig's base, DMF, rt; (ii) $LiAlH_4$, THF, 0 °C; (iii) acetone cyanohydrin, NEt_3, CH_2Cl_2, rt; (iv) NH_2OH 50 wt % in H_2O, EtOH, 50 °C; (v) a—PPh_3, DEAD, CH_2Cl_2; b—NaOH, THF/H_2O; (vi) EDC, HOBt, Hunig's base, DMF, rt; (vii) pyridine, 120 °C; (viii) a—Dess–Martin periodinane, CH_2Cl_2, rt; b—TFA, CH_2Cl_2, rt, preparative RP-HPLC (H_2O–acetonitrile, 0.1% HCl).

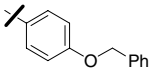
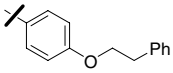
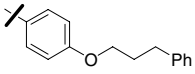
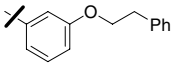
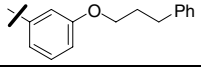
explore the phenyl 'end-cap' SAR in a subsequent series with an optimized linker moiety.

Examples **12a** and **12b** did indeed show a significant increase in potency against tryptase of 15× and 225×, respectively. Extending the end-cap phenyl moiety out by one more methylene group (**12c**) did not further improve the potency. Changing the trajectory from the *para* position to the *meta* position afforded the less potent compounds **12d** and **12e**. Also, the longer linker in **12e** is more potent than the shorter in **12d**, reversing the SAR trend seen in the analogs **12b** and **12c**. These observations suggest that the *para* trajectory points more directly toward the hydrophobic binding pocket than the *meta* trajectory (Table 1, Fig. 2).

The distinctive SAR of compounds **12a**–**e** for tryptase is sharply contrasted by the flat SAR for trypsin. The potency of all compounds against trypsin ranges from 24 to 92 nM. Thus, with **12b**, we have improved the potency and selectivity by a factor of 225× and 97×, respectively, over the lead compound **1**. This is strong evidence that the phenethyloxy group is extending and interacting with the P′-side hydrophobic binding pocket[30].

Table 1. Enzyme inhibition data for compounds **1** and **12a**–**e**[29]



Compound	R	βII-Tryptase K_i(app) [μM]	Trypsin K_i(app) [μM]	Selectivity
1		2.7	0.068	0.03
12a		0.180	0.024	0.13
12b		0.012	0.035	2.9
12c		0.025	0.041	1.6
12d		0.360	0.082	0.23
12e		0.120	0.092	0.76

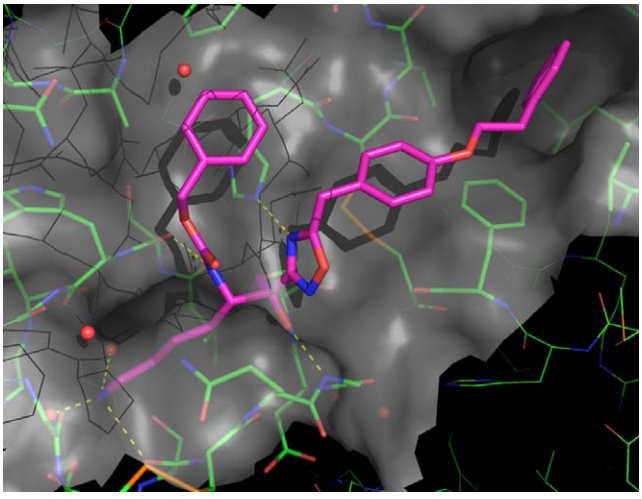
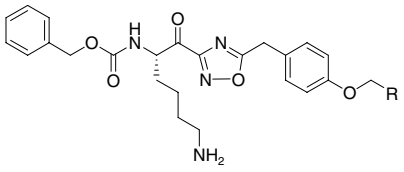
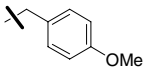
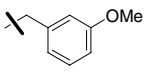
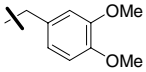
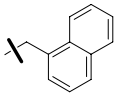
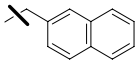
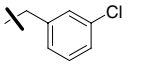
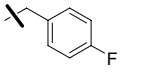
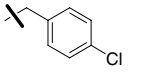
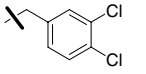
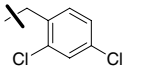


Figure 2. Docking studies of **12b** in tryptase showing the P′-side interaction of the phenyl end-cap.

Table 2. Enzyme inhibition data for compounds **12f**–**q**[29]



Compound	R	βII-Tryptase K_i(app) [μM]	Trypsin K_i(app) [μM]	Selectivity
12f		0.019	0.034	1.8
12g		0.041	0.059	1.4
12h		0.024	0.011	0.46
12i		0.078	0.095	1.2
12k		0.027	0.140	5.2
121		1.10	1.0	0.91
12m		0.013	0.190	14.6
12n		0.016	0.110	6.9
12o		0.014	0.270	19.3
12p		0.010	0.280	28.0
12q		0.096	0.410	4.3

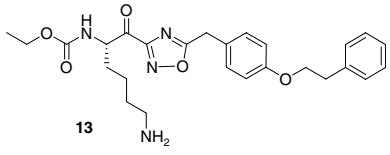
We next explored the SAR of the end-cap phenyl moiety using the optimized linker in compound **12b**. Elaboration of the end-cap phenyl moiety with electron-donating groups such as OMe (**12f–h**) did not improve tryptase potency or positively affect the selectivity against trypsin. Increasing steric bulk (**12i**,**k**) also had little effect, with the exception of **12l**, which lost significant potency against both tryptase and trypsin. However, electron-withdrawing groups deselected trypsin, while maintaining the tryptase potency, resulting in increased selectivity (**12m–q**). 3,4-Disubstitution with chlorine afforded compound **12p** with a selectivity against trypsin of 28× and a potency of 10 nM (Table 2).

We speculated that the Cbz group might be responsible for the low trypsin selectivity exhibited by the lead compound **1**. The very good density and low temperature factors in the X-ray structures of Cbz-containing molecules in trypsin suggested that this group was well suited for the S2 pocket of trypsin, while the crystal structures of related compounds with Cbz P2 elements in tryptase gave poor density and high temperature factors suggesting that it does not bind tightly to the P2-pocket in tryptase (data not shown). Deletion of the hydrophobic contact of the P2 Cbz phenyl moiety by replacement with an ethyl carbamate to give **13**, indeed, significantly reduced potency against trypsin to 190 nM (compared to 35 nM for **12b**), while preserving the potency against tryptase of 5.4 nM. Selectivity against several related trypsin-like serine proteases is striking (see Table 3). Modification of **13** to further improve the selectivity against trypsin is reported in the subsequent letter. Compound **13** has a modest oral absorption of 18% and an oral bioavailability of 8.4% in rat (data not shown).

Compound **13** was studied in a mast cell dependent mouse model of allergic asthma and was demonstrated to be efficacious in inhibiting ovalbumin-induced airway hyperresponsiveness and airway inflammation dose dependently.[31]

In summary, a novel series of α-keto[1,2,4]oxadiazoles was prepared using structure-guided design. These compounds were demonstrated to be potent inhibitors of tryptase when tested in biochemical assays and to show biologic activity in a mast cell dependent model of allergic asthma. In addition, when tested against a number of other related enzymes, these compounds exhibited exquisite selectivity for tryptase. Furthermore, this series of analogs will serve to guide the design of more refined therapeutic compounds with potential for the treatment of asthma.

Table 3. Enzyme inhibition and pharmacokinetics data for compound **13**[29]



13

Potency [K_i (μM)]	Human βII-tryptase	0.0054
	Mouse tryptase (m-MCP-6)	0.0435
	Rat tryptase (r-MCP6)	0.0055
	Dog tryptase	0.380
	Monkey tryptase	0.0465
	Trypsin	0.190
	Thrombin	64
	Plasmin	0.43
	Kallikrein	43
	APC	>150
	Chymotrypsin	>150
	Elastase	>150
	Chymase	>150
	Urokinase	>150
	Granzyme K	>150
PK (iv–po, rat)	C_{max} (μM)	0.59
	CL (ml/min/kg)	93
	V_c (ml/kg)	910
	V_{ss} (ml/kg)	7000
	MRT (min)	75
	AUC (μM min)	11
	Absorption (%)	18
	Bioavailability (%)	8.4
PK (ip, mouse)	Bioavailability (%)	68

References and notes

1. Cairns, J. A. *Pulm. Pharmacol. Ther.* **2005**, *18*, 55.
2. Payne, V.; Kam, P. C. A. *Anaesthesia* **2004**, *59*, 695.
3. Fiorucci, L.; Ascoli, F. *Cell. Mol. Life Sci.* **2004**, *61*, 1278.
4. Caughey, G. H. *Handbook of Proteolytic Enzymes*, 2nd ed. 2004; Vol. 2, p 1535.
5. Bradding, P. *Curr. Opin. Allergy Clin. Immunol.* **2003**, *3*, 45.
6. Newhouse, B. J. *IDrugs* **2002**, *5*, 682.
7. Abraham, W. M. *Am. J. Physiol.* **2002**, *282*, L193.
8. Gangloff, A. R. *Curr. Opin. Investig. Drugs* **2000**, *1*, 79.
9. Rice, K. D.; Sprengeler, P. A. *Curr. Opin. Drug Discovery Dev.* **1999**, *2*, 463.
10. Rice, K.; Spencer, J. *Exp. Opin. Ther. Patents* **1999**, *9*, 1537.
11. Elrod, K. C.; Numerof, R. P. *Emerg. Ther. Targets* **1999**, *3*, 203.
12. Rice, K. D.; Tanaka, R. D.; Katz, B. A.; Numerof, R. P.; Moore, W. R. *Curr. Pharm. Des.* **1998**, *4*, 381.
13. Clark, J. M.; Moore, W. R.; Tanaka, R. D. *Drugs Future* **1996**, *21*, 811.
14. Rice, K. D.; Moore, W. R. In *High Throughput Screening for Novel Anti-Inflammatories*; Kahn, M., Ed.; Birkhaeuser: Basel, 2000; pp 101–121.
15. Clark, J. M.; Abraham, W. M.; Fishman, C. E.; Forteza, R.; Ahmed, A.; Cortes, A.; Warne, R. L.; Moore, W. R.; Tanaka, R. D. *Am. J. Respir. Crit. Care Med.* **1995**, *152*, 2076.
16. Burgess, L. E. *Drug News Perspect.* **2000**, *13*, 147.
17. Pereira, P. J. B.; Bergner, A.; Macedo-Ribeiro, S.; Huber, R.; Matschiner, G.; Fritz, H.; Sommerhoff, C. P.; Bode, W. *Nature* **1998**, *392*, 306.
18. 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.
19. Maryanoff, B. E. *J. Med. Chem.* **2004**, *47*, 769.

20. Breslav, M.; Harris, B.; Kenney, B.; Maryanoff, C. A.; Villani, F. J.; Zhang-Plasket, F.; Zhong, H. M. *Abstracts of Papers*, 229th ACS National Meeting, San Diego, CA, USA, March 13–17, 2005, ORGN.
21. Katz, B. A.; Clark, J. M.; Finer-Moore, J. S.; Jenkins, T. E.; Johnson, C. R.; Ross, M. J.; Luong, C.; Moore, W. R.; Stroud, R. M. *Nature* **1998**, *391*, 608.
22. 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*, 1057.
23. Sutton, J. C.; Bolton, S. A.; Davis, M. E.; Hartl, K. S.; Jacobson, B.; Mathur, A.; Ogletree, M. L.; Slusarchyk, W. A.; Zahler, R.; Seiler, S. M.; Bisacchi, G. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2233.
24. Bisacchi, G. S.; Slusarchyk, W. A.; Bolton, S. A.; Hartl, K. S.; Jacobs, G.; Mathur, A.; Meng, W.; Ogletree, M. L.; Pi, Z.; Sutton, J. C.; Treuner, U.; Zahler, R.; Zhao, G.; Seiler, S. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2227.
25. 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.
26. 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.
27. Sutton, J.; Bisacchi, G. S.; Bolton, S. A.; Hartl, K. S.; Huang, M.-H.; Jacobs, G.; Ogletree, M. L.; Pi, Z.; Schumacher, W. A.; Slusarchyk, W. A.; Treuner, U.; Zhao, G.; Zahler, R.; Seiler, S. M. *Abstracts of Papers*, 221st ACS National Meeting, San Diego, CA, USA, April 1–5, 2001, MEDI-249.
28. Slusarchyk, W. A.; Bisacchi, G. S.; Hartl, K. S.; Huang, M.-H.; Jacobs, G.; Ogletree, M. L.; Sutton, J.; Treuner, U.; Zhao, G.; Zahler, R.; Seiler, S. M. *Abstracts of Papers*, 221st ACS National Meeting, San Diego, CA, USA, April 1–5, 2001, MEDI-250.
29. The velocity of the enzyme-catalyzed reaction was determined from the linear portion of the progress curve, typically the first five minutes of the reaction after initiation with the addition of substrate. The K_i values were determined by a non-linear least squares regression fit of the experimentally derived data to the Morrison equation for tight-binding inhibitors as described (Kuzmic, P., et al. *Anal. Biochem.* **2000**, *281*, 62). Example conditions for the tryptase and trypsin assays are provided below, other enzyme assays for which selectivity data are provided were performed in a similar manner using enzyme from human origin. Monkey tryptase: The enzyme was cloned from cynomolgus monkey, the cDNA used to generate recombinant monkey tryptase was isolated from CD34+ mast cell progenitors derived from cyno monkey blood. Example enzyme assay conditions: Tryptase–tryptase (recombinant human β-1 from Promega) was incubated at 2 nM with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 0.02% Tween 20, 1 mM EDTA, 50 μg/ml heparin, and 10% DMSO. The reaction was initiated with substrate, Tosyl-Gly-Pro-Lys-pNA (Centerchem), supplied at the K_m (400 μM). The change in absorbance as a function of time was monitored at 405 nm. Trypsin–trypsin (Athens Research Institute) was incubated at 10 nM with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 1.5 mM EDTA, 0.05% Tween 20 and 10% DMSO. The reaction was initiated with substrate, Tosyl-Gly-Pro-Lys-pNA (Centerchem), supplied at the K_m (25 μM). The change in absorbance as a function of time was monitored at 405 nm.
30. This flat SAR can be rationalized by rotamers about the methylene unit bridging the oxadiazole heterocycle and the end-cap linker moiety which afford conformations that have weak, non-specific surface interactions with the prime side of trypsin. This hypothesis is supported by the X-ray analysis of related compounds in trypsin: Attempts to soak these molecules into trypsin afforded good density only for the P1-Lys and the P2-Cbz moieties; no density was observed for the atoms after the heterocyclic ring (data not shown).
31. Scheerens, H.; Tan, M. D.; Sweeney, T. V.; Perea, T. V.; Baruch, A.; Beltman, J.; Graupe, D.; Liu, L.; Tai, V.; Lee, S.; Mendonca, R.; Sperandio, D.; Dalrymple, S. A. *Proc. Am. Thorac. Soc.* **2005**, *2*, A804.