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Synthesis of potent and selective 2-azepanone inhibitors of human tryptase

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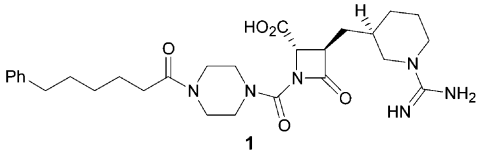
Dedicated to the memory of Steven M. Seiler, PhD (deceased 31 March 2003). Steve's untimely passing will not diminish the continuing impact of his drug discovery research or the admiration of his fellow co-workers

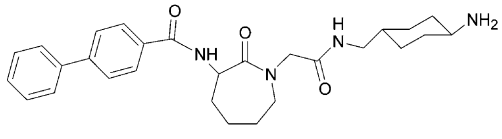
Abstract—The serine protease tryptase has been associated with a broad range of allergic and inflammatory diseases and, in particular, has been implicated as a critical mediator of asthma. The inhibition of tryptase therefore has the potential to be a valuable therapy for asthma. The synthesis, employing solution phase parallel methods, and SAR of a series of novel 2-azepanone tryptase inhibitors are presented. A member of this series, **8t**, was identified as a potent inhibitor of human tryptase ($IC_{50} = 38$ nM) with selectivity $\leqslant$330-fold versus related serine proteases (trypsin, plasmin, uPA, tPA, APC, alpha-thrombin, and FXa).

1. Introduction

Human tryptase is a structurally unique mast cell specific trypsin-like serine protease.[1] Recent biological studies have implicated tryptase as a mediator in the pathology of allergic and inflammatory conditions including rhinitis, conjunctivitis, and most notably asthma.[2] In asthma, tryptase has been associated with the acute bronchospastic response, the underlying inflammatory disease, and long-term airway remodelling. Approximately 150 million people suffer from asthma worldwide and the problem is growing.[3] The currently available therapy for asthma involves a stepwise approach in which patients are given increasingly more efficacious medications to achieve control at the risk of increased side effects.[4] Therefore new and effective drugs are urgently needed for the treatment of asthma. The design and evaluation of tryptase inhibitors for the treatment of asthma and other inflammatory diseases has been the subject of intense investigation.[5] Our previous report described BMS-363131 (**1**) as a potent, mechanism-based human tryptase inhibitor ($IC_{50} = 2$ nM) having exceptionally high selectivity (>1500-fold) versus related serine proteases including trypsin, thrombin, FXa, tPA and plasmin.[6] Intratracheally dosed BMS-363131 and related guanidine-containing azetidinones were shown to be efficacious at improving lung function and reducing lung inflammatory cell infiltration in an ovalbumin-sensitized guinea pig model. BMS-363131 and its analogues were designed for inhalation treatment. In a second phase of our program we sought potent and selective non-mechanism-based tryptase inhibitors having potential for oral administration.



1



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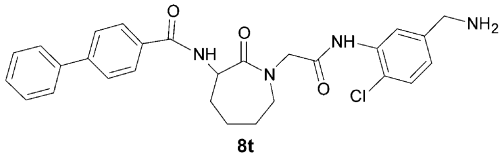

8t

Directed screening in our laboratories resulted in the discovery of azepanone **2** as a weak tryptase inhibitor ($IC_{50} = 4.43$ μM) which was utilized as a structural

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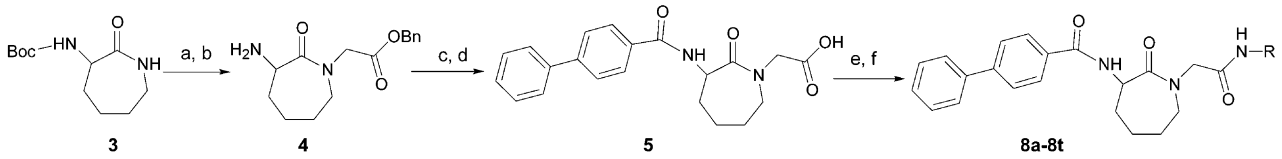


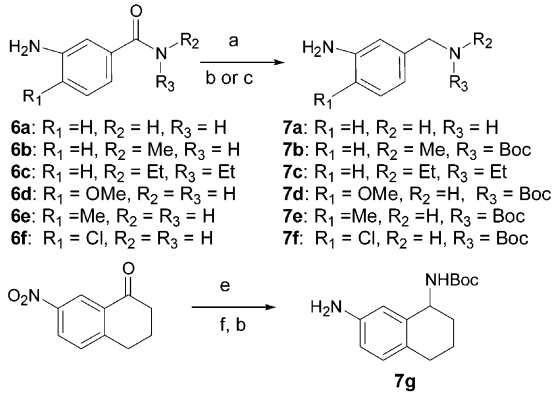
Scheme 1. (a) LiHMDS, $BrCH_2CO_2Bn$, THF, 83%; (b) TFA, CH_2Cl_2, 99%; (c) biphenyl-4-carboxylic acid, EDC, HOBT, $i Pr_2EtN$, CH_2Cl_2, 85%; (d) H_2, Pd/C, THF, 100%; (e) R-NH_2 amines, polystyrene-EDC, iPr_2NEt, DMF–$ClCH_2CH_2Cl$ (1:2), polystyrene-trisamine; (f) 20% TFA in $ClCH_2CH_2Cl$ for Boc protected amines; reverse phase preparative HPLC; 73–94% for two steps.

starting point in an attempt to improve tryptase inhibitory potency. In this letter, we report the solution phase parallel synthesis of a series of racemic 2-azepanone analogues of **2** exploring replacements to the aminocyclohexylmethyl and biphenyl substituents leading to the identification of analogue **8t** as a potent and selective non-mechanism-based inhibitor of human tryptase.

2. Chemistry

The preparation of biphenyl-4-carboxylic acid (1-carbamoylmethyl-2-oxo-azepan-3-yl)-amides **8a–t** listed in Table 1 is outlined in Scheme 1. Alkylation of 2-azapanone **3** with bromoacetic acid benzyl ester followed by Boc deprotection gave amine **4**. Acylation of **4** with biphenyl-4-carboxylic acid followed by reductive cleavage of the benzyl ester gave acid **5**. Acid **5** was then coupled under standard conditions with a variety of monoamines and diamines. Where required for selective coupling, mono-Boc protected diamines were used. Boc-protected penultimate intermediates were deprotected using TFA in methylene chloride to afford the final products **8a–e**, **8h–o** and **8r–t**. Coupling with unprotected aminoalkyl anilines afforded products **8f** and **8g** directly, while coupling with *m*-diethylaminomethyl aniline afforded product **8p**. Additionally, *m*-aminomethylbenzamide was coupled with **5** to afford product **8q**.

The Boc protected diamines **7a–b**, **7d–f**, and diamine **7c** used for the synthesis of **8l**, **8o**, **8r–t**, and **8p**, respectively, were prepared from the corresponding carboxamido anilines **6a–f** by reduction with lithium aluminum hydride in THF followed by Boc protection of the primary amine (Scheme 2). Additionally, the Boc protected diamine **7g** was synthesized from 7-nitro-tetralone via oxime mediated hydrogenation followed by Boc protection of the primary amine.



Scheme 2. (a) LAH, THF; (b) Boc_2O, THF: 90–95%; (c) 2 N HCl in Et_2O, 74–96%; (e) $HONH_2$, pyridine, 93%; (f) H_2, PtO_2, EtOH–$CHCl_3$ (10:1), 95%.

1-[(3-Aminomethyl-phenyl-carbamoyl)-methyl]-2-oxo-azepan-3-yl amides **12a–k** were synthesized from ester **9**, which was prepared using Skiles' procedure[7] (Scheme 3). The hydrolysis of ester **9** with TFA followed by the reaction with 3-*N*-Boc aminomethyl aniline (**7a**) gave acid **10**. Removal of the Cbz group via hydrogenolysis and coupling with a variety of acids ACO_2H generated amides **11** after purification with cation exchange resin. Subsequently, Boc deprotection provided amides **12a–k**.

3. Results and discussion

To broadly screen for replacements of the amino-cyclohexylmethyl moiety in **2** over a hundred corresponding amide analogues of **2** were initially synthesized using commercially available amines. Although neutral R (Scheme 1) groups were included, an emphasis was placed on R groups incorporating basic amines since our working hypothesis was that the aminocyclohexyl substituent of lead inhibitor **2** may be occupying the S1 pocket of tryptase with the primary amine forming a salt bridge to Asp 189.[1] A subset of these analogues is shown in Table 1 (compounds **8a–l**). Alkylamine analogues of various chain lengths (**8a–c**) as well as the aminomethylcyclohexyl (**8d**) or piperidinylmethyl (**8e**) analogues were poorly potent for tryptase, as were the aniline derivitives **8f** and **8g**. More interesting results were obtained from aminoalkylphenyl R groups. Whereas the *p*-aminoethylphenyl, *o*-aminomethylphenyl, and *m*-aminomethylphenylmethyl analogues (**8h–j**, respectively) were poorly potent, the *p*-aminomethylphenyl analogue **8k** showed activity ($IC_{50} = 3.64$ μM) at least as good as lead compound **2**. However, the corresponding *m*-aminomethyl analogue, **8l** had an IC_{50} of 0.25 μM, a 10-fold improvement in potency compared to **2** and **8k**.[8]

Next we decided to use **8l** as the starting point for a study of the replacement of the biphenylamide substitutent. Over 50 amide analogues **12** (Scheme 3) were prepared, and a subset of these is shown in Table 2. In this series, substitution on the terminal phenyl ring (**12a–c**) or extending the rings by insertion of a methylene group (**12d**,**e**) led to less potent compounds, suggesting a binding pocket of limited size. Replacement of the terminal phenyl ring by an aliphatic ring or chain (**12f**,**g**) also decreases the activity. Several other lipophilic mono- or bicyclic aromatic groups at various linker lengths also

Table 1. Tryptase inhibition for compounds **8a–t**

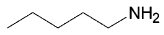
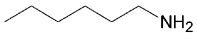
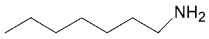
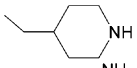
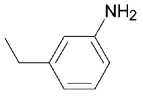
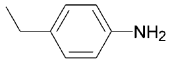
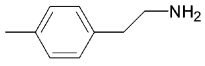
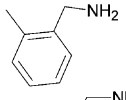
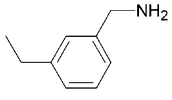
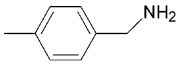
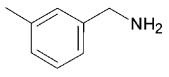
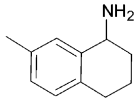
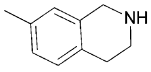
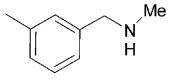
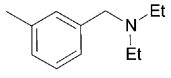
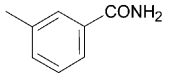
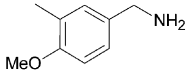
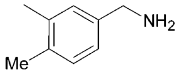
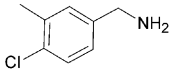
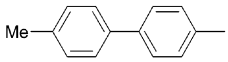
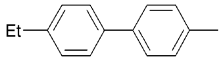
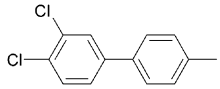
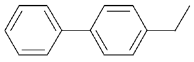
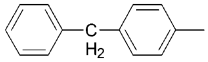
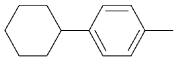
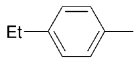
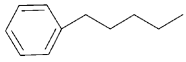
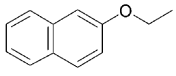
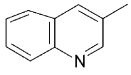
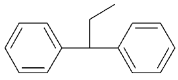
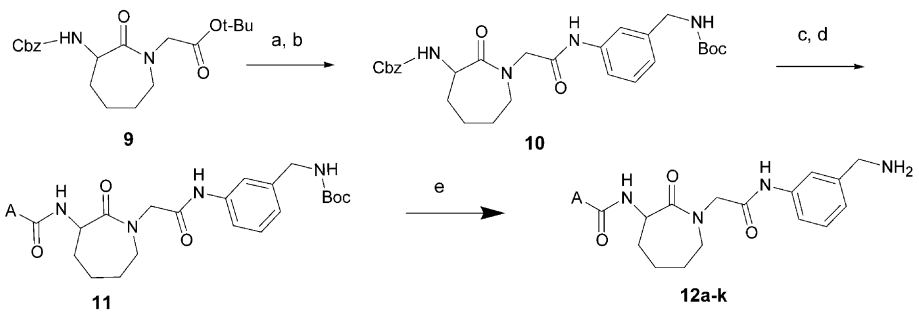
Compd	R	Tryptase IC_{50} (nM)
8a		>33,000
8b		12,000
8c		>33,000
8d		12,000
8e		>33,000
8f		>33,000
8g		>33,000
8h		11,000
8i		>33,000
8j		13,000
8k		3600
8l		250
8m		>33,000
8n		>33,000
8o		>33,000
8p		>33,000
8q		>33,000
8r		890
8s		74
8t		38

Table 2. Tryptase inhibition for compounds **12a–k**

Compd	A	Tryptase IC_{50} (nM)
12a		1300
12b		2900
12c		2100
12d		5200
12e		980
12f		980
12g		4600
12h		2900
12i		4600
12j		15,000
12k		200

afforded less potent compounds (**12h–j**). Interestingly, the branched 3,3-diphenylpropionamide analogue **12k** emerged as essentially equipotent to **8l**. However, in this limited study no clear improvement in potency was achieved by replacement of the biphenylamide.

At this point, we returned to **8l** to explore the effect of conformational constraints or substitution on the m-aminomethylphenyl substitutent. Constrained analogues **8m** and **8n** were inactive while small alkyl substitution on the terminal amino groups also resulted in loss of activity (**8o**,**p**). The corresponding neutral amide analogue (**8q**) of **8l** was also poorly potent, further suggesting the preference for a basic nitrogen on this part of the molecule. Small substitutents *para* position to the methyl amine group were tolerated. Methoxy substituted analogue **8r** (IC_{50} = 0.89 μM) was somewhat less potent than the unsubstituted parent **8l**. However, methyl and chloro substitution afforded a substantial boost in potency compared to **8l**. The methyl analogue **8s** had an IC_{50} of 74 nM and the chloro analogue **8t** had an IC_{50} of 38 nM, approximately a 10-fold improvement in potency compared to the unsubstituted parent **8l**, and 100-fold improvement compared with the original lead compound **2**.



Scheme 3. (a) TFA, CH_2Cl_2; (b) **7a**, EDC, HOBT, NMM, CH_2Cl_2, 78% for two steps; (c) H_2, Pd/C, THF, 100%; (d) acids, EDC, DMAP, DMF–$ClCH_2CH_2Cl$ (1:2); SCX cation exchange column purification; (e) TFA, $ClCH_2CH_2Cl$; reverse-phase preparative HPLC if necessary; 78–97% for two steps.

Table 3. Tryptase inhibition and selectivities for selected compounds

Enzyme	Compound			
	8l	**12k**	**8s**	**8t**
Tryptase IC_{50} (nM)	250	200	74	38
Selectivity ratio				
Trypsin	>130	>170	230	360
Plasmin	>130	>170	>450	330
uPA	>130	>170	>450	>870
tPA	>130	>170	>450	>870
APC	>130	>170	>450	>870
Thrombin	>130	>170	>450	>870
FXa	>130	>170	>450	>870

Compounds **8l**, **12k**, **8s**, and **8t** were screened for activity against a panel of related serine proteases (Table 3) including trypsin, plasmin, urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA), activated protein C (APC), alpha-thrombin (FIIa), and FXa. All compounds were selective against this panel. In particular, **8t**, the most potent compound of this series, exhibited >870-fold selectivity versus uPA, tPA, APC, thrombin, and FXa, and was 330-fold selective against plasmin and 360-fold selective against trypsin. Unlike the beta-lactam BMS-363131 and its analogues, these azepanones are not reactive mechanism-based inhibitors. Further they do not incorporate a highly basic guanidine group, and thus may have potential for oral administration.

In summary, we have studied the parallel synthesis and SAR of a series of N-1 and C-3 2-azepanone tryptase inhibitors. As the result of the preparation of three libraries, compound **8t** has been identified as a potent inhibitor of human tryptase with high selectivity against other serine protease including trypsin.

Acknowledgements

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References and notes

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