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Structure based design of 4-(3-aminomethylphenyl)piperidinyl-1-amides: novel, potent, selective, and orally bioavailable inhibitors of βII tryptase

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Abstract—Tryptase is a serine protease found almost exclusively in mast cells. It has trypsin-like specificity, favoring cleavage of substrates with an arginine (or lysine) at the P1 position, and has optimal catalytic activity at neutral pH. Current evidence suggests tryptase β is the most important form released during mast cell activation in allergic diseases. It is shown to have numerous pro-inflammatory cellular activities in vitro, and in animal models tryptase provokes broncho-constriction and induces a cellular inflammatory infiltrate characteristic of human asthma. Screening of in-house inhibitors of factor Xa (a closely related serine protease) identified β-amidoester benzamidines as potent inhibitors of recombinant human βII tryptase. X-ray structure driven template modification and exchange of the benzamidine to optimize potency and pharmacokinetic properties gave selective, potent and orally bioavailable 4-(3-aminomethyl phenyl)piperidinyl-1-amides.
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1. Introduction

Tryptase[1] is a serine protease found almost exclusively in mast cells. It has trypsin-like specificity, favoring cleavage of substrates with an arginine (or lysine) at the P1 position, and has optimal catalytic activity at neutral pH. It is stored primarily as a tetramer, stabilized by heparin. There are eight human tryptase homologues cloned and identified, to date—α, β (I), β (II), β (III), γ (hTMT), δ (mMCP-7-like) (two forms), and ε (epithelial cells). Current evidence suggests tryptase β is the most important form released during mast cell activation in allergic disease (βI and βII have only one amino acid difference—N103 in βI is K103 in βII). Unlike most other proteases there are no known endogenous inhibitors of β-tryptase—possibly due to its tetrameric structure.[2] Following allergen cross-linking of IgE on the IgE receptor, tryptase is exocytosed from mast cell granules and released extracellularly, where it has been shown to have a variety of pro-inflammatory functions.[1] Levels of tryptase are highly up-regulated in allergic disease states [e.g., 100- to 200-fold increase in human broncho alveolar lavage fluid (BALF) after antigen challenge (asthmatics vs control)].[1] It is shown to have numerous pro-inflammatory cellular activities in vitro, and in animal models tryptase provokes broncho-constriction and induces a cellular inflammatory infiltrate characteristic of human asthma.[1] Clinical evidence (APC-366) suggests that inhibition of the enzymatic activity of tryptase can attenuate the asthmatic response.[3] In chronic asthma and other long term respiratory disorders, all the known activities of tryptase could potentially drive the profound changes observed in the airways such as epithelial desquamation, fibrosis,

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Figure 1. Tryptase (TPSB2), factor Xa (F10), and trypsin (PRSS1) sequence comparison.[4] Highlighted residues represents trypsin homology, red βII-tryptase residues highlight the S1 pocket, magenta βII-tryptase residues—sequence variation versus αII tryptase. #—βII-tryptase S1 pocket sequence variation versus αII-tryptase (G vs D); *—K to N variation versus βI tryptase; †—Asp-189 and Gly-219.

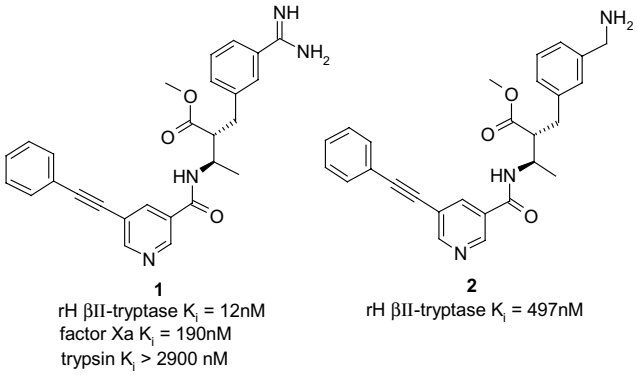


Figure 2. Initial hits from factor Xa library.

and thickening of underlying tissues.[1] These changes are not addressed by currently available therapeutics (e.g., steroids).

Our previous work on factor Xa, another serine protease with high homology to both trypsin and tryptase (Fig. 1),[4] offered us a more rational approach to screen for leads versus high throughput screening. The primary goal of the discovery program was an orally efficacious drug, so it was decided to focus on monomeric and nonpeptidic molecules so as to avoid some of the obvious liabilities of these templates, which have been generally targeted for inhaled delivery.[3,5]

2. Results and discussion

Approximately 2500 proprietary factor Xa inhibitors were screened in a medium throughput chromogenic tryptase assay against recombinant human (rH) β-II tryptase. A number of hits were identified and filtered for reasonable early absorption–distribution–metabolism–excretion (eADME) properties (e.g., solubility, S9 stability, and Caco-2 transport) and selectivity profiles (e.g., trypsin, factor Xa, chymase, thrombin). The β-amidoester **1** (tryptase K_i = 12 nM), was identified as a lead for optimization. The synthesis of this template and subsequent analogs was carried out according to the previously described protocols (Fig. 2).[6]

During our previous work on the factor Xa program[6] we investigated replacing the benzamidine moiety in order to improve the pharmacokinetic (PK) characteristics. Therefore, a small number of basic and nonbasic benzamidine replacements were also investigated for tryptase (a number of the potential replacements were also screened amongst the original 2500 compound set). A key amino acid difference between the factor Xa and tryptase S1 pockets (alanine-190 in factor Xa corresponds to serine-190 in tryptase) makes the S1 pocket smaller and more polar for tryptase, and limits the ability to parallel our factor Xa optimization rational with neutral P1 groups.[7] The only alternative P1 group that showed significant activity was the 40-fold less active *meta*-benzylamine analog **2** (tryptase K_i = 497 nM). In order to form a salt bridge to aspartate 189 at the base of the S1 pocket, the basic moiety needs to be protonated at physiological pH. The pK_a of benzamidine is ~11.6, therefore the equilibrium of protonated versus unprotonated form at pH 7.4 is about 10,000:1. Benzylamine has a pK_a of ~8.9, so should also favor the protonated form at pH 7.4, albeit in a lesser ratio (~50:1 at equilibrium).

A rat PK study for compound **2** showed a short half life and low bioavailability. A dual cannulated rat study showed that the compound was not found in the hepatic portal vein (HPV) or jugular vein post p.o. dosing. A number of explanations are possible, that is, the ester was poorly absorbed, metabolized in the GI epithelium or gut lumen (ester to acid transformation), not stable the GI tract (e.g., low stomach pH) or not stable in blood (hydrolytic enzymes). Plasma hydrolysis to the corresponding acid **3** (inactive vs β-tryptase) shows a conventional plasma conc./time profile. Benzamidine and benzylamine were retained in further small exploratory libraries, which varied the hydrophobic moiety, the ester functionality (amides etc.), and the α substituent (Fig. 3) in order to address the potency and also establish SAR for eADME and PK parameters. However, simplification of the template to give it improved metabolic stability (e.g., removal of the ester) resulted in a huge drop in activity versus tryptase. Compound **6** was the only analog to retain submicromolar K_i, presumably due to a hydrogen bond with the catalytic triad, but this compound was not followed up because of low metabolic stability.

Rational design based on crystallography of the β-amidoesters in factor Xa,[6] suggested that conformationally restricted analogs in the form of achiral piperidine derivatives could act as surrogates of this template (Scheme 1). This strategy had been previously employed for factor Xa inhibitors to good effect.[7d,e] Loss of potency from removal of the ester side chain could be com-

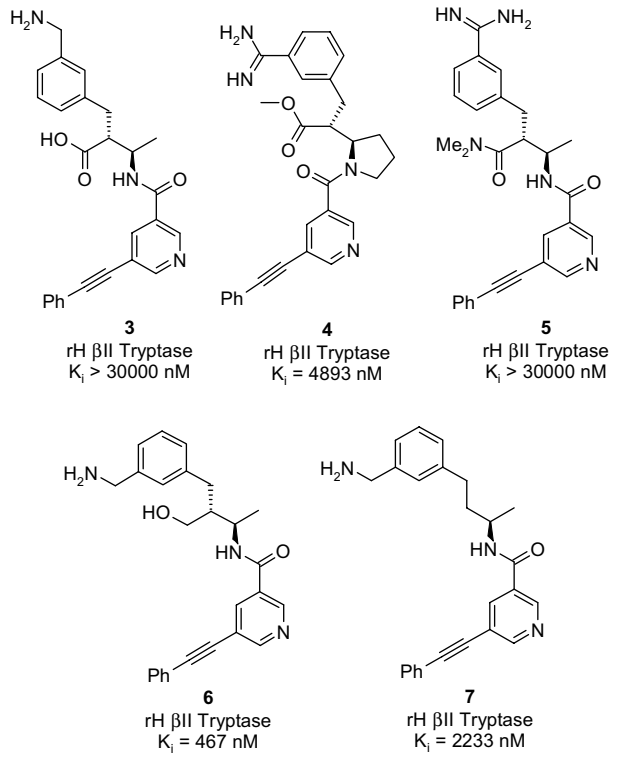
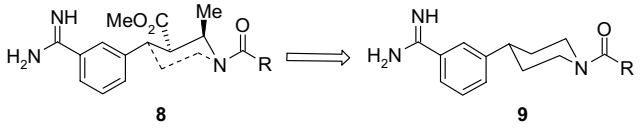


Figure 3. Selected phenylethynyl-pyridine amide substituted β-amidoester analogs.



Scheme 1. β-Amidoester to piperidine template modification.

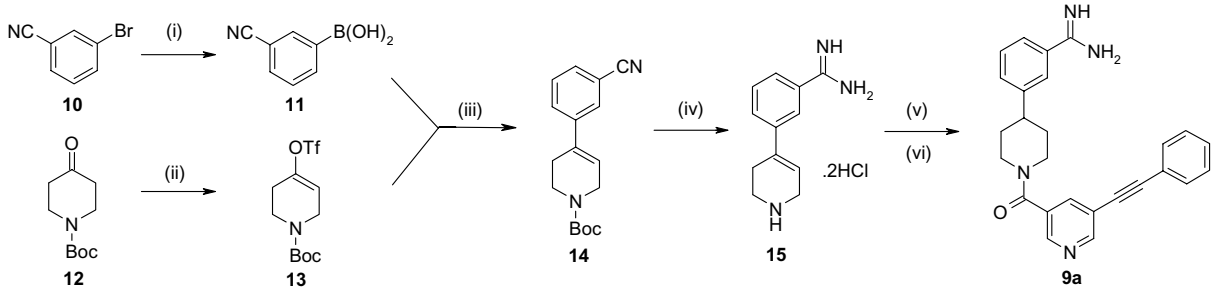
pensated for by the boost in potency from locking the molecule into its binding conformation.

The hydrophobic moiety which gave the best selectivity for tryptase on the β-amidoester template was the *meta*-substituted phenylethynyl-pyridine (**1**), so the corresponding benzamidine piperidine (**9**) was targeted.

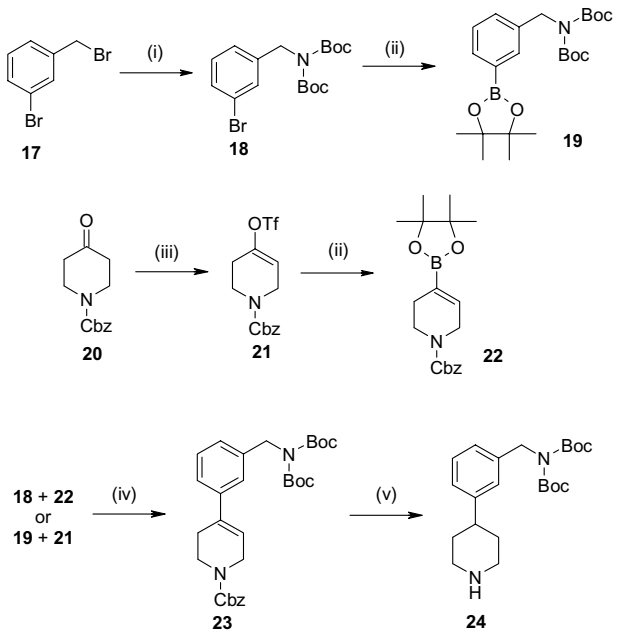
A convergent synthesis of the aforementioned phenylethynyl piperidine is highlighted in Scheme 2. Thus, Suzuki coupling of 3-cyanophenyl boronic acid (**11**) (from 3-bromobenzonitrile, **10**) with the triflate of *N*-Boc-piperidin-4-one (**13**) gave *N*-Boc-4-(3-cyanophenyl)-1,2,3,6-tetrahydropyridine (**14**). Conversion of the nitrile to benzamidine under Pinner conditions also facilitated removal of the Boc protecting group. Subsequent catalytic hydrogenation gave 3-piperidin-4-ylbenzamidine (**15**), which was selectively coupled at the piperidine nitrogen with the requisite carboxylic acid using TBTU as the coupling agent. These products could also be accessed by initial palladium coupling of the Cbz protected piperidine enol triflate with 3-cyanophenyl boronic acid, followed by tandem deprotection and catalytic hydrogenation of the endocyclic double bond. Subsequent amide coupling with the appropriate carboxylic acid, and conversion of the nitrile to benzamidine under Pinner conditions gave the desired materials. Two distinct routes were required because upon scale-up, certain carboxylic acids would couple to both the piperidine and the benzamidine groups. However, not all R groups would tolerate Pinner conditions at the end of the synthetic scheme.

Compound **9a** (tryptase K_i = 53 nM; factor Xa K_i = 380 nM) showed ~10-fold selectivity versus factor Xa and at least 40-fold versus trypsin. Re-analysis of the potential for replacing the benzamidine with alternative basic P1 groups and neutral P1 groups (e.g., targeting tyrosine 228) was once again unfruitful. However, replacing the benzamidine with benzylamine on the piperidine template resulted in only a 3-fold loss of activity (**16a**, tryptase K_i = 180 nM) rather than the 40-fold loss observed on the β-amidoester template (**2** vs **1**). Further profiling also revealed that **16a** (Fig. 5) was apparently inactive against a select panel of serine proteases, including both factor Xa and trypsin (Table 2).

The benzylamine template was accessed in a similar manner to the benzamidine, except that the coupling partners used in the Suzuki coupling were *N*,*N*-di-Boc-3-bromobenzylamine (**18**) and the pinacolato boronic ester derivative of the Cbz protected piperidine triflate (**22**) (Scheme 3).[8] Subsequent tandem reduction–deprotection to piperidine **24** and coupling, followed by



Scheme 2. Synthesis of benzamidinepiperidine template and subsequent coupling. Reagents and conditions: (i) a. *n*-BuLi, $B(OCHMe_2)_3$, THF, b. aq HCl; (ii) LDA, $PhNTf_2$, THF; (iii) $(Ph_3P)_4Pd$, Na_2CO_3, $MeCN/H_2O$; (iv) a. MeOH–HCl, b. MeOH–NH_3; (v) 5% Pd/C, H_2, MeOH; (vi) Et_3N, TBTU, 3-phenylethynyl-pyridinecarboxylic acid, DMF.



Scheme 3. Synthetic route to *N*,*N*-di-Boc protected 4-(3-aminomethylphenyl)piperidine template. Reagents and conditions: (i) NaH, $(Boc)_2NH$, THF; (ii) bis-pinacolato-diboron, $PdCl_2dppf{\cdot}DCM$ complex, dppf, KOAc, dioxane; (iii) LDA, $PhNTf_2$, THF; (iv) $PdCl_2dppf{\cdot}DCM$ complex, K_2CO_3, DMF; (v) H_2, Pd/C, EtOH.

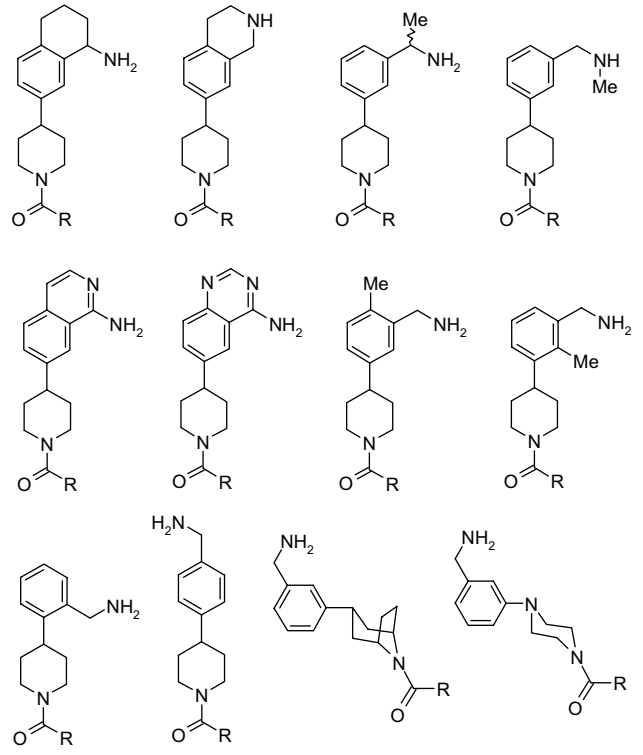


Figure 4. Inactive benzamidine, benzylamine, and piperidine analogs.

removal of the two Boc protecting groups gave the desired targets (**16**). Alternative Suzuki coupling protocols were also examined, e.g., **18** was converted to the pinacolato boronic ester (**19**) and coupled to the Cbz-protected piperidine triflate (**21**) to give **23**.

Many close analogs and simple derivatives of the benzylamine and piperidine moieties caused loss of activity (Fig. 4—all compounds were $K_i > 10$ μM). Analysis of potential piperidine replacements (e.g., 8-aza-nortropane and piperazine) also resulted in 10- to 20-fold loss of activity. Ester substitution on the 3 position of the piperidine (cf. closer to the β-amidoester parent) resulted in a 10- to 1000-fold loss in activity, depending on the diastereomer (the *trans*-piperidine mixture (3*R*,4*S* and 3*S*,4*R*) was the most active versus the *cis*-piperidine mixture (3*R*,4*R* and 3*S*,4*S*); the ester imparted metabolic instability, so the pure diastereomers were not pursued). Access to aspartate-189 is restricted by serine-190, and the angle of approach of the benzylamine is key to the ability of the molecule to form a good salt bridge to this residue. Presumably substitution *ortho* to the benzylamine causes a steric effect on the conformation of the benzylamine. Docking experiments also suggest that if the benzylamine were allowed to take up the preferred conformation, then there would be a steric clash of even small substituents *ortho* to the benzylamine (intramolecular as well as with the protein). Therefore, the template was fixed at 4-(3-aminomethylphenyl)piperidine, and the optimization for potency versus tryptase was addressed by varying the hydrophobic group.

A number of close analogs of the phenylethynyl-pyridine amide were synthesized on the benzylamine piperidine template (Fig. 5). The fully reduced phenethylpyridine (**16b**) gave a 12-fold boost in activity (tryptase $K_i = 15$ nM) versus the acetylene. Subsequent *N*-oxide formation on the phenethylpyridine (**16c**) reversed this (tryptase $K_i = 233$ nM). Replacement of the pyridine with a phenyl resulted in a 2-fold boost on the acetylene (**16d**, tryptase $K_i = 106$ nM), but 4-fold loss on the saturated ethyl analog (**16e**, tryptase $K_i = 53$ nM). Partial reduction of **16d** to the *meta*-*E*-alkene gave **16f** (tryptase $K_i = 367$). An attempt to replace the acetylene with a heterocyclic benzoxazole ring (**16g**) (Fig. 6) resulted in an 8-fold loss of activity (tryptase $K_i = 400$ nM).

Small diverse libraries of sulfonamide and urea derivatives of the piperidine benzylamine were prepared using solid phase synthesis techniques to investigate the potential for replacement of the piperidine amide. Unfortunately, almost all of the urea and sulfonamides were

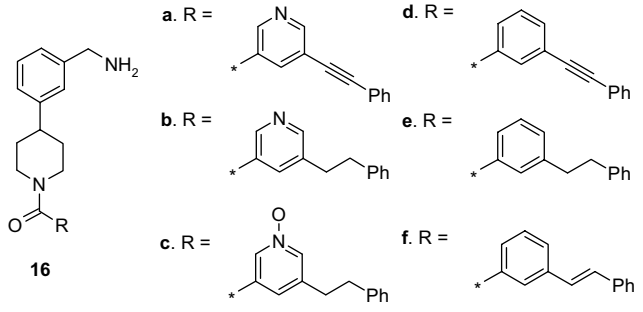


Figure 5. Compound **16a** and initial analogs.

inactive in the tryptase assay. The sulfonamides introduce an sp3 sulfur atom bridging the piperidine and the (usually aromatic) hydrophobic binding group. In factor Xa this amide would bind to glycine-219 and modeling into tryptase suggested that this would be an unfavorable hinge angle in order to point the benzylamine down into the S1 pocket. The urea introduces an extra chain atom between the piperidine and the hydrophobic group, which may not be easily tolerated. An alkyl bridge would probably give the correct angle, but would lack the hydrogen bonding capability for the crucial H-bond to glycine-219.

The initial rat PK study on **16b** showed low bioavailability and short i.v. half life (Fig. 6). However, further analysis in a dual cannulated rat study showed that the compound was 88% absorbed into the hepatic portal vein (HPV) post p.o. dosing, even though it was below the limit of quantitation in the jugular vein—that is, the compound was well absorbed, but efficiently cleared by first pass metabolism in the liver. The acid was the main metabolite identified, which was predictably inactive against tryptase.

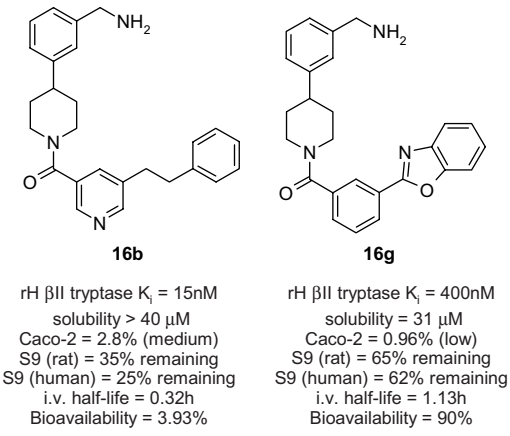


Figure 6. Initial eADME and PK.

Analysis of a carefully chosen subset of the benzylamines via a combi-PK cassette dosing regimen, showed that there was potential to improve the bioavailability to a good level with **16g**, even if the potency against the target was somewhat weaker for this compound. Rat PK showed ~90% bioavailability, although the i.v. half life was still rather short. This result suggested that the benzylamine was not necessarily a metabolic soft spot on the molecule. Furthermore, the hypothesis was supported by literature data on DuPont–Merck's benzylamine factor Xa inhibitor DPC423[9] (they have recently followed up with discussion of DPC602, another orally bioavailable factor Xa inhibitor with a benzylamine moiety).[10]

We were fortunate to be able to obtain crystals of our protein–inhibitor complex with compound **16b** by co-crystallization to give diffraction resolution at 2.2 Å (Fig. 7—in stereo). Only one molecule of **16b** was observed per monomer of the tetramer, and the electron density for the inhibitor is unambiguous. The benzylamine sits in the S1 specificity pocket and the amine group forms a salt bridge with aspartic acid-189 (ASP189) and three hydrogen bonds with the glycine-219 (GLY219) carbonyl oxygen atom, the serine-190 (SER190) carbonyl oxygen atom and a water molecule. The piperidine is in the chair conformation and the carbonyl oxygen atom of the amide forms a hydrogen bond

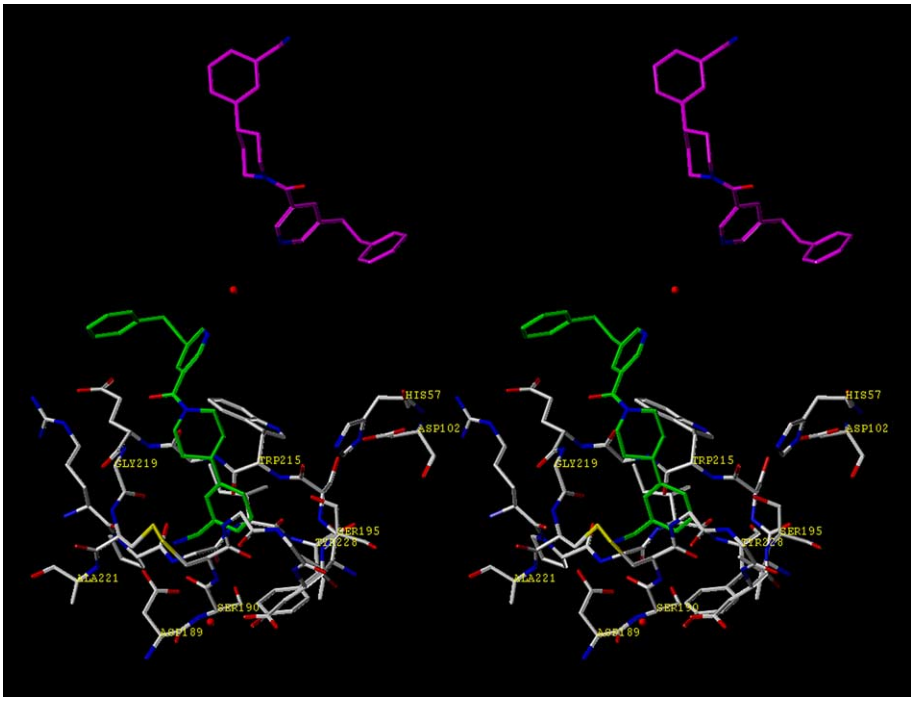


Figure 7. X-ray structure of **16b** in rH βII tryptase (stereo view).

with the GLY219 nitrogen. The pyridine nitrogen does not interact with the protein but is seen to form a hydrogen bond with a water molecule which bridges to another inhibitor molecule bound in the active site of the adjacent protein monomer. The distance between two inhibitor pyridine nitrogen atoms is ~5.5 Å. The phenylethyl plane is nearly 90° from the pyridine and makes Van der Waals contacts with proline-600_A, aspartic acid-601_A, and glutamic acid-217_C at the interface of two monomers.

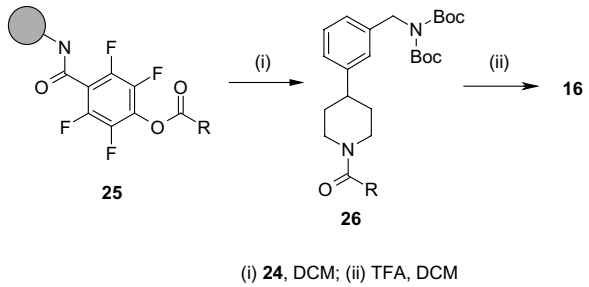
This crystal structure helped to explain much of the previously observed SAR:

- Size and nature of the S1 pocket was confirmed, which rationalized the intolerance of groups other than benzylamine/benzamidine.
- Loss of activity upon oxidation of **16b** to the *N*-oxide (**16c**); and the drop in activity of the phenethylphenyl versus phenethylpyridine amide was presumably due to the loss of an intermolecular water mediated hydrogen bond between two different inhibitor molecules bound into the active sites of adjacent protein monomers in the tetramer.
- Drop in activity when changing from piperidine to piperazine or aza-nortropane—the basic piperazine nitrogen was at an unfavorable location and changed the angle of access into the S1 pocket. The aza-nortropane bridging atoms could not be easily accommodated (steric clash with glutamine-92).

It was also clear from this X-ray structure that the surface hydrophobic pocket of the enzyme was quite large and should be tolerant of a wide variety of hydrophobic groups. A number of diversity oriented amide libraries were synthesized to explore this hydrophobic pocket.

Library synthesis: libraries were synthesized by two routes. The first involved treating TFP resin loaded carboxylic acids[11] with **24** in dichloromethane (DCM), followed by deprotection with TFA (Scheme 4).

The second route involved tethering the template to the solid phase (Scheme 5): *N*-Teoc-protected 4-piperidinone was converted to the triflate (**28**), and coupled, with the same Suzuki type methodology as previously described, to give the *N*-Teoc-protected 4-(3-cyanophenyl)-1,2,3,6-tetrahydropyridine (**29**). Tandem reduction of the nitrile and the endocyclic double bond was followed by loading this material onto Wang resin (via



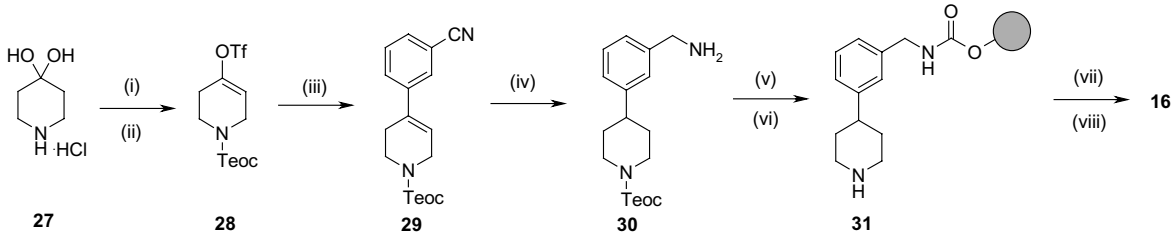
Scheme 4. Target synthesis via TFP-resin loaded carboxylic acids.

the *p*-nitrophenyl carbonate Wang resin) and removal of the Teoc protecting group with TBAF to give **31**. The resin bound template underwent standard amide coupling to carboxylic acids, and subsequent removal from the solid phase with TFA gave the target compounds (Table 1).

A representative sample of the compounds synthesized via these two methodologies is shown in Table 1. As was predicted from the crystal structure, a wide variety of hydrophobic groups are tolerated in this shallow surface pocket. This template allows a wide range of activities to be accessed. The salt bridge of the benzylamine and the hydrogen bonding network of the amide gives micromolar affinity, but optimization of the hydrophobe is required to achieve activity at the nanomolar level. This gave us the handle of diversity to allow parallel optimization for potency, toxicity, and pharmacokinetics.

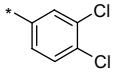
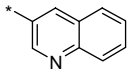
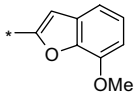
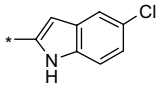
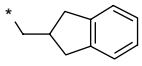
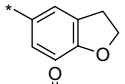
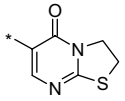
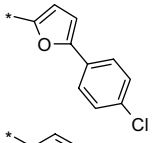
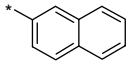
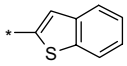
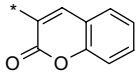
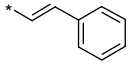
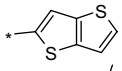
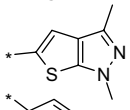
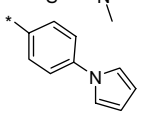
3. Conclusions

Knowledge from work on factor Xa provided a basis for identifying a series of moderately active (albeit unselective) tryptase inhibitors. Template and P1 binding group modification of the hit β-amidoester benzamidine gave achiral benzylamine piperidines with good potency and considerable selectivity versus many closely related serine proteases. Poor PK characteristics could be addressed whilst retaining a reasonable level of activity against the target. Initial in-house X-ray co-crystal structures have opened a number of viable ways forward for optimization of this series of novel potent, selective, and orally bioavailable inhibitors of βII tryptase. Subsequent synthesis of hydrophobe diversity explosion libraries has expanded the scope of this novel class of



Scheme 5. Target synthesis via resin bound template (i) 2-trimethylsilylethyl-*p*-nitrophenylcarbonate, Et_3N, DMAP, MeCN; (ii) $Li(TMS)_2$, $PhNTf_2$, THF; (iii) *m*-cyanophenyl boronic acid, $Na_2CO_{3(aq)}$, LiCl, $Pd(PPh_3)_4$, MeCN; (iv) H_2, Pd/C, concd HCl/EtOH; (v) *p*-nitrophenylcarbonate Wang resin, DIEA, DMAP, DMF; (vi) TBAF, THF; (vii) RCO_2H, DIC, HOBt, DMF; (viii) TFA, DCM.

Table 1. rH β-II tryptase K_i values for selected active, diversity oriented, amide hydrophobes

16h		>30 μM
16i		48 nM
16j		170 nM
16k		500 nM
16l		700 nM
16m		550 nM
16n		70 nM
16o		150 nM
16p		1000 nM
16q		933 nM
16r		270 nM
16s		70 nM
16t		750 nM
16u		130 nM
16v		330 nM
16w		300 nM
16x		290 nM
16y		733 nM

inhibitors, yielding opportunities for further structure based optimization.

4. Experimental

Atomic coordinates for the X-ray structure of rH βII tryptase with compound **16b** will be deposited at the Protein Data Bank upon publication.

Table 2. Selectivity data (IC_{50}/μM)

	Factor Xa	Thrombin	Trypsin	Elastase	Chymase
1	0.35	>100	5	ND	>100
9a	0.7	>100	20	>100	>100
16a	>100	>100	>100	>100	>100
16b	>100	>100	>100	>100	>100
16d	>100	>100	>100	>100	>100
16e	>100	>100	>100	>100	>100
16i	>100	>100	>100	>100	>100

4.1. Tryptase inhibition activity (in vitro)

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.[12]). Assays were performed at room temperature using 0.5 mM substrate ($2 \times K_m$) and the microplate was 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 temp 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. For a competitive inhibitor, the IC_{50} value was converted into K_i according to the equation: $K_i = IC_{50}/(1 + [S]/K_m)$.

4.2. In vitro permeability and metabolism assays

In vivo absorption through the intestine was modeled using the caco-2 cell line.[13] In vivo metabolism was modeled using the S9 homogenates from human and rat liver,[14] compounds were incubated for 15 min at 37 °C.

4.3. General experimental discussion

TLC analyses were performed with Merck DC-F254 silica gel plates, with visualization by UV light, iodine, alkaline permanganate, and/or molybdophosphoric

acid. Flash chromatography was performed with Merck silica gel 60 (0.040–0.063 μm). NMR spectra were recorded on Gemini-300 spectrometers in $CDCl_3$, unless otherwise stated. ^1H signals are reported in ppm from tetramethylsilane (s, d, t, m, and br for singlet, doublet, triplet, multiplet, and broad, respectively). Mass spectral data were collected at 70 eV on a Finnigan MAT 4600, Mat TSQ-700 or VG Analytical Ltd ZAB2-SE mass spectrometer and computerized for HRMS. Combustion analyses were performed using a Perkin–Elmer Model 2400 elemental analyzer and fell within ±0.4% of calculated values. The organic extracts were dried over magnesium sulfate or sodium sulfate prior to evaporation in vacuum on a rotary evaporator. Anhydrous solvents were purchased from Aldrich Chemical Co. or Acros organics and used as obtained. Any noncommercially available known starting materials were synthesized as referenced. TFP resin is commercially available from Polymer Labs. All polymer loaded tetrafluorophenoxy (PL-TFP) resin loaded carboxylic acids were prepared by the method of Salvino et al.[11] Any final products not specifically described herein were synthesized via the TFP resin protocol or the solid phase template protocol, and were isolated as library members characterized by LC/MS (overall purity >90%, no single impurity >5%). Solubility measurements: a 10 mM stock solution of the compound (in DMSO) was diluted 200-fold with LC/MS mobile phase to give a 50 μM standard solution; a separate portion of the 10 mM stock solution was diluted 200-fold with phosphate buffered saline, and this sample was mixed vigorously and centrifuged. Comparison of the area under the curve of the parent compound from LC/MS analysis of the two solutions gives a relative estimate of aqueous solubility (up to 50 μM).

CAUTION!—many intermediates prepared herein contain the 4-phenyl-1,2,5,6-tetrahydropiperidine substructure, which is closely related to the known Parkinson's inducing compound MPTP.[15] Although none of these particular unsaturated piperidine intermediates have known toxicity, extra care should be taken (especially upon scale-up).

4.3.1. 3-Cyanophenyl boronic acid (11). 3-Bromobenzonitrile (**10**) (3.6 g, 20 mmol) in anhydrous THF (100 mL) was treated with triisopropyl borate (13 mL, 56 mmol), followed by 1.6 M butyllithium in hexane (14 mL, 22 mmol) over 15 min at −78 °C. The reaction was stirred at −78 °C for 45 min then warmed to room temperature over 60 min. The reaction mixture was poured into 2 N HCl (40 mL) and the solid precipitate collected. The filter cake was washed with water and methylene chloride to yield **11** (2.3 g, 15.6 mmol). ^1H NMR δ 8.4 (br, 2H), 8.13 (s, 1H), 8.07 (d, 1H), 7.85 (d, 1H), 7.55 (t, 1H). MS(EI): 147 (M^+).

4.3.2. 4-(3-Cyanophenyl)-3,6-dihydro-2*H*-pyridin-1-carboxylic acid *tert*-butyl ester (14). Compound **11** (1.8 g, 12.2 mmol) and 4-(trifluoromethanesulfonyloxy)-3,6-dihydro-2*H*-pyridine-1-carboxylic acid *tert*-butyl ester (**13**) (5.0 g, 15.2 mmol) were dissolved in acetonitrile (60 mL) and 0.4 M aqueous sodium carbonate (60 mL). The solution was degassed and treated with palladium tetrakistriphenyl phosphine (0.81 g, 0.7 mmol) at 90 °C for 1 h. The reaction was cooled, filtered warm, and the filtrate concentrated to an oil. The oil was extracted with methylene chloride and the solvent removed under vacuum. The residue was chromatographed (15% ethyl acetate/hexane) to give **14** as a white solid (2.7 g, 9.5 mmol). ^1H NMR δ 7.63 (s, 1H), 7.55 (m, 2H), 7.44 (t, 1H), 6.10 (br, 1H), 4.09 (m, 2H), 3.63 (t, 2H), 2.50 (br, 2H). MS(EI): 285 (M^++1).

4.3.3. 3-(1,2,3,6-Tetrahydropyridin-4-yl)benzamidine dihydrochloride (15). Compound **14** (3.4 g, 12.0 mmol) was dissolved in anhydrous methanol (300 mL), cooled in an ice bath and saturated with a stream of HCl gas. The reaction vessel was sealed and the solution was stirred overnight at ambient temperature. The solvent was removed and the residue was redissolved in methanol (300 mL). The resulting solution was saturated with ammonia gas, the vessel was sealed and stirred overnight at room temperature. The solvent was removed in vacuo and the residue was dissolved in dilute aqueous HCl and purified by reverse phase HPLC (acetonitrile/H_2O, 2:98). Fractions containing the product were lyophilized to yield **15** as a white solid (1.7 g, 6.2 mmol). ^1H NMR (DMSO): δ 9.48 (s, 2H), 9.38 (s, 2H), 9.15 (s, 2H), 7.90 (s, 1H), 7.80 (d, 1H), 7.73 (d, 1H), 7.58 (t, 1H), 6.39 (s, 1H), 3.76 (br, 2H), 3.3 (br, 2H), 1.9 (m, 4H). MS(EI): 201 (M^+).

4.3.4. 3-Piperidin-4-ylbenzamidine dihydrochloride. Compound **15** (1.0 g, 5.0 mmol) was dissolved in methanol (50 mL) treated with 5% Pd on carbon and stirred overnight under an atmosphere of hydrogen gas. The reaction mixture was filtered; the filtrate was concentrated to give 3-piperidin-4-ylbenzamidine dihydrochloride as a white foam (1.30 g, 4.7 mmol). ^1H NMR (DMSO-d_6): δ 9.40 (s, 2H), 9.2 (br, 4H), 7.67 (br, 2H), 7.56 (br, 2H), 3.4 (br, 2H), 3.0 (m, 3H), 2.7 (br, 4H). MS(Ion spray): 204 (M^++1).

4.3.5. 3-(Piperidin-4-yl)-benzonitrile. A solution of benzyl 4-oxo-1-piperidinecarboxylate (**20**) (11.4 g, 49 mmol) in THF (100 mL) was cannulated dropwise into a cooled (−78 °C), vigorously stirring (overhead stirrer) solution of LDA (36.7 mL of 1.5 M solution in cyclohexanes, 55 mmol) in THF (100 mL), under N_2. The reaction mixture was left at −78 °C for 30 min before adding a solution of phenyl trifluorosulfonimide (19.1 g, 53.5 mmol) in THF (50 mL). Then the reaction mixture was warmed to room temperature and stirred for 3 h and concentrated directly in vacuo (water bath at 30 °C). The reaction mixture was flash columned on alumina with ramped dilution of neat hexanes to 4% EtOAc/hexanes. Product fractions were combined and concentrated to give benzyl 1,2,3,6-tetrahydro-4-(trifluoromethylsulfonyloxy)-pyridine-1-carboxylate (**21**) (11.34 g) as a white solid (after cooling overnight). ^1H NMR δ 7.30–7.38 (5H, m), 5.75 (H, br s), 5.15 (2H, s), 4.13 (2H, m), 3.70 (2H, t), 2.46 (2H, br s). A portion of this material (3.65 g, 10 mmol) was dissolved in anhydrous 1,2-dimethoxyethane (30 mL) and treated with **11** (1.47 g, 10 mmol), lithium chloride (1.27 g, 30 mmol), 2.0 M aqueous sodium carbonate (10 mL) and palla-

dium tetrakistriphenylphosphine (0.73 g, 0.6 mmol). The reaction mixture was heated at reflux for 2.5 h, cooled and concentrated under vacuum. The residue was partitioned between dichloromethane (2 × 100 mL) and 2.0 M aqueous sodium carbonate (100 mL) containing concd ammonium hydroxide (6 mL). The combined organic extracts were dried (magnesium sulfate), concentrated under vacuum and the resultant oil was chromatographed on silica gel (ethyl acetate/pentane 2:5) to yield a yellow oil (2.31 g). This material was dissolved in ethanol (70 mL), treated with 10% palladium on carbon (1.0 g) and stirred at ambient temperature under an atmosphere of hydrogen for 5 h. The reaction mixture was filtered through a short pad of hyflo and concentrated under vacuum to give the title compound as a colorless oil (1.26 g). ^{1}H NMR (500 MHz): δ 9.78 (br m, 1H), 7.50 (m, 4H), 4.49 (br m, 1H), 3.65 (br m, 1H), 3.00 (m, 2H), 2.82 (m, 1H), 2.21–1.75 (m, 4H). MS(EI): 187 (M^++H).

4.3.6. 3-Bromo-*N*,*N*-bis-(*tert*-butoxycarbonyl)benzylamine (18). A solution of 3-bromobenzylbromide (**17**) (7.5 g, 30 mmol) and di-*tert*-butyliminodicarboxylate (6.5 g, 30 mmol) in anhydrous tetrahydrofuran (80 mL) was treated portionwise with sodium hydride (1.2 g, 60% dispersion in mineral oil). After stirring at ambient temperature for 7 h the reaction mixture was partitioned between saturated aqueous ammonium chloride solution (90 mL) and ethyl acetate (two lots of 250 mL). The combined organic layers were washed with brine (75 mL), then dried over magnesium sulfate and concentrated under vacuum. The residue was subjected to chromatography on silica gel eluting with a mixture of pentane and dichloromethane (2:1, v/v) to give **18** as a pale yellow oil (9.52 g). ^{1}H NMR δ 7.43 (s, 1H), 7.36 (dt, 1H), 7.23–7.18 (m, 1H), 7.17 (dd, 1H), 4.72 (s, 2H), 1.45 (s, 18H). MS(EI): 386/388 (M^++H).

4.3.7. *B*-{3-[*N*,*N*-Bis-(*tert*-butoxycarbonyl)aminomethyl]-phenyl}-pinacolato-boron (19). Compound **18** (2.0 g, 5.2 mmol) was dissolved in anhydrous dimethylsulfoxide (30 mL) and the solution was treated with potassium acetate (1.52 g, 15.5 mmol), bis(pinacolato)diboron (1.45 g, 5.7 mmol), and [1,1′-bis-(diphenylphosphino)ferroceno]dichloropalladium(II)–dichloromethane complex (0.13 g, 0.16 mmol). This mixture was stirred at 80 °C under an atmosphere of nitrogen for 5 h, then cooled and then partitioned between water (100 mL) and diethyl ether (four lots of 75 mL). The combined organic layers were washed twice with brine (75 mL), then dried over magnesium sulfate and then concentrated under vacuum. The residue was subjected to chromatography on silica gel eluting with a mixture of pentane and dichloromethane (2:1, v/v) to give **19** as a colorless oil (1.08 g). ^{1}H NMR (500 MHz): δ 7.78 (s, 1H), 7.70 (m, 1H), 7.39 (m, 1H), 7.30 (m, 1H), 4.79 (s, 2H), 1.27 (s, 18H), 1.35 (s, 12H). MS(EI): 434 (M^++H).

4.3.8. 4-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-3,6-dihydro-2*H*-pyridine-1-carboxylic acid benzyl ester (22). Compound **21** (5.32 g, 14.58 mmol) was dissolved in dioxane (90 mL) and added under nitrogen to a degassed mixture of potassium acetate (4.29 g, 43.74 mmol, 3 equiv), 1,1′-bis-(diphenylphosphino)ferrocene (0.242 g, 0.437 mmol, 0.03 equiv), [1,1′-bis-(diphenylphosphino)ferroceno]dichloropalladium(II)–dichloromethane complex (0.32 g, 0.437 mmol, 0.03 equiv) and bis-pinacolato diborane (4.07 g, 16.04 mmol, 1.1 equiv) and the reaction mixture heated at 80 °C overnight under N_2. Added water (200 mL), extracted into EtOAc, dried ($MgSO_4$), and presorbed crude material onto silica and flash columned with ramped neat hexanes to 50% EtOAc/hexanes (5% steps)—product off at 20–25%. Combined pure product fractions and concentrated in vacuo to give a white solid (3.58 g, 72%). MS ESI: 385 (+MeCN), 344 (parent), 300 (base).^{1}H NMR δ 7.24–7.38 (m, 5H); 6.45 (d, H); 5.13 (s, 2H); 4.02 (q, 2H); 3.51 (t, 2H); 2.24 (br s, 2H); 1.25 (s, 12H).

4.3.9. 4-{3-[*N*,*N*-Bis-(*tert*-butoxycarbonyl)aminomethyl]-phenyl}-piperidine (24). Compound **21** (0.84 g, 2.3 mmol) was dissolved in anhydrous DMF (20 mL) and the solution was treated with **19** (1.0 g, 2.3 mmol), potassium carbonate (0.96 g, 6.7 mmol) and [1,1′-bis-(diphenylphosphino)ferroceno]dichloropalladium(II)–dichloromethane complex (0.1 g, 0.14 mmol). This mixture was heated at 80 °C under an atmosphere of nitrogen for 18 h, then cooled and then concentrated under vacuum. The residue was partitioned between ethyl acetate (two lots of 100 mL) and water (100 mL) containing concentrated ammonium hydroxide (6 mL). The combined organic extracts were dried over magnesium sulfate and then concentrated under vacuum. The resultant oil was subjected to chromatography on silica gel eluting with a mixture of ethyl acetate and pentane (1:4, v/v) to yield compound **23** as a yellow oil (0.9 g). ^{1}H NMR (300 MHz): δ 7.40–7.14 (m, 9H), 6.00 (d, H), 5.18 (s, 2H), 4.77 (s, 2H), 4.18–4.07 (m, 2H), 3.70 (t, 2H), 2.52 (br s, 2H), 1.43 (s, 18H). MS(EI): 545 (M^++Na). This material was dissolved in ethanol (20 mL) and the solution was treated with 10% palladium on carbon (20 mg) then stirred at ambient temperature under an atmosphere of hydrogen for 5 h. The reaction mixture was filtered through a short pad of hyflo and the filtrate was concentrated under vacuum to give **24** as colorless oil (0.54 g). ^{1}H NMR (500 MHz): δ 7.10 (m, 4H), 4.80 (s, 2H), 4.45 (br m, 1H), 3.20 (br m, 1H), 2.98 (br m, 1H), 2.75 (br m, 1H), 1.90 (m, 1H), 1.75–1.60 (m, 3H), 1.42 (s, 18H). MS(EI): 391 (M^++H). Compound **24** could also be synthesized by utilizing this same coupling methodology, but using **18** and **22** as reactants instead of **19** and **21** (all reactants and reagents were used in the same molar equivalents). The yield of compound **23** and **24** in this case were 80% and 85%, respectively, and gave identical analytical data to those above.

4.3.10. 5-Phenylethyl-pyridine-3-carboxylic acid. A solution of 5-phenylethynyl-pyridine-3-carboxylic acid (2 g, 8.9 mmol) in tetrahydrofuran (50 mL) was treated with 10% palladium on carbon (200 mg) and stirred at ambient temperature under an atmosphere of hydrogen for 5 h. The reaction mixture was filtered through a short pad of hyflo and the filtrate was concentrated under vacuum to give 5-phenylethyl-pyridine-3-carboxylic acid as

a white solid (2 g). ^{1}H NMR (DMSO-d_6, 500 MHz): 8.90 (m, 1H), 8.60 (m, 1H), 8.12 (m, 1H), 7.21 (m, 5H), 3.38 (br s, 1H), 2.95 (m, 4H).

4.3.11. 4-Oxo-piperidine-1-carboxylic acid (trimethylsilyl) ethyl ester. A solution of 4-piperidone monohydrate hydrochloride (**27**) (13.55 g, 88 mmol) and 2-trimethylsilylethyl-*p*-nitrophenylcarbonate (25.00 g, 88 mmol) in acetonitrile (300 mL) was treated with triethylamine (50 mL, 359 mmol) and dimethylaminopyridine (10.78 g, 88 mmol) and heated to reflux for 2 h. The solution was cooled and concentrated to an oil. The residue was dissolved in dichloromethane (300 mL) and washed twice with 1 N hydrochloric acid and twice with 1 N sodium hydroxide until all of the yellow color was removed from the organics. The organics were then washed with brine, dried over magnesium sulfate, and concentrated under vacuum to give 4-oxo-piperidine-1-carboxylic acid (trimethylsilyl) ethyl ester as colorless oil (19.35 g). ^{1}H NMR δ 4.24 (2H, t), 3.78 (4H, t), 2.45 (4H, t), 1.05 (2H, t), 0.05 (9H, s). MS(EI): 284 (M+CH_3CN).

4.3.12. 4-(3-Cyanophenyl)-3,6-dihydro-2*H*-pyridine-1-carboxylic acid 2-trimethylsilanyl-ethyl ester (29). A solution of lithium hexamethyldisilazide (60 mmol) in anhydrous tetrahydrofuran (150 mL), at −78 °C, was treated dropwise with a solution of 4-oxo-piperidine-1-carboxylic acid (trimethylsilyl) ethyl ester (13.30 g, 55 mmol) in anhydrous tetrahydrofuran (50 mL). The addition was over 20 min maintaining the internal temperature at −65 to −70 °C. This solution was stirred at −78 °C for 45 min and then treated with a solution of *N*-phenyltrifluoromethanesulfonimide (19.65 g, 55 mmol) in anhydrous tetrahydrofuran (75 mL). The solution was warmed to 0 °C, then stirred at 0 °C for 3 h and then concentrated under vacuum. The residue was dissolved in dichloromethane and washed with water, dried over magnesium sulfate, and concentrated to give 2-(trimethylsilyl) ethyl 1,2,3,6-tetrahydro-4-(trifluoromethylsulfonyloxy)-pyridine-1-carboxylate (**28**) as a yellow oil (22.1 g). The material was used crude, as column chromatography on silica gel or alumina caused the material to deteriorate. A portion of **28** (20.65 g, 55 mmol) was dissolved in acetonitrile (300 mL) and the solution was treated with **11** (8.90 g, 60 mmol), 2 M sodium carbonate (82.5 mL, 165 mmol) and lithium chloride (6.98 g, 165 mmol). The nonhomogenous mixture was stirred vigorously and flushed with nitrogen for 5 min, then added tetrakistriphenylphosphine palladium(0) (3.10 g, 3 mmol). The mixture was heated to reflux (90 °C oil bath) for 90 min, then cooled and filtered. The red filtrate was concentrated and the residue was partitioned between dichloromethane (three lots 100 mL) and 2 M sodium carbonate (200 mL). The combined organic extracts were dried over magnesium sulfate and then concentrated under vacuum. The resultant oil was subjected to chromatography silica gel eluting with a mixture of ethyl acetate, heptane, and dichloromethane (1:5:1, v/v) to give **29** as a yellow oil (10.46 g). ^{1}H NMR δ 7.40–7.65 (m, 4H), 6.10 (m, 1H), 4.23 (t, 2H), 4.15 (d, 2H), 3.70 (t, 2H), 2.45 (m, 2H), 1.12 (t, 2H), 0.05 (s, 9H).

4.3.13. 4-(3-Aminomethyl-phenyl)-piperidine-1-carboxylic acid 2-trimethylsilanyl-ethyl ester hydrochloride (30). Compound **29** (10.40 g, 32 mmol) was dissolved in ethanol (250 mL), concentrated hydrochloric acid (3 mL, 35 mmol) and 10% palladium on carbon (50% wet, 5.0 g) was added. The mixture was hydrogenated at 50 psi for 4 h then filtered through Celite and concentrated. The oily solid obtained was triturated with ether/pentane and **30** was obtained as a white solid (7.10 g). ^{1}H NMR (DMSO-d_6): δ 8.38 (br s, 2H), 7.20–7.40 (m, 4H), 4.10 (t, 4H), 3.98 (s, 2H), 2.63–3.00 (m, 3H), 1.75 (m, 2H), 1.50 (m, 2H), 0.94 (t, 2H), 0.02 (s, 9H). LC–MS(ES): 335 (M^++H), 93% TIC.

4.3.14. (3-Piperidin-4-yl-benzyl) carbamate Wang resin (31). *p*-Nitrophenyl carbonate Wang resin (11.00 g, 15 mmol) and anhydrous DMF (100 mL) were placed in a peptide synthesis vessel and the resin was allowed to swell for 15 min. This was then treated with **30** (7.50 g, 21 mmol) in DMF (50 mL), DMAP (0.72, 6 mmol) and diisopropylethylamine. The peptide vessel was shaken at room temperature for 24 h, then washed thoroughly with DMF (×5), methanol (×2), DMF (×2), methanol (×2), dichloromethane (×3), methanol (×2), dichloromethane (×2), methanol (×3), and dried. The resin was then treated with THF to swell the resin and then allowed to drain. Then anhydrous tetrahydrofuran (100 mL) and tetrabutylammonium fluoride (75 mL, 1 M in tetrahydrofuran) were added and the resin shaken for 18 h. The resin was drained and washed with tetrahydrofuran (×5), methanol (×3), dichloromethane (×3), methanol (×3), dichloromethane (×3), methanol (×3), and dried to give **31** (12.30 g).

4.4. General procedure for solution phase amide coupling of carboxylic acids to piperidine benzylamine and piperidine benzamidine templates, and subsequent deprotection

2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (120 mg, 0.38 mmol) was added to a room temperature solution of carboxylic acid (0.36 mmol) and diisopropylethylamine (140 mg, 1.08 mmol) dissolved/suspended in DMF (2 mL), under nitrogen. The reaction mixture was stirred for 10 min at room temperature before adding a solution of **24** (140 mg, 0.36 mmol) and diisopropylethylamine (140 mg, 1.08 mmol) dissolved in DMF (2 mL). The reaction mixture was stirred at room temperature for 16 h, concentrated to dryness in vacuo, and subjected to dry flash column chromatography on silica with 50:50, dichloromethane–ethyl acetate. The pure fractions were combined and concentrated to give the 1-acyl-4-(3-(*N*,*N*-di-*tert*-butoxycarbonylaminomethyl)phenyl)piperidine, which was generally isolated as a colorless oil. This intermediate was dissolved in dichloromethane (20 mL), cooled at 0 °C, and treated with trifluoroacetic acid (2 mL). The reaction mixture was stirred at room temperature under nitrogen for 2 h, and concentrated to dryness in vacuo. The residue was dissolved in 20% acetonitrile/water (containing 0.1% trifluoroacetic acid) (9 mL) and purified by preparative reverse-phase HPLC (C-18, 10 μm reverse-phase column), eluting with 10–100% acetonitrile/water (containing 0.1% trifluoroacetic

acid). The product fractions were combined and the acetonitrile removed in vacuo. The aqueous residue was frozen and lyophilized to give the products as amorphous white solids.

This procedure was also used for couplings with the benzamidine template.

TBTU was used interchangeably with *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU).

Deprotection could also be effected by dissolving the intermediate (0.25 mmol) in ethyl acetate (20 mL), cooling the solution to 0 °C, and saturating with $HCl_{(g)}$. After stirring for 4 h at room temperature the solvent was removed and the residue purified by preparative reverse-phase HPLC.

4.5. General procedure for coupling of TFP-resin bound carboxylic acids to piperidine benzylamine template, and subsequent deprotection

TFP resin (125 mg of 1.25 mmol/g resin, with 100% loading of acid, 156 mmol)[11] was swollen in dichloromethane (2.5 mL) for 15 min then treated with a solution of **24** (40 mg, 100 mmol) in dichloromethane (2.5 mL). The mixture was sealed in the reaction vessel then left shaking for 8 h. The resin was filtered, and washed with dichloromethane (2 mL) [TLC (5% MeOH/EtOAc) showed single product spot (no baseline amine)]. The filtrate was treated with trifluoroacetic acid (0.5 mL). After shaking/stirring for 2 h TLC showed no residual intermediate and the reaction mixture was evaporated to give the product directly as the trifluoroacetic acid salt.

4.6. General procedure for coupling Wang resin bound piperidine benzylamine template to carboxylic acids, and subsequent cleavage from the resin

Compound **31** (60 mg, 0.075 mmol) suspended in DMF (3 mL) and carboxylic acid (0.38 mmol), diisopropylcarbodiimide (48 mg, 0.38 mmol), and 1-hydroxybenzotriazole (50 mg, 0.38 mmol) were added. The mixture was shaken at room temperature overnight and washed with DMF (×5), methanol (×5), dichloromethane then methanol (repeat five times). The resin was treated with trifluoroacetic acid and dichloromethane (1:1 v/v, 4 mL) for 45 min and filtered. The filtrate was concentrated to give the product directly as the trifluoroacetic acid salt.

4.6.1. 3-[1-(5-Phenylethynyl-pyridine-3-carbonyl)-piperidin-4-yl]-benzonitrile. A solution of 5-phenylethynyl-pyridine-3-carboxylic acid (0.75 g, 3.3 mmol) in anhydrous DMF (9 mL) was treated with HATU (1.27 g, 3.3 mmol) and diisopropylethylamine (1.1 mL, 6.1 mmol). The reaction mixture was stirred 15 min at ambient temperature before being treated with a solution of 3-(piperidin-4-yl)-benzonitrile (0.6 g, 3 mmol) in DMF (6 mL). The reaction mixture was stirred at ambient temperature for 2 h, concentrated under vacuum and the residue partitioned between ethyl acetate (200 mL) and saturated aqueous sodium bicarbonate (45 mL). The organic layer was dried (magnesium sulfate), concentrated under vacuum and the residue chromatographed on silica gel (dichloromethane/methanol 39:1) to give the title compound as a yellow oil (0.53 g). ^{1}H NMR (500 MHz): δ 8.80 (s, 1H), 8.63 (s, 1H), 8.00 (s, 1H), 7.50 (m, 9H), 4.90 (br m, 1H), 3.85 (br m, 1H), 3.28 (m, 1H), 2.90 (m, 2H), 1.91 (m, 1H), 1.75 (m, 3H). MS(EI): 392 (M^++H).

4.6.2. 3-[1-(5-Phenylethynyl-pyridine-3-carbonyl)-piperidin-4-yl]-benzamidine hydrochloride (9a). A solution of 3-[1-(5-phenylethynyl-pyridine-3-carbonyl)-piperidin-4-yl]-benzonitrile (0.51 g, 1.3 mmol) in methanol (20 mL) was cooled to 0 °C and saturated with hydrogen chloride gas. The reaction vessel was sealed and allowed to stand at 0 °C for 18 h. The reaction mixture was concentrated to dryness under vacuum, redissolved in methanol (20 mL), and cooled to 0 °C. The solution was saturated with ammonia gas and the vessel sealed before being allowed to stand at 0 °C for 48 h. The reaction mixture was concentrated to dryness under vacuum and the residue chromatographed on silica gel (dichloromethane/methanol 9:1) to give the title compound as a light yellow amorphous solid (0.42 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.85 (s, 1H), 8.67 (s, 1H), 8.08 (s, 1H), 7.75 (s, 1H), 7.67 (m, 2H), 7.62 (m, 2H), 7.56 (m, 1H), 7.48 (m, 2H), 7.47 (m, 1H), 4.67 (br m, 1H), 3.65 (br m, 1H), 2.94 (m, 3H), 1.96 (m, 1H), 1.68 (m, 3H). MS(EI): 409 (M^++H).

4.6.3. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-(5-phenylethynyl-pyridin-3-yl)-methanone di-hydrochloride (16a). Solution phase HATU coupling of 5-phenylethynyl-pyridine-3-carboxylic acid—intermediate isolated as a yellow oil (0.25 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.82 (s, 1H), 8.62 (s, 1H), 8.02 (s, 1H), 7.61 (m, 2H), 7.45 (m, 3H), 7.30 (m, 1H), 7.20 (m, 1H), 7.15 (m, 1H), 7.04 (m, 1H), 4.68 (s, 2H), 4.62 (br m, 1H), 3.60 (br m, 1H), 3.23 (br m, 1H), 2.85 (m, 2H), 1.83 (br m, 1H), 1.65 (m, 3H), 1.39 (s, 18H). MS(EI): 596 (M^++H). HCl deprotection gave the product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.83 (s, 1H), 8.63 (s, 1H), 8.08 (s, 1H), 7.61 (m, 2H), 7.45 (m, 3H), 7.44 (s, 1H), 7.37 (m, 3H), 4.64 (br m, 1H), 4.00 (m, 2H), 3.62 (br m, 1H), 3.25 (br m, 1H), 2.90 (br m, 2H), 1.88 (br m, 1H), 1.70 (m, 3H). MS(EI): 396 (M^++H).

4.6.4. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-(5-phenethyl-pyridin-3-yl)-methanone di-hydrochloride (16b). Solution phase HATU coupling of 5-phenylethyl-pyridine-3-carboxylic acid—intermediate isolated as a white amorphous solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.50 (s, 1H), 8.41 (s, 1H), 7.61 (m, 1H), 7.30–7.05 (m, 9H), 4.68 (s, 2H), 4.62 (br m, 1H), 3.48 (br m, 1H), 3.35 (s, 4H), 3.18 (br m, 1H), 2.85 (m, 2H), 1.82 (br m, 1H), 1.65 (br m, 1H), 1.58 (br m, 2H), 1.39 (s, 18H). MS(EI): 600 (M^++H). HCl deprotection gave the product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.90 (m, 2H), 8.42 (s, 1H), 7.58 (m, 1H), 7.38–7.20 (m, 8H), 4.62 (br m, 1H), 4.00 (m, 2H), 3.45 (br m, 1H), 3.20 (br m, 1H), 3.17 (m, 2H), 3.00 (m,

2H), 2.90 (br m, 2H), 1.88 (br m, 1H), 1.70 (br m, 3H). MS(EI): 400 (M^++H).

4.6.5. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-(1-oxy-5-phenethyl-pyridin-3-yl)-methanone hydrochloride (16c). A solution of *N*,*N*-bis-(*tert*-butoxycarbonyl)-3-[1-(5-phenylethyl-pyridine-3-carbonyl)-piperidin-4-yl]-benzylamine (100 mg, 0.17 mmol) in dichloromethane (10 mL) was treated with *meta*-chloroperbenzoic acid (80%, 80 mg, 0.37 mmol). After stirring for 18 h at ambient temperature the reaction mixture was diluted with dichloromethane (40 mL) and then washed three times with saturated aqueous sodium bicarbonate solution (20 mL). The organic phase was dried over magnesium sulfate and then concentrated under vacuum. The residue was subjected to chromatography on silica gel eluting with a mixture of dichloromethane and methanol (98:2, v/v) to give *N*,*N*-bis-(*tert*-butoxycarbonyl)-3-[1-(1-oxy-5-phenylethyl-pyridine-3-carbonyl)-piperidin-4-yl]-benzylamine as a colorless oil (70 mg). This material was dissolved in ethyl acetate (10 mL) and the solution was cooled to 0 °C, then saturated with hydrogen chloride gas, then stirred at ambient temperature for 4 h and then concentrated to dryness under vacuum. The residue was treated with ethyl acetate (10 mL) and the solvent removed under vacuum. This process was repeated twice to leave **16c** as a white solid (45 mg). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.39 (s, 1H), 8.30 (s, 1H), 7.45 (s, 1H), 7.40 (s, 1H), 7.38–7.18 (m, 8H), 4.59 (br m, 1H), 4.00 (m, 2H), 3.50 (br m, 1H), 3.20 (br m, 1H), 2.98 (s, 4H), 2.85 (br m, 2H), 1.84 (br m, 1H), 1.68 (br m, 3H). MS(EI): 416 (M^++H).

4.6.6. 4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-quinolin-3-yl-methanone di-hydrochloride (16j). Solution phase HATU coupling of quinoline-3-carboxylic acid, followed by HCl deprotection gave the product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.10 (s, 1H), 8.78 (s, 1H), 8.20 (m, 2H), 7.98 (m, 1H), 7.80 (m, 1H), 7.50 (s, 1H), 7.35 (m, 3H), 4.72 (br m, 1H), 4.00 (m, 2H), 3.80 (br m, 1H), 3.30 (br m, 1H), 2.90 (br m, 2H), 1.90 (br m, 1H), 1.75 (br m, 3H). MS(EI): 346 (M^++H).

4.6.7. *N*,*N*-Bis-(*tert*-butoxycarbonyl)-3-[1-(3-ethynyl-benzoyl)-piperidin-4-yl]-benzylamine. Solution phase HATU coupling of 3-ethynyl-benzoic acid,[16] isolated as a white amorphous solid. ^{1}H NMR (500 MHz): δ 7.76 (m, 2H), 7.40 (m, 2H), 7.25 (m, 2H), 7.10 (m, 4H), 4.95 (br m, 1H), 4.79 (s, 2H), 3.80 (br m, 1H), 3.10 (br m, 1H), 2.84 (br m, 1H), 2.78 (br m, 1H), 1.90 (m, 1H), 1.75 (m, 3H), 1.42 (s, 18H). MS(EI): 541 (M^++Na).

4.6.8. *N*,*N*-Bis-(*tert*-butoxycarbonyl)-3-[1-(3-phenylethynyl-benzoyl)-piperidin-4-yl]-benzylamine. A mixture of *N*,*N*-bis-(*tert*-butoxycarbonyl)-3-[1-(3-ethynyl-benzoyl)-piperidin-4-yl]-benzylamine (0.24 g, 0.46 mmol), iodobenzene (95 mg, 0.46 mmol), dichlorobis(triphenylphosphine)palladium(II) (35 mg, 0.05 mmol), copper(I) iodide (26 mg, 0.14 mmol), triethylamine (0.57 mL, 4.1 mmol) and anhydrous DMF (8 mL) was stirred at ambient temperature under nitrogen for 18 h. The solvent was removed under vacuum and the residue was partitioned between ethyl acetate (three lots of 50 mL) and water (20 mL). The combined organic layers were washed with brine (50 mL), then dried over magnesium sulfate and then concentrated under vacuum. The residue was subjected to chromatography on silica gel eluting with a mixture of cyclohexane and ethyl acetate (3:2, v/v) to give *N*,*N*-bis-(*tert*-butoxycarbonyl)-3-[1-(3-phenylethynyl-benzoyl)-piperidin-4-yl]-benzylamine as a yellow oil (0.23 g). ^{1}H NMR (500 MHz): δ 7.58 (m, 4H), 7.40 (m, 5H), 7.25 (m, 1H), 7.10 (m, 3H), 4.90 (br m, 1H), 4.79 (s, 2H), 3.80 (br m, 1H), 3.15 (br m, 1H), 2.84 (br m, 1H), 2.78 (br m, 1H), 1.95 (m, 1H), 1.80 (m, 3H), 1.42 (s, 18H). MS(EI): 617 (M^++Na).

4.6.9. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-(3-phenylethynyl-phenyl)-methanone hydrochloride (16d). A solution of *N*,*N*-bis-(*tert*-butoxycarbonyl)-3-[1-(3-phenylethynyl-benzoyl)-piperidin-4-yl]-benzylamine (100 mg, 0.17 mmol) in methanol (10 mL), cooled to 0 °C, was saturated with hydrogen chloride gas. The mixture was stirred at ambient temperature for 4 h then concentrated to dryness under vacuum. The residue was triturated with a mixture of dichloromethane and diethyl ether to **16d** as a white amorphous solid (46 mg). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.61–7.30 (m, 13H), 4.62 (br m, 1H), 4.00 (s, 2H), 3.62 (br m, 1H), 3.25 (br m, 1H), 2.85 (br m, 2H), 1.88 (br m, 1H), 1.70 (br m, 3H). MS(EI): 395 (M^++H).

4.6.10. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-(3-phenethyl-phenyl)-methanone hydrochloride (16e). A solution of *N*,*N*-bis-(*tert*-butoxycarbonyl)-3-[1-(3-phenylethynyl-benzoyl)-piperidin-4-yl]-benzylamine (110 mg, 0.18 mmol) in ethanol (10 mL) was treated with 10% palladium on carbon (20 mg) and then stirred at ambient temperature under an atmosphere of hydrogen for 8 h. The reaction mixture was filtered through a short pad of hyflo and then concentrated under vacuum to give a white amorphous solid (0.54 g). HCl deprotection gave the product as a white amorphous solid (25 mg). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.40–7.15 (m, 13H), 4.62 (br m, 1H), 4.00 (s, 2H), 3.60 (br m, 1H), 3.10 (br m, 1H), 2.80 (br m, 2H), 1.85 (br m, 1H), 1.60 (br m, 3H). MS(EI): 399 (M^++H).

4.6.11. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-(3-benzoxazol-2-yl-phenyl)-methanone hydrochloride (16g). Solution phase HATU coupling of 3-(benzooxazo-2-yl)-benzoic acid,[17] followed by HCl deprotection gave the product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.28 (m, 1H), 8.19 (s, 1H), 7.81 (m, 2H), 7.50 (m, 2H), 7.43 (m, 3H), 7.30 (m, 3H), 4.66 (br m, 1H), 4.00 (m, 2H), 3.70 (br m, 1H), 3.25 (br m, 1H), 2.92 (br m, 1H), 2.82 (br m, 1H), 1.90 (br m, 1H), 1.70 (br m, 3H). MS(EI): 412 (M^++H).

4.6.12. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-[3-((*E*)-styryl)-phenyl]-methanone hydrochloride (16f). Solution phase HATU coupling of 3-(2-*E*-phenylethenyl)-benzoic acid,[18] followed by HCl deprotection gave the product as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.71 (m, 1H), 7.63 (m, 3H), 7.50 (m, 2H), 7.40–7.25 (m, 9H), 4.63 (br m, 1H), 4.00 (s, 2H), 3.71 (br m,

1H), 3.10 (br m, 1H), 2.84 (br m, 2H), 1.88 (br m, 1H), 1.70 (br m, 3H). MS(EI): 397 (M^++H).

4.6.13. 3-[4-(3-Aminomethyl-phenyl)-piperidine-1-carbonyl]-1-benzopyran-2-one trifluoroacetate (16t). Solution phase HATU coupling of coumarin-3-carboxylic acid, followed by TFA deprotection gave the product as a yellow oil. LC–MS: t_R = 3.15 min (>86% by ELSD). MS(ES^+): 363 (MH^+).

4.6.14. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-naphthalen-2-yl-methanone trifluoroacetate (16r). Solution phase HATU coupling of 2-naphthoic acid, followed by TFA deprotection gave the product as a yellow oil. LC–MS: t_R = 3.66 min (100% by ELSD). MS(ES^+): 345 (MH^+).

4.6.15. [4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-phenylmethanone trifluoroacetate (16q). Solution phase HATU coupling of benzoic acid, followed by TFA deprotection gave the product as a yellow oil. LC–MS: t_R = 3.39 min (>95% by ELSD). MS(ES^+): 295 (MH^+).

4.6.16. [4-(3-Aminomethylphenyl)piperidin-1-yl]-(3,4-dichlorophenyl)methanone trifluoroacetate (16i). TFP resin coupling and TFA deprotection gave the product as an amorphous white solid. ^{1}H NMR (DMSO-d_6): δ 8.15 (br s, 3H), 7.72–7.69 (m, 2H), 7.40 (dd, 1H), 7.36–7.25 (m, 4H), 4.66–4.51 (m, 1H), 4.05–3.96 (m, 2H), 3.69–3.48 (m, 1H), 3.30–3.11 (m, 1H), 2.91–2.73 (m, 2H), 1.90–1.54 (m, 4H). MS(Ion spray): 363 and 365 (M^++1).

4.6.17. 1-[4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-1-(5-chloro-1*H*-indol-2-yl)-methanone trifluoroacetate (16l). Solution phase TBTU coupling of 5-chloro-1*H*-indole-2-carboxylic acid, followed by TFA deprotection gave the product as an amorphous white solid. ^{1}H NMR (DMSO-d_6): δ 11.78 (s, H); 8.14 (br s, 3H, NH_3^+), 7.63 (s, H), 7.43–7.22 (m, 5H), 7.16 (dd, H), 6.76 (s, H), 4.55 (br d, 2H), 4.00 (q, 2H), 3.14 (br s, 2H), 2.94–2.80 (m, H), 1.90–1.79 (m, 2H), 1.73–1.54 (m, 2H). MS(Ion spray): 368 and 370 (M^++1).

4.6.18. 6-{1-[4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-methanoyl}-2,3-dihydro-thiazolo[3,2-*a*]pyrimidin-5-one (16o). Solution phase TBTU coupling of using 5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylic acid, followed by TFA deprotection gave the product as an amorphous white solid. ^{1}H NMR [CD_3OD]: δ 7.97 (s, H), 7.44–7.29 (m, 4H), 4.76 (br d, H), 4.59–4.51 (m, 2H), 4.07 (s, 2H), 3.83 (br d, H), 3.69–3.57 (m, 2H), 3.41–3.21 (m, H), 3.02–2.85 (m, 2H), 2.05–1.67 (m, 4H). MS(Ion spray): 371 (M^++1).

4.6.19. 1-[4-(3-Aminomethyl-phenyl)-piperidin-1-yl]-1-benzo[*b*]thiophen-2-yl-methanone-trifluoroacetate (16s). Solution phase TBTU coupling of benzo(*b*)thiophene-2-carboxylic acid, followed by TFA deprotection gave the product as an amorphous white solid. ^{1}H NMR (CD_3OD): δ 7.98–7.81 (m, 2H), 7.62 (s, 1H), 7.45–7.32 (m, 5H), 7.31–7.22 (m, 1H), 4.80–4.40 (m, 2H), 4.10 (s, 2H), 3.28–3.05 (m, 1H), 3.02–2.90 (m, 2H), 2.02–1.88 (m, 2H), 1.86–1.65 (m, 2H). MS(Ion spray): 351 (M^++1).

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