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Original article

Synthesis, preliminary biological evaluation and molecular modeling of some new heterocyclic inhibitors of TACE

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ABSTRACT

Central heteroaryl ring analogues belonging to a series of potent hydroxamate TACE inhibitors were synthesized. The TACE inhibitory activities of these compounds were evaluated by *in vitro* WBA and *in silico* molecular modeling studies using crystal structure of human TACE. Compound **14** showed very good *in vitro* inhibition, supported by the *in silico* docking studies.

1. Introduction

Tumor necrosis factor-α (TNF-α) is a pleitropic, pro-inflammatory cytokine produced by macrophages, monocytes, neutrophils, T-cells, mast cells, epithelial cells, osteoblasts and dendritic cells [1,2]. Overexpression of TNF-α is responsible for a number of pathological conditions such as Crohn's disease, ulcerative colitis [3], diabetes [4], multiple sclerosis [5] and atherosclerosis [6]. In addition to this, there is compelling data to support the fact that TNF-α plays a pivotal role in the origin and progression of rheumatoid arthritis (RA) and other immune-mediated disorders [7–11].

The anti-TNF-α biological therapeutics etanercept, infliximab and adalimumab have demonstrated clinical success in inflammatory and autoimmune diseases and have validated the hypothesis of TNF-α modulation as a drug discovery paradigm [12].

TNF-α exists in two forms, the membrane-bound proform comprising 233 amino acids with a molecular mass of 26 kDa and the soluble form of 17 kDa comprising 157 nonglycosylated amino acids. It has been shown recently that the shredding of the biologically active TNF-α from its membrane-anchored proform is mediated by a metalloproteinase called TNF-α converting enzyme (TACE) [13] and inhibition of TACE blocks the release of TNF-α [14]. TACE is a member of the ADAM (a disintegrin and a metalloprotease domain) subfamily of the metzincin superfamily that also includes the astacins, serralysins and matrix metalloproteinases (MMPs).

Since broad-spectrum MMP inhibitors have been found to cause side effects in oncology clinical trials [15], it is desirable to develop selective small molecule TACE inhibitors for the long-term treatment of TNF-α mediated disorders.

As evident from literature [16], various motifs have been used for developing small molecule TACE inhibitors which include succinate based compounds, macrocyclic inhibitors, sulfonamide inhibitors, γ-lactam inhibitors, β-benzamido inhibitors and benzothiadiazepine inhibitors. Majority of the potential new chemical entities failed in the phase I clinical trials due to their poor pharmacokinetic profile or lack of efficacy [17]. Apratastat (TMI-005), developed by Wyeth as a broad spectrum TACE inhibitor [18], and BMS-566394, a potent and selective orally bioavailable inhibitor of TACE, developed by Bristol-Myers Squibb (BMS) [19] had advanced to phase II clinical trials, but failed to progress further due to either lack of efficacy or associated side effects [20,21]. Thus, there is a requirement to develop orally bioavailable and efficacious small molecule inhibitors of TACE lacking side effects. Herein, we present a report towards development of new potent small-molecule hydroxamate inhibitors of TACE.

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2. Chemistry

Researchers from BMS have reported a series of orally bioavailable β-benzamidohydroxamates, amongst which BMS-566394 demonstrated exceptional potency for inhibition of pTACE as well as in the suppression of LPS induced TNF-α in human whole blood [22]. The PCT Application WO 03/24899 which discloses BMS-566394 generically claims analogues wherein the central ring system is specifically a phenyl. Although a prior art cited in this PCT Application includes claims for 5/6-membered monocyclic heteroaryl central ring, neither their synthesis nor their biological activity is reported therein [23]. While the hydroxamic acid part of these molecules attached to the central phenyl ring is responsible for interaction with the zinc binding site of TACE, the group in the *para* position imparts selectivity as well as cellular potency. It appears that the central phenyl ring remains dormant and does not contribute to any binding interaction with the active site of TACE [19,24]. Though considered to be a benign component of the molecule for the purpose of molecular modifications of BMS-566394, we intended to replace the central phenyl ring hitherto with 5/6-membered monocyclic heteroaryl rings with the expectation to improve the overall pharmacokinetic profile of the molecule without compromising the binding affinity to the enzyme.

Hence we prepared compounds wherein the central phenyl ring was replaced with monocyclic heteroaryl rings, viz. pyridine, thiophene and furan. These β–benzamidohydroxamates were synthesized in an analogous manner as previously described [24], involving amidation of substituted benzimidazolylmethyl heteroaryl acids with carbocyclic/heterocyclic β–amino acid esters, followed by conversion of the ester to the corresponding hydroxamic acids.

Scheme 1 outlines the general synthesis of the central pyridyl ring containing β-benzamidohydroxamic acids **10**–**15**. The benzimidazolylmethylpyridyl acids **3a**–**b** were coupled with the carbocyclic/heterocyclic β–amino esters **8a**–**f** using EDC [*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide] and HOBt. The resulting amido esters **9a**–**f** were treated with an excess of hydroxylamine to give the hydroxamates **10**–**15**.

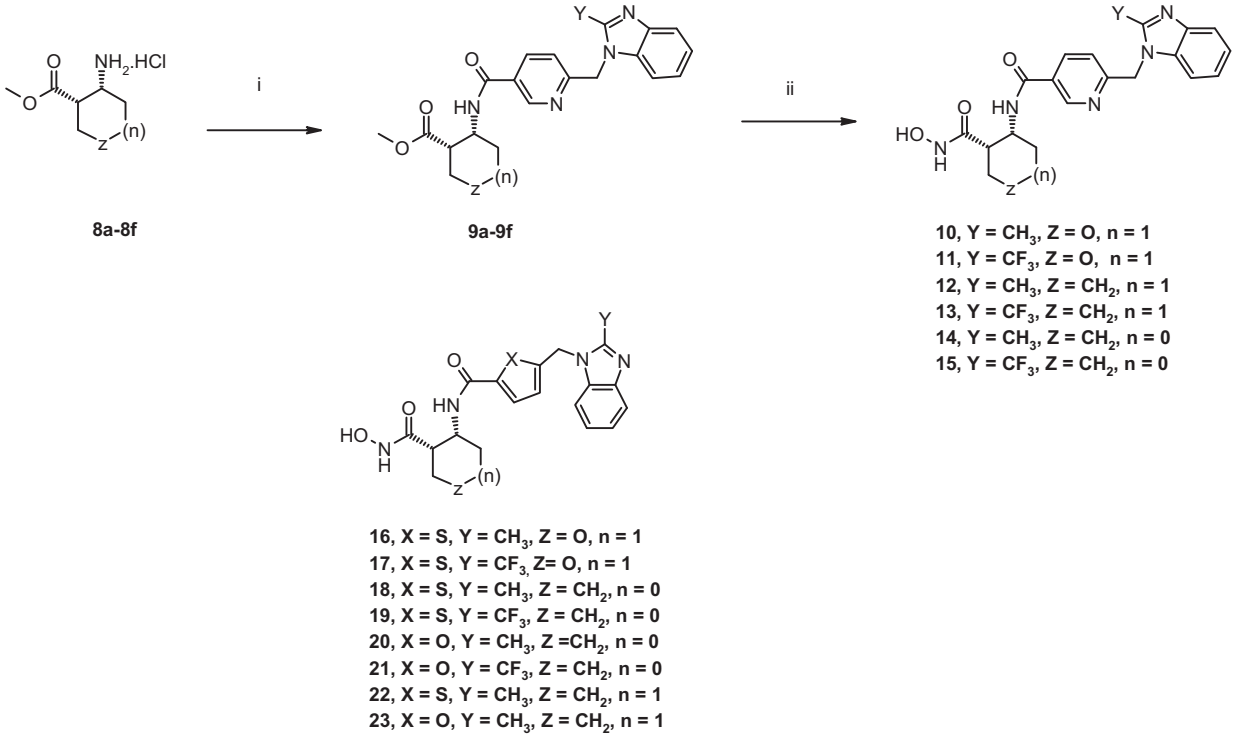
Similarly the central furan and thiophene ring containing β-benzamidohydroxamic acids **16**–**23** were prepared from the carbocyclic/heterocyclic β–amino esters **8a**–**f** and the acids **3c**–**f**.

The synthesis of the precursor benzimidazolylmethylheteroaryl acids for the proposed compounds is outlined in Scheme 2. Methyl 6-methylnicotinate **1** was photochemically chlorinated with *N*-chlorosuccinimide (NCS) to give ester **2**. Alkylation of **2** with 2-substituted benzimidazoles in presence of cesium carbonate in *N*,*N*-dimethylformamide provided the benzimidazole substituted esters which were saponified with sodium hydroxide in tetrahydrofuran-methanol to give the corresponding acids **3a**–**b**. The related furan and thiophene containing benzimidazolylmethylheteroaryl acids **3c**–**f** were analogously prepared.

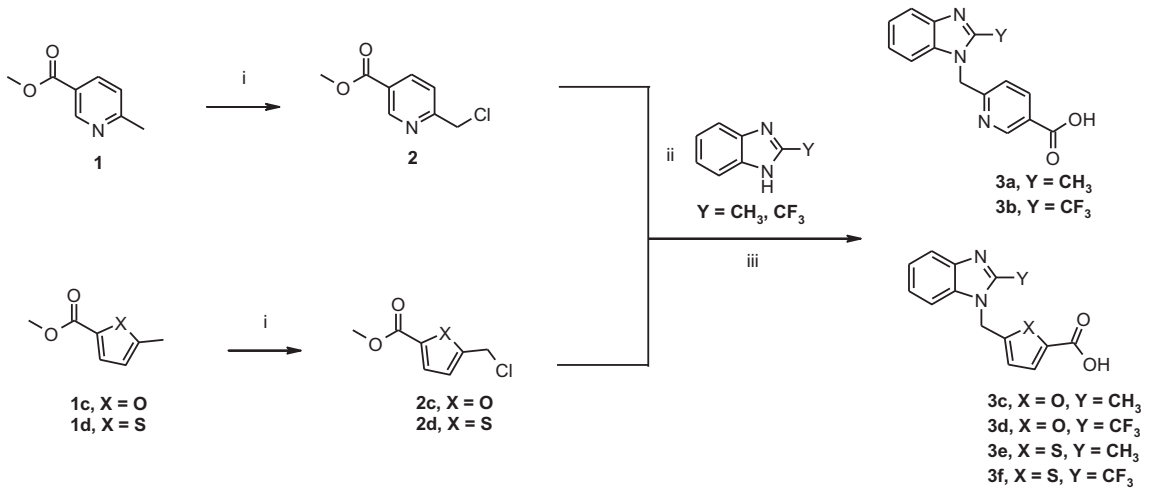
The β-keto esters **5a**–**f** were synthesized from the ketones **4a**–**f** using lithium hexamethyldisilazane (LHMDS), methyl cyanoformate and HMPA in THF at −78 °C. Condensation of β-ketoesters **5a**–**f** with (*R*)-α-methylbenzylamine in presence of ytterbium triflate afforded the corresponding enamine derivatives **6a**–**f**. Following a literature procedure [25], the enamines **6a**–**f** were reduced with sodium triacetoxyborohydride in acetonitrile and acetic acid at 0 °C to obtain **7a**–**f**, which upon hydrogenolysis using palladium hydroxide catalyst provided the desired carbocyclic/heterocyclic β–amino esters **8a**–**f** (Scheme 3).

3. Biological activity

The compounds prepared were tested *in vitro* by measuring cellular suppression of LPS-induced TNF-α using human whole blood assay (WBA) [26], and the inhibitory activities of these were compared with BMS-566394 (Table 1).



Scheme 1. Reagents and conditions: i) 3a–3b, EDC, HOBt, DMF; ii) NH_2OH, $NaOCH_3$, CH_3OH.



Scheme 2. Reagents and conditions: i) N-chlorosuccinimide, CCl_4; ii) Cs_2CO_3, DMF; iii) NaOH, THF-CH_3OH.

4. Molecular modeling studies

The synthesized compounds were energy minimized and docked in the active site of human TACE obtained from the Protein Data Bank (USA). The docking score (G score) for the potent compounds along with the experimentally determined IC_{50} values (from WBA assay) of the potent compounds is summarized in Table 2.

5. Results and discussion

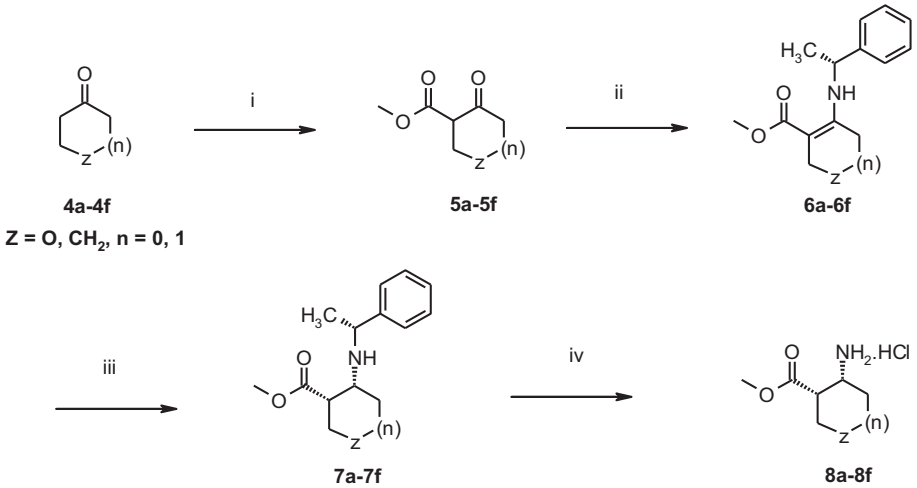
Tetrahydropyran containing β-benzamidohydroxamates **10** and **11** having central pyridyl ring proved to be better inhibitors than the corresponding cyclohexane analogues **12** and **13**. Similarly, substituting methyl group at 2-position of benzimidazole ring with trifluoromethyl group afforded higher inhibitory activity. The inhibitory potency increased substantially with cyclopentyl ring analogues **14** and **15**, either with methyl or trifluoromethyl groups at 2-position of the benzimidazole moiety.

The TACE inhibitory activity decreased remarkably for compounds **16**–**23** where the central six-membered pyridyl ring was replaced with five-membered thiophenyl and furyl ring systems, irrespective of changes that have been made in other parts of the molecule.

An alteration in the presumably dormant central ring system of the molecule leading to significant changes in the inhibitory activity was quite unexpected. Hence we undertook molecular modeling studies on the potent compounds **10**–**15** selected from Table 1 with human TACE enzyme so as to gather insight into their binding affinity.

Since compound **14** shows the highest binding score (G score −13.80) and is the most active compound in the series, its binding interactions within the binding site of the enzyme were extensively studied (Fig. 1).

The docking study for compound **14** suggested that the P1′ group (i.e. 2-methylbenzimidazol-1-ylmethyl moiety) is oriented towards the larger S1′ pocket of TACE and interacts with the active site residues (Ile438, Thr347 and Gly349). Oxygen atom of hydroxamic acid moiety of compound **14** binds with the catalytic zinc in the active site. Carbonyl group of hydroxamate interacts with His415 whereas the NH group interacts with Gly349, Leu350 and Glu406 active site residues (nitrogen of the hydroxamate interacts with carbonyl group of Glu406 and Gly349 and α-hydrogen of Leu350). Oxygen of the carbonyl group adjacent to



Scheme 3. Reagents and conditions: i) Methyl cyanoformate, LHMDS; ii) (*R*)-(α)-methylbenzylamine, Ytterbium (III) trifluoromethanesulfonate, benzene; iii) $NaBH(OAc)_3$, acetonitrile-acetic acid; iv) $H_2/Pd(OH)_2$-C, HCl, methanol.

Table 1
In vitro activity of the compounds for TNF-α inhibition.

Compound	% inhibition of TNF-α*	
	1 μM	0.3 μM
BMS-566394	77.78	66.26
10	80.09	72.45
11	83.07	66.40
12	78.10	66.48
13	64.23	40.61
14	84.90	80.53
15	81.20	77.98
16	58.50	24.90
17	3.98	0.00
18	0.00	0.00
19	55.80	29.20
20	3.26	9.57
21	3.50	7.06
22	0.00	0.00
23	6.40	13.20

*LPS induced TNF-α inhibition in WBA.

Table 2
In vitro TNF-α inhibition and docking scores for active compounds.

Compound	IC_{50} (μM)*	Glide score (G-score)
BMS-566394	0.130	−11.03
10	0.080	−12.54
11	0.160	−12.14
12	0.126	−12.41
13	0.280	−11.49
14	0.070	−13.80
15	0.074	−11.23

*LPS induced TNF-α inhibition in WBA.

the central pyridine ring of compound **14** interacts with the hydrogen of the NH group present on Gly349 and also with His415.

The nitrogen atom of the central pyridine ring (compound **14)** makes hydrogen bonding with the Val402 of active site residue (bond distance 2.54 Å). The α-hydrogen of Val402 interacts with the central pyridine ring and α-nitrogen of Val402 interacts with methyl group present on benzimidazole ring. Val434 interacts with the methylene group present between pyridine ring and benzimidazole ring. There is also a favorable hydrogen bonding between hydrogen of central pyridine and carbon of carbonyl group present on Ile438.

The most interesting outcome of the docking study is that central pyridine ring containing compound **14** (highest ranked compound in the present series) makes crucial hydrogen bonding with the α-hydrogen of Val402. Apart from this, three other vital interactions have been observed as mentioned below:

- Between central pyridine ring and carbon of the carbonyl group present on Glu406 (2.754 Å)
- Between the −NH of CONHOH and the oxygen of the carbonyl group of Gly349 (2.496 Å) and
- Between oxygen of the carbonyl group attached to the pyridine ring and the NH group of Gly349 (2.182 Å).

Briefly, the docking data suggests that the hydroxamic acid moiety of compound **14** binds with the zinc ion in the active site. Nitrogen present on the central pyridine ring interacts with the α-hydrogen of Val402 (2.54 Å) through hydrogen bond, which affords an extra stabilization to the compound **14**. Such stabilizing interaction however does not exist for compounds having central phenyl ring or five-membered heterocyclic rings (furan and thiophene). In fact, docking studies reveal that compounds having central five-membered rings (compounds **16**–**23**) adopt a pose in which the zinc binding group (hydroxamate) moves away from the catalytic zinc. This could probably be the reason for poor TACE inhibition of the compounds having five-membered central rings.

6. Conclusions

In summary, a new series of central heteroaryl containing hydroxamates as inhibitors of TACE have been synthesized. It was envisaged to make changes in the unexplored central phenyl ring of

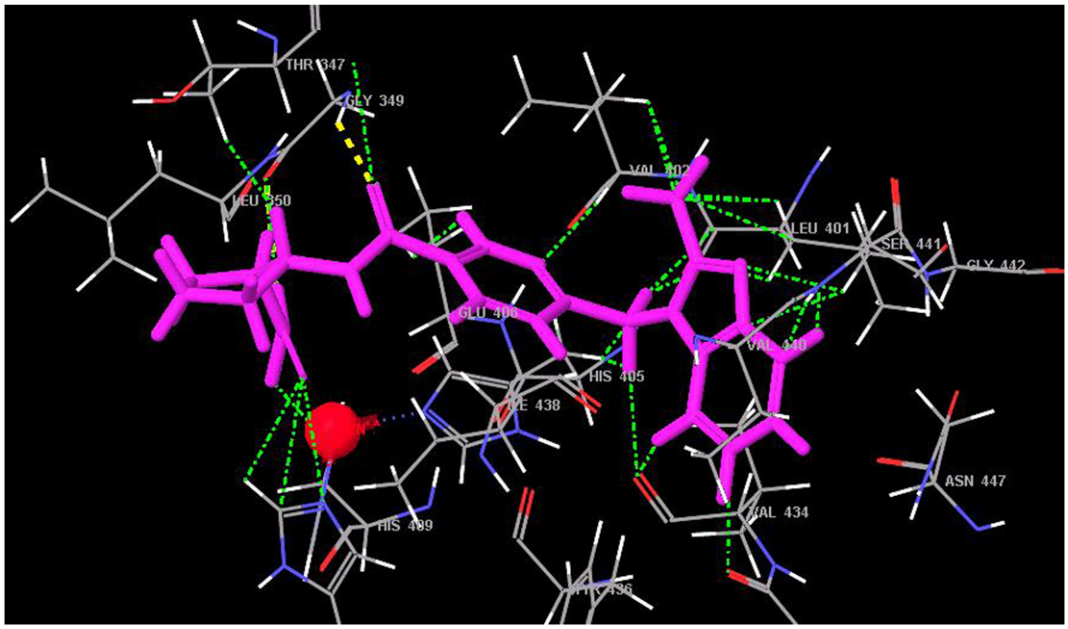


Fig. 1. Docking of compound **14** (shown in violet color) in the active site of TACE. Interactions are shown in green lines, zinc is shown in red color (for sake of clarity only important amino acids are given). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

BMS-566394 primarily with monocyclic five and six-membered aromatic heterocyclic rings viz. furan, thiophene and pyridine. The tetrahydropyranyl ring of the lead compound BMS-566394 was also replaced with five and six-membered carbocyclic rings. The synthesized compounds were evaluated by *in vitro* WBA for their TACE inhibitory activity. Some of the compounds (**10, 14** and **15**) showed promising TACE inhibitory activity.

In order to assess the role played by central heteroaryl ring of the synthesized compounds for binding to the active site, molecular modeling studies were carried out using crystal structure of human TACE obtained from protein data bank. Compound **14** having the highest activity was found to have an extra binding to the active site in the form of a hydrogen bond between the pyridine nitrogen and Val402. Compounds **16**–**23** possessing central five-membered heterocyclic rings were shown to have a "different pose" which disallowed interaction between the zinc-binding hydroxamate group and the catalytic zinc ion site in these modeling studies. This study throws new light on the role played by the central aromatic ring in benzamidohydroxamate series of compounds belonging to BMS-566394 which was hitherto unexplored. The *in silico* crystal structure of these new heterocyclic inhibitors bound to TACE enhances our understanding of the binding interactions with the TACE active site which can facilitate the structure-based design of inhibitors with improved potency for the treatment of disorders mediated by TNF-α.

7. Experimental

7.1. Chemistry

Melting points were determined in open cavities and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C in DMSO-d_6. The mass spectrum was obtained on a JEOL SX-102 mass spectrometer. Thin layer chromatography was performed on silica gel G (Merck). Column chromatography was performed with silica gel (100–200 mesh).

7.2. General procedure for the synthesis of acids ***3a–f***

A solution of ester (33 mmol), *N*-chlorosuccinimide (33 mmol) and benzoyl peroxide (0.82 mmol) in carbon tetrachloride (50 ml) was refluxed for 12 h and then filtered. The filtrate was washed with saturated solution of sodium metabisulfite, dried with sodium sulfate and concentrated under vacuum to afford the crude product. Flash chromatography (hexane/ethyl acetate, 8:2) provided the desired compound as colorless crystalline solid.

A solution of this chloromethyl ester (1.8 mmol) in *N,N*-dimethylformamide (4 ml) was treated with the 2-substituted benzimidazoles (1.8 mmol) and cesium carbonate (2.8 mmol). The mixture was stirred for 3 h at r.t. and partitioned with water and ethyl acetate (20 ml). The aqueous layer was further extracted with ethyl acetate (20 ml) and the combined organic layers washed with brine (20 ml), dried (sodium sulfate), filtered and concentrated. Flash chromatography with ethyl acetate provided the desired product as colored oil.

The compound obtained from the previous reaction was taken in tetrahydrofuran-methanol (1:1, 10 ml) and to this was added sodium hydroxide (10N, 0.4 ml) and stirred for 1 h at r.t. The reaction was concentrated under reduced pressure and the residue was acidified (pH 5–6) with dil. hydrochloric acid. The white solid was filtered, washed with water and dried on vacuum.

7.3. General procedure for the synthesis of amines ***8a–f***

A tetrahydrofuran solution of LHMDS (5.2 mmol) was added dropwise to a solution of the cyclic ketone (5.0 mmol) in tetrahydrofuran (20 ml) at −78 °C and the resulting solution was stirred at −20 °C for 1 h. The mixture was again cooled to −78 °C and methyl cyanoformate (6.0 mmol) was added dropwise. After 10 min, the reaction was quenched with aqueous ammonium chloride and extracted with ether. The organic layer was washed with brine, dried (sodium sulfate) and concentrated. Column chromatography on silica gel (ether-hexane, 1:4 to 3:2) yielded the ester as an oil.

The ester obtained from previous step (3.4 mmol) was dissolved in benzene (20 ml) and treated with (*R*)-α-methylbenzylamine (2.3 mmol) and ytterbium (III) trifluoromethanesulfonate (0.34 mmol). The mixture was heated to reflux under Dean–Stark conditions for 2 h, concentrated and purified by column chromatography on silica gel (ethyl acetate-hexane, 1:4) to yield the desired enamine as a white solid.

The enamine obtained (1.3 mmol) was taken in acetonitrile-acetic acid (1:1, 10 ml) and treated with sodium triacetoxyborohydride (1.3 mmol) and stirred for 2 h at 0 °C. Following concentration *in vacuo*, the residue was dissolved in ether, washed with saturated sodium bicarbonate until the aqueous phase was basic, dried (magnesium sulfate) and concentrated to yield an oil.

The intermediate (7 mmol) in methanol (10 ml) was treated with 10% palladium hydroxide on carbon (0.6 g) and aqueous 1N hydrochloric acid (10 ml) and hydrogenated for 72 h at r.t. The catalyst was removed by filtration; concentration of filtrate provided the desired amine as a hydrochloric acid salt.

7.4. General procedure for the synthesis of hydroxamic acids ***10–23***

The amine (**8a**–**f**) (0.7 mmol) and the acid (**3a**–**f**) (0.7 mmol) in *N,N*-dimethylformamide (2 ml) was treated with EDC (1.12 mmol) and 1-hydroxybenzotriazole (1.12 mmol) and stirred for 12 h at r.t. The reaction was quenched with saturated aqueous sodium bicarbonate and extracted with ethyl acetate, washed with water, dried and concentrated to furnish the desired ester.

Hydroxylamine hydrochloride (75 mmol) was suspended in dry methanol (10 ml) and a solution of potassium hydroxide (77 mmol) in methanol (10 ml) was added. The reaction mixture was stirred at r.t. for 30 min. Ester derivative (**9a**–**f**, 0.7 mmol) was added to the above reaction mixture, stirred at r.t for 3 h and quenched with ice-water (15 ml). The mixture was cooled to 0 °C, conc. hydrochloric acid was added dropwise until pH = 6 and extracted with chloroform. The organic extract was dried over anhydrous sodium sulfate and concentrated to afford an oil which upon trituration with ether afforded the desired products.

7.4.1. *N-[(3R, 4R)-3-(hydroxycarbamoyl)tetrahydro-2H-pyran-4-yl]-6-[(2-methyl-1H-benzimidazol-1-yl)methyl]nicotinamide (**10**)*

R$_f$: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.51 (s, 1H), 8.90 (s, 1H), 8.76 (s, 1H), 8.48 (d, 1H, *J* = 7.0 Hz), 8.15 (d, 1H, *J* = 7.5 Hz), 7.61–7.57 (m, 1H), 7.51–7.47 (m, 1H), 7.39 (d, 1H, *J* = 8.0 Hz), 7.20–7.16 (m, 2H), 5.67 (s, 2H), 4.33 (m, 1H), 4.07–3.98 (m, 1H), 3.83–3.72 (m, 1H), 3.68–3.55 (m, 3H), 2.21–2.10 (m, 1H), 1.72–1.64 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 170.23, 166.44, 159.37, 153.89, 149.53, 142.56, 137.31, 136.07, 130.64, 123.24, 123.02, 122.08, 118.78, 110.91, 67.37, 66.05, 48.97, 47.64, 42.64, 29.71, 13.67; ESI-MS (*m/z*): 410.1 $(M + H)^+$.

7.4.2. *N-[(3R, 4R)-3-(hydroxycarbamoyl)tetrahydro-2H-pyran-4-yl]-6-[(2-trifluoromethyl-1H-benzimidazol-1-yl)methyl] nicotinamide (**11**)*

R$_f$: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.50 (s, 1H), 8.81 (s, 1H), 8.75 (s, 1H), 8.49 (d, 1H, *J* = 7.3 Hz), 8.18 (d, 1H,

J = 6.8 Hz), 7.92 (d, 1H, J = 7.7 Hz), 7.75 (d, 1H, J = 7.8 Hz), 7.50–7.42 (m, 3H), 5.93 (s, 2H), 4.33 (m, 1H), 4.04–3.99 (m, 1H), 3.83–3.81 (m, 1H), 3.64–3.61 (m, 1H), 3.56–3.55 (m, 1H), 2.72–2.68 (m, 1H), 2.10–2.09 (m, 1H), 1.68–1.67 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 171.39, 167.69, 159.15, 150.02, 142.16, 140.18, 138.03, 131.44, 127.39, 125.69, 122.16, 122.09, 122.05, 113.08, 68.23, 67.14, 50.60, 48.61, 43.43, 30.11; ESI-MS (m/z): 464.43 $(M + H)^+$.

*7.4.3. N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclohexyl]-6-[(2-methyl-1H-benzimidazol-1-yl)methyl]nicotinamide (**12**)*

R_f: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.51 (s, 1H), 8.90 (s, 1H), 8.76 (s, 1H), 8.48 (d, 1H, J = 7.0 Hz), 8.15 (d, 1H, J = 7.5 Hz), 7.61–7.57 (m, 1H), 7.51–7.47 (m, 1H), 7.39 (d, 1H, J = 8.0 Hz), 7.20–7.16 (m, 2H), 5.67 (s, 2H), 4.33 (m, 1H), 4.07–3.98 (m, 1H), 3.83–3.72 (m, 1H), 3.68–3.55 (m, 3H), 2.21–2.10 (m, 1H), 1.72–1.64 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 170.06, 164.38, 158.26, 152.29, 148.59, 142.37, 136.31, 135.31, 129.72, 121.51, 121.28, 120.96, 118.20, 109.90, 48.22, 47.87, 41.23, 28.97, 24.91, 22.90, 21.71, 13.70; ESI-MS (m/z): 408.15 $(M + H)^+$.

*7.4.4. N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclohexyl]-6-[(2-trifluoromethyl-1H-benzimidazol-1-yl)methyl]nicotinamide (**13**)*

R_f: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.51 (s, 1H), 8.90 (s, 1H), 8.76 (s, 1H), 8.48 (d, 1H, J = 7.0 Hz), 8.15 (d, 1H, J = 7.5 Hz), 7.61–7.57 (m, 1H), 7.51–7.47 (m, 1H), 7.39 (d, 1H, J = 8.0 Hz), 7.20–7.16 (m, 2H), 5.67 (s, 2H), 4.33 (m, 1H), 4.07–3.98 (m, 1H), 3.83–3.72 (m, 1H), 368–3.55 (m, 3H), 2.21–2.10 (m, 1H), 1.72–1.64 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 170.04, 164.30, 156.99, 148.43, 140.45, 140.16, 139.86, 136.35, 135.95, 129.88, 125.43, 123.64, 120.81, 120.66, 120.12, 117.97, 112.06, 64.91, 48.82, 48.19, 41.27, 29.00, 24.82, 22.93, 21.55, 15.16; ESI-MS (m/z): 462.09 $(M + H)^+$.

*7.4.5. N-[(1S, 2R)-2-(hydroxycarbamoyl)cyclopentyl]-6-[(2-methyl-1H-benzimidazol-1-yl)methyl]nicotinamide (**14**)*

R_f: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.48 (s, 1H), 8.85 (s, 1H), 8.69 (s, 1H), 8.34 (d, 1H, J = 7.7 Hz), 8.11 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.0 Hz), 7.59–7.57 (m, 1H), 7.50–7.48 (m, 1H), 7.36 (d, 1H, J = 8.1 Hz), 7.19–7.17 (m, 2H), 5.65 (s, 2H), 4.45–4.40 (m, 1H), 2.74 (dd, 1H, J_1 = 15.3 Hz, J_2 = 7.7 Hz), 2.62 (s, 3H), 2.00–1.93 (m, 1H), 1.86–1.78 (m, 4H), 1.56–1.55 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 169.50, 164.28, 158.28, 152.29, 148.46, 142.37, 136.16, 135.31, 129.59, 121.53, 121.29, 120.95, 118.21, 109.91, 52.60, 47.87, 43.90, 31.57, 27.74, 22.56, 13.71; ESI-MS (m/z): 394.13 $(M + H)^+$.

*7.4.6. N-[(1S, 2R)-2-(hydroxycarbamoyl)cyclopentyl]-6-[(2-trifluoromethyl-1H-benzimidazol-1-yl)methyl]nicotinamide (**15**)*

R_f: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.47 (s, 1H), 8.76 (s, 1H), 8.68 (s, 1H), 8.35 (d, 1H, J = 7.8 Hz), 8.14 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.8 Hz), 7.91 (d, 1H, J = 7.7 Hz), 7.75 (d, 1H, J = 7.9 Hz), 7.50–7.42 (m, 3H), 5.92 (s, 2H), 4.47–4.40 (m, 1H), 2.73 (dd, 1H, J_1 = 15.2 Hz, J_2 = 7.7 Hz), 1.98–1.93 (m, 1H), 1.89–1.78 (m, 4H), 1.55–1.51 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 169.45, 164.21, 156.99, 148.28, 140.44, 140.15, 139.85, 136.71, 135.93, 129.80, 125.44, 123.64, 120.81, 120.64, 120.11, 117.96, 112.35, 52.60, 48.81, 43.94, 31.55, 27.69, 22.53; ESI-MS (m/z): 448.07 $(M + H)^+$.

*7.4.7. N-[(3R, 4R)-3-(hydroxycarbamoyl)tetrahydro-2H-pyran-4-yl]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]thiophene-2-carboxamide (**16**)*

R_f: 0.14 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.46 (s, 1H), 8.76 (s, 1H), 8.25 (d, 1H, J = 7.0 Hz), 7.73–7.58 (m, 2H), 7.26–7.19 (m, 2H), 7.16–7.15 (m, 1H), 5.72 (s, 2H), 4.19–4.18 (m, 1H), 4.01–3.97 (m, 1H), 3.87–3.84 (m, 1H), 3.63–3.59 (m, 1H), 3.53–3.49 (m, 1H), 2.66–2.64 (m, 1H), 2.62 (s, 3H), 2.25–2.23 (m, 1H), 1.61–1.58 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 168.26, 160.23, 151.41, 144.34, 142.32, 139.20, 134.72, 128.40, 126.81, 121.73, 121.53, 118.34, 109.99, 66.69, 64.85, 41.67, 28.81,13.55; ESI-MS (m/z): 415.1 $(M + H)^+$.

*7.4.8. N-[(3R, 4R)-3-(hydroxycarbamoyl)tetrahydro-2H-pyran-4-yl]-5-[(2-trifluoromethyl-1H-benzimidazol-1-yl)methyl]thiophene-2-carboxamide (**17**)*

R_f: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.47 (s, 1H), 8.75 (s, 1H), 8.29 (d, 1H, J = 7.0 Hz), 7.93–7.89 (m, 1H), 7.74–7.73 (m, 1H), 7.58–7.54 (m, 1H), 7.49–7.45 (m, 1H), 7.19–7.18 (m, 1H), 5.95 (s, 2H), 4.19 (m, 1H), 4.01–3.97 (m, 1H), 3.87–3.84 (m, 1H), 3.53–3.49 (m, 1H), 2.66–2.61 (m, 1H), 2.25–2.22 (m, 1H), 1.61–1.59 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 168.25, 160.16, 142.66, 140.48, 139.76, 139.07, 135.06, 128.30, 127.54, 125.75, 124.00, 121.04, 112.12, 66.69, 64.86, 43.36, 28.80; ESI-MS (m/z): 469.0 $(M + H)^+$.

*7.4.9. N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclopentyl]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]thiophene-2-carboxamide (**18**)*

R_f: 0.1 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.46 (s, 1H), 8.69 (s, 1H), 8.15 (d, 1H, J = 6.9 Hz), 7.63–7.58 (m, 3H), 7.26–7.19 (m, 2H), 7.14–7.13 (m, 1H), 5.71 (s, 2H), 4.31 (d, 1H, J = 7.0 Hz), 2.71–2.67 (m, 1H), 2.62 (s, 3H), 1.90–1.84 (m, 5H), 1.54–1.52 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 169.71, 160.20, 151.41, 144.36, 142.31, 139.48, 134.73, 128.03, 126.75, 123.73, 121.52, 118.33, 110.00, 52.74, 43.54, 41.66, 31.55, 28.04, 22.36, 13.55; ESI-MS (m/z): 399.10 $(M + H)^+$.

*7.4.10. N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclopentyl]-5-[(2-trifluoromethyl-1H-benzimidazol-1-yl)methyl]thiophene-2-carboxamide (**19**)*

R_f: 0.1 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.45 (s, 1H), 8.68 (s, 1H), 8.19 (d, 1H, J = 7.3 Hz), 7.93–7.90 (m, 2H), 7.65–7.64 (m, 1H), 7.58–7.54 (m, 1H), 7.49–7.45 (m, 1H), 7.17–7.16 (m, 1H), 5.94 (s, 2H), 4.34–4.27 (m, 1H), 2.72–2.67 (m, 1H), 1.92–1.79 (m, 5H), 1.56–1.52 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 169.69, 160.10, 142.35, 140.47, 140.00, 135.03, 127.90, 127.46, 125.72, 123.97, 121.01, 112.10, 52.75, 43.56, 43.32, 31.52, 28.02, 22.35; ESI-MS (m/z): 453.04 $(M + H)^+$.

*7.4.11. N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclopentyl]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]furan-2-carboxamide (**20**)*

R_f: 0.5 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.64 (s, 1H), 8.91 (s, 1H), 7.95 (d, 1H, J = 6.9 Hz), 7.70 (d, 1H, J = 7.7 Hz), 7.57 (d, 1H, J = 7.7 Hz), 7.28–7.18 (m, 2H), 7.07 (d, 1H, J = 3.3 Hz), 6.66 (d, 1H, J = 3.2 Hz), 5.56 (s, 2H), 4.31–4.24 (m, 1H), 2.74 (s, 3H), 2.72–2.68 (m, 1H), 2.00–1.70 (m, 6H); ^{13}C NMR (δ, DMSO-d_6): 170.02, 156.74, 151.93, 151.82, 147.54, 142.16, 134.82, 121.78, 121.54, 118.23, 114.10, 110.63, 110.13, 52.23, 43.14, 32.52, 28.33, 22.28, 13.55; ESI-MS (m/z): 383.13 $(M + H)^+$.

*7.4.12. N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclopentyl]-5-[(2-trifluoromethyl-1H-benzimidazol-1-yl)methyl]furan-2-carboxamide (**21**)*

R_f: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.61 (s, 1H), 8.84 (s, 1H), 8.04 (d, 1H, J = 8.2 Hz), 7.90 (d, 2H, J = 7.6 Hz), 7.60 (m, 1H), 7.47 (m, 1H), 7.09 (d, 1H, J = 2.8 Hz), 6.66 (d, 1H, J = 2.5 Hz), 5.79 (s, 2H), 4.31–4.28 (m, 1H), 2.72–2.67 (m, 1H), 1.92–1.76 (m, 5H), 1.60–1.50 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 169.88, 156.63, 150.40, 147.73, 140.32, 135.22, 125.83, 123.92, 120.87, 114.10, 112.32, 110.23, 52.26, 43.19, 41.13, 32.42, 28.16, 22.18; ESI-MS (m/z): 437.07 $(M + H)^+$.

*7.4.13. N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclohexyl]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]thiophene-2-carboxamide (**22**)*

R_f: 0.1 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.43 (s, 1H), 8.65 (s, 1H), 7.98–7.96 (m, 1H), 7.85–7.72 (m, 1H), 7.63–7.58

(m, 2H), 7.23 (d, 1H, $J = 7.5$ Hz), 7.14 (m, 1H), 5.71 (s, 2H), 4.02 (s, 1H), 2.62 (s, 3H), 2.38–2.23 (m, 1H), 2.13–2.04 (m, 1H), 1.96–1.88 (m, 2H), 1.68–1.53 (m, 2H), 1.49–1.37 (m, 2H), 1.29–1.14 (m, 1H); ^{13}C NMR (δ, DMSO-d_6): 170.13, 160.19, 151.40, 144.13, 142.32, 139.53, 134.71, 128.21, 126.74, 121.71, 121.50, 118.32, 109.99, 48.66, 41.67, 40.77, 28.59, 25.73, 22.43, 22.22, 13.54; ESI-MS (m/z): 413.09 $(M + H)^+$.

7.4.14. *N-[(1S, 2R)-3-(hydroxycarbamoyl)cyclohexyl]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]furan-2-carboxamide (**23**)*

R_f: 0.3 (CH_2Cl_2-methanol, 9:1); ^{1}H NMR (δ, DMSO-d_6): 10.57 (s, 1H), 8.83 (s, 1H), 8.04 (d, 1H, $J = 8.2$ Hz), 7.70 (d, 2H, $J = 7.3$ Hz), 7.57 (d, 1H, $J = 7.6$ Hz), 7.27–7.18 (m, 2H), 7.10 (d, 1H, $J = 3.1$ Hz), 6.67 (d, 1H, $J = 3.0$ Hz), 5.58 (s, 2H), 4.00 (m, 1H), 2.73 (s, 3H), 2.52 (m, 1H), 2.12–2.11 (m, 1H), 1.87–1.85 (m, 1H), 1.60–1.57 (m, 3H), 1.49–1.38 (m, 3H); ^{13}C NMR (δ, DMSO-d_6): 170.21, 156.61, 151.78, 151.73, 147.47, 142.14, 134.80, 121.68, 121.42, 118.17, 114.00, 110.43, 109.96, 47.83, 40.92, 28.76, 25.76, 22.59, 21.73, 13.41; ESI-MS (m/z): 397.12 $(M + H)^+$.

7.5. Biological screening

Blood was drawn from normal donors into tubes containing 143 USP units of heparin/10 ml. The heparinized human blood (225 μl) was plated directly into 1 ml sterile polypropylene tubes. Compounds were diluted in DMSO/serum-free medium and added to the blood samples so the final concentrations of the compounds were 50, 10, 5, 1, 0.5, 0.1 and 0.01 μM. The final concentration of DMSO did not exceed 0.5%. Compounds were preincubated for 15 min before the addition of 100 ng/ml LPS. Plates were incubated for 5 h in an atmosphere of 5% CO_2 in air. At the end of 5 h, 750 μl of serum-free medium was added to each tube and the samples were spun at 1200 rpm for 10 min. The supernatant was collected off the top and assayed for TNF-α production by a standard sandwich ELISA. The ability of the compounds to inhibit TNF-α production by 50% compared to DMSO-treated cultures was given by the IC_{50} value.

7.6. Molecular modeling studies

All the molecular modeling studies reported herein were performed on a Silicon Graphics Fuel Workstation running on the IRIX 6.5 operating system using SYBYL 6.9 molecular modeling software from Tripos, Inc., USA [27] and GLIDE from Schröndinger Inc., USA [28,29]. All compounds used for docking were built from the fragments in the SYBYL database, keeping the hydroxamic acid in its singly deprotonated form, and the carboxylate of the catalytic Glu 406 was protonated following the finding from the early publication of Cross et al. [30]. All compounds used for docking were built from the fragments in the SYBYL database. Each structure was fully geometry optimized using the standard Tripos force field employing the Gasteiger Huckel charges with a distance-dependent dielectric function until a root mean square deviation (RMSD) of 0.01 kcal/mol Å was achieved. Conformational search was carried out using MULTISEARCH option in SYBYL 6.9. The lowest energy conformer thus obtained was further minimized using the Tripos force field and was subsequently used in docking. As number of human crystal structures of TACE are available in the Protein Data Bank (USA), we have used crystal structure of human TACE complexed with IK682 (pdb code: 2FV5) since it is a highly tight binding hydroxamate inhibitor of TACE besides having chemical similarity with the synthesized compounds. Docking protocol was validated by docking IK682, which was built, minimized and docked in a similar manner like the compounds under study. Further it was superimposed on the co-crystallized structure of IK682 (extracted one) and the RMSD was found to be in the acceptable range. The bond orders and formal charges of IK682 were adjusted prior to docking. Formal charge of +2 was assigned to the zinc metal. Docking was performed using GLIDE software according to reported protocol [26,28].

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.09.018.

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