

RSC Advances



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

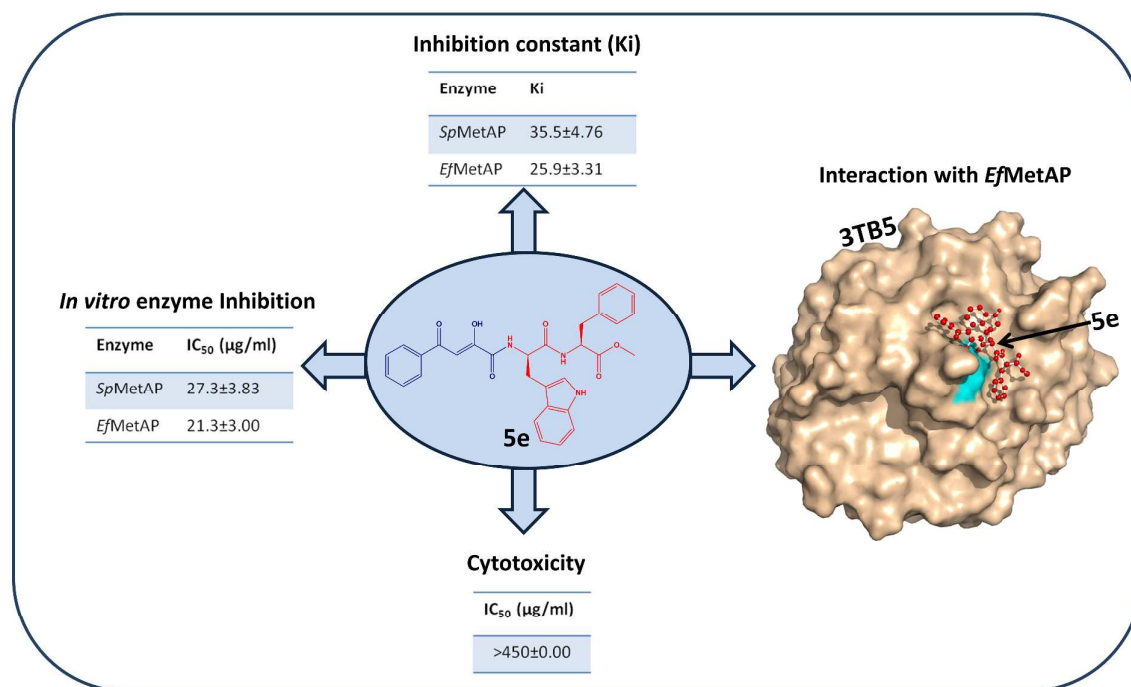
You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Diketo acids and their amino acid/dipeptidic analogues as promising scaffolds for the development of bacterial methionine aminopeptidase inhibitors

Mir Mohammad Masood^a, Vijay K. Pillalamarri^b, Mohammad Irfan^a, Babita Aneja^a, Mohamad Aman Jairajpuri^c, Md. Zafaryab^d, M. Moshahid A. Rizvi^d, Umesh Yadava^c, Anthony Addlagatta^b, Mohammad Abid^{a*},

Diketo acids and their peptidic analogues were designed and synthesised as bacterial MetAPs inhibitors. In the enzymatic assay, the representative compound **5e** showed excellent inhibition of bacterial MetAPs with no cytotoxicity.



Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Diketo acids and their amino acid/dipeptidic analogues as promising scaffolds for the development of bacterial methionine aminopeptidase inhibitors

Mir Mohammad Masood^a, Vijay K. Pillalamarri^b, Mohammad Irfan^a, Babita Aneja^a, Mohamad Aman Jairajpuri^c, Md. Zafaryab^d, M. Moshahid A. Rizvi^d, Umesh Yadava^e, Anthony Addlagatta^b, Mohammad Abid^{a*},

Received (in XXX, XXX) XthXXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXXXX 20XX

DOI: 10.1039/b000000x

Abstract: Using diketoesters as the template, various derivatives were designed and the selected compounds were synthesized as bacterial methionine aminopeptidase (MetAP) inhibitors. The results of *in vitro* antibacterial screening revealed fifteen compounds (**1a-c**, **1e-h**, **1j**, **1l**, **2a-c**, **3d**, **5c** and **5e**) as potent against different bacterial strains. By using MTT assay on human cell line (HepG2), the viability of cell proliferation was evaluated and nine compounds (**1c**, **1e**, **1j**, **1l**, **2a-b**, **3d**, **5c** and **5e**) showed no cytotoxic effect at the concentration range of 50-450 µg/ml. In the biochemical evaluation against methionine aminopeptidase (MetAPs) from *Streptococcus pneumonia* (*SpMetAP*), *Mycobacterium tuberculosis* (*MtMetAP*), *Enterococcus faecalis* (*EfMetAP*) and human (*HsMetAP*), compounds displayed differential behaviour against these four enzymes. Moreover, compounds **1g** showed 84% inhibition of *SpMetAP*, while compound **3d** selectively inhibit *MtMetAP* with 79% inhibition and little effect on *HsMetAP* at 100 µM concentration. At the same concentration, compound **5e** exhibited 87% and 85% inhibition of *EfMetAP* and *SpMetAP*, respectively. Understanding the mode of binding through modeling at the active site provided the structural basis for the possible mode of inhibition. Together, these data will be useful for further development of diketo acid based inhibitors with improved potency and selectivity.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

1. Introduction

Bacterial infections are responsible for some of the most deadly diseases and widespread epidemics in the world.^{1,3} Due to the rise in resistance of bacteria to current antibacterial chemotherapeutics, it is necessary to develop novel approaches and new inhibitors against resistant bacterial pathogens.^{1,3} To overcome this problem, new enzyme targets must be identified which can be targeted with small molecules. MetAPs represent one such important enzyme family, which is essential for bacteria.⁴ MetAPs cleave the initiator methionine from about 70% of all ribosome assisted newly synthesized proteins. The cleavage of *N*-terminal methionine by MetAP is an important event during protein synthesis and maturation.^{5,6} Since, the *N*-terminal methionine excision process is crucial for the metabolism and cell survival, MetAP is an ideal drug target for designing new inhibitors against bacterial pathogens.

MetAPs are first-row transition metallo-enzymes with five conserved metal ion-binding residues in the active site. Therefore, we have designed organic scaffolds that can serve as the metal chelators. Compounds with β -diketo pharmacophoric motif are believed to function as chelators of dinuclear Mn(II) or Mg(II) active site of HIV-1 integrase enzyme.⁷ Very recently, a diketo acid coupled with L-alanine methyl ester has been reported as *Ec*MetAP inhibitor.⁸ Considering the large pharmacological importance of β -diketo acids and in continuation to our efforts to explore novel biologically active molecules,^{9,10} in-house database of 201 compounds virtual library was screened against *Escherichia coli* MetAP (*Ec*MetAP). Based on this preliminary

data, diketoesters (**1a-m**), diketo acids (**2a-f**, **2h**, **2m**) were selected and synthesized in good yields. Selected diketo acids were also coupled with methyl ester of L-Ala, L-Phe and a dipeptide to get their novel amino acid/dipeptidic analogues (**5a-e**). All the synthetic compounds were well characterized using various spectroscopic techniques. Antibacterial activity was carried out against gram positive *Staphylococcus aureus* (*S. aureus*) and gram negative *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Salmonella typhimurium* (*S. typhimurium*). MIC and MBC values were calculated. The cytotoxicity by MTT assay on active compounds was also performed on a human cell line (HepG2). Most active compounds against bacteria were screened against *Sp*MetAP, *Mt*MetAP, *Ej*MetAP and *Hs*MetAP.

2. Results and discussion

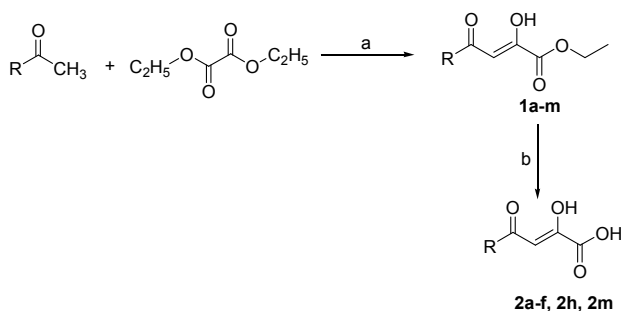
2.1. Screening using molecular docking

We modelled 201 diketo esters, acids and their amino acid/peptidic analogues and did a virtual screening using docking against *Ec*MetAP. Not all ligands showed binding and a great variation in the binding affinities was observed using Auto dock Vina¹¹ and Gilde docking.¹² Appreciable binding affinity in kcal/mol was observed for 14 compounds (**1a** (-6.6), **1b** (-6.1), **1c** (-6.5), **1e** (-7.0), **1f** (-7.0), **1g** (-6.2), **1h** (-6.4), **1j** (-7.0), **2a** (-7.1), **2b** (-8.1), **2c** (-7.0), **3d** (-6.4), **5c** (-6.7) and **5e** (-8.6). The main active site residues which take part in H-bonding interactions and ionic interactions are Glu204, His171, His178, His79, Asp97, and Asp108 while several residues were involved in hydrophobic interactions. Compounds showing good binding affinity and

interactions were further tested for antibacterial potential and their ability to inhibit various MetAPs.

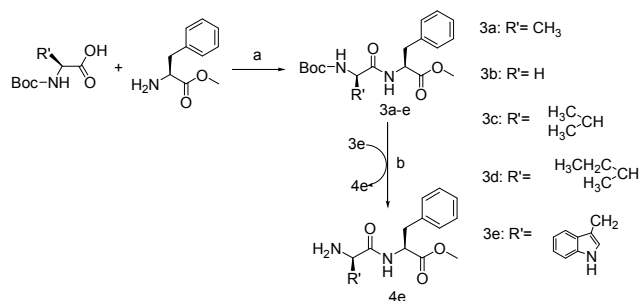
2.2. Chemistry

The synthesis of diketoesters (**1a-m**) and diketo acids (**2a-f**, **2h**, **2m**) was accomplished as outlined in Scheme 1. The oxalylolation of variously substituted aryl, heteroaryl or alicyclic methyl ketones by diethyl oxalate in the presence of freshly prepared sodium ethoxide afforded β -diketoesters (**1a-m**).¹³ These compounds were purified by column chromatography (petroleum ether/ethyl acetate: 9:1) to give desired compounds in moderate to high yields as confirmed by spectroscopic analysis (Table 1).



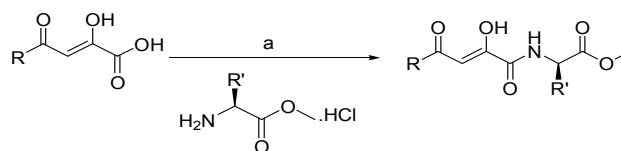
Scheme 1 Reagents and conditions: (a) Na metal, C₂H₅OH, 0 °C-r.t., 3-4 hr; (b) LiOH (2M), THF:H₂O, r.t., 2 hr.

Treatment of the selected β -diketoesters with freshly prepared lithium hydroxide solution (2M) in THF/H₂O mixture (1:4) for 2 hr at room temperature yielded corresponding β -diketo acids (**2a-f**, **2h**, **2m**) in quantitative yields (Table 2).¹⁴ All the acids obtained showed a single spot on TLC, therefore used without any further purification. Purity of the acids was also checked by elemental analyses and the structures were confirmed by FT-IR, ¹H, ¹³C-NMR and mass analyses. For the synthesis of dipeptides (**3a-e**), desired boc-protected amino acid was coupled with methyl ester of L-Phe in acetonitrile at room temperature using EDC.HCl as coupling reagent with HOBt and N-methyl morpholine (NMM) as a base (Scheme 2).¹⁵



Scheme 2 Reagents and conditions: (a) EDC.HCl, HOBt, NMM, CH₃CN, r.t., 2 hr; (b) DCM, TFA, r.t., 2 hr.

The crude product obtained was purified by column chromatography using ethyl acetate (30-40%) in petroleum ether to give pure dipeptides (**3a-e**) in quantitative yields. Boc group of a dipeptide (**3e**) was cleaved using TFA:DCM mixture (1:1). The reaction mixture was stirred for 2 hr at room temperature to give the desired dipeptide Trp-Phe-OCH₃ (**4e**) in quantitative yield which was used without further purification.¹⁶ As shown in Scheme 3, the coupling of diketo acids (**2a**, **2e**, **2h**, **2m**) with methyl ester of L-Ala/L-Phe was done in anhyd. DMF using PyBOP and HOBt in presence of triethyl amine to give novel amino acid conjugated to diketo acids (**5a-d**) in moderate to good yields.¹⁷ Compound **2a** was coupled with Trp-Phe-OCH₃ (**4e**) using the same methodology to give the dipeptidic analogue of diketo acid (**5e**) in excellent yield (Scheme 4).



	5a	5b	5c	5d
R	<i>p</i> -Cl-Ph	<i>o</i> -CH ₃ -Ph	<i>p</i> -OCH ₃ Ph	2,3-dihydrobenzo[<i>b</i>][1,4]dioxine
R'	CH ₂ -Ph	CH ₂ -Ph	CH ₃	CH ₃

Scheme 3 Reagents and conditions (a) HOBt, DMF, Et₃N, PyBOP, r.t., 24 hr.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Table 1 Structure of various diketoesters (1a-m)


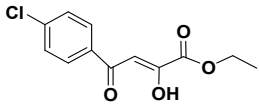

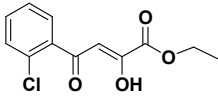

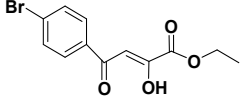

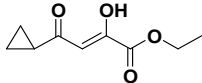

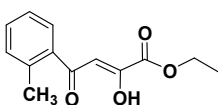

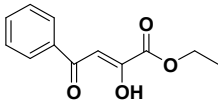

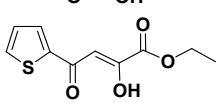

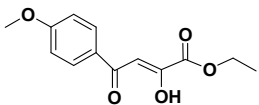

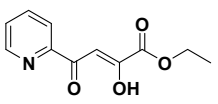

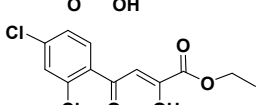

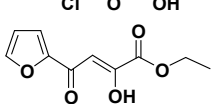

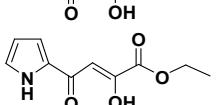

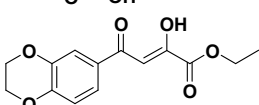

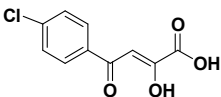

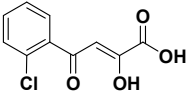

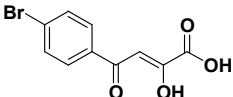

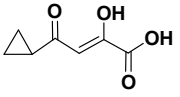

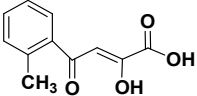

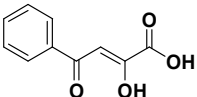
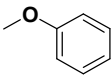
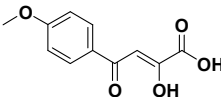
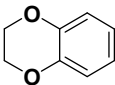
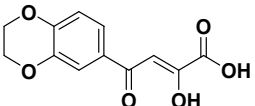
Compound	R	Chemical structure	Mol. formula	Ref.
1a			C ₁₂ H ₁₁ ClO ₄	[18]
1b			C ₁₂ H ₁₁ ClO ₄	[19]
1c			C ₁₂ H ₁₁ BrO ₄	[18]
1d			C ₉ H ₁₂ O ₄	-
1e			C ₁₃ H ₁₄ O ₄	[19]
1f			C ₁₂ H ₁₂ O ₄	[19]
1g			C ₁₀ H ₁₀ O ₄ S	[19]
1h			C ₁₃ H ₁₄ O ₅	-
1i			C ₁₁ H ₁₁ NO ₄	[19]
1j			C ₁₂ H ₁₀ Cl ₂ O ₄	-
1k			C ₁₀ H ₁₀ O ₅	[19]
1l			C ₁₀ H ₁₁ NO ₄	-
1m			C ₁₄ H ₁₄ O ₆	-

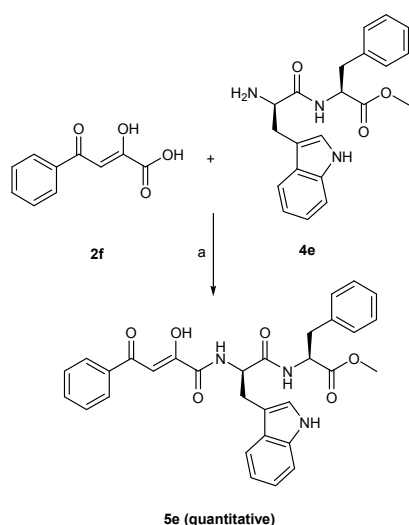
Table 2 Structure of various diketo acids (2a-f, 2h, 2m)

Compound	R	Structure	Mol. formula	Ref.
2a			C ₁₀ H ₇ ClO ₄	-
2b			C ₁₀ H ₇ ClO ₄	-
2c			C ₁₀ H ₇ BrO ₄	-
2d			C ₇ H ₈ O ₄	-
2e			C ₁₁ H ₁₀ O ₄	-
2f			C ₁₀ H ₈ O ₄	[20]
2h			C ₁₁ H ₁₀ O ₅	[20]
2m			C ₁₂ H ₁₀ O ₆	-

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE



Scheme 4 Reagents and conditions (a) HOBt, DMF, Et₃N, PyBOP, r.t., 24 hr.

Spectral analysis

The structure of β-diketoesters (**1a-m**) was in accordance to their spectroscopic and analytical data. In the IR spectrum, absorption bands in the region 1520-1605 cm⁻¹ suggested the presence of β-diketoesters in enolic form and the peaks in the region 1721-1739 cm⁻¹ corresponding to α, β-unsaturated ester also indicated the formation of β-diketoesters. In the ¹H-NMR spectrum, the proton adjacent to enolic hydroxyl group showed a sharp singlet in the range δ 6.48-7.57 ppm which confirmed that the β-diketoester is present in enolic form. The ¹³C-NMR spectral data were in good agreement with the assigned structures. The carbons of β-diketone group (in enolic form) exhibited chemical shift values in the range δ 189.22- 200.80 ppm and δ 180.38- 193.26 ppm. The mass spectra of all the compounds exhibited molecular ion peaks and contain fragments which further confirmed the formation of desired compounds. In case of diketo acids (**2a-f**, **2h**, **2m**), peaks

for the enolic form occur at their corresponding positions as in the case of β-diketoesters. But the disappearance of peaks for α, β-unsaturated ester group and the appearance of peaks in the region 3410-3494 cm⁻¹ (broad) and 1684-1709 cm⁻¹ corresponding to acid functionality and α, β-unsaturated acid indicate the conversion of diketoesters into their corresponding acids. All the compounds displayed either [M+H]⁺ or [M-H]⁻ corresponding to their molecular formulae. The coupling between diketo acid with amino acid (L-Ala-OCH₃/L-Phe-OCH₃) or dipeptide (Trp-Phe-OCH₃) can be monitored based on the disappearance of peaks corresponding to free amino and carboxylic groups. Two distinct bands were also observed in the region 1650- 1687cm⁻¹ and 1510-1570 cm⁻¹ related to -CONH for C=O stretching and N-H bending vibrations, respectively. In the ¹H-NMR spectra, broad singlet for the carboxylic group disappeared and peaks for the amino group was also absent while the aromatic protons as well as protons belonging to alicyclic system appeared at their usual chemical shift and integral values. The ¹³C-NMR spectra clearly showed coupling between diketoacids and amino acids or dipeptide as peaks corresponding to amide bond appeared at the expected chemical shift values δ 169.23-160.85 ppm. The mass spectra of some of the coupled compounds showed either [M+H]⁺ and [M-H]⁻ or [M-COOCH₃]⁺ and [M-COOCH₃]⁻ which further provided evidence for the formation of desired compounds.

2.3. Antibacterial activity

Agar well diffusion assay was carried out to access the susceptibility of one gram positive (*S. aureus* MTCC737) and three gram negative (*E. coli* MTCC739, *K. pneumoniae* MTCC109, and *S. typhimurium* MTCC98) bacterial strains.

Twenty two compounds that displayed significant zone of inhibition at 0.5 mg/ml concentration were further evaluated to calculate their MIC and MBC values against respective strains by micro dilution method. Ciprofloxacin was used as the positive control and the results are summarized in Table 3. Most of the synthesized compounds showed good antibacterial effect against *E. coli* and *S. aureus*. Among all the tested compounds, **1c** showed most potent antibacterial effect against all the strains with MIC value in the range 9.9-39.6 $\mu\text{g/ml}$. Compounds **1b**, **11**, **2a**, and **5c** selectively inhibited *E. coli* with MIC value of 9.9 $\mu\text{g/ml}$. Moreover **1a-c**, **1f-g**, **1j**, and **1l** also showed good activity against *S. aureus* with MIC value of 19.8 $\mu\text{g/ml}$. The **5e** was the only compound with MIC value of 19.8 $\mu\text{g/ml}$ against *S. typhimurium*. The MBC values of potent compounds were also calculated which showed their bactericidal nature.

Methionine aminopeptidase inhibition studies

Compounds with good antibacterial properties were screened for inhibition against four selected methionine aminopeptidases (MetAP's); *HsMetAP*, *SpMetAP*, *EfMetAP*, and *MtMetAP*. IC_{50} and K_i values were determined for the compounds showing greater than 70% inhibition of MetAP at 100 μM concentration (Tables 4 and 5). The results showed that the enzyme *SpMetAP* was inhibited by compound **5e** ($K_i = 35.5$) and **1g** ($K_i = 33.9$), *EfMetAP* was inhibited by compound **5e** ($K_i = 21.3$) and *MtMetAP* was inhibited by compound **3d** ($K_i = 56.9$).

In vitro cytotoxicity profile

To examine the toxicity of most active compounds based on MIC and MBC on cell proliferation, the cytotoxicity was checked by MTT assay on HepG2 cell line and the results are shown in Table 3. Nine compounds (**1c**, **1e**, **1j**, **1l**, **2a-b**, **3d**, **5c** and **5e**) were found to be non cytotoxic at concentration range 50-450 $\mu\text{g/ml}$. Compound **1g**, **1h** and **2c** showed cytotoxicity in the range of 195-

250 $\mu\text{g/ml}$. The effect of compounds on % viability of cell proliferation versus concentration shown in Figure 1, indicates toxicity at a very high concentration range to MIC and MBC values of the lead compounds in antibacterial assay. These results encouraged us to further examine enzymatic and *in vivo* studies to give a better lead in the antibacterial potential.

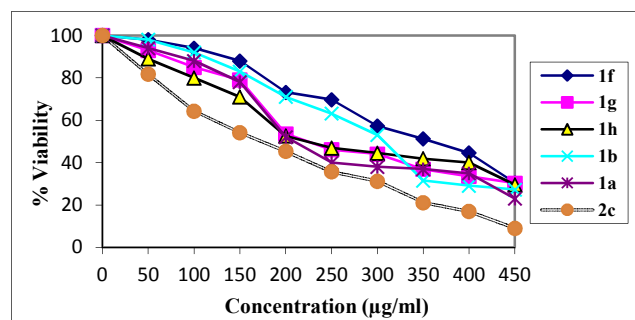


Figure 1 Cell Viability Assay on HepG2 Cell Line

Interactions of Lead molecules with MetAPs

Comprehensive inhibitory studies conducted on MetAPs with identified leads (**1g**, **3d** and **5e**) showed appreciable inhibition on purified proteins. We did a comprehensive docking of these leads with *MtMetAP*, *EfMetAP* and compared it with *HsMetAP*. The results shown in Figure 2 indicated high binding affinity and tight binding. Compound **1g** showed significant increase in the % inhibition with *SpMetAP*, however its inhibitory activity against *HsMetAP* and *EfMetAP* were comparable, consequent to this it also showed similar binding affinities. Compound **5e** was found with best binding affinity with all MetAPs but it showed significant increase in % inhibition with *EfMetAP* as compared to *HsMetAP*. It also showed a reduced IC_{50} value and a very low K_i . Significantly **5e** binds to three different sites on *EfMetAP*, *MtMetAP* and *HsMetAP*. Compound **3d** interacts with the active site residues (Ser109 and Val111) of *MtMetAP* and inhibits it with low IC_{50} (Table 6).

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Table 3 *In vitro* antibacterial activity (MIC and MBC) and cytotoxicity of synthesized compounds (in µg/ml)

Compound	<i>E. coli</i>		<i>S. aureus</i>		<i>K. pneumonia</i>		<i>S. typhimurium</i>		Cytotoxicity
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	IC ₅₀ ±S.D.
1a	>312.5	n.d.	19.8	78.1	156.2	>312.5	>312.5	n.d.	300±0.02
1b	9.9	9.9	19.8	78.1	>312.5	n.d.	>156.2	n.d.	300±0.01
1c	19.8	39.6	9.9	19.8	19.8	78.1	39.6	156.2	>450±0.0
1d	>312.5	n.d.	>312.5	n.d.	>156.2	n.d.	>312.5	n.d.	n.d.
1e	>312.5	n.d.	39.6	78.1	>156.2	n.d.	>156.2	>312.5	>450±0.0
1f	>312.5	n.d.	19.8	78.1	>312.5	n.d.	>312.5	n.d.	350±0.03
1g	39.6	156.2	19.8	39.6	39.6	>312.5	78.1	>312.5	250±0.03
1h	78.1	>312.5	39.6	>156.2	>312.5	n.d.	39.6	>156.2	200±0.01
1j	>312.5	n.d.	19.8	78.1	78.1	>312.5	>312.5	n.d.	>450±0.0
1l	9.9	19.8	19.8	19.8	>78.1	>312.5	39.6	156.2	>450±0.0
1m	>312.5	n.d.	>312.5	n.d.	312.5	n.d.	312.5	n.d.	n.d.
2a	9.9	19.8	39.6	39.6	156.2	312.5	39.6	156.2	>450±0.0
2b	39.6	312.5	78.1	>312.5	312.5	n.d.	>312.5	n.d.	>450±0.0
2c	19.8	78.1	78.1	>312.5	312.5	n.d.	>312.5	n.d.	195±0.02
2e	>312.5	n.d.	78.1	312.5	>312.5	n.d.	156.2	312.5	n.d.
3b	39.6	312.5	78.1	>312.5	>312.5	n.d.	156.2	>312.5	n.d.
3c	>312.5	n.d.	>312.5	n.d.	>312.5	n.d.	>312.5	n.d.	n.d.
3d	>312.5	n.d.	>312.5	n.d.	78.1	156.2	39.6	39.6	>450±0.0
5a	>312.5	n.d.	78.1	>312.5	>312.5	n.d.	>312.5	n.d.	n.d.
5c	9.9	19.8	>312.5	n.d.	>312.5	n.d.	>312.5	n.d.	>450±0.0
5d	312.5	n.d.	>312.5	n.d.	>312.5	n.d.	>312.5	n.d.	n.d.
5e	>312.5	n.d.	>312.5	n.d.	39.6	312.5	19.8	19.8	>450±0.0
Cip	<1	<1	<1	<1	<1	<1	<1	<1	n.d.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

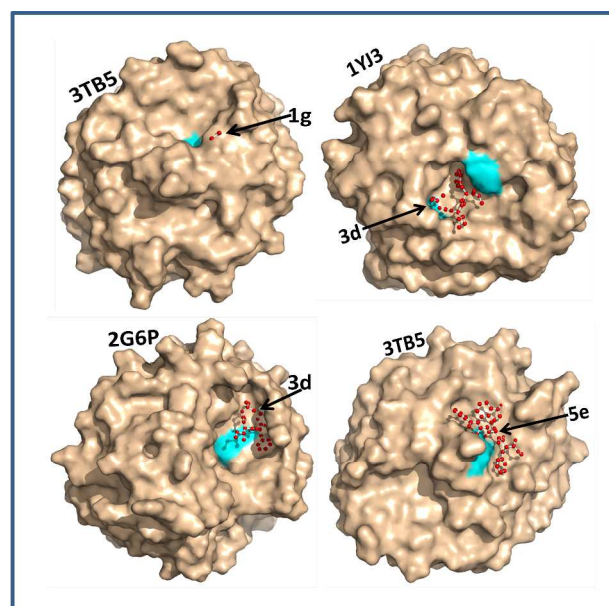
Table 4 Inhibition of different MetAPs (in % age) at 100 µg/ml concentration

Comp.	<i>Sp</i> MetAP	<i>Mt</i> MetAP	<i>Hs</i> MetAP	<i>Ef</i> MetAP
1a	52±0.32	33±0.3	54±3.58	34±1.23
1b	63±2.52	-29±2.21	59±3.71	26±26.22
1c	29±3.00	-12±3.76	19±5.54	20±0.75
1e	25±0.79	26±3.39	24±2.29	27±2.97
1f	37±0.91	47±2.39	40±3.20	32±2.93
1g	84±2.71	22±0.87	60±3.01	57±1.60
1h	68±2.24	29±2.54	50±4.15	27±2.80
1j	53±1.59	20±2.09	38±2.76	30±2.22
2a	35±0.45	-3±3.28	38±2.83	40±0.40
2b	30±1.23	-2±1.06	38±1.22	22±2.3
2c	49±0.82	4±2.22	40±4.41	31±2.04
3d	19±0.41	79±1.41	9±2.85	32±3.73
5c	41±1.52	49±5.32	39±1.71	55±2.63
5e	85±1.7	40±4.53	43±2.55	87±1.11

Table 5 IC₅₀ and Ki values of lead compounds against bacterial MetAPs

Compound	Protein	IC ₅₀	Ki
1g	<i>Sp</i> MetAP	28.7±3.97	33.9±2.44
3d	<i>Mt</i> MetAP	28.5±1.69	56.9±3.2
5e	<i>Sp</i> MetAP	27.3±3.83	35.5±4.76
5e	<i>Ef</i> MetAP	21.3±3.00	25.9±3.31

However in *Hs*MetAP, its binds away from the active site and there is significant reduction the % inhibition. Based on the binding affinity, MIC values, % inhibition, Ki and IC₅₀ values, **3d** and **5e** are significant lead and can be explored with much more specific synthetic designs around these two compounds.

**Figure 2** Interactions of **1g**, **3d** and **5e** with different MetAPs**Table 6** Docking score and interaction of lead with *Mt*MetAP (1YJ3), *Hs*MetAP (2G6P), and *Ef*MetAP (3TB5)

Protein	Ligand		
	1g	3d	5e
1YJ3	-5.4 kcal ⁻¹	-6.7 kcal ⁻¹	-7.5 kcal ⁻¹
	His212	Ala58, Arg164	Thr203
2G6P	-5.6 kcal ⁻¹	-6.9 kcal ⁻¹	-8.9 kcal ⁻¹
	Tyr196	His310	Glu128, Thr311, Ala312, Asn314
3TB5	-5.3 kcal ⁻¹	-5.8 kcal ⁻¹	-7.6 kcal ⁻¹
	His108	Arg28, Ser109, Val111, Tyr231	Glu251

3. Experimental protocols

3.1. Screening of ligands by Molecular docking studies

AutoDock Vina was used for screening of 201 designed compounds against *E. Coli* MetAP enzyme. The three dimensional coordinate (2MAT) of *EcMetAP* was retrieved from PDB library. AutoDock Vina 4.2 is advanced version which is much faster and accurate in binding mode prediction if compared with its older version AutoDock 4.0.¹⁰ Default parameters of AutoDock Vina were used with slight modification. The affinity grid box was centred to the whole protein and the grid spacing fixed to 1 Å. The program automatically calculates the grid map and grid centre coordinates (X = 44, Y = 46, Z = 44). After generating receptor and all ligands' PDB in PyMOL AutoDock/Vina plugin, Vina was ran to obtained docking score (binding affinity in kcal/mol) and RMSD value for all compounds. PyMOL was used for visualization of the polar contacts in between ligands and binding site of protein.²¹ The best docking result can be considered to be the conformation with the lowest docking score and lowest RMSD. The screening of ligands was also performed by glide docking using GLIDE 5.8 software with standard procedure.^{22,23}

3.2. Materials and methods

All the chemicals purchased from Sigma-Aldrich, Spectrochem and Hi Media were used without further purification. Precoated Aluminium sheets (Silica gel 60 F₂₅₄, Merck Germany) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. The IR spectra of compounds were taken on Agilent Cary 630 FT-IR spectrometer. ¹H and ¹³C NMR spectra were obtained at ambient temperature using Bruker Spectrospin DPX-300 MHz, 400 MHz, Agilent 500 MHz FT-NMR in CDCl₃ using tetramethylsilane (TMS) as an internal standard. Splitting

patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in parts per million (ppm) and coupling constants (*J*) in Hertz (Hz). Mass spectra were recorded on a Q Star XL hybrid electron spray ionization high resolution mass spectrometer (Applied biosystems) in a scan range of 100 to 1000 atomic mass units (amu). Melting points were recorded on a digital Buchi melting point apparatus (M-560) and were reported uncorrected. Purification of the compounds was done by column chromatography using silica gel (230-400 mesh size) with cyclohexane/ethyl acetate as eluent. The optical rotation of compounds **5a-e** was recorded on Anton Paar MCP-200 polarimeter at 20 °C using sodium D light.

3.3. General procedure for the synthesis of diketo esters (**1a-m**)

In a two neck round bottom flask, sodium metal (21 mmol) was dissolved in anhydrous ethanol at 0 °C to give sodium ethoxide solution. To this freshly prepared solution, a mixture of diethyl oxalate (20 mmol) and ketone (21 mmol) was added slowly with the help of dropping funnel over a period of 20 minutes. Thick precipitate was formed and reaction mixture was stirred for 3-4 hr at room temperature. After completion of the reaction checked by TLC, the precipitate obtained was dissolved in 2N sulphuric acid (72 ml) and the compound was extracted with diethyl ether, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The characterization of compounds **1a**, **1b**, **1c**, **1e**, **1f**, **1g**, **1i**, and **1k** is previously reported.^{18,19}

(*Z*)-Ethyl 4-(4-chlorophenyl)-2-hydroxy-4-oxobut-2-enoate (**1a**)

Yield: 72%.

(*Z*)-Ethyl 4-(2-chlorophenyl)-2-hydroxy-4-oxobut-2-enoate (**1b**)

Yield: 69%.

(Z)-Ethyl 4-(4-bromophenyl)-2-hydroxy-4-oxobut-2-enoate (1c)

Yield: 54%.

(Z)-Ethyl 4-cyclopropyl-2-hydroxy-4-oxobut-2-enoate (1d)Colourless oil, yield: 52%, R_f (cyclohexane:ethyl acetate = 7:3):

0.47, Anal ($C_9H_{12}O_4$) calc. C 58.69 H 6.57, found: C 58.72 H 6.54. IR (neat): 2948, 2872, 1719, 1678, 1655, 1579, 1508, 1430, 1350, 1322, 1290, 1207, 1130, 1065, 1013, 901, 879, 843, 767, 707 cm^{-1} . 1H -NMR (400MHz, $CDCl_3$) (δ , ppm): 14.62 (s, 1H, OH) 6.48 (s, 1H, CH), 4.34 (q, 2H, $J= 2.4$ Hz, CH_2), 1.92-1.86 (m, 1H, cyclopropyl ring), 1.38 (t, 3H, $J= 5.2$ Hz, CH_3), 1.24 (q, 2H, $J= 4.4$ Hz, cyclopropyl ring), 1.07 (q, 2H, $J= 4.4$ Hz, cyclopropyl ring). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 198.89, 185.24, 161.98, 99.76, 62.84, 14.03, 10.04. LC-MS: m/z $[M+H]^+$ 185.34.

(Z)-Ethyl 2-hydroxy-4-oxo-4-o-tolylbut-2-enoate (1e)

Yield: 78%.

(Z)-Ethyl 2-hydroxy-4-oxo-4-phenylbut-2-enoate (1f)

Yield: 43%.

(Z)-Ethyl 2-hydroxy-4-oxo-4-(thiophen-2-yl)but-2-enoate (1g)

Yield: 43%.

(Z)-Ethyl-2-hydroxy-4-(4-methoxyphenyl)-4-oxobut-2-enoate (1h)

Orange solid, M.pt.: 92 °C, yield: 55%, R_f (petroleum ether:ethyl acetate = 7:3): 0.60, Anal ($C_{13}H_{14}O_5$) calc. C 62.39 H 5.64, found: C 62.40 H 5.66. IR (neat): 2948, 2844, 2386, 2125, 1898, 1676, 1596, 1512, 1462, 1423, 1361, 1289, 1240, 1175, 1140, 1119, 1020, 851, 830, 778, 696 cm^{-1} . 1H -NMR (300MHz, $CDCl_3$) (δ , ppm): 7.99-7.90 (m, 2H, Ar-H), 7.18-7.08 (m, 2H, Ar-H), 6.91 (s, 1H, CH), 4.42 (q, 2H, $J= 6.9$ Hz, CH_2), 3.89 (s, 3H, OCH_3), 1.42 (t, 3H, $J= 7.2$ Hz, CH_3). ^{13}C -NMR (75MHz, $CDCl_3$) (δ ,

ppm): 196.94, 190.34, 168.06, 162.47, 130.61, 127.66, 114.21, 97.73, 62.50, 55.45, 14.09. LC-MS: m/z $[M+H]^+$ 251.2.

(Z)-Ethyl 2-hydroxy-4-oxo-4-(pyridin-2-yl)but-2-enoate (1i)

Yield: 63%.

(Z)-Ethyl 4-(2, 4-dichlorophenyl)-2-hydroxy-4-oxobut-2-enoate (1j)

Brown solid, M.pt.: 60 °C, yield: 70%, R_f (cyclohexane:ethyl acetate = 7:3): 0.86, Anal ($C_{12}H_{11}ClO_4$) calc. C 49.85 H 3.49, found: C 49.86 H 3.46. IR (neat): 3101, 2983, 1737, 1628, 1583, 1475, 1456, 1391, 1385, 1253, 1225, 1106, 1089, 1039, 1020, 883, 819, 778, 687 cm^{-1} . 1H -NMR (400MHz, $CDCl_3$) (δ , ppm): 14.94 (s, 1H, OH) 7.92 (d, 1H, $J= 8.4$ Hz, Ar-H), 7.76 (s, 1H, Ar-H), 7.68 (d, 1H, $J=8.4$ Hz, Ar-H), 7.57 (s, 1H, CH), 4.71 (q, 2H, $J= 7.2$ Hz, CH_2), 1.72 (t, 3H, $J= 7.2$ Hz, CH_3). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 194.78, 189.22, 161.88, 140.14, 136.76, 135.45, 133.12, 129.09, 128.73, 98.67, 63.57, 13.93. LC-MS: m/z $[M-H]^-$ 288.9.

(Z)-Ethyl 4-(furan-2-yl)-2-hydroxy-4-oxobut-2-enoate (1k)

Yield: 72%.

(Z)-Ethyl 2-hydroxy-4-oxo-4-(1H-pyrrol-2-yl)but-2-enoate (1l)

Black solid, M.pt.: 62 °C, yield: 68%, R_f (petroleum ether:ethyl acetate = 7:3) : 0.60, Anal ($C_{10}H_{11}NO_5$) calc. C 57.41 H 5.30 N 6.70 found: C 57.42 H 5.32 N 6.71. IR (neat): 3265, 3116, 1721, 1633, 1547, 1510, 1428, 1398, 1324, 1264, 1130, 1078, 1045, 1022, 974, 929, 843, 767, 750 cm^{-1} . 1H -NMR (400MHz, $CDCl_3$) (δ , ppm): 7.03 (d, 1H, $J= 6$ Hz, Ar-H), 6.91 (d, 1H, $J= 6.3$ Hz, Ar-H), 6.63 (s, 1H, CH), 6.29 (t, 1H, $J= 7.2$ Hz, Ar-H), 4.25 (q, 2H, $J= 7.23$ Hz, CH_2), 1.54 (t, 3H, $J= 7.23$ Hz, CH_3). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 200.80, 193.26, 161.67, 131.3, 128.9,

125.09, 113.74, 98.67, 61.87, 14.96. LC-MS: m/z $[M+2H]^+$ 211.3.

(Z)-Ethyl 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-hydroxy-4-oxobut-2-enoate (1m)

Light yellow oil, yield: 54%, R_f (cyclohexane:ethyl acetate = 7:3): 0.40, Anal ($C_{14}H_{14}O_6$) calc. C 60.43 H 5.07, found: C 60.48 H 5.10. IR: 2958, 2920, 2853, 1736, 1605, 1577, 1508, 1460, 1369, 1330, 1289, 1253, 1184, 1127, 1024, 912, 890, 819, 780, 726 cm^{-1} . 1H -NMR (300MHz, $CDCl_3$) (δ , ppm): 7.49 (s, 1H, CH), 7.21(s, 1H, Ar-H), 6.91 (d, 2H, $J=9$ Hz, Ar-H), 4.93 (q, 2H, $J=15.3$ Hz, CH_2), 4.21 (d, 4H, $J=17.7$ Hz, alicyclic ring), 1.22 (t, 3H, CH_3). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 190.93, 189.46, 161.24, 153.53, 148.37, 129.88, 121.90, 114.81, 99.84, 64.34, 61.71, 14.52. LC-MS: m/z $[M+H]^+$ 279.3, $[M-H]^-$ 277.2.

3.4. General procedure for the synthesis of diketo acids (2a-f, 2h, 2m)

To a solution of diketo ester (1.0 mmol) in THF/water mixture (1:4), freshly prepared 2M LiOH solution (4.5 mmol) was added with the help of a dropping funnel. The reaction was continued for 2 hr until white precipitate is obtained. After completion of the reaction, pH of the reaction was adjusted to 2-3 by adding 1N HCl. The compound was extracted with diethyl ether, dried over sodium sulphate, concentrated to give diketo acids (**2a-f**, **2h**, **2m**) in moderate to excellent yields (42-99%). The characterization of compounds **2f** and **2h** is previously reported.²⁰

(Z)-4-(4-Chlorophenyl)-2-hydroxy-4-oxobut-2-enoic acid (2a)

Light yellow solid, M.pt.: 102 °C yield: 75%, R_f (cyclohexane:ethyl acetate = 7:3): 0.0, Anal ($C_{10}H_7ClO_4$) calc. C 53.00 H 3.11, found C 53.01 H 3.09, IR (neat) : 3410, 2974, 2866, 1684, 1585, 1395, 1358, 1256, 1090, 1010, 956, 907, 823, 762, 620, 584, 519 cm^{-1} . 1H -NMR (400 MHz, $CDCl_3$) (δ , ppm):

15.07 (s, 1H, OH), 7.90 (d, 2H, $J=8.4$ Hz, Ar-H), 7.45 (d, 2H, $J=8.8$ Hz, Ar-H), 7.14 (s, 1H, CH). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 197.04, 189.24, 163.12, 138.96, 133.47, 129.27, 128.63, 97.99. LC-MS: m/z $[M+H]^+$ 227.2, $[M-H]^-$ 225.0.

(Z)-4-(2-Chlorophenyl)-2-hydroxy-4-oxobut-2-enoic acid (2b)

Light yellow solid, M.pt.: 154 °C, yield: 86%, R_f (cyclohexane:ethyl acetate = 7:3): 0.0, Anal ($C_{10}H_7ClO_4$) calc. C 53.00 H 3.11, found C 53.02 H 3.12 .IR : 3457, 2925, 1685, 1585, 1358, 1261, 1127, 1067, 1001, 823, 746, 475 cm^{-1} . 1H -NMR (300 MHz, Acetone- D_6) (δ , ppm): 8.05 (d, 1H, $J=8.4$ Hz, Ar-H), 7.93 (d, 1H, $J=8.7$ Hz, Ar-H), 7.79 (d, 1H, $J=8.4$ Hz, Ar-H) 7.71 (d, 1H, $J=8.7$ Hz, Ar-H), 7.15 (s, 1H, CH). ^{13}C -NMR (75MHz, Acetone- D_6) (δ , ppm): 196.32, 189.62, 162.38, 134.00, 132.26, 131.75, 130.06, 129.68, 128.34, 97.54. LC-MS: m/z $[M+H]^+$ 227.45, $[M-H]^-$ 225.18.

(Z)-4-(4-Bromophenyl)-2-hydroxy-4-oxobut-2-enoic acid (2c)

Orange oil, yield: 82%, R_f (cyclohexane:ethyl acetate = 7:3): 0.0, Anal ($C_{10}H_7BrO_4$) calc. C 44.31 H 2.60, found C 44.35 H 2.63 .IR: 3494, 1721, 1613, 1583, 1485, 1451, 1398, 1233, 1183, 1139, 1107, 1069, 1051, 903, 819, 773 cm^{-1} . 1H -NMR (300 MHz, Acetone- D_6) (δ , ppm): 8.15 (d, 2H, $J=8.7$ Hz, Ar-H), 7.31 (d, 2H, $J=8.7$ Hz, Ar-H), 7.02 (s, 1H, CH). ^{13}C -NMR (75MHz, Acetone- D_6) (δ , ppm): 205.03, 186.49, 162.24, 142.06, 136.02, 133.53, 129.09, 99.04. LC-MS: m/z $[M+H]^+$ 270.87.

(Z)-4-Cyclopropyl-2-hydroxy-4-oxobut-2-enoic acid (2d)

Light yellow oil, yield: 43%, R_f (cyclohexane:ethyl acetate = 7:3): 0.0, Anal ($C_7H_8O_4$) calc. C 53.85 H 5.16, found C 53.88 H 5.20 .IR : 3341, 2963, 2920, 2851, 1745, 1669, 1520, 1449, 1438, 1393, 1371, 1300, 1274, 1242, 1222, 1168, 1018, 979, 883, 754, 674 cm^{-1} . 1H -NMR (300 MHz, $CDCl_3$) (δ , ppm): 14.95 (s, 1H, OH), 6.50 (s, 1H, CH), 1.85-1.78 (m, 1H, cyclopropyl ring), 1.22

(q, 2H, $J = 4.6$ Hz, cyclopropyl ring), 1.08 (q, 2H, $J = 4.6$ Hz, cyclopropyl ring). $^{13}\text{C-NMR}$ (75MHz, CDCl_3) (δ , ppm): 198.54, 185.96, 161.39, 98.89, 43.09, 11.13. LC-MS: m/z $[\text{M-H}]^-$ 155.0.

(Z)-2-Hydroxy-4-oxo-4-o-tolylbut-2-enoic acid (2e)

5 Yellow oil, yield: 99%, R_f (cyclohexane:ethyl acetate = 5:5): 0.0, Anal ($\text{C}_{11}\text{H}_{10}\text{O}_4$) calc. C 64.07 H 4.89, found C 64.09 H 4.88. IR : 2931, 2507, 1709, 1596, 1492, 1456, 1387, 1233, 1169, 1127, 1028, 914, 832, 763, 728, 693 cm^{-1} . $^1\text{H-NMR}$ (400 MHz, Acetone- D_6) (δ , ppm): 7.66 (d, 1H, $J = 7.2$ Hz, Ar- H), 7.45 (t, 1H, $J = 7.2$ Hz, Ar- H), 7.33-7.26 (m, 2H, Ar- H), 6.93 (s, 1H, CH), 2.53 (s, 3H, CH_3). $^{13}\text{C-NMR}$ (75MHz, Acetone- D_6) (δ , ppm): 194.02, 187.52, 163.74, 138.60, 134.64, 132.46, 132.09, 129.21, 126.13, 100.75, 21.20. LC-MS: m/z $[\text{M-H}]^+$ 205.1.

(Z)-2-Hydroxy-4-oxo-4-phenylbut-2-enoic acid (2f)

15 Yield: 83%.

(Z)-2-Hydroxy-4-(4-methoxyphenyl)-4-oxobut-2-enoic acid (2h)

Yield: 71%.

(Z)-4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-2-hydroxy-4-oxobut-2-enoic acid (2m)

20 Lemon yellow oil, yield: 91%, R_f (cyclohexane: ethyl acetate = 5:5): 0, Anal ($\text{C}_{12}\text{H}_{10}\text{O}_6$) calc. C 57.60 H 4.03, found: C 57.55 H 4.01. IR: 3408, 2967, 2912, 2847, 1732, 1610, 1575, 1504, 1455, 1364, 1321, 1284, 1247, 1181, 1121, 1021, 908, 887, 816, 765, 723 cm^{-1} . $^1\text{H-NMR}$ (300MHz, CDCl_3) (δ , ppm): 7.55 (s, 1H, CH), 7.19 (s, 1H, Ar- H), 6.89 (d, 2H, $J = 8.8$ Hz, Ar- H), 4.33 (d, 4H, $J = 8.7$ Hz, alicyclic ring). $^{13}\text{C-NMR}$ (75MHz, CDCl_3) (δ , ppm): 189.97, 189.56, 163.24, 152.53, 148.74, 130.88, 122.90, 115.81, 114.90, 99.84, 64.54, 62.71, 14.52. LC-MS: m/z $[\text{M+H}]^+$ 251.97.

30 **3.5. General procedure for the synthesis of boc-dipeptides (3a-e)**

In a 50 ml round bottom flask, L-phenylalanine methyl ester hydrochloride (1.0 mmol) and desired Boc amino acid (1.2 mmol), were dissolved in acetonitrile (10 ml) and cooled to 0 °C. To this solution, NMM (1.2 mmol), HOBt (2.0 mmol) and EDC.HCl (1.2 mmol) were added in small portions. The reaction mixture was brought to room temperature and the progress of the reaction was checked by TLC. After completion of the reaction, solvent was removed under vacuum and ethyl acetate was added to the residue, washed with 10% citric acid solution, 5% sodium bicarbonate solution, water and then with brine and finally dried over anhydrous MgSO_4 . The organic layer was concentrated under vacuum. The dipeptide obtained was purified by column chromatography using cyclohexane:ethyl acetate (7:3) as eluent to obtain pure dipeptides in 83-97% yields.

Boc-Ala-Phe-OCH₃ (3a)

White powder, M.pt.: 80 °C, yield: 91%, R_f (cyclohexane:ethyl acetate = 7:3): 0.24, Anal ($\text{C}_{18}\text{H}_{26}\text{N}_2\text{O}_5$) calc. C 61.70 H 7.48 N 7.99, found C 61.72 H 7.46 N 7.80, IR (neat) : 3326, 2978, 2931, 1737, 1691, 1654, 1520, 1447, 1391, 1367, 1335, 1251, 1207, 1162, 1127, 1080, 1056, 1035, 968, 907, 871 cm^{-1} . $^1\text{H-NMR}$ (400MHz, CDCl_3) (δ , ppm): 7.29-7.22 (m, 3H, Ar- H), 7.10 (d, 2H, $J = 6.8$ Hz, Ar- H), 6.49 (s, 1H, NH), 4.85 (q, 1H, $J = 6$ Hz, CH), 4.12 (q, 1H, $J = 7.2$ Hz, CH), 3.72 (s, 3H, OCH_3), 3.12 (d, 2H, $J = 10.8$ Hz, CH_2), 1.43 (s, 9H, CH_3), 1.32-1.22 (d, 3H, $J = 4.2$ Hz, CH_3). $^{13}\text{C-NMR}$ (75MHz, CDCl_3) (δ , ppm): 172.84, 171.83, 154.90, 137.01, 129.09, 128.11, 126.53, 78.05, 53.39, 51.81, 49.51, 36.66, 28.16, 18.09. LC-MS: m/z $[\text{M+H}]^+$ 351.3, $[\text{M-H}]^+$ 349.3, $[\text{M-Boc}]^+$ 251.3.

60 **Boc-Gly-Phe-OCH₃ (3b)**

Colourless oil, yield: 92%, R_f (cyclohexane:ethyl acetate = 5:5) : 0.65, Anal ($C_{18}H_{26}N_2O_5$) calc. C 60.70 H 7.19 N 8.33, found C 60.72 H 7.18 N 8.34, IR (neat) : 3339, 2978, 2933, 1655, 1510, 1439, 1369, 1279, 1249, 1216, 1166, 1052, 1032, 944, 866, 747, 702 cm^{-1} . 1H -NMR (400MHz, $CDCl_3$) (δ , ppm): 7.29-7.26 (m, 3H, Ar-H), 7.09 (d, 2H, $J=7.2$ Hz, Ar-H), 6.45 (s, 1H, NH), 5.02 (s, 1H, NH), 4.89(m, 1H, CH), 3.82-3.76 (m, 2H, CH_2), 3.72(s, 3H, OCH_3), 3.11(d, 2H, $J=8.4$ Hz, CH_2), 1.44 (s, 9H, CH_3). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 171.69, 171.30, 155.73, 135.70, 129.23, 128.62, 127.16, 79.89, 53.12, 52.28, 37.99, 30.84, 28.30. LC-MS: m/z $[M+H]^+$ 337.4, $[M-H]^+$ 335.2.

***Boc-Val-Phe-OCH₃* (3c)**

White solid, M.pt.: 94 °C, yield: 84%, R_f (cyclohexane: ethyl acetate = 5:5): 0.74, Anal ($C_{20}H_{30}N_2O_5$) calc. C 63.47 H 7.99 N 7.40, found C 63.48 H 7.98 N 7.41, IR (neat) : 3343, 2963, 2929, 2874, 1745, 1669, 1518, 1449, 1371, 1300, 1274, 1244, 1222, 1168, 1121, 1082, 1018, 981, 938, 883, 804, 754, 702, 672 cm^{-1} . 1H -NMR (400MHz, $CDCl_3$) (δ , ppm): 7.31-7.20 (m, 3H, Ar-H), 7.11 (d, 2H, $J=6.8$ Hz, Ar-H), 6.26 (s, 1H, NH), 4.99 (s, 1H, NH), 4.88(t, 1H, $J=5.6$ Hz, CH), 3.88 (d, 1H, $J=8$ Hz, CH), 3.71(s, 3H, OCH_3), 3.17-3.07(m, 2H, CH_2), 1.44 (s, 9H, CH_3). 0.89 (d, 6H, $J=6.8$ Hz, CH_3). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 191.31, 156.43, 145.30, 137.90, 136.17, 133.07, 130.15, 128.69, 128.58, 122.16, 120.82, 118.03, 115.23. LC-MS: m/z $[M+H]^+$ 379.4, $[M-Boc]^+$ 279.4.

***Boc-Ile-Phe-OCH₃* (3d)**

White solid, M.pt.: 112 °C, yield: 98%, R_f (cyclohexane: ethyl acetate = 7:3): 0.71, Anal ($C_{21}H_{32}N_2O_5$) calc. C 64.26 H 8.22 N 7.14, found C 64.27 H 8.24 N 7.16, IR (neat) : 3343, 3328, 2965, 1743, 1667, 1514, 1454, 1447, 1367, 1277, 1235, 1168, 1102, 1048, 1022, 889, 752, 700 cm^{-1} . 1H -NMR (500MHz, $CDCl_3$) (δ , ppm): 7.29-7.25 (m, 3H, Ar-H), 7.11 (d, 2H, $J=9.84$ Hz, Ar-H),

6.35 (s, 1H, NH), 5.0 (s, 1H, NH), 4.88(q, 1H, $J=5.6$ Hz, CH), 3.88 (t, 1H, $J=5.6$ Hz, CH), 3.17 (s, 3H, OCH_3), 3.17(d, 2H, $J=5.3$ Hz, CH_2), 2.87 (m, 1H, CH), 1.44 (m, 11H, CH_3 , CH_2), 0.89-0.85 (m, 6H). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 171.62, 171.24, 155.61, 135.63, 129.21, 128.57, 127.12, 77.31, 59.20, 53.05, 52.28, 37.94, 37.15, 28.26, 24.60, 15.38, 11.36. LC-MS: m/z $[M+H]^+$ 393.6, $[M-Boc]^+$ 293.6.

***Boc-Trp-Phe-OCH₃* (3e)**

Brown solid, M.pt.: 110 °C, yield: 96%, R_f (petroleum ether:ethyl acetate = 7:3): 0.35, Anal ($C_{26}H_{31}N_3O_5$) calc. C 67.08 H 6.71 N 9.03, found C 67.10 H 6.72 N 9.04, IR : 3404, 3065, 2959, 2127, 1737, 1665, 1544, 1536, 1501, 1465, 1359, 1141, 1015, 855, 842, 801, 745, 726, 704 cm^{-1} , 1H -NMR (400MHz, $CDCl_3$) (δ , ppm): 8.08 (s, 1H, NH), 7.66 (d, 1H, $J=7.6$ Hz, Ar-H), 7.33 (d, 1H, $J=14.8$ Hz, Ar-H), 7.22-7.11 (m, 5H, Ar-H), 7.02(s, 1H, Ar-H), 6.80 (d, 2H, $J=6.8$ Hz, Ar-H), 6.20 (s, 1H, NH), 4.73 (m, 1H, CH), 4.49 (m, 1H, CH), 3.61 (s, 3H, OCH_3), 3.30-3.08 (m, 2H, CH_2), 2.94 (d, 2H, $J=5.6$ Hz, CH_2), 1.42 (s, 9H, CH_3). ^{13}C -NMR (75MHz, $CDCl_3$) (δ , ppm): 172.67, 171.98, 156.61, 139.86, 135.63, 129.64, 128.57, 127.32, 126.56, 122.57, 119.62, 111.42, 78.31, 56.20, 53.05, 51.28, 37.82, 31.15, 28.26. LC-MS: m/z $[M+H]^+$ 466.1, $[M-H]^+$ 464.1 $[M-Boc]^+$ 366.0.

3.6. Synthesis of *Trp-Phe-OCH₃* (4e)

To a solution of **3e** (1.0 mmol) in 5 ml of anhydrous dichloromethane was added 5 ml of trifluoroacetic acid and stirred the reaction mixture for 2 hr at room temperature. Evaporation to dryness of the mixture led to yellowish oil. This residue was triturated in ether and the precipitate was recovered as a white powder after drying in quantitative yield.

Scarlet brown solid, M.pt.: 108 °C, yield: 99%, R_f (petroleum ether: ethyl acetate = 7:3):0.42, Anal ($C_{21}H_{23}N_3O_3$) calc. C 69.02

H 6.34 N 11.50, found C 69.05 H 6.37 N 11.47, IR : 1726, 1687, 1655, 1524, 1453, 1426, 1359, 1343, 1292, 1244, 1166, 1099, 1045, 1013, 868, 778, 735, 704 cm⁻¹, ¹H-NMR (400MHz, CDCl₃) (δ, ppm): 8.35 (s, 1H, NH), 7.45 (d, 1H, *J* = 7.6 Hz, Ar-*H*), 7.31 (d, 1H, *J* = 8 Hz, Ar-*H*), 7.15 (s, 4H, Ar-*H*), 7.06 (t, 2H, *J* = 7.6 Hz, Ar-*H*), 6.96-6.90 (m, 2H, Ar-*H*), 6.74 (s, 1H, NH), 5.48 (s, 2H, NH₂), 4.60 (t, 1H, *J* = 6.8 Hz, CH), 4.28 (t, 2H, *J* = 7 Hz, CH), 3.63 (s, 3H, OCH₃), 3.25 (t, 2H, *J* = 7.6 Hz, CH₂), 2.99 (t, 2H, *J* = 5.6 Hz, CH₂). ¹³C-NMR (75MHz, CDCl₃) (δ, ppm): 172.89, 171.50, 157.10, 139.56, 136.23, 129.89, 128.91, 127.12, 126.31, 122.52, 120.62, 110.98, 56.45, 53.25, 51.37, 37.76, 31.23. LC-MS: m/z [M+ H]⁺ 366.0, [M- H]⁻ 364.0.

3.7. General procedure for the synthesis of amino acid/dipeptide analogues of diketo acids (5a-e)

To a solution of desired diketo acid (1.0 mmol) in anhydrous DMF under argon were added HOBT (2.0 mmol), PyBOP (1.2 mmol), L-amino acid methyl ester hydrochloride/dipeptide (4e) (1.0 mmol) and Et₃N (1.6 mmol) and the reaction mixture was stirred for 24 hr at room temperature. Solvent was removed and the residue was dissolved in 50 ml of ethyl acetate. The organic phase was washed with a solution of 10% citric acid, 5% NaHCO₃, water and finally with brine solution (each 50 ml). The organic phase then dried over Na₂SO₄ and evaporated to dryness. Purification by column chromatography on silica gel (cyclohexane:ethyl acetate = 7:3) provided desired compound in 70-99% yields.

(*S,Z*)-Methyl-2-(4-(4-chlorophenyl)-2-hydroxy-4-oxobut-2-enamido)-3-phenyl propanoate (5a)

Brown solid, M.pt.: 98 °C yield: 70%, R_f (cyclohexane : ethyl acetate = 5:5): 0.50, [α]_D²⁰ = -47.19 (c 0.45, C₂H₅OH), Anal (C₂₀H₁₈ClNO₅) calc. C 61.94 H 4.68 N 3.61, found C 61.89 H 4.72 N 3.64, IR (neat) : 1737, 1655, 1579, 1572, 1558, 1439,

1400, 1363, 1309, 1246, 1222, 1175, 1113, 1091, 1024, 1015, 775, 747, 702 cm⁻¹. ¹H-NMR (300MHz, CDCl₃) (δ, ppm): 10.83 (s, 1H, OH), 7.90 (d, 2H, *J* = 8.4 Hz, Ar-*H*), 7.70 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.44 (d, 2H, *J* = 8.7 Hz, Ar-*H*), 7.28 (t, 3H, *J* = 9.6 Hz, Ar-*H*), 6.96 (s, 1H, CH), 4.76 (t, 1H, *J* = 5.4 Hz, CH), 3.78 (d, 2H, *J* = 6.3 Hz, CH₂), 3.74 (s, 3H, OCH₃). ¹³C-NMR (75MHz, CDCl₃) (δ, ppm): 191.67, 189.95, 170.97, 166.56, 139.67, 135.41, 129.75, 129.25, 128.91, 128.69, 127.42, 53.23, 52.59, 37.20. LC-MS: m/z [M+ H]⁺ 388.95.

(*S,Z*)-Methyl-2-(2-hydroxy-4-(4-methoxyphenyl)-4-oxobut-2-enamido)-3-phenylpropanoate (5b)

Yellow oil, yield: 76%, R_f (cyclohexane : ethyl acetate = 5:5): 0.60, [α]_D²⁰ = -0.06 (c 0.75, C₂H₅OH), Anal (C₂₁H₂₁NO₆) calc. C 65.79 H 5.52 N 3.65, found C 65.83 H 5.58 N 3.67, IR (neat) : 2937, 2842, 1671, 1595, 1508, 1456, 1420, 1357, 1309, 1247, 1169, 1114, 1023, 956, 831, 753, 699, 566, 478 cm⁻¹. ¹H-NMR (300MHz, CDCl₃) (δ, ppm): 7.93 (d, 2H, *J* = 8.7 Hz, Ar-*H*), 7.24-7.15 (m, 5H, Ar-*H*), 7.04 (d, 2H, *J* = 8.7 Hz, Ar-*H*), 6.81 (s, 1H, CH), 4.45 (q, 1H, *J* = 7.6 Hz, CH), 3.84 (s, 3H, OCH₃), 3.76 (s, 3H, OCH₃), 3.54 (d, 2H, *J* = 5.6 Hz, CH₂). ¹³C-NMR (75MHz, CDCl₃) (δ, ppm): 196.30, 189.43, 172.02, 163.09, 139.07, 130.46, 129.88, 128.93, 127.53, 126.06, 113.81, 108.41, 55.50, 53.79, 51.83, 38.66. LC-MS: m/z [M+ H]⁺ 384.59.

(*Z*)-Methyl 2-(2-hydroxy-4-oxo-4-*o*-tolylbut-2-enamido)propanoate (5c)

Yellow oil, yield: 70%, R_f (cyclohexane:ethyl acetate = 5:5): 0.66, [α]_D²⁰ = -37.65 (c 0.80, C₂H₅OH), Anal (C₃₁H₂₉N₃O₆) calc. C 61.85 H 5.88 N 4.81, found C 61.86 H 5.89 N 4.82, IR (neat) : 2952, 2861, 2872, 1687, 1678, 1605, 1510, 1451, 1439, 1208, 761, 737 cm⁻¹. ¹H-NMR (400MHz, CDCl₃) (δ, ppm): 7.63 (d, 1H, *J* = 7.6 Hz, Ar-*H*), 7.42 (t, 1H, *J* = 7.6 Hz, Ar-*H*), 7.31-7.28 (m,

2H, Ar-*H*), 6.83 (s, 1H, *CH*), 4.39 (q, 1H, *J*= 7.6 Hz, *CH*), 3.57 (s, 3H, *OCH*₃), 2.55 (s, 3H, *CH*₃), 1.41 (t, 3H, *J*= 7.2 Hz, *CH*₃). ¹³C-NMR (125 MHz, CDCl₃) (δ, ppm): 191.80, 175.82, 172.48, 160.85, 138.10, 134.78, 131.89, 131.82, 129.11, 125.99, 98.28, 52.65, 48.17, 21.12, 18.19. LC-MS: m/z [M- COOCH₃]⁺ 235.2, [M- COOCH₃]⁻ 233.1.

(*Z*)-Methyl-2-(4-(2,3-dihydrobenzo[*b*]1,4)dioxin-6-yl)-2-hydroxy-4-oxobut-2-enamido)propanoate (5d)

Golden yellow oil, yield: 75%, R_f (cyclohexane:ethyl acetate = 5:5): 0.47, [α]_D²⁰ = -11.75 (c 0.40, C₂H₅OH), Anal (C₁₆H₁₇NO₇) calc. C 57.31 H 5.11 N 4.18, found C 57.37 H 5.14 N 4.15, IR (neat) : 2965, 2868, 1745, 1678, 1607, 1581, 1508, 1462, 1430, 1350, 1322, 1292, 1207, 1132, 1065, 1013, 901, 845, 767, 707 cm⁻¹. ¹H-NMR (400 MHz, CDCl₃) (δ, ppm): 7.51 (s, 1H, *CH*), 7.49(s, 1H, Ar-*H*), 6.93 (d, 2H, *J*= 8.7 Hz, Ar-*H*), 4.87 (q, 1H, *J*= 5.4 Hz, *CH*), 4.33 (d, 4H, *J*= 5.1 Hz, alicyclic ring), 3.59 (s, 3H, *OCH*₃), 1.26 (d, 3H, *J*= 5.4 Hz, *CH*₃). ¹³C-NMR (125 MHz, CDCl₃) (δ, ppm): 196.60, 189.47, 171.62, 166.56, 147.95, 143.21, 131.05, 122.39, 117.75, 115.88, 110.02, 64.62, 64.06, 48.54, 46.26, 18.61. LC-MS: m/z [M+H]⁺ 336.42.

(*Z*)-Methyl-2-(2-(2-hydroxy-4-oxo-4-phenylbut-2-enamido)-3-(1*H*-indol-3-yl) propanamido)-3-phenylpropanoate (5e)

Light yellow solid, yield: 99%, M.pt.: 73 °C, R_f (ethyl acetate : cyclohexane = 6:4): 0.59, [α]_D²⁰ = -16.47 (c 0.60, C₂H₅OH), Anal (C₃₁H₂₉N₃O₆) calc. C 69.00 H 5.42 N 7.79, found C 69.01 H 5.43 N 7.78, IR (neat) : 2926, 2853, 1743, 1655, 1601, 1510, 1458, 1439, 1343, 1253, 1214, 1182, 1078, 1028, 819, 775, 745, 702 cm⁻¹. ¹H-NMR (300 MHz, DMSO) (δ, ppm): 8.67 (d, 1H, *J*= 7.5 Hz, Ar-*H*), 8.51 (d, 1H, *J*= 8.4 Hz, Ar-*H*), 8.01 (d, 2H, *J*= 7.5 Hz, Ar-*H*), 7.68-7.54 (m, 4H, Ar-*H*), 7.33- 7.23 (m, 6 H, Ar-*H*, *NH*), 7.10-6.93 (m, 4H, Ar-*H*, *CH*), 4.68 (t, 1H, *J*= 5.7 Hz, *CH*) 4.54 (t,

1H, *J*= 6.3 Hz, *CH*), 3.57 (s, 3H, *OCH*₃), 3.15-2.97(m, 4H, *CH*₂). ¹³C-NMR (125 MHz, CDCl₃) (δ, ppm): 176.92, 170.19, 169.23, 159.28, 135.19, 134.87, 132.28, 132.14, 127.70, 127.50, 126.85, 126.03, 125.22, 122.32, 119.76, 117.10, 116.97, 110.02, 107.89, 92.78, 52.36, 52.19, 50.52, 35.56, 30.22. LC-MS: m/z [M+ H]⁺ 540.4, [M-H]⁺ 538.3.

3.8. Antibacterial Activity

In vitro bacterial susceptibility assay

In vitro antibacterial susceptibility of all the compounds against four different bacterial strains was determined by standard agar well diffusion assay.²⁴ Petri dishes (size 100 mm diameter) containing 18 ml of cool and molten Mueller Hinton Agar (MHA) (at 40 °C) were seeded with 50 μl inoculums of bacterial strain (inoculums size was adjusted so as to deliver a final inoculums of approximately 1.0 x 10⁵ CFU/ml). Wells of 6 mm diameter were cut into solidified agar media with the help of sterilized cork borer. An aliquot of 50 μl of each concentration was poured in the respective well and the plates were incubated at 37 °C for overnight. DMSO was used as negative control while ciprofloxacin (10 μg/ml) was used as positive control. The experiment was performed in triplicate under strict aseptic conditions. The antibacterial activity for each of the compound evaluated was expressed in terms of the average of the diameter of zone of inhibition (in mm) produced by the respective concentration of each compound at the end of incubation period.

Determination of MIC and MBC of the susceptible compounds

A micro broth dilution technique was employed to determine the MIC (minimum inhibitory concentration) of synthesized compounds along with positive (Ciprofloxacin) and negative (10% DMSO) controls.²⁵ The concentration of DMSO were maintained less than 10% in the final test volume. Various

concentrations (312.5... 2.475 µg/ml) of test compounds and (512.0... 1.0 µg/ml) of ciprofloxacin were dispensed into wells, then inoculated with test organisms with approx. 2.5×10^6 cells/ml (McFarland standard) and incubated at 37 °C for 24 hr. The effect of 10% DMSO was also checked on all the strains separately. The MIC values were determined as the lowest concentration resulting in no growth. The MBC of potent compounds were also determined by transferring of 10 µl aliquot on sterile MH agar plate from wells with no growth. The plates were incubated at 37 °C for 24 hr. After incubation, the MBC was determined as the lowest concentration of test compound that results no growth. All the experiments were done in triplicate in separate time.

Enzymatic inhibition assay

All compounds were dissolved in DMSO to a stock concentration of 10 mM. Initially the compounds were screened for inhibition with each of the four MetAP's (*HsMetAP*, *MtMetAP*, *SpMetAP*, *EfMetAP*). Expression, purification and biochemical assays are performed as reported previously.^{26,27} The enzyme assays were performed using Met-AMC as substrate. IC₅₀ values were determined by using compound concentration range of 1 µM to 120 µM. The Ki values were determined by Dixon method. The reaction mixture contained 50 mM HEPES (pH-7.5 for *SpMetAP*, *EfMetAP*, *MtMetAP* and pH-8.0 for *HsMetAP*), 150 mM KCl, CoCl₂ (three molar equivalents of the enzyme concentration), 4 µM enzymes (*HsMetAP*, *MtMetAP*, *SpMetAP* or *EfMetAP*) and 50, 100, 150 µM concentrations of L-Met-AMC. All reactions were performed in triplicate. 2, 2' Bipyridine was used as positive control.

In vitro cytotoxicity

HepG2 cells were cultured and maintained as a monolayer in Dulbecco's modified Eagle's medium (DMEM, Sigma)

supplemented with 10% of fetal calf serum (Sigma) and antibiotics (100 IU/ml of penicillin and 100 mg/ml of streptomycin, Sigma). All cells were cultured at 37 °C in a 100% humidity atmosphere and 5% CO₂. Exponentially growing viable cells were plated at 1.2×10^4 cells per well into 96-well plates and incubated for 48 hr before the addition of the compounds/metronidazole. Stock solutions of compounds were initially dissolved in 20% (v/v) DMSO and further diluted with fresh complete medium. The growth-inhibitory effects of the compounds were measured using standard tetrazolium MTT assay. After 48 hr of incubation at 37 °C, the medium was removed and 25 ml of MTT (5 mg/ml) in serum free medium was added to each well. The plates were incubated at 37 °C for 4 hr. At the end of the incubation period, the medium was removed and 100 µl DMSO added to all wells. The metabolized MTT product dissolved in DMSO was quantified by reading the absorbance at 570 nm with a reference wavelength of 655 nm in an ELISA plate reader. All assays were performed in triplicate. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

4. Conclusion

In this study, we successfully designed and synthesized some diketo acids, their amino acid/dipeptidic analogues as novel antibacterial agents targeting bacterial MetAPs. The results of *in silico* screening and *in vitro* antibacterial activity support the above findings and suggest their candidature to act as small molecule inhibitors of MetAPs. Compounds **1c**, **1e**, **1j**, **1l**, **2a**, **2b**, **3d**, **5c** and **5e** showed moderate to excellent antibacterial activity with no cytotoxic effect in the concentration range of 50-450 µg/ml. In the biochemical evaluation, compounds **1g** and **3d** showed 84% and 79% inhibition of *SpMetAP* and *MtMetAP*, respectively at 100 µM concentration. At the same concentration,

compound **5e** exhibited 87% and 85% inhibition of *EfMetAP* and *SpMetAP* respectively. Furthermore, efforts in modifying these and other lead structures with the aim of improving potency as well as specificity are in progress.

5 Conflicts of interest

All authors declare no competing interests.

Acknowledgement

Mohammad Abid gratefully acknowledges funding support in the form of Young Scientist from Science & Engineering Research Board (SR/FT/LS-03/2011), New Delhi, India. AA acknowledges the support from DST (SR/SO/BB-55/2008) and DBT (BT-BRB-TF-2-2011), India. VKP, MI and BA acknowledge the fellowship support from UGC, INDIA. The authors are thankful to Prof. Umar Farooq, Faculty of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Solan, H.P. India, for providing laboratory facilities for antibacterial activities.

Notes and References

^aMedicinal Chemistry Lab, Department of Biosciences, Jamia Millia Islamia (A Central University), Jamia Nagar, New Delhi 110025, India

^bCentre for Chemical Biology, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500607, India

^cProtein Conformation and Enzymology Lab, Department of Biosciences, Jamia Millia Islamia (A Central University), Jamia Nagar, New Delhi 110025, India

^dGenome Biology Lab, Department of Biosciences, Jamia Millia Islamia (A Central University), Jamia Nagar, New Delhi 110025, India

^eDepartment of Biochemistry 409 Ullman Building, Albert Einstein College of Medicine of Yeshiva University 1300 Morris Park Avenue, Bronx, NY

³⁰*Corresponding author: M. Abid, Email: mabid@jmi.ac.in, Phone: +91-8750295095, Fax: +91-11-2698 0229

Electronic Supplementary Information (ESI) available: [].

References

1. J. Chin, *New Sci.*, 1996, **2051**, 32-35.
2. R. A. Howe, K. E. Bowker, T. R. Walsh, T. G. Feest and A. P. MacGowan, *Lancet*, 1998, **351**, 602.
3. S. B. Levy, *Sci. Am.*, 1998, **278**, 32-9.
4. R. A. Bradshaw, W. W. Brickey and K. W. Walker, *Trends Biochem. Sci.*, 1998, **23(7)**, 263-267.
5. W. T. Lowther and B. W. Matthews, *Biochim. Biophys. Acta. (BBA)-Protein Structure and Molecular Enzymology*, 2000, **1477**, 157-167.
6. W. T. Lowther and B. W. Matthews, *Chem. Rev.*, 2002, **102**, 4581-4608.
7. C. Fossey, A. H. Vu, A. Vidu, I. Zarafu, D. Laduree, S. Schmidt, G. Laumond and A. M. Aubertin, *J. Enzym. Inhib. Med. Ch.*, 2007, **22**, 591-607.
8. E. Goemaere, A. Melet, V. R. Larue, A. L. Lieutaud, R. A. De Sousa, J. Chevalier, L. Yimga-Djapa, C. Giglione, F. Huguet and M. Alimi, *J. Antimicrob. Chemoth.*, 2012, dks058.
9. M. Abid, A. R. Bhat, F. Athar and A. Azam, *Eur. J. Med. Chem.*, 2009, **44(1)**, 417-425.
10. M. Irfan, B. Aneja, U. Yadav, S. I. Khan, N. Manzoor, C. G. Daniliuc and M. Abid, *Eur. J. Med. Chem.*, 2015, **93**, 246-254.
11. O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455-461.
12. L. L. C. Schrödinger, 2012, *New York, NY*.
13. D. W. Ribbons, *New J. Chem.*, 1999, **23**, 437-446.
14. R. N. Patel, L. Chu, R. Chidambaram, J. Zhu and J. Kant, *Tetrahedron-Asymmetr*, 2002, **13**, 349-355.
15. S. P. Chakrabarty, R. Ramapanicker, R. Mishra, S. Chandrasekaran and H. Balaram, *Bioorgan. Med. Chem.*, 2009, **17**, 8060-8072.
16. Y. Zhou, X. C. Guo, T. Yi, T. Yoshimoto and D. Pei, *Anal. Biochem.*, 2000, **280**, 159-165.
17. M. Rowley, *Progr. Med. Chem.*, 2008, **46**, 1-28.
18. D. Geffken, R. Soliman, F. S. G. Soliman, M. M. Abdel-Khalek and D. A. E. Issa, *Med. Chem. Res.*, 2011, **20**, 408-420.
19. S. S. Zimmerman, A. Khatri, E. C. Garnier-Amblard, P. Mullasseril, N. L. Kurtkaya, S. Gyoneva, K. B. Hansen, S. F. Traynelis and D. C. Liotta, *J. Med. Chem.*, 2014, **57**, 2334-2356.
20. B. Chen, H. F. Yin, Z. S. Wang, J. H. Xu, L. Q. Fan and J. Zhao, *Adv. Synth. Catal.*, 2009, **351**, 2959-2966.
21. W. L. Delano, DeLano Scientific, San Carlos, CA, USA, 2002.
22. R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley and J. K. Perry, *J. Med. Chem.*, 2004, **47**, 1739-1749.
23. L. L. C. Schrodinger, *New York, NY: Schrodinger, LLC*.
24. C. Perez, M. Pauli and P. Bazerque, *Acta Biol. Med. Exp.*, 1990, **15**, 113-115.
25. M. A. Wikler, Performance standards for antimicrobial susceptibility testing: Seventeenth informational supplement, Clinical and Laboratory Standards Institute, 2007.
26. C. Kishor, T. Arya, R. Reddi, X. Chen, V. Saddanapu, A. K. Marapaka, R. Gumpena, D. Ma, J. O. Liu and A. Addlagatta, *J. Med. Chem.*, 2013, **56** (13), 5295-5305.
27. T. Arya, C. Kishor, V. Saddanapu, R. Reddi, and A. Addlagatta, *PLoS One*, 2013, **8(10)**, -75207.