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Design, synthesis and evaluation of novel metalloproteinase inhibitors based on L-tyrosine scaffold

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ABSTRACT

A series of novel L-tyrosine derivatives were designed, synthesized and assayed for their inhibitory activities on matrix metalloproteinase 2 (MMP-2) and histone deacetylase 8 (HDAC-8). The results showed that these L-tyrosine derivatives exhibited inhibitory profiles against MMP-2 and HDAC-8. The compounds **6h** (IC_{50} = 0.013 ± 0.001 μM) and **6j** (IC_{50} = 0.017 ± 0.001 μM) were equal potent MMP-2 inhibitors to the positive control NNGH (IC_{50} = 0.014 ± 0.001 μM). As for HDAC-8 inhibition, some of the hydroxamate compounds, such as **6d** (IC_{50} = 3.6 ± 0.2 μM) and **6c** (IC_{50} = 5.8 ± 0.5 μM), were equal potent to the positive control SAHA (IC_{50} = 1.6 ± 0.1 μM). Structure–activity relationships were also briefly discussed.

1. Introduction

The matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endoproteinases that degrade and remodel structural proteins in the extracellular matrix.[1] They include more than 20 subtypes, among which MMP-2 is highly involved in the process of tumor invasion and metastasis and has been considered as a promising target for cancer therapy.[2,3]

It has been reported that besides the catalytic activity center zinc (II) ion of MMP-2, there are two hydrophobic domains, which are called S1′ pocket and S1 pocket, respectively. S1′ pocket, the key domain of MMP-2, is deeper and narrower than that of most other MMP subtypes, and S1 pocket is solvent exposed.[4,5] Currently identified MMP-2 inhibitors shared the following structural character and binding mode: (1) a zinc binding group (ZBG, such as hydroxamate and carboxylate) capable of chelating the active site zinc ion; (2) one at least functional group which provides a hydrogen bond interaction with the enzyme backbone; (3) one or more side chains which undergo effective interactions with the enzyme subsites, such as S1′ pocket and S1 pocket.[6,7]

In our previous work, the L-hydroxyproline scaffold-based MMP-2 inhibitors were studied, and some active compounds were reported in the literature.[8–10] Some of these compounds (see Fig. 1) showed low nanomolar inhibitory activity for MMP-2. In order to identify more potent MMP-2 inhibitors, using scaffold hopping strategy, we replaced L-hydroxyproline with L-tyrosine scaffold to form the new integrated structural pattern (see Fig. 1). R_1 group can be various alkyl groups, such as benzyl and phenylethyl group. R_2 group can be various acyl groups, such as benzoyl, acetyl or methylsulfonyl group. COR_3 group can be hydroxamate, carboxylate or carboxyl group. R_1 group and R_2 group might occupy the S1′ and S1 pockets, respectively, while the COR_3 group might chelate the active site zinc ion. The tyrosine scaffold might bond to the enzyme backbone.

2. Results and discussion

The target compounds were synthesized via the route as shown in Scheme 1. Starting from N-Boc-L-tyrosine methyl ester (**1**) as a chiral template, the important intermediate (S)-methyl 2-amino-3-(4-alkyloxyphenyl)propanoate (**3a–b**) was prepared via etherization[11] and deprotection of Boc by CF_3COOH.[12] Acylation of **3a–b** with various acyl chlorides produced compounds **4a–j**.[13] Further hydrolysis of **4a–j** provided the carboxyl acid compounds **5a–j**,[10] and ammonolysis of **4a–j** with NH_2OK provided the final hydroxamate compounds **6a–j**.[9] These target compounds have not been reported in literature and their chemical structures were confirmed by IR, ^1H NMR, ^{13}C NMR and ESI-MS.

The newly synthesized L-tyrosine derivatives were assayed for the inhibitory activities on MMP-2 and histone deacetylase 8 (HDAC-8). Similarly as MMP-2, HDAC-8 is also a zinc-dependent metalloproteinase involved in the process of tumor invasion and

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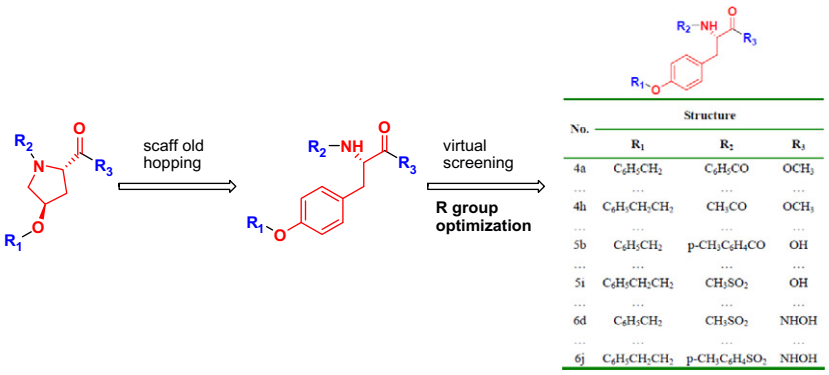
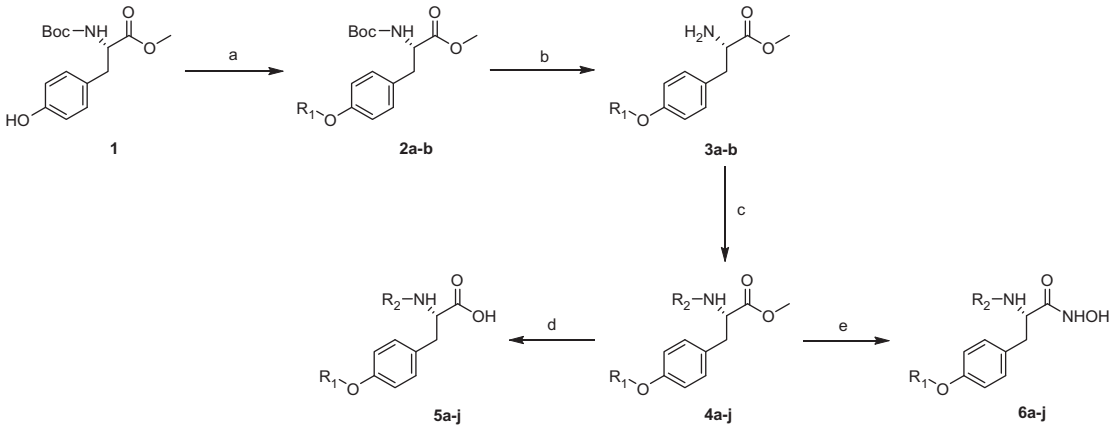


No.	Structure		
	R_1	R_2	R_3
4a	$C_6H_5CH_2$	C_6H_5CO	OCH_3
...	...	...	...
4h	$C_6H_5CH_2CH_2$	CH_3CO	OCH_3
...	...	...	...
5b	$C_6H_5CH_2$	p-$CH_3C_6H_4CO$	OH
...	...	...	...
5i	$C_6H_5CH_2CH_2$	CH_3SO_2	OH
...	...	...	...
6d	$C_6H_5CH_2$	CH_3SO_2	NHOH
...	...	...	...
6j	$C_6H_5CH_2CH_2$	p-$CH_3C_6H_4SO_2$	NHOH

Figure 1. Construction of L-tyrosine based compounds database.



Scheme 1. Reagents: (a) R_1Cl, K_2CO_3, NaI; (b) CF_3COOH; (c) R_2Cl, Na_2CO_3; (d) NaOH, HCl; (e) NH_2OK.

metastasis.[14] Thus the assay was performed on both of MMP-2 and HDAC-8 so as to identify the compound selectivity. NNGH (*N*-isobutyl-*N*-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid), a potent inhibitor of MMP-2, was used as the positive control of MMP-2 assay. SAHA (suberoylanilide hydroxamic acid), a potent HDACs inhibitor that has been registered as antitumor drug, was used as the positive control of HDAC-8 assay.

The results showed that these L-tyrosine derivatives exhibited inhibitory activities against MMP-2 and HDAC-8 (Table 1). Most of these compounds, especially the hydroxamate compounds, could inhibit MMP-2 at nanomolar concentrations and HDAC-8 at micromolar concentrations, thus exhibiting selective inhibition of MMP-2 against HDAC-8.

As for MMP-2 inhibition, the compounds **6h** and **6j** were equal potent to the positive control NNGH. The Flexible docking of the compounds **6h** and **6j** with MMP-2 was done using Discovery Studio 3.0 software and the results were shown in Figures 2 and 3. The phenylethyloxyl group occupied the deep S1′ pocket of MMP-2, and the hydroxamate group chelated the active site zinc ion 166. Compounds **6h** and **6j** interacted well with MMP-2 active site, especially the deep S1′ pocket and zinc ion 166, consistent with the binding mode of the original ligand I52, thus showing high inhibitory activities against MMP-2.

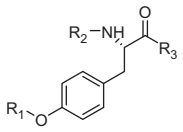
Compounds **6a–j** were more potent than their predecessors **5a–j**. This activity difference was caused by the ZBG (COR_3), which was the only structural difference between **6a–j** and their predecessors. The ZBG is hydroxamate (CONHOH) for **6a–j** and carboxyl group for their predecessors, respectively. Both of these two groups could chelate zinc ion at catalytic activity center of the enzyme. However, the hydroxamate group was a more potent ZBG than carboxyl group as shown in the activity order of **6a-j** and their predecessors.

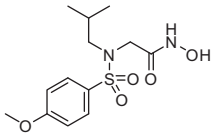
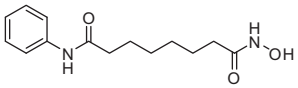
Among compounds **6a–j**, while fixing R_2 group, the R_1 group was altered as benzyl and phenylethyl group. So the differences in the inhibitory activities of these compounds were caused by the R_1 group length. Introduction of longer group (**6f–j**) displayed higher activity. The same rule could also be seen among compounds **5a–j** and **4a–j**.

As for HDAC-8 inhibition, some of the hydroxamate compounds, such as **6d** and **6c**, were equal potent to the positive control SAHA. Figure 4 showed the docking result of compound **6d** with HDAC-8 active site (the broken line showing SAHA). The molecule inserted into the pocket and the hydroxamate group was found to be close to zinc ion 237 in the active site and chelated well with the distance of 1.82 and 2.15 Å, respectively. The binding mode of **6d** with HDAC-8 active site was similar to that of SAHA, which could explain the high potency of **6d**. Among compounds **6a–j**, shorter R_1 group (benzyloxyl) and R_2 group (acetyl and methanesulfonyl) exhibited higher activity, and this principle could also be seen among compounds **5a–j** (except compounds **5b** and **5g**).

The different inhibitory profile might be explained by the active site structural differences. MMP-2 was a zinc-dependent endopeptidase that could cut the peptide to parts from the specific amino acid residue of peptide. However, HDAC-8 was a class of enzyme that removed acetyl groups (O=C–CH_3) from an ε-*N*-acetyl lysine amino acid on a histone. Due to the structural differences between MMP-2 and HDAC-8, there were different structural requirements for their respective inhibitors.

Table 1
The structures and IC_{50} values of L-tyrosine derivatives



No.	Structure			IC_{50}[a] (μM)	
	R_1	R_2	R_3	MMP-2	HDAC-8
4a	$C_6H_5CH_2$	C_6H_5CO	OCH_3	67.5 ± 6.9	>1000
4b	$C_6H_5CH_2$	p-$CH_3C_6H_4CO$	OCH_3	83.7 ± 9.5	>1000
4c	$C_6H_5CH_2$	CH_3CO	OCH_3	27.6 ± 2.5	871.5 ± 73.2
4d	$C_6H_5CH_2$	CH_3SO_2	OCH_3	17.8 ± 1.6	753.7 ± 72.6
4e	$C_6H_5CH_2$	p-$CH_3C_6H_4SO_2$	OCH_3	10.7 ± 1.1	>1000
4f	$C_6H_5CH_2CH_2$	C_6H_5CO	OCH_3	74.6 ± 7.4	>1000
4g	$C_6H_5CH_2CH_2$	p-$CH_3C_6H_4CO$	OCH_3	85.5 ± 7.8	>1000
4h	$C_6H_5CH_2CH_2$	CH_3CO	OCH_3	12.5 ± 1.3	825.1 ± 82.9
4i	$C_6H_5CH_2CH_2$	CH_3SO_2	OCH_3	2.4 ± 0.2	>1000
4j	$C_6H_5CH_2CH_2$	p-$CH_3C_6H_4SO_2$	OCH_3	6.3 ± 0.5	961.8 ± 85.4
5a	$C_6H_5CH_2$	C_6H_4CO	OH	9.8 ± 0.9	110.5 ± 10.9
5b	$C_6H_5CH_2$	p-$CH_3C_6H_4CO$	OH	8.1 ± 0.8	>1000
5c	$C_6H_5CH_2$	CH_3CO	OH	3.0 ± 0.3	36.9 ± 5.2
5d	$C_6H_5CH_2$	CH_3SO_2	OH	1.4 ± 0.1	47.2 ± 5.1
5e	$C_6H_5CH_2$	p-$CH_3C_6H_4SO_2$	OH	2.5 ± 0.3	504.7 ± 48.9
5f	$C_6H_5CH_2CH_2$	C_6H_5CO	OH	7.6 ± 0.7	253.4 ± 33.6
5g	$C_6H_5CH_2CH_2$	p-$CH_3C_6H_4CO$	OH	4.5 ± 0.5	385.6 ± 40.5
5h	$C_6H_5CH_2CH_2$	CH_3CO	OH	0.75 ± 0.07	120.9 ± 14.6
5i	$C_6H_5CH_2CH_2$	CH_3SO_2	OH	0.12 ± 0.01	102.3 ± 12.7
5j	$C_6H_5CH_2CH_2$	p-$CH_3C_6H_4SO_2$	OH	0.27 ± 0.02	>1000
6a	$C_6H_5CH_2$	C_6H_5CO	NHOH	1.7 ± 0.1	9.3 ± 1.0
6b	$C_6H_5CH_2$	p-$CH_3C_6H_4CO$	NHOH	0.46 ± 0.04	30.7 ± 4.1
6c	$C_6H_5CH_2$	CH_3CO	NHOH	0.037 ± 0.002	5.8 ± 0.5
6d	$C_6H_5CH_2$	CH_3SO_2	NHOH	0.067 ± 0.006	3.6 ± 0.2
6e	$C_6H_5CH_2$	p-$CH_3C_6H_4SO_2$	NHOH	0.025 ± 0.002	42.4 ± 4.3
6f	$C_6H_5CH_2CH_2$	C_6H_5CO	NHOH	0.9 ± 0.1	27.8 ± 3.2
6g	$C_6H_5CH_2CH_2$	p-$CH_3C_6H_4CO$	NHOH	3.9 ± 0.3	52.5 ± 4.9
6h	$C_6H_5CH_2CH_2$	CH_3CO	NHOH	0.013 ± 0.001	14.4 ± 1.1
6i	$C_6H_5CH_2CH_2$	CH_3SO_2	NHOH	0.029 ± 0.002	9.5 ± 1.0
6j	$C_6H_5CH_2CH_2$	p-$CH_3C_6H_4SO_2$	NHOH	0.017 ± 0.001	82.5 ± 9.3
NNGH				0.014 ± 0.001	/
SAHA				/	1.6 ± 0.1

[a] IC_{50} values are mean of three experiments, standard deviation is given.

The above structure–activity relationship studies encouraged us to further design novel-scaffold-based MMP-2 and HDAC-8 inhibitors, which would be reported later.

3. Conclusions

In conclusion, a series of novel L-tyrosine derivatives was designed and synthesized. These compounds exhibited inhibitory activities against MMP-2 and HDAC-8. The compounds **6h** and **6j** were equal potent to the positive control NNGH. As for HDAC-8 inhibition, some of the hydroxamate compounds, such as **6d** and **6c**, were equal potent to the positive control SAHA. SAR studies indicated that introduction of longer R_1 group favored the inhibitory activity against MMP-2, while shorter R_1 group and R_2 group exhibited higher HDAC-8 inhibitory activity. The flexible docking was consistent with the above SAR results. Further assays of these compounds on cell culture and animal models were underway.

4. Experimental

4.1. Synthetic methods and spectroscopic details

Melting points were determined using X-6 digital display binocular microscope (uncorrected). Infrared spectra were measured on a nicolet nexus 470 FT-IR spectrometer using smear KBr crystal or KBr plate. ^1H NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometer; *J* values are in Hz. ^{13}C NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometer. Mass spectra were recorded on an electro-spray ionization mass spectrometer as the value *m*/*z*. Flash column chromatography was performed using 300 mesh silica gel. The yields were calculated by the last step reaction.

4.1.1. (*S*)-Methyl 3-(4-(substituted oxy)phenyl)-2-(*tert*-butoxycarbonylamino)propanoate (2)

N-Boc-L-tyrosine methyl ester (**1**) (29.5 g, 100 mmol) and benzyl chloride (or phenylethyl chloride) (200 mmol) were dissolved

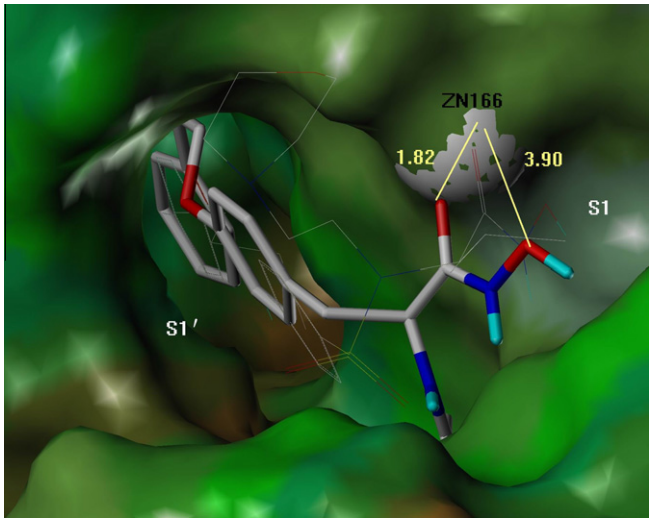


Figure 2. Flexible docking result of compound **6h** with MMP-2 (the broken line showing the original ligand I52).

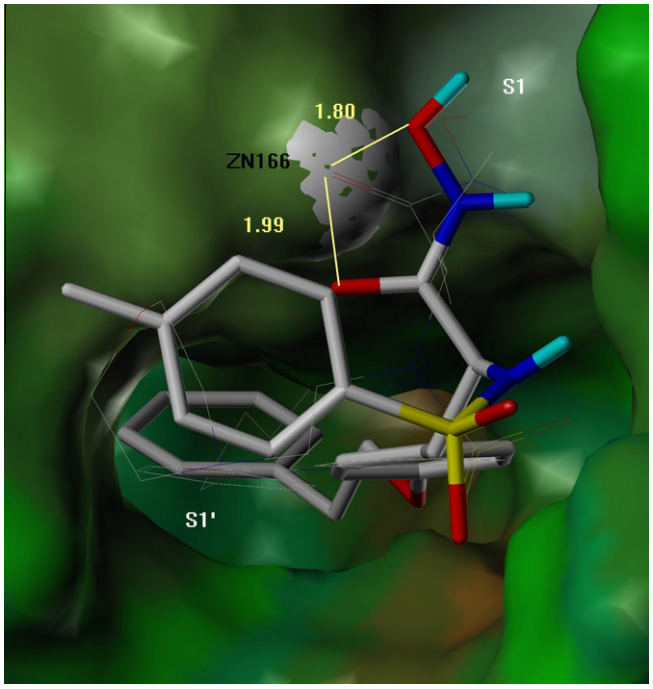


Figure 3. Flexible docking result of compound **6j** with MMP-2 (the broken line showing the original ligand I52).

in acetone (1000 ml), then potassium carbonate (27.6 g, 200 mmol) and sodium iodide (catalytic amount) were added to the solution. The mixture was rufluxed for 10 h, and TLC was used to monitor the reaction. The resulting mixture was filtrated and the filtrate was evaporated to give yellow oil. The oil was further purified by flash column chromatography (ethyl acetate/*n*-hexane = 1/5) and **2** was obtained.

4.1.1.1. (*S*)-Methyl 3-(4-(benzyloxy)phenyl)-2-(*tert*-butoxycarbonylamino)propanoate (2a). Yellow solid, 96%, mp 52.0–54.2 °C. ^{1}H NMR ($CDCl_3$, δ): 7.45–7.31 (m, 5H, Ar-H), 7.08 (d, 2H, Ar-H, J = 8.00 Hz), 6.93 (d, 2H, Ar-H, J = 8.00 Hz), 5.21 (d, 1H, NH, J = 8.00 Hz), 5.03 (s, 2H, CH_2O), 4.59 (d, 1H, CH, J = 7.20 Hz), 3.70 (s, 3H, OCH_3), 3.12–2.98 (m, 2H, CH_2), 1.47 (s, 9H, $C(CH_3)_3$). ESI-MS: 401.6 (M+NH_4).

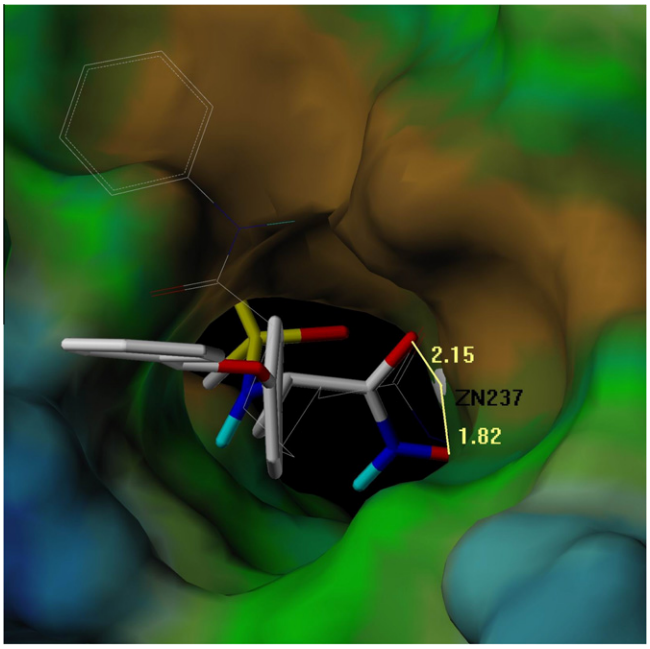


Figure 4. Flexible docking result of compound **6d** with HDAC-8 (the broken line showing SAHA).

4.1.1.2. (*S*)-Methyl 2-(*tert*-butoxycarbonylamino)-3-(4-phenethoxyphenyl)propanoate (2b). White solid, 84.2%, mp 78.3–80.4 °C. ^{1}H NMR ($CDCl_3$, δ): 7.290 (d, 1H, N–H, J = 6.8 Hz), 7.248–7.223 (m, 5H, Ar-H), 7.052 (d, 2H, Ar-H, J = 8.0 Hz), 6.846 (d, 2H, Ar-H, J = 8.4 Hz), 5.024 (s, 1H, CH), 3.962 (t, 2H, CH_2, J = 6.0 Hz), 3.737 (s, 3H, CH_3O), 3.056 (q, 2H, CH_2, J = 4.8 Hz), 2.833 (t, 2H, CH_2O, J = 7.6 Hz), 1.455 (s, 9H, $(CH_3)_3C$). ESI-MS: 438.2 (M+K).

4.1.2. (*S*)-Methyl 2-amino-3-(4-(substituted oxy)phenyl) propanoate (3)

On an ice bath, **2a** or **2b** (10 mmol) was added to a solution of trifluoroacetic acid and dichloromethane (1:1, 10 ml). The mixture was reacted for 5 h and TLC was used to monitor completion. The solvent was evaporated to give yellow oil, which was dissolved in saturated $NaHCO_3$ solution to PH7. After extraction with dichloromethane, the organic layer was evaporated to give oil, which was purified by flash column chromatography (ethyl acetate:*n*-hexane = 1:1) and **3a** or **3b** was obtained as white crystal.

4.1.2.1. (*S*)-Methyl 2-amino-3-(4-(benzyloxy)phenyl)propanoate (3a). 84%, mp 158–160 °C, ^{1}H NMR (DMSO-d_6, δ): 8.52 (s, 2H, NH_2), 7.46–7.33 (m, 5H, Ar-H), 7.14 (d, 2H, Ar-H, J = 8.80 Hz), 6.98 (d, 2H, Ar-H, J = 8.80 Hz), 5.09 (s, 2H, OCH_2), 4.21 (t, 1H, CH, J = 7.00 Hz), 3.68 (s, 3H, OCH_3), 3.20–3.00 (m, 2H, CH_2). ESI-MS: 286.1 (M+1).

4.1.2.2. (*S*)-Methyl 2-amino-3-(4-phenethoxyphenyl)propanoate (3b). 93.6%, mp: 109.5–112.1 °C. ^{1}H NMR (DMSO-d_6, δ): 7.275 (d, 2H, Ar-H, J = 7.6 Hz), 7.222 (d, 3H, Ar-H, J = 6.8 Hz), 7.080 (d, 2H, Ar-H, J = 8.8 Hz), 6.835 (d, 2H, Ar-H, J = 8.8 Hz), 3.920 (t, 2H, NH_2, J = 6.4 Hz), 3.642 (s, 1H, CH), 3.588 (s, 1H, CH_3), 3.175 (s, 2H, CH_2), 2.832–2.710 (m, 4H, CH_2CH_2). ESI-MS: 300.2 (M+1).

4.1.3. (*S*)-Methyl 2-substituted amido-3-(4-(substituted oxy) phenyl)propanoate (4)

Compound **3a** or **3b** (10 mmol) was dissolved in dichloromethane (100 ml) and Na_2CO_3 (2.12 g, 20 mmol) was added. Acyl chloride (10 mmol) in dichloromethane (100 ml) was added to the solution. The mixture was reacted for 18 h and TLC was used to monitor the completion. The filtrate was evaporated to give oil, which was purified by flash column chromatography (ethyl acetate:*n*-hexane = 1:1) and **4** was obtained.

4.1.3.1. (*S*)-Methyl 2-benzamido-3-(4-(benzyloxy)phenyl)propanoate (4a). Yellow crystal, 79%, mp 128.3–130.5 °C, ^{1}H NMR (DMSO-d_6, δ): 8.83 (d, 1H, NH, J = 8.00 Hz), 7.80 (d, 2H, Ar-H, J = 8.00 Hz), 7.54 (t, 1H, Ar-H, J = 7.20 Hz), 7.48–7.29 (m, 7H, Ar-H), 7.22 (d, 2H, Ar-H, J = 8.00 Hz), 6.92 (d, 2H, Ar-H, J = 8.00 Hz), 5.04 (s, 2H, OCH_2), 4.60 (m, 1H, CH), 3.64 (s, 3H, OCH_3), 3.12–2.99 (m, 2H, CH_2). ESI-MS: 390.3 (M+1).

4.1.3.2. (*S*)-Methyl 2-(*p*-methyl)benzamido-3-(4-(benzyloxy) phenyl)propanoate (4b). White crystal, 66%, mp: 157.4–159.3 °C. ^{1}H NMR (DMSO-d_6, δ): 8.727 (d, 1H, N–H, J = 8.0 Hz), 7.723 (d, 2H, Ar-H, J = 8.4 Hz), 7.429–7.203 (m, 9H, Ar-H), 6.913 (d, 2H, Ar-H, J = 8.8 Hz), 5.040 (s, 2H, CH_2O), 4.619–4.562 (m, 1H, CH), 3.630 (s, 3H, CH_3O), 3.112–2.994 (m, 2H, CH_2), 2.352 (s, 3H, CH_3). ESI-MS: 402.2 (M-1).

4.1.3.3. (*S*)-Methyl 2-acetamido-3-(4-(benzyloxy)phenyl)propanoate (4c). Yellow crystal, 55.7%, mp: 133.6–134.7 °C. ^{1}H NMR (DMSO-d_6, δ): 8.275 (d, 1H, NH, J = 8.0 Hz), 7.363–7.442 (m, 2H, Ar-H), 7.300–7.336 (t, 3H, Ar-H, J = 7.2 Hz), 7.130–7.109 (d, 2H, Ar-H, J = 8.4 Hz), 6.927–6.906 (d, 2H, Ar-H, J = 8.4 Hz), 5.060 (s, 2H, OCH_2), 4.358–4.414 (m, 1H, CH), 3.580 (s, 3H, OCH_3), 2.768–2.951 (m, 2H, CH_2), 1.787 (s, 3H, $COCH_3$). ESI-MS: 328.2(M+1).

4.1.3.4. (*S*)-Methyl 2-methanesulfonyl amido-3-(4-(benzyloxy) phenyl)propanoate (4d). Yellow crystal, 80.7%, mp: 122.3–124.9 °C. ^{1}H NMR (DMSO-d_6, δ): 7.811 (d, 1H, NH, J = 8.8 Hz), 7.441–7.302 (m, 5H, Ar-H), 7.185 (d, 2H, Ar-H, J = 8.1 Hz), 6.943 (d, 2H, J = 7.9 Hz, Ar-H), 5.083 (s, 2H, CH_2), 4.096 (q, 1H, J = 8.7 Hz, CH), 3.628 (s, 3H, CH_3O), 2.975–2.748 (m, 2H, CH_2), 2.564 (s, 3H, CH_3). ESI-MS: 749.4 (2 M+Na).

4.1.3.5. (*S*)-Methyl 2-(4-methylbenzene-1-sulfonyl amido)-3-(4-(benzyloxy)phenyl)propanoate (4e). White crystal, 55%, mp: 143.5–146.6 °C. ^{1}H NMR (DMSO-d_6, δ): 8.350 (s, 1H, N–H), 7.449–7.380 (m, 6H, Ar-H), 7.354–7.332 (m, 1H, Ar-H), 7.260 (d, 2H, Ar-H, J = 8.4 Hz), 7.011 (d, 2H, Ar-H, J = 8.4 Hz), 6.840 (d, 2H, Ar-H, J = 8.4 Hz), 5.058 (s, 2H, CH_2O), 3.898–3.838 (m, 1H, CH), 3.333 (s, 3H, CH_3O), 2.831 (q, 1H, CH_2, J = 6.8 Hz), 2.680 (q, 1H, CH_2, J = 6.8 Hz), 2.348 (s, 3H, $ArCH_3$). ESI-MS: 438.2 (M-1).

4.1.3.6. (*S*)-Methyl 2-benzamido-3-(4-(phenylethyloxy)phenyl) propanoate (4f). White crystal, 60.3%, mp: 130.1–131.7 °C. ^{1}H NMR (DMSO-d_6, δ): 8.810–8.830 (d, 1H, NH, J = 8.0 Hz), 7.944–7.963 (d, 2H, Ar-H, J = 7.8 Hz), 7.800–7.963 (d, 2H, Ar-H), 7.799–7.817 (d, 2H, Ar-H, J = 7.6 Hz), 7.438–7.542 (m, 4H, Ar-H), 7.171–7.268 (m, 4H, Ar-H), 6.823–6.845 (d, 2H, Ar-H, J = 8.8 Hz), 5.047–5.103 (m, 1H, CH), 3.889–3.920 (t, 2H, OCH_2, J = 6.4 Hz), 3.642 (s, 3H, CH_3), 3.030–3.179 (m, 2H, CH_2), 2.691–2.730 (t, 2H, CH_2, J = 8.0 Hz). ESI-MS: 402.3(M-1).

4.1.3.7. (*S*)-Methyl 2-(*p*-methylbenzamido)-3-(4-(phenylethyloxy)phenyl)propanoate (4g). White crystal, 83.4%, mp: 111.5–113.8 °C. ^{1}H NMR (DMSO-d_6, δ): 8.701 (d, 1H, NH, J = 8.0 Hz), 7.721 (d, 2H, Ar-H, J = 7.6 Hz), 7.273–7.184 (m, 9H, Ar-H), 6.827 (d, 2H, Ar-H, J = 7.6 Hz), 4.591 (q, 1H, CH, J = 8.0 Hz), 3.907 (t, 2H, CH_2, J = 6.4 Hz), 3.634 (s, 3H, CH_3), 3.061 (m, 2H, CH_2), 2.712 (t, 2H, CH_2, J = 7.6 Hz), 2.352 (s, 3H, CH_3). ESI-MS: 418.2(M+1).

4.1.3.8. (*S*)-Methyl 2-acetamido-3-(4-(phenylethyloxy)phenyl) propanoate (4h). White crystal, 56.9%, mp: 117.7–119.1 °C. ^{1}H NMR (DMSO-d_6, δ): 8.277–8.296 (d, 1H, NH, J = 7.6 Hz), 7.101–7.307 (m, 7H, Ar-H), 6.829–6.848 (d, J = 7.6 Hz, 2H, Ar-H), 4.381–4.399 (d, 1H, CH, J = 7.2 Hz), 3.914–3.945 (m, 2H, CH_2), 3.591 (s, 3H, CH_3), 2.508–2.954 (m, 4H, CH_2CH_2), 1.796 (s, 3H, CH_3). ESI-MS: 342.2(M+1).

4.1.3.9. (*S*)-Methyl 2-methanesulfonyl amido-3-(4-(phenylethyloxy)phenyl)propanoate (4i). Yellow crystal, 81.2%, mp: 64.8–65.2 °C. ^{1}H NMR (DMSO-d_6, δ): 7.829 (s, 1H, NH), 7.299–7.161 (m, 7H, Ar-H), 6.857 (d, 2H, Ar-H, J = 8.4 Hz), 4.100 (t, 1H, CH, J = 6.0 Hz), 3.924 (t, 2H, CH_2, J = 6.4 Hz), 3.629 (s, 3H, CH_3O), 2.971–2.499 (m, 4H, CH_2CH_2), 2.765 (s, 3H, SO_2CH_3). ESI-MS: 376.2(M-1).

4.1.3.10. (*S*)-Methyl 2-(4-methylbenzene-1-sulfonyl amido)-3-(4-(phenylethyloxy)phenyl)propanoate (4j). White crystal, 61%, mp: 89.0–91.4 °C. ^{1}H NMR ($CDCl_3$, δ): 7.622 (d, 2H, Ar-H, J = 8.4 Hz), 7.265 (d, 2H, Ar-H, J = 7.6 Hz), 7.203 (q, 6H, Ar-H and N–H, J = 4.4 Hz), 6.951 (d, 2H, Ar-H, J = 8.4 Hz), 6.736 (d, 2H, Ar-H, J = 8.4 Hz), 4.125 (m, 1H, C–H), 3.896 (t, 2H, CH_2, J = 6.4 Hz), 3.467 (s, 3H, CH_3O), 2.945 (m, 2H, CH_2), 2.788 (t, 2H, CH_2, J = 7.6 Hz), 2.366 (s, 3H, Ar-CH_3). ESI-MS: 452.2(M-1).

4.1.4. (*S*)-3-(4-(Substituted oxy)phenyl)-2-(substituted amido) propanoic acid (5)

Compound **4** (2.75 mmol) was dissolved in 14 ml methanol and 28 ml NaOH (1 mol/L) was added. The solution was stirred at room temperature for 4 h and TLC was used to monotor the completion. The solvent was evaporated to give yellow solid. The yellow solid was dissolved in 50 ml water and 1 mol/L HCl was added to pH 2-3. The yellow solid was precipitated, which was washed by water to give **5**.

4.1.4.1. (*S*)-3-(4-(Benzyloxy)phenyl)-2-(benzamido)propanoic acid (5a). Yellow crystal, 90.7%, mp: 163.9–165.4 °C. ^{1}H NMR (DMSO-d_6, δ): 12.712 (s, 1H, OH), 8.649–8.669 (d, 1H, NH, J = 8.0 Hz), 7.797–7.815 (d, 2H, Ar-H, J = 7.2 Hz), 7.228–7.529 (m, 10H, Ar-H), 6.903–6.922 (d, 2H, Ar-H, J = 7.6 Hz), 5.034 (s, 2H, OCH_2), 4.574–4.631 (m, 1H, CH), 2.979–3.145 (m, 2H, CH_2). ESI-MS: 750.5 (2M-1).

4.1.4.2. (*S*)-3-(4-(Benzyloxy)phenyl)-2-(*p*-methylbenzamido) propanoic acid (5b). Yellow crystal, 90.6%, mp: 141.2–143.1 °C. ^{1}H NMR (DMSO-d_6, δ): 8.562 (d, 1H, N–H, J = 8.0 Hz), 7.718 (d, 2H, Ar-H, J = J = 8.0 Hz), 7.428–7.215 (m, 9H, Ar-H), 6.905 (d, 2H, Ar-H, J = 8.4 Hz), 5.031 (s, 2H, CH_2O), 4.561–4.515 (m, 1H, CH), 3.098–3.004 (m, 2H, CH_2), 2.350 (s, 3H, CH_3). ESI-MS: 388.1 (M-1).

4.1.4.3. (*S*)-3-(4-(Benzyloxy)phenyl)-2-(acetamido)propanoic acid (5c). White crystal, 81.2%, mp: 183.4–185.2 °C. ^{1}H NMR (DMSO-d_6, δ): 12.712 (s, 1H, OH), 8.124–8.143 (d, 1H, NH, J = 7.6 Hz), 7.328–7.452 (m, 5H, Ar-H), 7.136–7.154 (d, 2H, Ar-H, J = 7.2 Hz), 6.912–6.931 (d, 2H, Ar-H, J = 7.6 Hz), 5.063 (s, 2H, OCH_2), 4.350-4.362 (m, 1H, CH), 2.745–2.993 (m, 2H, CH_2), 1.789 (s, 3H, CH_3). ESI-MS: 625.4 (2M-1).

4.1.4.4. (*S*)-3-(4-(Benzyloxy)phenyl)-2-(methanesulfonyl amido) propanoic acid (5d). White crystal, 94.4%, mp: 146.3–147.0 °C. ^{1}H NMR (DMSO-d_6, δ): 12.890 (s, 1H, OH), 7.628 (d, 1H, NH, J = 8.8 Hz), 7.448–7.321 (m, 5H, Ar-H), 7.200 (d, 2H, Ar-H, J = 8.8 Hz), 6.943 (d, 2H, Ar-H, J = 8.8 Hz), 5.080 (s, 2H, CH_2), 3.969 (m, 1H, CH), 3.000–2.706 (m, 2H, CH_2), 2.539 (s, 3H, CH_3). ESI-MS: 697.5 (2 M-1), 721.3 (2 M+Na).

4.1.4.5. (*S*)-3-(4-(Benzyloxy)phenyl)-2-(4-methylbenzene-1-sulfonyl amido)propanoic acid (5e). White crystal, 71.4%, mp: 96.0–98.3 °C. ^{1}H NMR (DMSO-d_6, δ): 7.650 (s, 1H, N–H), 7.460–7.333 (m, 7H, Ar-H), 7.241 (d, 2H, Ar-H, J = 8.0 Hz), 7.023 (d, 2H, Ar-H, J = 8.4 Hz), 6.813 (d, 2H, Ar-H, J = 8.8 Hz), 5.043 (s, 2H, CH_2O), 3.593 (t, 1H, CH, J = 5.6 Hz), 3.342 (s, 1H, OH), 2.853-2.699 (m, 2H, CH_2), 2.334 (s, 3H, CH_3). ESI-MS: 424.1 (M-1).

4.1.4.6. (*S*)-3-(4-(Phenylethyloxy)phenyl)-2-(benzamido)propanoic acid (5f). Yellow crystal, 84.2%, mp: 181.5–183.3 °C. ^{1}H NMR (DMSO-d_6, δ): 8.315–8.333 (d, 1H, NH, J = 7.2 Hz), 7.755 (s, 2H, Ar-H), 6.872–7.755 (m, 12H, Ar-H), 7.170–7.440 (m, 10H, Ar-H), 6.767–6.894 (m, 2H, Ar-H), 4.419–4.429 (s, 1H, CH), 3.886 (s, 2H, OCH_2), 3.003–3.128 (m, 2H, CH_2), 2.706 (m, 2H, CH_2). ESI-MS: 388.2 (M-1).

4.1.4.7. (*S*)-3-(4-(Phenylethyloxy)phenyl)-2-(p-methylbenzamido)propanoic acid (5g). White crystal, 91.7%, mp: 96.2–98.9 °C. ^{1}H NMR (DMSO-d_6, δ): 8.116 (s, 1H, NH), 7.646 (d, 2H, Ar-H, J = 7.6 Hz), 7.289–7.104 (m, 9H, Ar-H), 6.756 (d, 2H, Ar-H, J = 8.0 Hz), 4.321 (d, 1H, CH, J = 4.8 Hz), 3.886 (t, 2H, CH_2, J = 6.4 Hz), 3.140–2.986 (m, 2H, CH_2), 2.708 (t, 2H, CH_2, J = 7.6 Hz), 2.340 (s, 3H, CH_3). ESI-MS: 404.2 (M+1).

4.1.4.8. (*S*)-3-(4-(Phenylethyloxy)phenyl)-2-(acetamido)propanoic acid (5h). White crystal, 78.3%, mp: 172.8–174.3 °C. ^{1}H NMR (DMSO-d_6, δ): 12.585–12.598 (d, 1H, OH, J = 7.2 Hz), 8.093–8.111 (d, 1H, NH, J = 7.6 Hz), 7.107–7.282 (m, 7H, Ar-H), 6.817–6.835 (d, 2H, Ar-H, J = 7.2 Hz), 4.328 (s, 1H, CH), 3.923 (s, 2H, CH_2), 2.711–2.784 (m, 3H, CH_3), 1.994–2.010 (m, 2H, CH_2), 1.780 (m, 4H, CH_2). ESI-MS: 326.5 (M-1).

4.1.4.9. (*S*)-3-(4-(Phenylethyloxy)phenyl)-2-(methanesulfonyl amido)propanoic acid (5i). White crystal, 93.9%, mp:113.4–115.3 °C. ^{1}H NMR (DMSO-d_6, δ): 12.867 (s, 1H, OH), 7.615 (d, 1H, NH, J = 8.8 Hz), 7.287–7.176 (m, 7H, Ar-H), 6.859 (d, 2H, Ar-H, J = 8.4 Hz), 4.004–3.918 (m, 3H, CH+CH_2), 2.996–2.713 (m, 4H, CH_2CH_2), 2.573 (s, 3H, CH_3). ESI-MS: 362.1 (M-1).

4.1.4.10. (*S*)-3-(4-(phenylethyloxy)phenyl)-2-(4-methylbenzene-1-sulfonyl amido)propanoic acid (5j). Yellow crystal, 49.5%, mp: 126.5–128.5 °C. ^{1}H NMR (DMSO-d_6, δ): 12.780 (s, 1H, O–H), 8.361 (d, 1H, N–H, J = 8.8 Hz), 8.000 (s, 1H, Ar-H), 7.459 (t, 2H, Ar-H, J = 8.0 Hz), 7.440–7.173 (m, 6H, Ar-H), 6.999 (t, 2H, Ar-H, J = 8.4 Hz), 6.740 (t, 2H, Ar-H, J = 8.0 Hz), 3.917 (m, 1H, C–H), 3.321 (m, 2H, CH_2), 2.853–2.650 (m, 4H, CH_2CH_2), 2.324 (s, 3H, CH_3). ESI-MS: 440.1 (M+1).

4.1.5. (*S*)-N-hydroxy-3-(4-substituted oxyphenyl)-2-(substituted amino)propanamide (6)

To a solution of compound **5** (2 mmol) in methanol (7 ml) at room temperature, was added dropwise a solution of NH_2OK (6 mmol) in methanol (3.4 ml). The mixture was stirred at room temperature for 24 h and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (ethyl acetate:*n*-hexane = 1:1) to give **6**.

4.1.5.1. (*S*)-*N*-Hydroxy-3-(4-benzyloxyphenyl)-2-(benzamido) propanamide (6a). Yellow crystal, 30%, mp:167.2–168.8 °C. IR (KBr, cm^{-1}): 3294.50 (OH and NH), 2919.85 (CH), 1658.49 (C=O), 1176.39 (C–O). ^{1}H NMR (DMSO-d_6, δ): 10.80 (s, 1H, OH), 8.91 (s, 1H, ONH), 8.63 (d, 1H, NH, J = 8.40 Hz), 7.81 (d, 2H, Ar-H, J = 7.20 Hz), 7.50 (d, 1H, Ar-H, J = 7.20 Hz), 7.46–7.31 (m, 7H, Ar-H), 7.24 (d, 2H, Ar-H, J = 8.40 Hz), 6.90 (d, 2H, Ar-H, J = 8.40 Hz), 5.02 (s, 2H, OCH_2), 4.54 (m, 1H, CH), 3.00–2.80 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ): 170.2 (C=O), 168.7 (C=O), 154.5 (C aro), 137.8 (C aro), 135.4 (C aro), 131.3 (C aro), 129.5 (4 × C aro), 128.3 (4 × C aro), 126.4 (4 × C aro), 112.7 (2 × C aro), 72.6 (CH_2), 55.6 (CH), 35.2 (CH_2). ESI-MS: 391.1 (M+1), 413.0 (M+Na), 389.3 (M-1).

4.1.5.2. (*S*)-*N*-Hydroxy-3-(4-benzyloxyphenyl)-2-(*p*-methylbenzamido)propanamide (6b). Yellow crystal, 22.5%, mp: 126.5–128.0 °C. IR (KBr, cm^{-1}): 3422.46 (OH and NH), 2921.71 (CH), 1632.60 (C=O), 1177.08 (C–O). ^{1}H NMR (DMSO-d_6, δ): 10.769 (s, 1H, N*H*OH), 8.889 (s, 1H, NHO*H*), 8.509 (d, 1H, CONH, J = 8.0 Hz), 7.730 (d, 2H, Ar-H, J = 7.2 Hz), 7.428–7.231 (m, 9H, Ar-H), 6.900 (d, 2H, Ar-H, J = 8.4 Hz), 5.042 (s, 2H, CH_2O), 4.530 (d, 1H, CH, J = 5.6 Hz), 2.944 (s, 2H, CH_2), 2.344 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, δ): 168.9 (C=O), 167.2 (C=O), 155.8 (C aro), 142.1 (C aro), 135.6 (C aro), 132.1 (C aro), 129.5 (4 × C aro), 128.3 (3 × C aro), 126.7 (5 × C aro), 114.8 (2 × C aro), 71.2 (CH_2), 55.1 (CH), 37.5 (CH_2), 22.3 (CH_3). ESI-MS: 403.2 (M-1).

4.1.5.3. (*S*)-*N*-Hydroxy-3-(4-benzyloxyphenyl)-2-(acetamido) propanamide (6c). Yellow crystal, 19.8%, mp: 169.2–170.9 °C. IR (KBr, cm^{-1}): 3314.27 (OH and NH), 3046.70 (CH), 1636.15 (C=O), 1181.77 (C–O). ^{1}H NMR (DMSO-d_6, δ): 10.630 (s, 1H, OH), 8.816 (s, 1H, NH–O), 8.123-8.144 (d, 1H, NHCO, J = 8.4 Hz), 7329–7.452 (m, 5H, Ar-H), 7.124–7.143 (d, 2H, Ar-H, J = 7.6 Hz), 6.898–6.917 (d, 2H, Ar-H, J = 7.6 Hz), 5.056 (s, 2H, OCH_2), 4.305–4.325 (m, 1H, CH), 2.658–2.863 (m, 2H, CH_2), 1.757 (s, 3H, $COCH_3$). ^{13}C NMR (DMSO-d_6, δ): 170.4 (C=O), 168.7 (C=O), 156.9 (C aro), 136.1 (C aro), 129.1 (2 × C aro), 128.6 (3 × C aro), 126.9 (3 × C aro), 114.5 (2 × C aro), 71.1 (CH_2), 54.5 (CH), 37.2 (CH_2), 22.6 (CH_3). ESI-MS: 679.6 (2 M+Na).

4.1.5.4. (*S*)-*N*-Hydroxy-3-(4-benzyloxyphenyl)-2-(methanesulfonyl amido)propanamide (6d). White crystal, 23.2%, mp: 153.3–156.0 °C. IR (KBr, cm^{-1}): 3423.89 (OH and NH), 2929.82 (CH), 1627.91 (C=O), 1150.38 (C–O). ^{1}H NMR (DMSO-d_6, δ): 7.427–7.322 (m, 6H, Ar-H+NH), 7.174 (d, 2H, Ar-H, J = 8.4 Hz), 6.939 (d, 2H, Ar-H, J = 8.4 Hz), 5.076 (s, 2H, CH_2), 3.800 (q, 1H, CH, J = 8.4 Hz), 3.340 (s, 1H, CH_3), 2.843–2.676 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ): 168.9 (C=O), 156.4 (C aro), 136.5 (C aro), 129.4 (2 × C aro), 128.4 (3 × C aro), 127.2 (3 × C aro), 114.1 (2 × C aro), 71.5 (CH_2), 54.8 (CH), 44.1 (CH_3), 35.2 (CH_2). ESI-MS: 363.1 (M-1).

4.1.5.5. (*S*)-*N*-Hydroxy-3-(4-benzyloxyphenyl)-2-(4-methylbenzene-1-sulfonyl amido)propanamide (6e). White crystal, 21.6%, mp: 170.7–173.4 °C. IR (KBr, cm^{-1}): 3356.87 (OH), 3261.25 (NH), 2922.13 (CH), 1609.44 (C=O), 1156.73 (C–O). ^{1}H NMR (DMSO-d_6, δ): 7.770 (d, 1H, SO_2NH, J = 8.0 Hz), 7.500–7.317 (m, 7H, Ar-H), 7.201 (d, 2H, Ar-H, J = 8.4 Hz), 6.943 (d, 2H, Ar-H, J = 8.4 Hz), 6.794 (t, 2H, Ar-H, J = 8.0 Hz), 5.041 (s, 2H, CH_2O), 3.700 (m, 1H, OH), 3.175–2.600 (m, 2H, CH_2), 2.357 (t, 3H, CH_3, J = 8.0 Hz). ^{13}C NMR (DMSO-d_6, δ): 168.5 (C=O), 156.1 (C aro), 141.9 (C aro), 137.2 (C aro), 136.3 (C aro), 129.1 (4 × C aro), 128.3 (5 × C aro), 127.8 (3 × C aro), 114.4 (2 × C aro), 71.1 (CH_2), 54.4 (CH), 35.9 (CH_2), 21.8 (CH_3). MS *m*/*z*: 439.2 (M-1).

4.1.5.6. (*S*)-*N*-Hydroxy-3-(4-phenylethyloxyphenyl)-2-(benzamido)propanamide (6f). Yellow crystal, 17.9%, mp: 168.5–170.2 °C. IR (KBr, cm^{-1}): 3271.53 (OH and NH), 2922.55 (CH), 1633.33 (C=O), 1177.90 (C–O). ^{1}H NMR (DMSO-d_6, δ): 10.783 (s, 1H, OH), 8.892 (s, 1H, CONH), 8.590–8.611 (d, 1H, Ar-CONH, J = 8.4 Hz), 7.802–7.821 (d, 2H, Ar-H, J = 7.6 Hz), 7.170–7.513 (m, 10H, Ar-H), 6.809–6.829 (d, 2H, Ar-H, J = 8.0 Hz), 4.531–4.546 (t, 1H, CH, J = 6.0 Hz), 3.883–3.914 (t, 2H, OCH_2), 2.943 (t, 2H, CH_2), 2.689–2.727 (m, 2H, CH_2), 1.970 (t, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ): 169.5 (C=O), 167.9 (C=O), 156.2 (C aro), 138.5 (C aro), 134.3 (C aro), 132.3 (C aro), 129.5 (2 × C aro), 128.6 (5 × C aro), 127.8 (4 × C aro), 125.5 (C aro), 114.2 (2 × C aro), 67.1 (CH_2), 54.2 (CH), 37.9 (CH_2), 35.3 (CH_2). ESI-MS: 403.3 (M-1).

4.1.5.7. (*S*)-*N*-Hydroxy-3-(4-phenylethyloxyphenyl)-2-(*p*-methylbenzamido)propanamide (6g). White crystal, 51.2%, mp: 149.9–152.2 °C. IR (KBr, cm^{-1}): 3291.14 (OH and NH), 2921.45 (CH), 1629.84 (C=O), 1177.63 (C–O). ^{1}H NMR (DMSO-d_6, δ): 10.739 (s, 1H, OH), 8.863 (s, 1H, N*H*OH), 8.479 (d, 1H, CONH, J = 8.4 Hz), 7.725 (d, 2H, Ar-H, J = 8.0 Hz), 7.286–7.152 (m, 9H, Ar-H), 6.810 (d, 2H, Ar-H, J = 8.0 Hz), 4.528 (d, 1H, CH, J = 6.0 Hz), 3.898 (t, 2H, CH_2, J = 6.4 Hz), 2.947 (d, 2H, CH_2, J = 10.0 Hz), 2.708 (t, 2H, CH_2, J = 7.2 Hz), 2.342 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, δ): 169.4 (C=O), 167.6 (C=O), 156.6 (C aro), 141.3 (C aro), 138.3 (C aro), 131.3 (C aro), 129.3 (4 × C aro), 128.5 (3 × C aro), 127.4 (4 × C aro), 125.3 (C aro), 114.3 (2 × C aro), 67.6 (CH_2), 54.1 (CH), 37.3 (CH_2), 35.2 (CH_2), 21.2 (CH_2). ESI-MS: 417.3 (M-1).

4.1.5.8. (*S*)-*N*-Hydroxy-3-(4-phenylethyloxyphenyl)-2-(acetamido)propanamide (6h). Yellow crystal, 20.5%, mp: 144.6–146.1 °C. IR (KBr, cm^{-1}): 3294.83 (OH and NH), 2936.75 (CH), 1635.42 (C=O), 1162.83 (C–O). ^{1}H NMR (DMSO-d_6, δ): 10.660 (s, 1H, OH), 8.852 (s, 1H, CONH), 8.148–8.168 (d, 1H, CONH, J = 8.0 Hz), 7.112–7.285 (m, 7H, Ar-H), 6.806–6.825 (d, 2H, Ar-H, J = 7.6 Hz), 4.311-4.347 (m, 1H, CH), 3.903–3.918 (m, 2H, OCH_2), 3.177 (m, 2H, CH_2), 2.658–2.747 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, δ): 170.2 (C=O), 169.2 (C=O), 156.2 (C aro), 138.3 (C aro), 129.4 (2 × C aro), 128.4 (3 × C aro), 127.2 (2 × C aro), 125.5 (C aro), 114.4 (2 × C aro), 67.4 (CH_2), 54.0 (CH), 37.7 (CH_2), 35.3 (CH_2), 22.6 (CH_3). ESI-MS: 341.2 (M-1).

4.1.5.9. (*S*)-*N*-Hydroxy-3-(4-phenylethyloxyphenyl)-2-(methanesulfonyl amido)propanamide (6i). Yellow crystal, 26.4%, mp: 137.1–139.9 °C. IR (KBr, cm^{-1}): 3440.47 (OH and NH), 2930.63 (CH), 1583.57 (C=O), 1149.06 (C–O). ^{1}H NMR (DMSO-d_6, δ): 7.287–7.132 (m, 8H, Ar-H and SO_2N–H), 6.798 (d, 2H, Ar-H, J = 8.0 Hz), 3.911 (t, 2H, CH_2, J = 6.4 Hz), 3.707 (s, 1H, CH), 2.916 (s, 1H, CH_2), 2.749–2.687 (m, 6H, CH_2+CH_3). ^{13}C NMR (DMSO-d_6, δ): 170.0 (C=O), 156.5 (C aro), 138.1 (C aro), 129.4 (2 × C aro), 128.3 (3 × C aro), 127.1 (2 × C aro), 125.6 (C aro), 114.7 (2 × C aro), 67.6 (CH_2), 55.1 (CH), 43.8 (CH_3), 35.9 (CH_2), 35.1 (CH_2). ESI-MS: 377.2 (M-1).

4.1.5.10. (*S*)-*N*-Hydroxy-3-(4-phenylethyloxyphenyl)-2-(4-methylbenzene-1-sulfonyl amido)propanamide (6j). Yellow crystal, 24%, mp: 129.3–131.7 °C. IR (KBr, cm^{-1}): 3261.92 (OH and NH), 2924.78 (CH), 1611.93 (C=O), 1156.30 (C–O). ^{1}H NMR (DMSO-d_6, δ): 7.581 (d, 1H, SO_2N–H, J = 6.4 Hz), 7.436 (s, 1H, Ar-H), 7.311–7.124 (m, 8H, Ar-H), 7.052 (d, 1H, Ar-H, J = 7.2 Hz), 6.813 (d, 1H, Ar-H, J = 7.2 Hz), 6.668 (s, 2H, Ar-H), 3.894 (s, 2H, CH_2), 3.700 (s, 1H, CH), 2.725 (s, 3H, CH_3), 2.314 (t, 4H, CH_2CH_2, J = 8.0 Hz). ^{13}C NMR (DMSO-d_6, δ): 170.2 (C=O), 156.4 (C aro), 141.2 (C aro), 138.1 (C aro), 137.4 (C aro), 129.7 (4 × C aro), 128.3 (5 × C aro), 127.4 (2 × C aro), 125.4 (C aro), 114.8 (2 × C aro), 67.4 (CH_2), 55.6 (CH), 35.8 (CH_2), 35.1 (CH_2), 21.8 (CH_3). ESI-MS: 453.3 (M-1).

4.2. Biological evaluation

4.2.1. MMP-2 inhibition assay

The L-tyrosine derivatives were assayed for the inhibitory activities against MMP-2 using MMP-2 Colorimetric Drug Discovery Kit. The assay buffer was pipetted into each desired well of the microplate and equilibrated to 37 °C. 20 μl MMP-2 was added to the control, inhibitor NNGH, and test inhibitor wells. Then 20 μl NNGH inhibitor was added to the inhibitor NNGH wells only, and desired volume of test inhibitor was added to appropriate wells. The plate was incubated for 30 min at 37 °C to allow inhibitor/enzyme interaction. Then 10 μl BML-P125-9090 substrate was added to start reaction. Continuously read plates at $A_{412\ nm}$ in the microplate reader and record data at 1 min time intervals for 10 min. Data analysis was performed using OriginPro 7.5 software and the IC_{50} values were obtained.

4.2.2. HDAC-8 inhibition assay

The L-tyrosine derivatives were further assayed for the inhibitory activities against HDACs using HDAC-8 Fluorimetric Drug Discovery Kit. Firstly, HDAC-8 assay buffer, diluted SAHA or test inhibitors were added to appropriate wells of the microplate. Then substrate solution and the HDAC-8 assay buffer were warmed for diluting the enzyme to assay temperature. The chilled and undiluted HDAC-8 was added to the warmed buffer, then the diluted HDAC-8 was added to all wells except those that are to be 'No Enzyme Controls'. By adding diluted substrate (25 μl) to each well and mixing thoroughly, HDAC-8 reactions were initiated and proceeded for desired length of time, which were then stopped by addition of Fluor de Lys® Developer II (50 μl). The plate was incubated at room temperature (30 °C) for at least 45 min. Signal was stable for at least 60 min. Finally, the samples were read in a microplate reading fluorimeter capable of excitation at a wavelength in the range 350–380 nm and detection of emitted light in the range 440–460 nm. The IC_{50} values were obtained using OriginPro 7.5 software.

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