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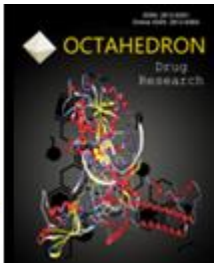

Design, synthesis *in vitro* and *in vivo* evaluation of new diaryltriazoles carboxylic and hydroxamic acid derivatives as inhibitors of tumor necrosis alpha converting enzyme (TACE)

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ABSTRACT

A series of vicinal diaryltriazoles carboxylic and hydroxamic acid derivatives (**4a-4x, 5a-5x, 6a, 6b**) were prepared with proving their structures by different spectroscopic techniques. They showed good to moderate anti-inflammatory activity (30-117% % of indomethacin activity). In testing gastric ulceration, the synthetized compounds showed a low incidence of gastric ulceration, (0-3). Histopathological investigation of compounds **5a** and **5i** showed that stomach tissue integrity was normal without any damage. The mechanistic study through inhibition of TACE showed noticeable inhibition of TACE, with IC_{50}'s ranging from 1.1 to 4.6 μM. Docking studies showed that compounds **5a**, **5f** and **5h** have good binding with TACE enzyme that was in agreement with *in vitro* inhibitory activity towards the TACE enzyme. These results could be a reasonable explanation for their good anti-inflammatory activity in compare to reference drug indomethacin. Evaluation of IC_{50} of inhibition of tested compounds on MMP-1 showed slight selectivity (2-6 folds) of tested compounds towards TACE enzyme.

1. **Introduction.** The cytokine tumor necrosis alpha (TNF-α) is a pro-inflammatory protein produced by macrophages, neutrophiles, eosinophiles, natural killer cells (NK cells) and neurons.[1-3] TNF-α is produced as pro-TNF and then converted by the zinc containing proteinase TNF-α converting enzyme (TACE) to a soluble form (sTNF). The soluble form interacts with two receptors TNF-RI and TNF-RII [4] and mediates several inflammatory disorders [5-7] (as rheumatoid arthitis) and autoimmune diseases[2] (psoriasis and Crohn's disease[8]).

TNF-α is considered a drug target for treatment of the diseases associated with its overexpression. Monoclonal antibodies have been developed to bind to the released sTNF thus preventing it from interaction with its receptor and stop cell signaling.[9] There are thee approaches to inhibit TNF-α which are inhibition of TNF-α synthesis e.g. through interference with transcription, translation or mRNA half-life. The second approach was through TNF antagonism as monoclonal antihuman TNF-α antibody, infliximab (Remicade®) and Entanercept (Enbrel®).[10] The third approach is inhibition of TNF-α shedding through TNF-α converting enzyme inhibitors (TACEI) that is considered attractive because of reduced cost of treatment, ease of administration (orally active), high patient compliance and being potential for more precise control of TNF-α level.[10]

Several classes of small molecules and structurally variable TACE inhibitors have also been developed. The most useful groups were those containing hydroxamic acids as they are the most potent motif for zinc binding in the enzyme.[11] Many classes have been developed that contain hydroxamate moiety as linear succinate based inhibitors (Marimastat **I**, Ro 31-9790 [12] **II** and BB-1101 **III** [13, 14]), macrocyclic succinate based inhibitors (Prinomastat **IV**) [15], non-succinate hydroxamate inhibitors (**V**) [16], and non-hydroxamate inhibitors (**VI**) [17] (**Fig. 1**).

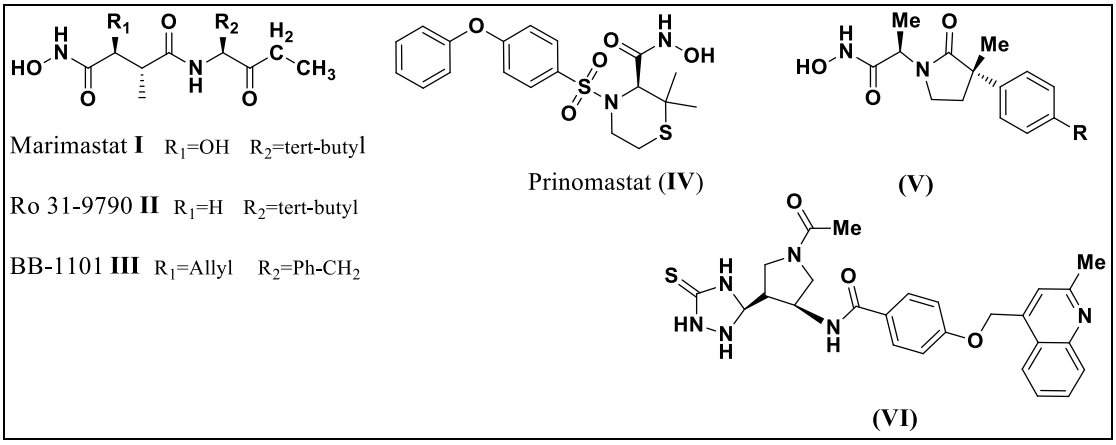


Fig. 1: Chemical structures of developed small molecule TACE inhibitors

The design of a TACE inhibitor is very challenging because structure TACE and matrix metalloproteinase (MMP) enzymes are very similar. The structure of both have a similar catalytic site and also they are zinc endopeptidases.[18] Therefore, TACE inhibitors are associated with several musculoskeletal side effects resulting from MMP inhibition.[19] Marimastat and prinomastat as previosly used MMP inhibitors were found to also inhibit TACE. Some structural modifications of TACE inhibitors were done and resulted in improved selectivity. These modifications are summarized in **Fig. 2**.[20]

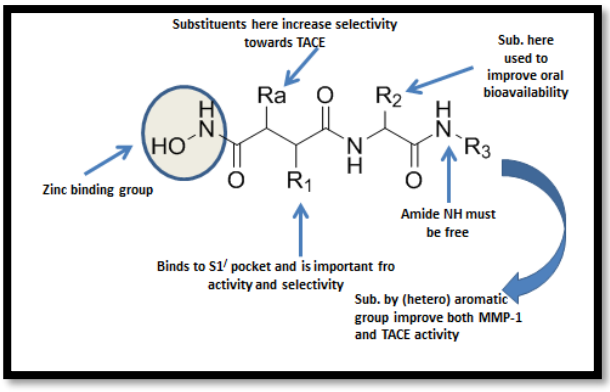
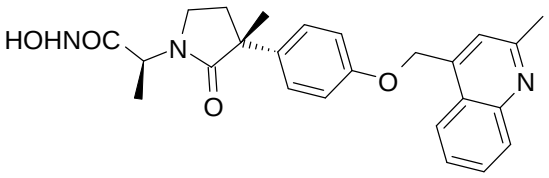


Fig.2: SAR of TACE inhibitors

The structure of TACE is different from MMP-3 in the shape of S1`pocket, which is straight and deep in MMP-3 and L-shaped in the TACE model.[21] Bristol Mayers Squib developed new series of $\gamma$-lactam **(VII)** adapted the S1`pocket of TACE, so presence of bulky group at S1 site will be more selective TACE inhibitor over MMPs, so derivative with benzyloxy group at R is more potent 250 times than isobutyl (IC_{50} values are 4 and 1000 nM,

respectively) (**Fig. 3**). The ether linkage is essential for potency. Presence of biphenyl group at R has IC_{50} value 1.3 μM while substitution with phenoxy group has IC_{50} value 185 nM.[22] Linear succinate compounds represent a promising scaffold for developing selective TACE inhibitors. Structure activity relationship studies (SAR) of linear succinates revealed that they fit to the enzyme subsites in a manner like the enzyme substrate.



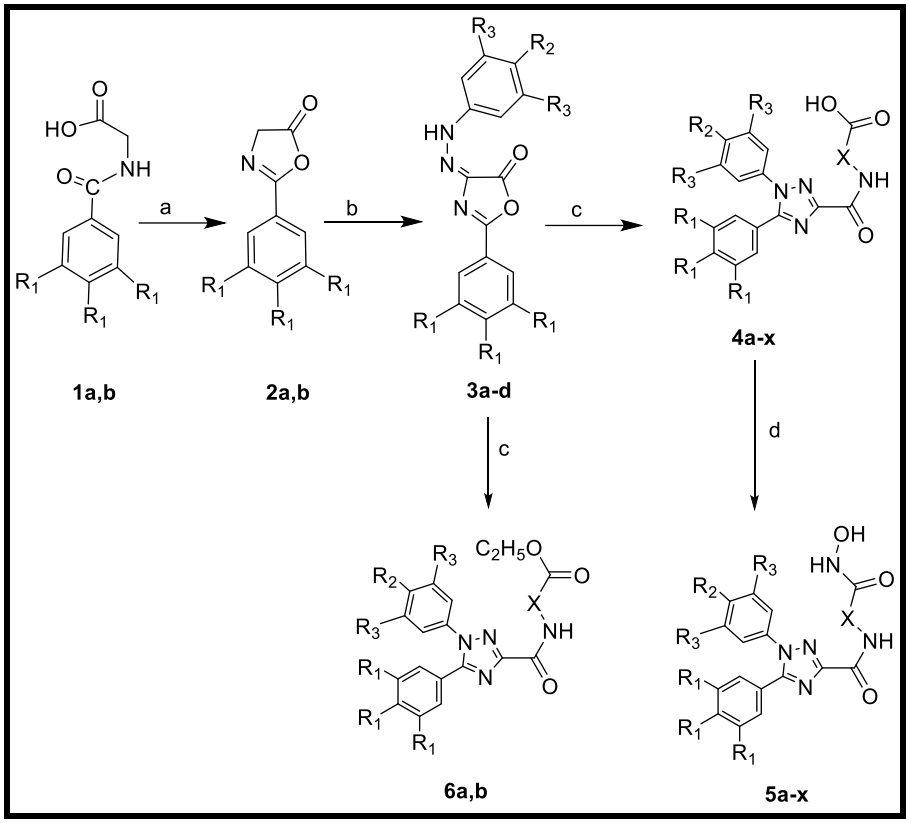
(VII)

Fig. 3: Structure of selective TACE inhibitor IK682. In our previous work a new series of novel 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamides was synthetized that exhibited remarkable anti-inflammatory activity (38% - 100% of indomethacin activity) and (44% - 115% of celecoxib activity) and with almost no side effect on GIT. Compounds **VIII** and **IX** (**Fig.4**) were the most potent anti-inflammatory with minimal GIT side effects.[23] It was stupendous that the safety of compounds on the GIT is not related to the selectivity toward COX II. Inspired by the above discussion, the target here was the discovery of new compounds that can be used in the treatment of intractable diseases like autoimmune diseases and with lesser side effects. A new series of vicinal diaryltriazoles (**4,5a-x**) was designed as potential anti-inflammatory agents that are both potent and with high safety profile on the GIT (**Fig.4**). The designed compounds would show low ulcer index and good stomach tissue integrity on the histopathological investigation. TACE inhibition is used as a parameter for the anti-inflammatory activity. The target compounds were designed to fulfil the structural requirements for TACE inhibition; presence of hydroxamic acid group (zinc binding group), different linkers (aliphatic and aromatic) to occupy enzyme subsite, and presence of substituted amide bond (essential for activity) [20](**Fig. 2**). Two series were synthesized with different substitution on diaryl rings (R_1= H or OCH_3, R_2= Cl or OCH_3, R_3= H or OCH_3, **Fig. 4**) to assess the effect of EDGs and EWGs and their ability to inhibit TACE was evaluated (**Fig.4**). Also, their ability to inhibit MMP-1 was evaluated and compared with their activity towards TACE. Guiding with the performed docking studies, the mechanistic results were in great match and it explained the plausible binding interactions in the enzyme subsite.

2. Results and discussion

2.1. Chemistry

Scheme 1 discusses the preparation of hydroxamic acid derivatives of 1,5-diphenyl-1*H*-1,2,4-triazole-3-carboxamides **5a-x**. 2-benzamidoacetic acid **1a** and 2-(3,4,5-trimethoxybenzamido) acetic acid **1b** were synthetized by the reaction of glycine with benzoyl chloride or 3,4,5- trimethoxybenzoyl chloride, respectively.[24] Then, compounds **1a** or **1b** were refluxed with acetic anhydride to afford the compounds **2a** and **2b**, respectively. Further, compounds **3a-d** were prepared by coupling of the diazonium salt of aniline, 4-chloroaniline, or 3,4,5-trimethoxyaniline with compounds **2a** or **2b** in presence of NaOAc.[24] Reaction of compounds **3a-d** with different amino acid in glacial acetic acid and sodium acetate by Sawdey rearrangement [25] yield the corresponding amides **4a-x** in moderate percentage yield [55-71%] **(Scheme 1)**. In the last step, the target hydroxamic acid derivatives **5a-x** was done by activation of carboxylic acids derivatives **4a-x** using CDI followed by reaction with $NH_2OH.HCl$ **(Scheme 1)**. Alternatively, synthesis of ester derivatives **6a** and **6b** was carried out by the reaction of **3c** and **3d** with benzocaine **(Scheme 1)**. The structure formulae of all compounds were confirmed via different spectroscopic as all protons and carbons appeared in the expected shift.



Scheme 1: Synthesis of 1,5-diphenyl-1*H*-1,2,4-triazole-3-carboxamide derivatives 5a-x

Regents and conditions:

a) Ac_2O, 60ºc, 40 min, b) Aniline, 4-chloroaniline or 3,4,5-trimethoxyaniline, HCl, NaOAc, 2-8 ^{0}C, 2 h, c) $H_2NXCOOH$ or $H_2NXCOOC_2H_5$, AcOH, NaOAc, reflux 2 h. d) CDI then $NH_2OH.HCl$, stirring 6 h.

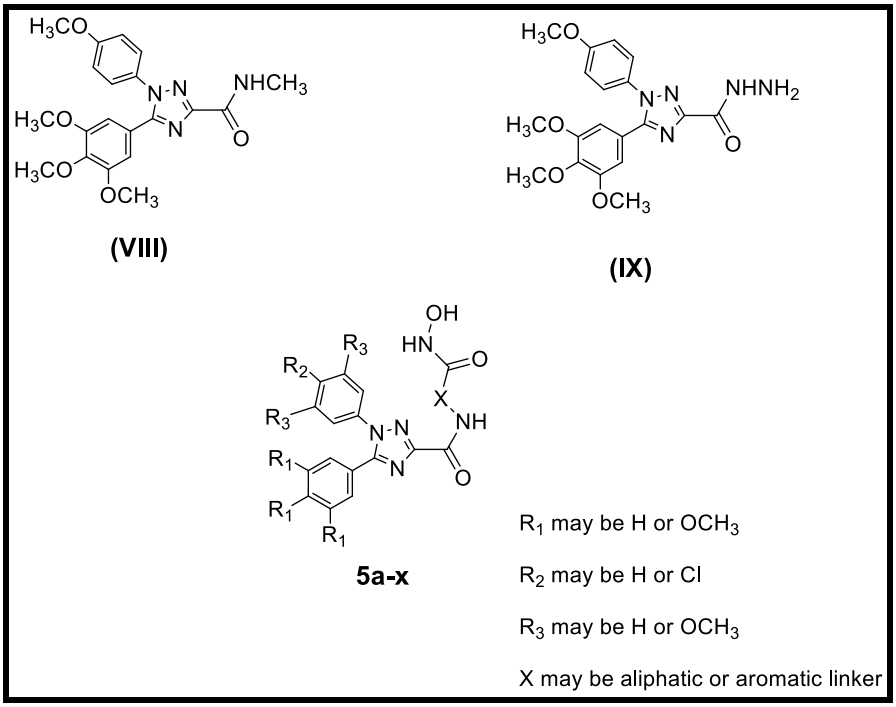


Fig. 4: Design of previous triazole derivatives (**VIII** and **IX**) and newly synthetized compounds **5a-x**

Table 1. Variable attachment points and substituents of 1-2a,b, 3a-d, 4,5a-x, and 6a,b

Compound	R_1	R_2	R_3	X	Compound	R_1	R_2	R_3	X
1a	H	-	-	-	**1b**	OCH_3	-	-	-
2a	H	-	-	-	**2b**	OCH_3	-	-	-
3a	H	H	H	-	**3b**	H	OCH_3	OCH_3	-
3c	H	Cl	H	-	**3d**	OCH_3	Cl	H	-
4,5a	H	H	H	$-CH_2-$	**4,5b**	H	H	H	$-(CH_2)_2-$
4,5c	H	H	H	$4-C_6H_4-$	**4,5d**	H	OCH_3	OCH_3	$-CH_2-$
4,5e	H	OCH_3	OCH_3	$-(CH_2)_2-$	**4,5f**	H	OCH_3	OCH_3	$-(CH_2)_3-$
4,5g	H	OCH_3	OCH_3	$4-C_6H_4-$	**4,5h**	H	OCH_3	OCH_3	$4-CH_2-C_6H_4-$
4,5i	H	Cl	H	$-CH_2-$	**4,5j**	H	Cl	H	$-(CH_2)_2-$
4,5k	H	Cl	H	$-(CH_2)_3-$	**4,5l**	H	Cl	H	$-(CH_2)_4-$
4,5m	H	Cl	H	$-(CH_2)_5-$	**4,5n**	H	Cl	H	$4-C_6H_4-$
4,5o	H	Cl	H	$4-CH_2-C_6H_4-$	**4,5p**	H	Cl	H	$4-C_6H_4-CH_2-$
4,5q	OCH_3	Cl	H	$-CH_2-$	**4,5r**	OCH_3	Cl	H	$-(CH_2)_2-$
4,5s	OCH_3	Cl	H	$-(CH_2)_3-$	**4,5t**	OCH_3	Cl	H	$-(CH_2)_4-$
4,5u	OCH_3	Cl	H	$-(CH_2)_5-$	**4,5v**	OCH_3	Cl	H	$4-C_6H_4-$
4,5w	OCH_3	Cl	H	$4-CH_2-C_6H_4-$	**4,5x**	OCH_3	Cl	H	$4-C_6H_4-CH_2-$
6a	H	Cl	H	$4-C_6H_4-$	**6b**	OCH_3	Cl	H	$4-C_6H_4-$

2.2. Biological investigations

2.2.1. Screening of anti-inflammatory activity

Winter et al described a robust method to evaluate the anti-inflammatory activities of new compounds by using carrageenan-induced paw edema.[26] We used this traditional method to detect the anti-inflammatory activity of the synthesized compounds (**4a-x, 5a-x** and **6a-b**). The selected compounds and indomethacin were tested by the afore mentioned method according to previously described procedure.[27] Test compounds

and indomethacin were administered to albino male rats intraperitonially at a dose of 0.20 mmol/Kg, 30 min that is prior carrageenan injection at the right hind paw. By measuring the thickness of the two paws at various time intervals, calculations were done to get the % decrease in edema thickness caused by administration of carrageenan as previously described.[27] The anti-inflammatory activity of the target compounds and indomethacin was calculated and presented in (**Table 2)** The results showed the strong edema inhibition of indomethacin at both the 3rd h and 4th h, with percentage of inhibition about 68 % and 80%, respectively.

The results founded in (**Table 2)** exhibited that carboxylic acid derivatives **4a-x** showed good to moderate anti-inflammatory activity with maximum activity was after 3 or 4 h, and then the activity decreased gradually. Compared to indomethacin, only compounds **4i, 4p, 4q,** and **4v** showed weak anti-inflammatory activity (10~30% of indomethacin activity). Compounds of aliphatic short linker (1-3 carbons) showed good anti-inflammatory activity with % of edema inhibition equals 75~105% of indomethacin activity. Compounds **4a, 4d, 4f, 4k**, **4m**, **4s**, and **4u** showed the best anti-inflammatory activity at the 4th h of 80%, 80%, 91%, 91%, 96%, 105%, and 96%, respectively of indomethacin activity. Compounds with aromatic linker; **4o, 4p**, and **4v** showed moderate to weak anti-inflammatory activity with % of edema inhibition equals to 65%, 30%, and 30% of indomethacin activity at 4thh, respectively. Only compounds **4w** and **4x** with aromatic linkers that showed good activity (80 and 96%, respectively). Also, it was observed that compounds with Cl substituent at benzene ring on position No.1 on triazole ring showed weaker anti-inflammatory activity than with compounds of OCH_3 substituent (**4d** versus **4i**, 80% and 10%, respectively). Also, addition of methoxy groups in the phenyl ring attached at position 5 of the triazole ring slightly increased the anti-inflammatory activity (**4j** and **4k** versus **4r** and **4s**, 65% and 91% versus 75% and 105%, respectively).

Compounds of hydroxamic acid derivatives showed better activity than carboxylic acid derivatives especially after the 3rd h. Compounds **5a, 5d-f, 5h-i, 5k-l, 5p, 5q, 5s,** and **5u** showed significant anti-inflammatory activity with % of edema inhibition equals 96%, 88%, 100%, 100%, 96%, 98%, 90%, 83%, 94%, 90%, 100% and 117% , respectively of indomethacin activity (**Table 2)**. Other compounds showed moderate to weak activity with % edema inhibition (16-76%) compared to indomethacin. Hydroxamic acid derivatives showed similar structure activity relationship as in carboxylic acid derivatives with aliphatic linkers (**5a**, 91%) more active than aromatic linkers (**5c**, 73%) and compounds with Cl substituent at ring on position No.1 on triazole ring showed weaker anti-inflammatory activity than compounds of OCH_3 substituent. Only compound **5u** is more active than indomethacin (117%).

Compounds **6a,b** with ester group showed weak anti-inflammatory activity (29%, and 33%, respectively of indomethacin activity).

From the afore mentioned results, we can summarize that most of the synthesized compounds exhibited good anti-inflammatory activity but at different time intervals (3h for hydroxamic acid derivatives or 4h for carboxylic acid derivatives). In general, hydroxamic acid derivative have anti-inflammatory activity better than their corresponding carboxylic acid: **5a**>**4a**, **5f**>**4f,** and **5i**>**4i**. The activity of hydroxamic acid derivatives may be due to chelation of this group with zinc atom of TACE enzyme which will be further confirmed by TACE inhibition assay and docking studies. Trimethoxyphenyl substitution on C5 of 1,2,4-triazole instead of phenyl slightly increased the anti-inflammatory activity. Also, addition of methoxy groups in the phenyl ring attached at position 5 of the triazole ring increased the anti-inflammatory activity. It is obvious that electron donating groups are better than electron withdrawing groups (OCH_3 > H > Cl). Compounds with aliphatic chain linker are slightly potent than compounds with aromatic linker in carboxylic or hydroxamic acid. The optimum aliphatic linker length is about 1-3 carbons ester group of compounds (**6a,b**) abolished the anti-inflammatory activity.

Table 2. The anti-inflammatory activity of **4a-x, 5a-x** and **6a-b** relative to that of indomethacin using carrageenan-induced paw edema in rats.

Comp.	3 h	4 h		3 h	4 h
4a	76.47%	80.00%	**5d**	88.24%	80.00%
4b	82.40%	75.04%	**5e**	100.00%	80.00%
4c	88.24%	85.00%	**5f**	100.00%	80.00%
4d	76.47%	80.00%	**5g**	82.35%	75.00%
4f	73.53%	91.35%	**5h**	96.11%	67.26%
4i	18.38	10.42	**5i**	98.04	97.22
4j	70.59	65.00	**5j**	36.76	36.46
4k	67.87	91.35	**5k**	90.50	86.54
4l	73.53	57.29	**5l**	83.33	83.33
4m	107.47	96.15	**5m**	41.18	50.00
4n	70.59	80.00	**5n**	29.41	25.00
4o	64.71	65.00	**5o**	56.56	62.50
4p	47.06	30.00	**5p**	94.12	90.00
4q	36.76	26.04	**5q**	90.50	67.31
4r	82.35	75.00	**5r**	41.18	35.00
4s	100.00	105.00	**5s**	100.00	90.00
4t	52.94	50.00	**5t**	76.47	70.00
4u	107.47	96.15	**5u**	117.65	105.00
4v	35.29	30.00	**5v**	41.18	40.00
4w	100.00	80.00	**5w**	65.36	64.81
4x	101.81	96.15	**5x**	16.97	14.42
5a	96.15%	91.35%	**6a**	29.41	20.00
5b	73.53%	81.73%	**6b**	33.94	24.04
5c	79.66%	72.92%			

2.2.2. Screening of ulcerogenicity

In order to prove the safety profile of the newly synthetized compounds **4a-d**, **4f-x**, **5a-x** and **6a,b**, the *in vivo* ulcerogenic liability was evaluated relative to indomethacin and celecoxib using a previously reported procedure.[28] The ulcer index (UI) could be calculated after classification of ulcers area into different levels as following: level I, ulcer area less than 1 mm^2, level II, ulcer area from 1-3 mm^2 and level III, ulcer area more than 3 mm^2. Then by using the following equation, ulcer index could be calculated.

UI = 1× (no. of ulcers level I) + 2 × (no. of ulcers level II) + 3 × (no. of ulcers level III), etc.

The UI of compounds **4a-d**, **4f** and **6a,b** was calculated (**Table 3)** as (mean ± S.E.M). The results of ulcerogenic liability exhibited that indomethacin (in equimolar dose to prepared compounds) caused remarkable ulcerogenic toxicity with UI of **29.8**, while celecoxib showed very low UI of **0.4**. Many of the target compounds were with much lower UIs relative to indomethacin. Compounds **4b, 4c, 4q, 4r, 4v, 5k, 5m, 5q,** and **5u** showed ulcer index of 0.4 like celecoxib (**Table 3)**. Compounds **4d, 5a, 5g, 5i, 5j** and **5s** showed ulcer index lower than celecoxib (0.2). Compounds **5b** and **5d** did not show any ulcerogenicity.

The calculated data showed that almost of the test compounds were with safer ulcerogenic liability relative to indomethacin. Also, some compounds had UI similar or lesser than celecoxib. Ulcer indices measured post administration of compounds **4a-d**, **4f-x**, **5a-x** and **6a,b** compared to indomethacin and celecoxib.

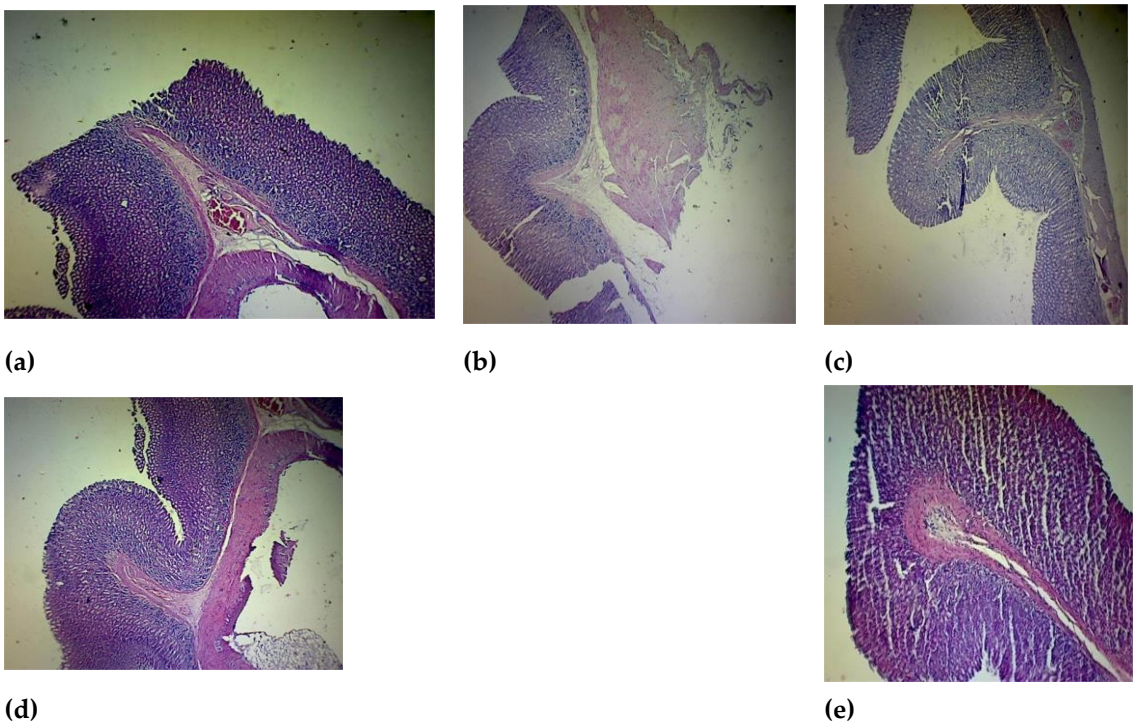
Table 3. Ulcer indices measured post administration of compounds **4a-d**, **4f, 4i-x** 5**a-x** and **6a,b** compared to indomethacin and celecoxib.

Comp.	UI Mean± SE	Comp.	UI Mean± SE	Comp.	UI Mean± SE
Control	0.6±0.24	**5r**	0.4±0.24	**5k**	0.4±0.24
Indomethacin	29.8±1.02	**5s**	0.6±0.24	**5l**	1.2±0.37
Celecoxib	0.4±0.24	**5t**	0.6±0.24	**5m**	0.4±0.24
4a	0.8±0.018	**5u**	1.2±0.20	**5n**	1.2±0.20
4b	0.4±0.022	**5v**	0.4±0.24	**5o**	1.2±0.20
4c	0.4±.022	**5w**	1.6±0.24	**5p**	0.6±0.24
4d	0.2±0.018	**5x**	0.6±0.24	**5q**	0.4±0.24
4f	1±0.028	**5a**	0.2±0.18	**5r**	1.4±0.24
4i	4.2±0.58	**5b**	0±0	**5s**	0.2±0.2
4j	3.2±0.37	**5c**	1±0.029	**5t**	0.6±0.24
4k	1.6±0.24	**5d**	0±0	**5u**	0.4±0.24
4l	2.6±0.24	**5e**	3.2±0.066	**5v**	1.6±0.24
4m	1.4±0.24	**5f**	0.8±0.018	**5w**	2±0.32
4n	1±0.00	**5g**	0.2±0.018	**5x**	1±0.00
4o	0.8±0.20	**5h**	0.6±0.022	**6a**	1.4±0.24
4p	1.6±0.24	**5i**	0.2±0.20	**6b**	1.2±0.20
4q	0.4±0.24	**5j**	0.2±0.20		

2.2.3. Histopathological investigation

Different stomach sections of the ulcers including the control and the treated groups with indomethacin or prepared compounds were stained by standard hematoxylin and eosin stains. Microscopical examination was done for the prepared slides and pictures were taken for these slides.

By examining the control (**Fig. 5a**), no lesions were detected, and the mucosal layer was continuous. Indomethacin (**Fig. 5b**) showed high loss of mucosal membrane detected at the ulcer area, some areas of fundic glands were completely damaged and with great loss of cellular details. Capillary inflammatory cells were also found, and apoptotic glandular epithelial cells could be detected. On the other hand, by examining the stomach sections of the ulcers after treatment with compounds **5a** and **5i** normal morphology for the fundic glands was observed and the results were in agreement with the previous results.[28] The observed edema was very low and with very low vasodilatation of blood confirmed by the low UI of 0.2 of compounds **5a** and **5i** (**Fig. 5c and 5d,** respectively). A significant incidence of gastric ulceration was induced by the compound **5e** where a small loss mucosal layer was observed with the presence of capillary inflammatory cells and the ulcerative damage of the gastric mucosa was markedly increased, which was proved by the UI of 3.20 **(Fig. 5e).**



(a) **(b)** **(c)**

(d) **(e)**

Fig. 5: Photomicrograph of the mucosa of fundic stomach of (a) control, (b) indomethacin, (c) compound **5a**, (d) compound **5i**, (e) compound **5e**

2.2.4. TACE inhibitory assay.

To go deeper into the mechanism of action of the target compounds, compounds **5a, 5f, 5h, 5i, 5k, 5p,** and **5u** were evaluated for *in vitro* TACE inhibitory activity in Jurkat, Clone E6-1 cells using Human TACE ELISA (Enzyme-Linked Immunosorbent Assay) kit. This ELISA kit shows no cross-reactivity with any of the cytokines tested, and the % inhibition of TACE was calculated for each sample as a % of control and listed in **Table 4.** It was noticed a good correlation of compounds TACE inhibitory activity and their *in vitro* anti-inflammatory activity. The compounds showed good % inhibition of TACE (78 - 89%). As previously mentioned in the introduction part that the design of a TACE inhibitor is very challenging because there are structural similarities between TACE and matrix metalloproteinase (MMP) that may lead to many skeletomuscular side effects.[19] In order to present our compounds as specific TACE inhibitors, IC_{50} of TACE

and MMP-1 inhibition of compounds **5a, 5f, 5h, 5i, 5k, 5p,** and **5u** were calculated in Jurkat, Clone E6-1 cells and the results are listed in **Table 5.** The results showed that the synthetized compounds are 2-6 folds more active as TACE inhibitor than MMP-1 inhibitor.

2.2.5. Docking studies

Molecular modeling study was performed for compounds **5a, 5f**, and **5h** and reference compound in the binding site of TACE (pdb: 2A8H) [29] using Molecular Operating Environment (MOE®) version 2014.09. Molecular docking was carried out at Assuit University. Reference compound is *N*-((2R)-2-[2-(hydroxyamino)-2-oxoethyl]-4-methylpentanoyl)-3-methyl-L-valyl-*N*-(2-aminoethyl)-L-alaninamide, which belongs to a linear succinate hydroxamate TACE inhibitor (Fig. 6).

Table 4: *In vitro* TACE inhibitory activity of **5a, 5f, 5h, 5i, 5k, 5p,** and **5u** at 10 μM concentration.

Comp.	OD[a]	TACE residual conc. Pg/mL[b]	% Inhibition
5a	0.69475	919.556	78.91
5f	0.62875	812.261	81.37
5h	0.38625	442.706	89.85
5i	0.576	728.397	83.29
5k	0.44625	530.081	87.84
5p	0.55475	695.114	84.06
5u	0.68325	900.679	79.35
Control	2.43575	4361.65	00.0

Table 5: IC_{50} results for inhibition of TACE and MMP-1 in Jurkat, Clone E6-1 cells for compounds **5a, 5f, 5h, 5i, 5k, 5p,** and **5u**

Compound No.	**IC_{50} of TACE** μM	**IC_{50} of MMP-1** μM
5a	4.16	7.3
5f	2.57	5.2
5h	1.1	4.17
5i	2.13	4.9
5k	1.83	3.51
5p	1.85	4.09
5u	1.67	6.06

All the three tested compounds have high binding affinity to the enzyme as showed from the binding free energy (dG) values. The binding score dg values of them was around (-6) Kcal/mole comparable with the reference compound (-5.5) as shown in **Table 6**.

Results showed good correlation between the binding score dG values of the test compounds and their *in vivo* and *in vitro* anti-inflammatory activity. The most active compound as TACE inhibitor **5h** showed high binding score dG value of (-6.8) comparable to **5f** and **5a** that proved better binding to the enzyme **Table 6**.

Table 6: Energy of binding of tested compounds **5a, 5f**, and **5h** and reference compound with TACE enzyme.

Compound	dG (TACE) Kcal/mole
Reference	-5.5197
5a	-5.8992
5f	-6.3909
5h	-6.8324

Reference compound forms an expected zinc chelation though the hydroxamic carbonyl group. Also, it forms different hydrogen bonding with Gly 394 and His 415, with hydroxamic N and hydroxamic carbonyl. Another example of hydrogen bonding is carbonyl with Th 347 and Leu 348 and N with Pro 437 and Ala 439 at P2′ of the compound. Furthermore, carbonyl of P3′ of the compound binds with Tyr 390 of the enzyme (**Fig. 6)**.

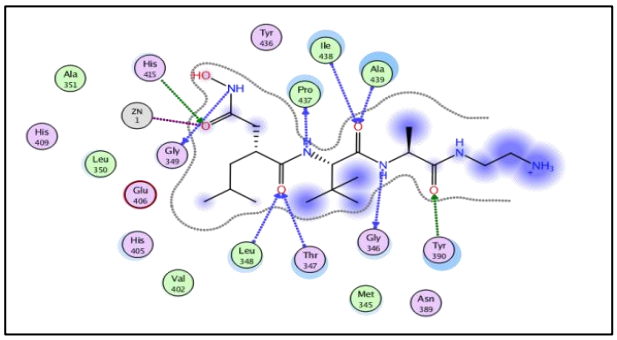


Fig. 6: 2D representation of docking of reference compound into the TACE active site**.**

Target compounds bind to the enzyme in a manner like that of the reference. Compound **5a** forms hydrogen bonding with Glu 406 and Gly 349 amino acids of TACE.

Also, zinc atom chelates the oxygen of hydroxamic group. Moreover, phenyl ring interacts with both Ile 438 and Ala 439 by Van der Waal's bonding (**Fig. 7)**. Compound **5f** binds with TACE in different modes; zinc atom chelates with carbonyl oxygen of the amide and N4 of triazole ring. Hydroxyl group of hyroxamic acid forms hydrogen bonding with Glu 404. Also, there is a hydrophobic bonding of unsubstituted phenyl ring with His 405 (**Fig. 8)**. Compound **5h** showed higher binding with the enzyme than compounds **5a** and **5f**. It showed different types of bonds; zinc chelation with hydroxamic acid carbonyl, hydrogen bonds with Glu 406, His 415, Gly 346 and Gly 349. It also has hydrophobic bonding with Th 347, Asn 389 and Ala 439 by phenyl ring of *p*-methylbenzoic acid side chain, unsubstituted phenyl ring and triazole ring, respectively (**Fig. 9)**. All compounds showed higher interactions with the enzyme than the reference drug. Triazole ring showed a good role in binding with the enzyme especially in compounds **5f** and **5h** that may explain their high binding score.

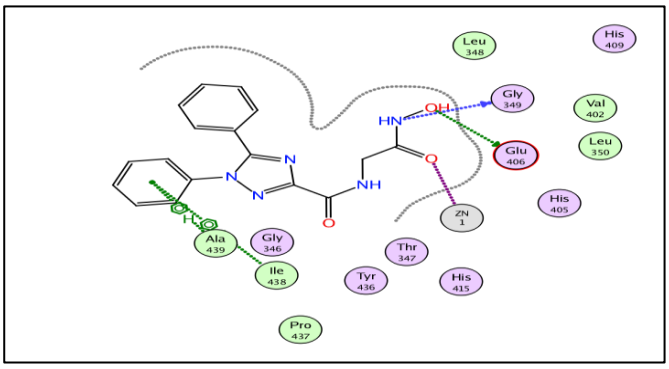


Fig. 7: 2D representation of docking of compound **5a** into the TACE active site.

Conclusion

A new series of vicinal diaryltriazoles (**4a-4x,5a-5x, 6a, 6b**) has been synthetized and their anti-inflammatory activities were evaluated. Compound **5u** showed better activity than indomethacin. All compounds showed good GIT safety profile compared to indomethacin. The selected compounds exhibited good inhibition of TACE

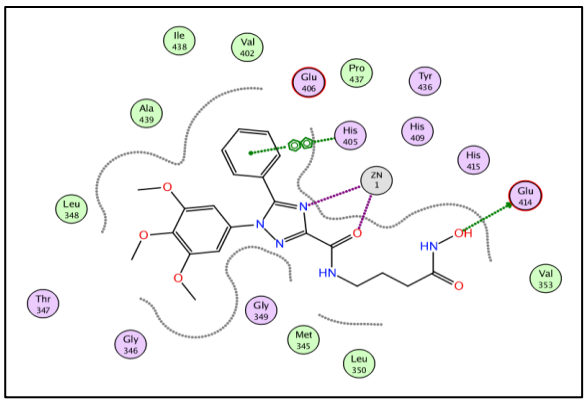


Fig. 8: 2D representation of docking of compound **5f** into the TACE active site.

enzyme with IC_{50} of 1.1 for compound **5h**. Docking studies on TACE active site was in agreement with the *in vitro* studies. The compounds showed slight selectivity towards TACE than MMP-1. Further study is needed in order to increase selectivity towards TACE inhibition.

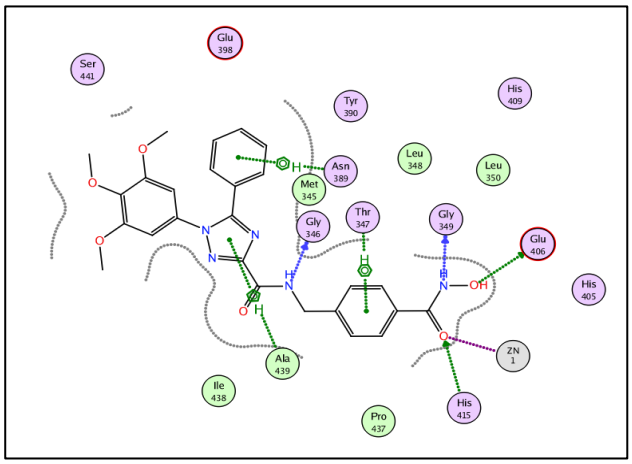


Fig. 9: 2D representation of docking of compound **5h** into the TACE active site

4. Experimental

4.1. Chemistry

Materials and methods

Benzoyl chloride, 3,4,5-trimethoxybenzoyl chloride, aniline, 3,4,5-trimethoxyaniline, 4-chloroaniline, glycine, β-alanine, 4-aminobutyric acid, 5-aminovaleric acid, 6-aminohexanoic acid, *p*-aminobenzoic acid, 4-aminomethylbenzoic acid, 2-(4-aminophenyl)acetic acid, benzocaine, hydroxylamine hydrochloride, CDI, anhydrous sodium acetate, sodium hydroxide, hydrochloric acid, acetic anhydride, 1,4-dioxan, glacial acetic acid, anhydrous sodium acetate, sodium nitrite

and different solvents used in the preparation of the intermediate and final compounds are of commercial grade, purchased from El-Nasr pharmaceutical chemicals, Aldrich, Merck, and Fluka.

Thin-layer chromatography (TLC) using Merck 9385 pre-coated aluminium plate silica gel (Kieselgel 60) 5 x 20 cm plates with a layer thickness of 0.2 mm, was used to examine purity of compounds and spots were visualized by exposure to UV-lamp at λ= 254 nm. Melting points were determined on Stuart electro-thermal melting point apparatus and are uncorrected.

IR spectra were recorded on Nicolet iS5 FT-IR spectrometer, Faculty of Pharmacy, Minia University. ^{1}H-NMR spectra were carried out using Bruker apparatus 400 MHz spectrometer, Faculty of Pharmacy, Beni Suef University. High resolution mass spectra (HMS) were obtained on a Thermo Scientific Q Exactive™ Orbitrap mass spectrometer, Faculty of Pharmaceutical sciences, University of British Columbia, Canada.

4.1.1. Synthesis of 2-benzamidoacetic acid (hippuric acid) (1a) and 2-(3,4,5-trimethoxybenzamido)acetic acid (1b)[30] [24]

Compound **1a** was crystallized from water in 92% yield and compound **1b** was crystallized from aqueous ethanol in 65% yield.

4.1.2. Synthesis of 4-phenylhydrazono-2-phenyl-4*H*-oxazol-5-one (3a), 4-[(4-chlorophenyl)hydrazono]-2-phenyl-4*H*-oxazol-5-one (3b), 4-[(3,4,5-trimethoxyphenyl)hydrazono]-2-phenyl-4*H*-oxazol-5-one (3c) and 4-[(4-chlorophenyl)hydrazono]-2-(3,4,5-trimethoxyphenyl)-4*H*-oxazol-5-one (3d)[31] [24]

Compounds **3a**, **3b**, **3c** and **3d** were crystallized from acetone as yellow crystals (crude yield 90%, 72%, 85%, 77% respectively); IR (cm^{-1}): 1795 (C=O), 1630 (C=N), 1520 (C=C), 1230 (C-O-C).

4.1.3. General procedure for the synthesis of carboxylic acid derivatives of 1,2,4-triazole-3-carboxamides (4a-x)

Compound **3a** (0.01 mol, 2.65 g), **3b** (0.01 mol, 2.99 g), **3c** (0.01 mol, 3.55 g) or **3d** (0.01 mol, 3.89 g) were mixed with appropriate amino acid (0.01 mol). The mixture was heated under reflux for 2 h in acetic acid (50 mL) and in the presence of anhydrous sodium acetate (0.018 mol, 1.5 g). Then, the mixture was cooled and poured into ice water (100 mL). The formed precipitate was filtered off, dried and crystallized from aqueous methanol.

2-(1,5-Diphenyl-1*H*-1,2,4-triazole-3-carboxamido)acetic acid (4a) [24]

Pale yellow crystals (2.41 g, 75%); mp 210-212 °C

3-(1,5-Diphenyl-1*H*-1,2,4-triazole-3-carboxamido)propionic acid (4b)

Reaction of **3a** (0.01, 2.66 g) with β-alanine (0.012 mol, 1.06 g) yielded pale yellow crystals (2.45g ,73%); mp 183-185 °C; IR (cm^{-1}): 3640-2540 (OH), 3301 (NH), 1690 (C=O), 1669 (C=O), 1592 (C=N);^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.76 (t, 2H, *J* = 6.0 Hz, C$\underline{H_2}$-COOH), 3.82 (q, 2H, *J* =5.4 Hz, NH-C$\underline{H_2}$), 7.35-7.69 (m, 10H, Ar-$\underline{H}$), 7.97 (t, 1H, *J* = 5.2 Hz, N$\underline{H}$); HRMS: m/z calculated for $C_{18}H_{16}N_4O_3$ [M-H]$^-$: 335.11496, found: 335.11526.

4-(1,5-Diphenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid (4c) [24]

Brown crystals (2.95 g, 77%); mp 279 °C

2-((1-(3,4,5-Trimethoxyphenyl)-5-phenyl)-1*H*-1,2,4-triazole-3-carboxamido) acetic acid (4d) [24]

White powder (2.91g, 70%); mp161-163 °C

3-((1-(3,4,5-Trimethoxyphenyl)-5-phenyl)-1*H*-1,2,4-triazole-3-carboxamido) propionic acid (4e) [24]

Yellowish white powder (3.1g, 73%) ; mp 161-163 °C

4-((1-(3,4,5-Trimethoxyphenyl)-5-phenyl)-1*H*-1,2,4-triazole-3-carboxamido)butanoic acid (4f) [24]

Yellowish white powder (3.17g, 73%); mp 135-137 °C

4–(1-(3,4,5-Trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid (4g) [24]

Brown colored powder (3.65g, 77%); mp 269-271 °C

4–((1-(3,4,5-Trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)methyl) benzoic acid (4h) [24]

Brownish white powder (3.51,72%); mp 189-191 °C.

2-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)acetic acid (4i) [24] Pale yellow crystals; 45% yield; m.p 190 °C.

3-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid (4j) [24]

Pale yellow crystals; 47% yield; m.p 193°C.

4-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)butanoic acid (4k)

Reaction of **3c** (0.01 mol, 2.99 g) with 4-aminobutyric acid (0.01 mol, 1.03 g) yielded pale yellow crystals; 61% yield; mp 199°C; IR (cm^{-1}): 3510-2555 (OH), 3360 (NH), 1730 (carboxylic C=O), 1680 (amidic C=O),1608 (C=N);^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm):2.00 (p,2H, $CH_2\underline{CH_2}CH_2$), 2.47 (t,2H,*J* = 6.00 Hz,$\underline{CH_2}CO$), 3.58 (t, 2H,*J* = 6.00 Hz,$\underline{CH_2}NH$),7.33 (d, 2H, *J* = 8.00 Hz, Ar-H),7.41 (d, 2H, *J* = 8.00 Hz, Ar-H) , 7.46-7.49 (m, 5H, Ar-H),7.63 (s, 1H, CONH); HRMS: m/z calculated for $C_{19}H_{17}ClN_4O_3$[M-H]$^-$: 383.09164, found: 383.09073.

5-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)pentanoic acid (4l)

Reaction of **3c** (0.01 mol, 2.99 g) with 5-aminovaleric acid (0.01 mol, 1.17 g) yielded pale yellow crystals; 52% yield; mp 192°C; IR (cm^{-1}): 3242-2535 (OH), 3320 (NH), 1710 (carboxylicC=O), 1660 (amidic C=O), 1560 (C=N);^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm):1.69-1.74 (m, 4H, $CH_2\underline{CH_2CH_2}CH_2$), 2.42 (t,2H,*J* = 6.00 Hz,$\underline{CH_2}CO$), 3.54 (t, 2H,*J* = 5.60 Hz,$\underline{CH_2}NH$),7.31 (d, 2H, *J* = 8.00 Hz, Ar-H),7.41 (d, 2H, *J* = 8.00 Hz, Ar-H) , 7.41-7.53 (m, 5H, Ar-H),7.68 (s, 1H, CONH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 22.34, 29.03, 33.78, 38.75, 119.93, 127.38, 128.20, 129.27, 130.06, 130.98, 134.55, 136.81, 155.10, 157.06, 158.98; 174.89; HRMS: m/z calculated for $C_{20}H_{19}ClN_4O_3$ [M-H]$^-$: 397.10729, found: 397.10785.

6-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)hexanoic acid (4m)

Reaction of **3c** (0.01 mol, 2.99 g) with 6-aminohexanoic acid (0.01 mol, 1.31 g) yielded pale yellow crystals; 57% yield; mp 195°C; IR (cm^{-1}): 3240-2535 (OH), 3350 (NH), 1715 (carboxylic C=O), 1675 (amidic C=O), 1570 (C=N);^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.30 (p, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.53 (p, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.21 (t, 2H,*J*= 6.00 Hz,$\underline{CH_2}CO$), 3.26 (t, 2H,*J*= 6.00 Hz,$\underline{CH_2}NH$), 7.61 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.69 (d, 2H, *J*= 8.40 Hz, Ar-H) , 7.43-7.75(m, 5H, Ar-H), 8.72 (s, 1H, CONH), 12.03 (s, 1H , COOH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 25.37, 26.49, 29.33, 32.70, 49.07, 127.38, 128.19, 129.18, 129.37, 130.06, 130.98, 134.55, 136.81, 155.08, 157.09 , 158.92, 169.53; HRMS: m/z calculated for $C_{21}H_{21}ClN_4O_3$ [M-H]$^-$: 411.12294, found: 411.12225.

4-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid (4n)

Reaction of **3c** (0.01 mol, 2.99 g) with PABA (0.01 mol, 1.37 g) yielded pale yellow crystals; 75% yield; m.p 211°C; IR (cm^{-1}): 3280-2550 (OH), 3310 (NH), 1720 (carboxylic C=O), 1670 (amidic C=O), 1588 (C=N);^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.25 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.49 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.51-7.57 (m, 5H, Ar-H), 7.62 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.40 Hz, Ar-H),9.12 (s, 1H, CONH), 12.66 (s, 1H, COOH); HRMS: m/z calculated for $C_{22}H_{15}ClN_4O_3$[M-H]$^-$: 417.07599, found: 417.07532.

4-((1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)methyl)benzoic acid (4o)

Reaction of **3c** (0.01 mol, 2.99 g) with 4-aminomethylbenzoic acid (0.01 mol, 1.51 g) yielded pale yellow crystals; 76.80% yield; mp 209°C; IR (cm^{-1}): 3242-2535 (OH), 3329 (NH), 1718 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.51 (s, 2H, CH_2), 7.46 (d, 2H, *J* = 8.00 Hz, Ar-H),7.51 (d, 2H, *J* = 8.00 Hz, Ar-H)7.53-7.55 (m, 5H, Ar-H), 7.60 (d, 2H, *J* = 8.40 Hz, Ar-H) , 7.92 (d, 2H, *J*= 8.40 Hz, Ar-H), 9.22 (s, 1H, CONH); HRMS: m/z calculated for $C_{23}H_{17}ClN_4O_3$[M-H]$^-$: 431.09164, found: 431.09171.

4-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)phenylacetic acid (4p) [24]

Pale yellow crystals; 74.80% yield; mp 209°C.

2-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)acetic acid (4q)

Reaction of **3d** (0.01 mol, 3.89 g) with glycine (0.01 mol, 0.75 g) yielded pale yellow crystals; 43% yield; m.p 202°C; IR (cm^{-1}): 3242-2535 (OH), 3329 (NH), 1718 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.63 (s, 6H, 2-OCH_3), 3.70 (s, 3H, OCH_3), 3.95 (d, 2H,*J* = 6.00 Hz, CH_2), 6.74 (s, 2H, Ar-H), 7.57 (d, 2H, *J* = 8.80 Hz, Ar-H), 7.66 (d, 2H, *J* = 8.80 Hz, Ar-H) , 8.88 (t, 1H, *J* = 6.00 Hz, CONH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ (ppm): 49.06, 56.27, 60.64, 107.02, 122.24, 128.53, 130.05, 134.73, 136.92, 139.64, 153.20, 155.08, 156.38, 159.27, 171.31; HRMS: m/z

calculated for $C_{20}H_{19}ClN_4O_6$[M-H]$^-$: 445.09204, found: 445.09241.

3-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid (4r) [24]

Pale yellow crystals; 47% yield; mp 185°C.

4-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)butanoic acid (4s)

Reaction of **3d** (0.01 mol, 3.89 g) with 4-aminobutyric acid (0.01 mol, 1.03 g) yielded pale yellow crystals; 66% yield; mp 199°C; IR (cm^{-1}): 3340-2500 (OH), 3310 (NH), 1715 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N);^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.94 (p, 2H, $CH_2\underline{CH_2}CH_2$), 2.4 (t, 2H,*J* = 6.40 Hz,$\underline{CH_2}$CO), 3.53 (t, 2H,*J* = 6.40 Hz,$\underline{CH_2}$NH), 3.75 (s, 6H, 2-OCH_3), 3.85 (s, 3H, OCH_3), 7.28 (s, 2H, Ar-H), 7.38 (d, 2H, *J* = 8.00 Hz,Ar-H), 7.44 (d, 2H*J* = 8.00 Hz, Ar-H), 7.54 (s, 1H, CONH) ; ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 25.4, 31.34, 38.82, 56.10, 60.97, 106.41, 121.40, 127.03, 129.64, 135.49, 136.21, 140.09, 153.27, 154.97, 156.29, 159.38, 174.51; HRMS: m/z calculated for $C_{22}H_{23}ClN_4O_5$[M-H]$^-$: 473.12391, found:473.12347.

5-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)pentanoic acid (4t)

Reaction of **3d** (0.01 mol, 3.89 g) with 5-aminovaleric acid (0.01 mol, 1.17 g) yielded pale yellow crystals ; 55% yield; mp 188°C; IR (cm^{-1}): 3242-2535 (OH), 3329 (NH), 1718 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N);^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.55-1.76 (m, 4H, $CH_2\underline{CH_2CH_2}CH_2$), 2.44 (t, 2H, *J* = 6.00 Hz, $\underline{CH_2}$CO), 3.55 (t, 2H, *J* = 5.60 Hz, $\underline{CH_2}$NH), 3.75 (s, 6H, 2-OCH_3), 3.89 (s, 3H, OCH_3), 6.71 (s, 2H, Ar-H), 7.39 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.47 (d, 2H, *J* = 8.00 Hz, Ar-H) , 7.88 (s, 1H, CONH) ; ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 21.96, 29.70, 33.28, 39.04, 56.12, 61.00, 106.41, 121.44, 122.55, 126.97, 129.66, 134.76, 139.25, 153.32, 156.44, 158.99, 163,31, 178.12; HRMS: m/z calculated for $C_{23}H_{25}ClN_4O_6$ [M-H]$^-$: 487.13905, found: 487.13953.

6-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)hexanoic acid (4u)

Reaction of **3d** (0.01 mol, 3.89 g) with 6-aminohexanoic acid (0.01 mol, 1.31 g) yielded pale yellow crystals; 61% yield; mp 201°C; IR (cm^{-1}): 3242-2535 (OH), 3329 (NH), 1718 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N);^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.34 (p, 2H, $CH_2CH_2\underline{CH_2}CH_2CH$), 1.56 (p, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.22 (t, 2H,*J* = 6.00 Hz, $\underline{CH_2}$CO), 3.29 (q, 2H, *J* = 5.60 Hz,$\underline{CH_2}$NH), 3.64 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3),6.78 (s, 2H, Ar-H), 7.55 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.64 (d, 2H, *J*= 8.40 Hz, Ar-H), 8.72 (t, 1H, *J* = 5.60 Hz, CONH),12.14 (s, 1H, COOH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 24.71, 26.45, 29.27, 34.12, 39.10, 56.45, 60.67, 107.44, 120.23, 122.33, 129.99, 13.67, 137.02, 140,07, 153.28, 154.95, 157.02, 159.01, 174.74; HRMS: m/z calculated for $C_{24}H_{27}ClN_4O_6$ [M-H]$^-$: 501.15464, found: 501.1553.

4-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid (4v) [24]

Pale yellow crystals; 77.90% yield; mp 213°C.

4-((1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)methyl)benzoic acid (4w) [24]

Pale yellow crystals; 73.40% yield; mp 213°C.

4-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)phenylacetic acid (4x)

Reaction of **3d** (0.01 mol, 3.89 g) with 2-(4-aminophenyl)acetic acid (0.01 mol, 1.51 g) yielded pale yellow crystals; 78.60% yield; mp 214°C; IR (cm^{-1}) 3230-2590 (OH), 3380 (NH), 1725 (carboxylic C=O), 1685 (amidic C=O), 1650 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.56 (s, 2H, $\underline{CH_2}$CO), 3.20 (s, 6H, 2-OCH_3), 3.66 (s, 3H, OCH_3),6.83 (s, 2H, Ar-H), 7.26 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.60 (d, 2H, *J* = 8.40 Hz, Ar-H) , 7.66 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.00 Hz, Ar-H), 9.13 (s, 1H, CONH); HRMS: m/z calculated for $C_{26}H_{23}ClN_4O_6$[M-H]$^-$: 521.12334, found: 521.12366.

4.1.4. General procedure for the synthesis of hydroxamic acid derivatives of 1,2,4-triazole-3-carboxamides (5a-x)

In acid washed glassware, the appropriate carboxylic acid (**4a-x**) (0.010 mol) was dissolved in dry 1,4-dioxan (20 mL) then CDI (0.015 mol, 2.43 g) was added in 3 portions. The reaction mixture was stirred for 1 h. An aqueous solution of $NH_2OH.HCl$ (0.02 mol, 1.39 g) was added dropwise and the resulting mixture was stirred

overnight (16 h). Water (100 mL) was added to reaction mixture and the formed precipitate was filtered off, washed with water, dried and crystallized from water.

2-(1,5-diphenyl-1*H*-1,2,4-triazole-3-carboxamido)acetic acid hydroxyamide (5a)

Reaction of **4a** (0.01 mol, 3.22 g) with hydroxylamine hydrochloride (0.02 mol, 1.39 g) yielded white crystals (2.76g, 82%); mp 214-217 ºC; IR (cm^{-1}): 3578-2730 (OH), 3310 (NH), 1720 (C=O), 1672 (C=O), 1591 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.96 (d, 2H, *J* = 6.5 Hz, NH –C$\underline{H}_2$), 7.40-7.55 (m, 10H, Ar-$\underline{H}$), 8.8 (t, 1H, *J* = 6.5 Hz, N$\underline{H}$); Anal. Calc for $C_{17}H_{15}N_5O_3$: C, 60.53; H, 4.48; N, 20.76. Found: C, 60.74; H, 4.56; N, 20.98.

3-(1,5-diphenyl-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid hydroxyamide (5b)

Reaction of **4b** (0.01 mol, 3.35) with hydroxylamine hydrochloride (0.02 mol, 1.39 g) yielded white crystals (3.02g, 86%); mp 190-192 ºC; IR (cm^{-1}): 3670(OH), 3322 (NH), 1705 (C=O), 1633 (C=O), 1610 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.15 (t, 2H, *J* = 6.8 Hz, C$\underline{H}_2$-CONHOH), 3.72 (q, 2H, NH –C$\underline{H}_2$), 7.41-7.55 (m, 10H, Ar-$\underline{H}$), 8.71 (t, 1H, *J* = 5.2 Hz, N$\underline{H}$), 8.86 (s, 1H, NHO$\underline{H}$), 10.61 (s, 1H, N$\underline{H}$OH); Anal. calcd for $C_{18}H_{17}N_5O_3$: C, 61.53; H, 4.88; N, 19.93. Found: C, 61.80; H, 4.95; N, 20.17.

4-(1,5-diphenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid hydroxyamide (5c)

The acid **4c** (0.01 mol, 3.64 g) reacted with hydroxylamine hydrochloride (0.02 mol, 1.39 g) yielded pale brown crystals (3.52 g, 88%); mp 280-283 ºC; IR (cm^{-1}): 3620-2793 (OH), 3390 (NH), 1720 (C=O), 1688 (C=O), 1602 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 7.44-7.58 (m, 10H, Ar-$\underline{H}$), 7.96 (d, 2H, *J* = 8.8 Hz, $\underline{H}_{2,6}$ of benzoic acid ring), 8.02 (d, 2H, *J* = 8.8 Hz, $\underline{H}_{3,5}$ of benzoic acid ring), 10.79 (s, 1H, N$\underline{H}$); Anal. calculated for $C_{22}H_{17}N_5O_3$ (384.14): C, 66.16; H, 4.29; N, 17.53. Found: C, 66.39; H, 4.35; N, 17.80.

2-(1-(3,4,5-trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)acetic acid hydroxyamide (5d)

Reaction of **4d** (0.01 mol, 4.11g) with hydroxylamine hydrochloride (0.02 mol, 1.39 g) yielded white crystals (3.54 g, 83%); mp 132-135 ºC; IR (cm^{-1}): 3556-2773(OH), 3332 (NH), 1721 (C=O), 1660(C=O), 1590 (C=N); NMR (400 MHz, DMSO-d_6) δ (ppm): 3.69 (s, 6H, 2 OC$\underline{H}_3$), 3.73 (s, 3H, OC$\underline{H}_3$), 3.98 (d, 2H, , *J* =6.0 Hz, HN-C$\underline{H}_2$), 6.83 (s, 2H, $\underline{H}_{2,6}$ of Ar-$\underline{H}$), 7.47-7.589 (m, 5H, $\underline{H}_{2,6}$ of Ar-$\underline{H}$), 7.79 (t, 2H, , *J* = 6.1 Hz, N$\underline{H}$); Anal. calculated for $C_{20}H_{21}O_5N_6$ C, 56.20; H, 4.95; N, 16.39. Found: C, 56.43; H, 4.98; N, 16.62.

3-(1-(3,4,5-trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid hydroxyamide (5e)

Reaction of **4e** (0.01 mol 4.25 g) with hydroxylamine hydrochloride (0.02 mol, 1.39 g) yielded white crystals (3.75 g, 85%); mp 159-162 ºC; IR (cm^{-1}): 3615-2635 (OH), 3310 (NH), 1727 (C=O), 1633 (C=O), 1590 (C=N); NMR (400 MHz, DMSO-d_6) δ (ppm): 2.92 (t, 2H, *J* = 7.1 Hz, C$\underline{H}_2$-CONHOH), 3.73 (s, 6H, 2 OC$\underline{H}_3$), 3.78 (t, *J* = 6.9 Hz 2H, HN-C$\underline{H}_2$), 3.82 (s, 3H, OC$\underline{H}_3$), 6.63 (s, 2H, $\underline{H}_{2,6}$ of Ar-$\underline{H}$), 7.44-7.56 (m, 5H, Ar-$\underline{H}$), 8.58 (t, H, *J* = 5.8 Hz, N$\underline{H}$); Anal. calculated for $C_{21}H_{23}N_5O_6$: C, 57.14; H, 5.25; N, 15.86. Found: C, 57.40; H, 5.28; N, 16.01.

4-(1-(3,4,5-trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)butanoic acid hydroxyamide (5f)

Reaction of **4f** (0.01 mol, 4.39 g) with hydroxylamine hydrochloride (0.02 mol, 1.39 g) yielded white crystals (4 g, 88%); mp134-136ºC; IR (cm^{-1}): 3698-2774 (OH), 3322 (NH), 1717 (C=O), 1688 (C=O), 1590 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.57 (p, 2H, *J* = 7.1 Hz, HN-CH$_2$-C$\underline{H}_2$), 2.05 (t, 2H, *J* = 7.4, Hz, C$\underline{H}_2$-CONHOH), 3.11 (t, *J* = 6.5, Hz, 2H, HN-C$\underline{H}_2$), 3.46 (s, 6H, 2 OC$\underline{H}_3$), 3.46 (s, 3H, OC$\underline{H}_3$), 6.51 (s, 2H, $\underline{H}_{2,6}$ of Ar-$\underline{H}$), 7.24-7.34 (m, 5H, Ar-$\underline{H}$), 8.44 (t, H, *J* = 5.9 Hz, N$\underline{H}$); Anal. calculated for $C_{22}H_{25}N_5O_6$: C, 58.01; H, 5.53; N, 15.38. Found: C, 58.29; H, 5.57; N, 15.57.

4-(1-(3,4,5-trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid hydroxyamide (5g)

Reaction of **4g** (0.01 mol, 4.73 g) with hydroxylamine hydrochloride (0.02 mol, 1.39 g) yielded white crystals (4.26 g, 87%); mp 264-266 ºC; IR (cm^{-1}): 3617-2887 (OH), 3313 (NH), 1733 (C=O), 1675 (C=O), 1613 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.69 (s, 6H, 2 OC$\underline{H}_3$), 3.73 (s, 3H, OC$\underline{H}_3$), 6.90 (s, 2H, H$_2$ of Ar-$\underline{H}$),7.43 (d, 2H, *J* = 8.6 Hz, $\underline{H}_{3,5}$ of benzoic acid ring), 7.45-7.60

(m, 5H, Ar-H), 7.96 (d, 2H, *J* = 8.6 Hz, $H_{2,6}$ of benzoic acid ring), 8.59 (s, 1H, CH_2NH); HRMS: m/z calculated for $C_{25}H_{23}N_5O_6$ $[M+H]^+$: 490.17211, found: 513.11615 for $C_{25}H_{22}N_5O_6Na$.

4-((1-(3,4,5-trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)methyl)benzoic acid hydroxyamide (5h)

Reaction of **4h** (0.01mol, 4.87 g) with hydroxyamine hydrochloride (0.02 mol, 1.39 g) yielded white crystals (4.26 g, 87%); mp 181-183 ºC; IR (cm^{-1}): 3610-2737 (OH), 3315 (NH), 1732 (C=O), 1677 (C=O), 1590 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.65 (s, 6H, 2 OCH_3), 3.70 (s, 3H, OCH_3), 3.52 (d, 2H, NH-CH_2), 6.81 (s, 2H, $H_{2,6}$ of Ar-H), 7.42-7.48 (m, 5H, Ar-H), 7.54 (d, 2H, *J* = 8.5 Hz, $H_{2,6}$ of benzoic acid ring), 7.90 (d, 2H, *J* = 8.5 Hz, $H_{3,5}$ of benzoic acid ring), 9.33(t, 1H, NH); HRMS: m/z calculated for $C_{26}H_{25}N_5O_6$ $[M+H]^+$: 504.18776, found: 527.133397 for $C_{26}H_{24}N_5O_6Na$.

2-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)acetic acid hydroxyamide (5i)

Reaction of **4i** (0.01 mol, 3.57 g)with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 72% yield; mp 201°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1685 (amidic C=O), 1550 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 4.30 (s, 2H, CH_2), 7.32 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.36 (d, 2H, *J* = 8.40 Hz, Ar-H) , 7.43-7.48 (m, 5H, Ar-H), 8.02 (s, 1H, CONH); HRMS: m/z calculated for $C_{17}H_{14}ClN_5O_3[M-H]^-$: 370.07124, found: 370.07037; Elemental Analysis: Calculated: C, 54.92; H, 3.80; N, 18.84. Found: C, 55.10; H, 3.92; N, 18.93.

3-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid hydroxyamide (5j)

Reaction of **4j** (0.01 mol, 3.71 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 77% yield; mp 191°C; IR (cm^{-1}): 3320-2550 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1665 (amidic C=O), 1510 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.75 (t, 2H,*J* = 6.00 Hz, CH_2CO), 3.80 (q, 2H,*J* = 6.00 Hz, CH_2NH), 7.33 (d, 2H, *J* = 8.80 Hz, Ar-H), 7.41 (d, 2H, *J* = 8.80 Hz, Ar-H) , 7.45-7.49 (m, 5H, Ar-H), 7.97 (t, 1H, *J* = 5.60 Hz, CONH)), 9.06 (s, 1H, CONHOH); HRMS: m/z calculated for $C_{18}H_{16}ClN_5O_3[M+H]^+$: 386.09435, found: 386.09125; Elemental Analysis: Calculated: C, 56.04; H, 4.18; N, 18.15.Found:C, 56.31; H, 4.22; N, 18.42.

4-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)butanoic acid hydroxyamide (5k)

Reaction of **4k** (0.01 mol, 3.85 g)with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 82% yield; mp 191°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1715 (hydroxamic C=O), 1685 (amidic C=O), 1570 (C=N);^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 1.98 (p,2H, $CH_2CH_2CH_2$), 2.47 (t, 2H, *J* = 6.00 Hz, CH_2CO), 3.58 (q,*J* = 6.00 Hz, 2H, CH_2NH), 7.33 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.41 (d, 2H, *J* = 8.40 Hz, Ar-H) , 7.46-7.50 (m, 5H, Ar-H), 7.63 (t, 1H,*J* = 5.60 Hz, CONH); HRMS: m/z calculated for $C_{19}H_{18}ClN_5O_3[M+H]^+$: 400.11709, found: 423.06134 as sodium salt; Elemental Analysis: Calculated: C, 57.07; H, 4.54; N, 17.52. Found: C, 57.40; H, 4.72; N, 17.83.

5-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)pentanoic acid hydroxyamide (5l)

Reaction of **4l** (0.01 mol, 3.99 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 75% yield; m.p 187°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1685 (amidic C=O), 1650 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.49-1.55 (m, 4H, $CH_2CH_2CH_2CH_2$),2.27 (t, 2H, *J* = 6.40 Hz, CH_2CO), 3.29 (t, 2H, *J* = 6.40 Hz, CH_2NH),7.51(d, 2H, *J* = 8.00 Hz, Ar-H),7.61(d, 2H, *J* = 7.60 Hz, Ar-H), 7.48-7.52(m, 5H, Ar-H), 8.70 (t, 1H,*J* = 6.40 Hz, CONH),8.98 (s, 1H,CONHOH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 22.34, 29.03, 33.78, 38.75, 119.93, 127.38, 128.20, 129.27, 130.06, 130.98, 134.55, 136.81, 155.10, 157.06, 158.98; 174.89; HRMS: m/z calculated for $C_{20}H_{20}ClN_5O_3$ $[M+H]^+$: 414.13274, found: 437.0769 as sodium salt; Elemental Analysis: Calculated: C, 58.04; H, 4.87; N, 16.92. Found: C, 58.40; H, 5.12; N, 17.13.

6-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)hexanoic acid hydroxyamide (5m)

Reaction of **4m** (0.01 mol, 4.13 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals ; 79% yield; mp 195°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1730 (hydroxamic C=O), 1665 (amidic C=O), 1530 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.30 (p, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.41-1.52 (m,4H, $CH_2CH_2CH_2CH_2CH_2$), 2.33 (t,2H,*J* = 6.00 Hz, CH_2CO),

3.16 (t, 2H, *J* = 6.00 Hz, $\underline{CH_2}$NH), 7.51 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.61 (d, 2H, *J* = 8.00 Hz, Ar-H) , 7.69-7.74 (m, 5H, Ar-H), 8.74 (s, 1H, CONH); 10.46 (s, 1H, CO$\underline{NH}$OH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 25.37, 26.49, 29.33, 32.70, 49.07, 127.38, 128.19, 129.18, 129.37, 130.06, 130.98, 134.55, 136.81, 155.08, 157.09 , 158.92, 169.53; HRMS: m/z calculated for $C_{21}H_{22}ClN_5O_3$[M-H]$^-$: 426.13384, found: 426.13446; Elemental Analysis: Calculated: C, 58.95; H, 5.18; N, 16.37. Found: C, 59.20; H, 5.22; N, 16.53.

4-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid hydroxyamide (5n)

Reaction of **4n** (0.01 mol, 4.19 g)with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 92% yield; m.p 205°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1720 (hydroxamic C=O), 1685 (amidic C=O), 1580 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.64 (d, 2H, *J* = 8.40 Hz, Ar-H) , 7.53-7.61 (m, 5H, Ar-H), 7.95 (d, 2H, *J* = 8.40 Hz, Ar-H), 8.01 (d, 2H, *J* = 8.40 Hz, Ar-H), 9.12(s, 1H, CONH), 10.89(s, 1H, CO$\underline{NH}$OH); Elemental Analysis: Calculated: C, 60.91; H, 3.72; N, 16.14. Found: C, 61.23; H, 3.79; N, 16.38.

4-((1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)methyl)benzoic acid hydroxyamide (5o)

Reaction of **4o** (0.01 mol, 4.33 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 77.40% yield; mp 212°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1685 (amidic C=O), 1570 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 4.57 (s, 2H, CH_2), 7.46 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.51 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.53-7.56 (m, 5H, Ar-H), 7.61 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.92 (d, 2H, *J* = 8.40 Hz, Ar-H), 9.21(s, 1H, CONH); HRMS: m/z calculated for $C_{23}H_{18}ClN_5O_3$[M+H]$^+$: 448.20709, found: 448.20981; Elemental Analysis: Calculated: C, 61.68; H, 4.05; N, 15.64. Found: C, 62.01; H, 4.09; N, 15.89.

4-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)phenyl acetic acid hydroxyamide (5p)

Reaction of **4p** (0.01 mol, 4.33 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g)) yielded white crystals; 76.50% yield; mp 207°C; IR (cm^{-1}): 3320-2580 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1685 (amidic C=O), 1550 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.56 (s, 2H, CH_2), 7.26 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.48 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.51-7.55 (m, 5H, Ar-H), 7.62 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.40 Hz, Ar-H), 8.68 (s, 1H, CONH), 10.54 (s, 1H, CO$\underline{NH}$OH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 66.86, 107.23, 121.09, 128.19, 129.19, 129.44, 129.97, 130.08, 131.07, 133.67, 134.74, 136.79, 137.26, 155.42, 157.08, 157.59, 172.99; HRMS: m/z calculated for $C_{23}H_{18}ClN_5O_3$ [M+H]$^-$: 448.20709, found: 448.20981; Elemental Analysis: Calculated: C, 61.68; H, 4.05; N, 15.64. Found: C, 61.92; H, 4.12; N, 15.97.

2-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)acetic acid hydroxyamide (5q)

Reaction of **4q** (0.01 mol, 4.47 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 72% yield; mp 187°C; IR (cm^{-1}): 3300-2560 (OH), 3350 (NH), 1710 (hydroxamic C=O), 1685 (amidic C=O), 1550 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.63 (s, 6H, 2-OCH_3), 3.70 (s, 3H, OCH_3), 3.95 (d, 2H,*J* = 6.00 Hz,CH_2), 6.75 (s, 2H, Ar-H), 7.57 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.66 (d, 2H, *J* = 8.40 Hz, Ar-H) , 8.88 (t, 1H,*J* = 6.00 Hz, CONH) , 10.60 (s, 1H, CO$\underline{NH}$OH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 49.06, 56.27, 60.64, 107.02, 122.24, 128.53, 130.05, 134.73, 136.92, 139.64 , 153.20, 155.08, 156.38, 159.27, 171.31; HRMS: m/z calculated for $C_{20}H_{20}ClN_5O_6$[M+H]$^+$: 462.10749, found: 462.10706; Elemental Analysis: Calculated: C, 52.01; H, 4.36; N, 15.16. Found: C, 52.17; H, 4.60; N, 15.40.

3-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid hydroxyamide (5r)

Reaction of **4r** (0.01 mol, 4.61 g)with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 75.30% yield; mp 184°C; IR (cm^{-1}): 3230-2500 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1680 (amidic C=O), 1530 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.56 (t, 2H,*J* = 6.00 Hz, $\underline{CH_2}$CO), 3,53 (t, 2H, *J* = 6.00 Hz, $\underline{CH_2}$NH), 3.64 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 6.78 (s, 2H, Ar-H), 7.55 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.64 (d, 2H, *J* = 8.00 Hz, Ar-H) , 8.52 (t, 1H,*J* = 6.00 Hz, CONH) , 9.16 (s, 1H, CONHOH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 34.22, 35.52, 56.46, 60.67, 107.43, 122.26, 128.42, 130.00, 134.71, 136.98, 140.08, 153.28, 155.02, 156.74, 159.01,

173.21; Elemental Analysis: Calculated: C, 53.00; H, 4.66; N, 14.72. Found: C, 53.21; H, 4.69; N, 14.89.

4-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)butanoic acid hydroxyamide (5s)

Reaction of **4s** (0.01 mol, 4.75 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g)yielded white crystals; 76% yield; mp 191°C; IR (cm^{-1}): 3310-2540 (OH), 3380 (NH), 1720 (hydroxamic C=O), 1685 (amidic C=O), 1650 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.81 (p, 2H, $CH_2\underline{CH_2}CH_2$), 2.28 (t, 2H,*J* = 6.00 Hz, $\underline{CH_2}CO$), 3,34 (q,*J* = 6.00 Hz, 2H, $\underline{CH_2}NH$), 3.65 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 6.78 (s, 2H, Ar-H), 7.55 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.64 (d, 2H, *J* = 6.00 Hz, Ar-H) , 8.59 (t, 1H,*J* = 6.00 Hz,CONH) , 11.84 (s, 1H, CO$\underline{NH}$OH); Elemental Analysis: Calculated: C, 53.94; H, 4.94; N, 14.30. Found: C, 54.04; H, 5.22; N, 14.33.

5-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)pentanoic acid hydroxyamide (5t)

Reaction of **4t** (0.01 mol, 4.89 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 77.90% yield; mp 196°C; IR (KBr, cm^{-1}): 3290-2550 (OH), 3380 (NH), 1715 (hydroxamic C=O), 1685 (amidic C=O), 1560 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.45-1.57 (m, 4H, $CH_2\underline{CH_2CH_2}CH_2$), 2.26 (t, 2H, *J* = 5.60 Hz, $\underline{CH_2}CO$), 3,31 (q, 2H, *J* = 5.60 Hz, $\underline{CH_2}NH$), 3.64 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 6.78 (s, 2H, Ar-H), 7.56 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.63 (d, 2H, *J* = 8.00 Hz, Ar-H) , 8.54 (t, 1H,*J* = 5.60 Hz, CONH) , 11.85 (s, 1H, CO$\underline{NH}$OH); Elemental Analysis: Calculated: C, 54.82; H, 5.20; N, 13.90. Found: C, 54.90; H, 5.42; N, 14.13.

6-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)hexanoic acid hydroxyamide (5u)

Reaction of **4u** (0.01 mol, 5.03 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 75.90% yield; mp 199°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1685 (amidic C=O), 1650 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.34 (p, 2H, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.56-189 (m, 4H, $CH_2\underline{CH_2}CH_2\underline{CH_2}CH_2$), 2.22 (t, 2H,*J* = 6.00 Hz, $\underline{CH_2}CO$), 3,21 (q, 2H, *J* = 5.60 Hz, $\underline{CH_2}NH$), 3.64 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 6.78 (s, 2H, Ar-H), 7.55 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.6 (d, 2H, *J* = 8.00 Hz, Ar-H) , 8.52 (t, 1H,*J* = 5.60 Hz, CONH) , 11.85 (s, 1H, CO$\underline{NH}$OH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 24.71, 26.45, 29.27, 34.12, 39.10, 56.46, 60.67, 107.44, 122.33, 128.42, 129.99, 134.67, 137.02, 140.07, 153.28, 154.95, 157.02, 159.01, 174.74; Elemental Analysis: Calculated: C, 55.65; H, 5.45; N, 13.52. Found: C, 55.89; H, 5.53; N, 13.67.

4-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid hydroxyamide (5v)

Reaction of **4v** (0.01 mol, 5.09 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 77.05% yield; mp 203°C; IR (cm^{-1}): 3330-2590 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1670 (amidic C=O), 1580 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.64 (s, 6H, 2-OCH_3), 3.71 (s, 3H, OCH_3), 6.82 (s, 2H, Ar-H), 7.61 (d, 2H, *J* = 8.00 Hz, Ar-H),7.68 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.96 (d, 2H, *J* = 8.40 Hz, Ar-H) , 8.01 (d, 2H, *J* = 8.40 Hz, Ar-H), 8.69 (s, 1H, CONH), 10.86 (s, 1H, CONHOH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 56.32, 60.67, 107.17, 120.41, 122.10, 126.52, 128.61, 130.08, 130.67, 134.87, 136.81, 139.75, 142.8, 153.22, 155.39, 156.53,157.96, 167.36; Elemental Analysis: Calculated: C, 57.31; H, 4.23; N, 13.37. Found: C, 57.45; H, 4.30; N, 13.52.

4-((1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)methyl)benzoic acid hydroxyamide (5w)

Reaction of **4w** (0.01 mol, 5.23 g)with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 75.30% yield; mp 214°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1685 (amidic C=O), 1570 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.64 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 4.57 (s, 2H, CH_2), 6.79 (s, 2H, Ar-H), 7.46 (d, 2H, *J* = 8.00 Hz, Ar-H),7.56 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.64 (d, 2H, *J* = 8.00 Hz, Ar-H) , 7.92 (d, 2H, *J* = 8.00 Hz, Ar-H), 9.21 (s, 1H, CONH), 10.86 (s, 1H, CONHOH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 56.46, 60.67, 66.86, 107.45, 122.26, 127.83, 128.44, 129.23, 129.91, 130.00, 134.73, 136.99, 140.10, 144.85, 153.29, 155.10, 156.74, 159.28, 167.60; Elemental Analysis: Calculated: C, 58.05; H, 4.50; N13.02. Found: C, 57.92; H, 4.48; N, 13.38.

4-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)phenylacetic acid hydroxyamide (5x)

Reaction of **4x** (0.01 mol, 5.23 g) with $NH_2OH.HCl$ (0.02 mol, 1.39 g) yielded white crystals; 76.20% yield; mp 213°C; IR (cm^{-1}): 3230-2590 (OH), 3380 (NH), 1725 (hydroxamic C=O), 1685 (amidic C=O), 1550 (C=N);^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.21 (s, 2H, CH_2), 3.66 (s, 6H, 2-OCH_3), 3.73 (s, 3H, OCH_3), 6.83 (s, 2H, Ar-H), 7.27 (d, 2H, *J* = 8.00 Hz, Ar-H),7.61 (d, 2H, *J* = 8.00 Hz, Ar-H), 7.66 (d, 2H, *J* = 8.00 Hz, Ar-H) , 7.78 (d, 2H, *J* = 8.00 Hz, Ar-H), 9.23 (s, 1H, CONH), 10.38 (s, 1H, CONHOH);^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 56.46, 61.77, 65.98, 107.75, 122.26, 127.83, 128.44, 129.23, 129.91, 131.42, 133.71, 136.99, 141.33, 144.85, 153.29, 155.10, 156.74, 159.28, 167.60; Elemental Analysis: Calculated: C, 58.05; H, 4.50; N, 13.02.Found: C, 58.34; H, 4.57; N, 13.41.

4.1.5. General procedure for the synthesis of ester derivatives of 1,2,4-triazole-3-carboxamide (6a,b)

A mixture of compound **3c** (0.01 mol, 2.99 g) or **3d** (0.01 mol, 3.89 g) and benzocaine (0.01 mol, 1.65 g) was refluxed in acetic acid (50 mL) in the presence of anhydrous sodium acetate (1.5 g, 0.018 mol) for 2 h. The reaction mixture was cooled and poured into ice water (50 mL). The formed precipitate was filtered off, dried and recrystallized from methanol.

Ethyl 4-(1-(4-chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoate (6a) [24]

Pale yellow crystals; 85.10% ; mp 173°C.

Ethyl4-(1-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)benzoate (6b) [24]

Pale yellow crystals; 87.60% yield; mp 177°C.

3.2. Biology

3.2.1. Screening of anti-inflammatory activity

Using carrageenan, the anti-inflammatory activity of the newly synthetized compounds was studied (Supplementary Data).

3.2.2. Screening of ulcerogenicity

The ulcerogenicity of test and reference compounds was evaluated (Supplementary Data).

3.2.3. Histopathological investigation

The histopathological investigations of ulcers induced by test and reference compounds were carried out (Supplementary Data).

3.2.4. TACE inhibitory activity

The *in vitro* TACE inhibitory activities of compounds **5a, 5f, 5h, 5i, 5k, 5p,** and **5u** were measured using colorimetric assay kit (Biovision, Inc.) on Jurkat Clone E6-1 cell line according to manufacturer's directions (Supplementary Data).

3.2.5. Docking study at TACE active site

Docking simulation study is performed in Medicinal Chemistry Department, Faculty of pharmacy, Assiut University using Molecular Operating Environment (MOE®) version 2014.09, Chemical Computing Group Inc., Montreal, Canada (Supplementary Data).

Supplementary:

https://odr.journals.ekb.eg/article_281182.html

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