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Synthesis and structure-activity relationship of elastase inhibiting novel ethylated thiazole-triazole acetamide hybrids: Mechanistic insights through kinetics and computational contemplations

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ABSTRACT

Keeping in mind the pharmacological importance of 2-aminothiazole and 1,2,4-triazole heterocyclic moieties, a series of novel ethylated bi-heterocyclic acetamide hybrids, **9a–p**, was synthesized in a multi-step protocol. The structures of newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and EI-MS spectral studies. The inhibitory effects of these bi-heterocyclic acetamides (**9a–n**) were evaluated against elastase and all these molecules were identified as potent inhibitors relative to the standard used. The Kinetics mechanism was analyzed by Lineweaver-Burk plots which revealed that, **9h**, inhibited elastase competitively by forming an enzyme-inhibitor complex. The inhibition constants K_i calculated from Dixon plots for this compound was 0.9 µM. The computational study was articulate with the experimental results and these ligands unveiled good binding energy values (kcal/mol). So, these molecules can be considered as promising medicinal scaffolds for the treatment of skin melanoma, wrinkle formation, uneven pigmentation, and solar elastosis.

1. Introduction

Many heterocyclic compounds are distinguished as active products pharmaceutically and are gaining consistent attention in the development of new drugs [1]. Heterocyclic ring contains elements other than carbon; most frequently are sulfur, nitrogen and oxygen [2]. The physicochemical properties of ring depend on the type and size of ring structure, and these properties also vary by changing the substituent attached with it [3,4]. Heterocyclic molecules have various clinical applications and play an active role as antitumor [5,6], antibacterial [7], anti-inflammatory [8], antiviral [9], and antifungal agents [10].

Thiazoles are very important compounds and are the significant components of large number of medicinal molecules containing important biological activities, such as antibacterial [11], anthelmintic [12], antitubercular [13], antiprotozoal [14], antifungal [15], anti-inflammatory, cardiotonic, bacterial, fungicidal, anesthetic and sedatives [16].

2-Aminothiazole derivatives have been reported to contain antiviral [17], anti-inflammatory [18], and antimicrobial [19] activities. The *in vitro* anticancer studies showed that different 2-aminothiazole derivatives possess selective and potent inhibitory effect against wide-ranging human cancerous cell such as prostate, leukemia, renal, breast, CNS, lung, ovarian, colon, melanoma cell lines [20–23]. Some of the 2-aminothiazole derivatives have also been reported as enzymes inhibitors, such as kynurenine-3-hydroxylase or cyclin-dependent kinase [24].

1,2,4-Triazole is the organic heterocyclic compound containing two carbon atoms and three nitrogen atoms at nonadjacent position in five membered di-unsaturated ring. It is aromatic and containing molecular formula $C_2H_3N_3$. This parent compound itself has been screened for antimicrobial and anticancer activity [25,26]. Over the last decade, different 1,2,4-triazole derivatives have taken attention due to their considerable antiviral, anti-inflammatory, antitumor [27–29], anticancer [30], antifungal [31,32], analgesic [33] and antibacterial

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activities [34]. 1,2,4-Triazole compounds also possess remarkable applications in agriculture [35–37].

Skin aging whether intrinsic or extrinsic is a natural process which occurs due to exposure of skin by chronic ultra violet radiation. The continuous exposure of ultra violet radiations becomes the cause of physical changes in the connective tissues and skin by the formation of reactive oxygen species and lipid peroxides [38]. This can lead to the loss of skin elasticity which can ultimately become the cause of wrinkle formation, brown spots, skin cancer, uneven pigmentation, leathery appearance, solar elastosis, laxity and melanoma [39–41]. The major responsible enzyme for wrinkle formation and dehydration on the skin is elastase [42]. Elastase is responsible for delayed wound healings, increased inflammation progress and tissue permeability. Elastin is the protein widely distributed in the vertebrate tissues of human beings especially abundant in ligaments, lungs and skin, which is being hydrolyzed or cleaved by elastase enzyme belonging to the class of serine proteases [43]. It is also the key enzyme which attacks the all major matrix protein of connective tissue [44].

Recently, some medicinal plant's extracts [45,46] have been reported as elastase inhibiting cosmeceuticals. In addition, some synthesized compounds, such as safranal [47], aryl and heteroaryl oxime ester derivatives [48], *S*-substituted perhalo-2-nitrobuta-1,3-dienes [49], and thiazol-2-(3*H*)-ones [50] have been reported as elastase inhibitors, but still the need for the development of superior and novel inhibitors is evident. Therefore, in continuation of previous efforts to explore the enzyme inhibitory potential of related bi-heterocyclic bi-amides [51], the present investigation was designed to seek some novel ethylated thiazole-triazole acetamide hybrids as elastase inhibitors to devastate the problems of skin melanoma, wrinkle formation, uneven pigmentation, and solar elastosis.

2. Results and discussion

2.1. Chemistry

The designed multi-step synthesis was initiated by the reaction of ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (**1**) with hydrazine hydrate in methanol to produce its hydrazide, **2**, which by refluxing with ethyl isothiocyanate (**3**) was transformed into 2-[2-(2-amino-1,3-thiazol-4-yl) acetyl]-*N*-ethyl-1-hydrazinecarbothioamide (**4**). This intermediate compound **4** was further cyclized to a bi-heterocyclic nucleophile, 5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4*H*-1,2,4-triazole-3-thiol (**5**). In a parallel set of reactions, different electrophiles, **8a–p**, were synthesized by reacting un/substituted anilines (**6a–p**) with 2-bromoacetyl bromide (**7**) in an aqueous medium. Finally, the desired ethylated bi-heterocyclic acetamides, **9a–p**, were synthesized by coupling **5** with different electrophiles, **8a–p**, in DMF using LiH as an activator. This synthesis has been outlined in Scheme 1 and various groups involved in the scheme have been tabulated in Table 1. The structures of newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR and EI-MS spectral analysis.

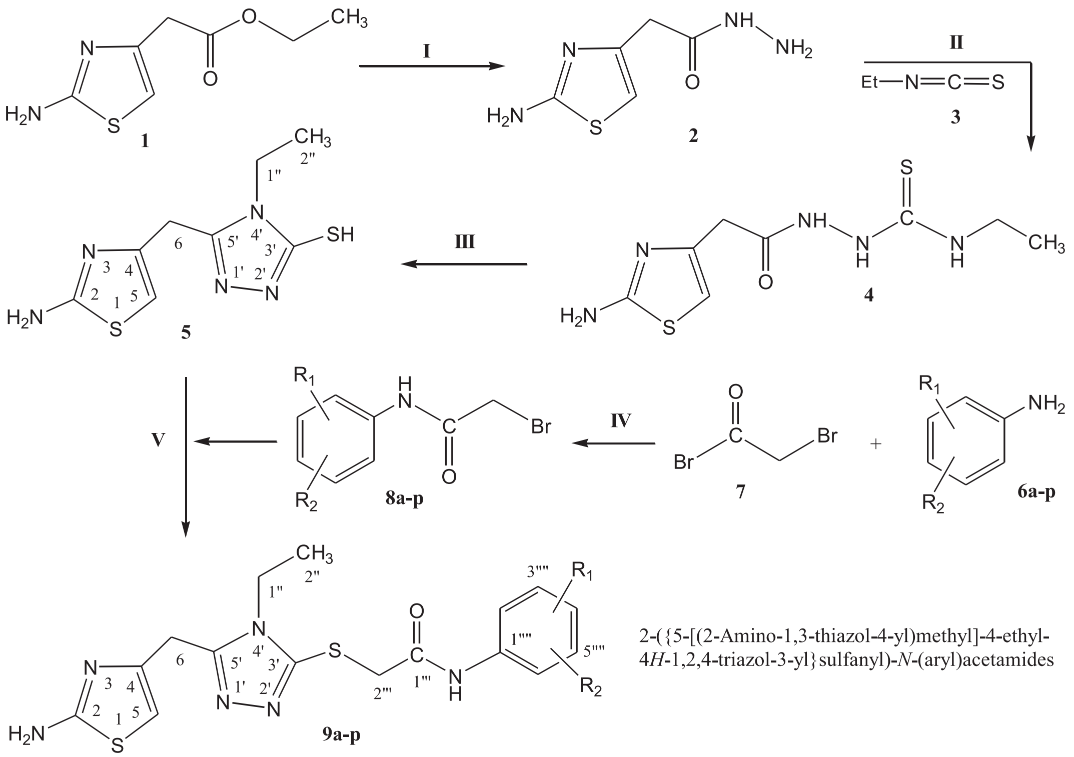
For the benefit of reader, structural analysis of one compound is discussed hereby. The molecule **9k** was synthesized as light pink amorphous solid having melting point of 108–109 °C. Its molecular formula, $C_{18}H_{20}N_6O_3S_2$, was accredited by its CHN analysis data, counting the resonance of proton in its ^{1}H NMR spectrum and carbon in its ^{13}C NMR spectrum. Various functionalities in the molecule were recognized by IR spectrum. The characteristic peaks appeared at ν 3334 (N—H stretch), 3022 (C—H stretch), 2948 (—CH_2— stretch), 1667 (C═O stretch), 1587 (C═C stretch), 1535 (C═N stretch), 1162 (C—N—C bond stretch), 622 (C—S stretch) cm^{-1}. In its ^{1}H NMR spectrum, the nitrogen (4′) atom of 1,2,4-triazole heterocyclic ring was connected with ethyl group, this ethyl group was identified by two prominent peaks, a quartet and a triplet. Quartet was located at δ 3.98 (q, J = 7.2, 2H, CH_2-1″) while triplet was at δ 1.13 (t, J = 7.2, 3H, CH_3-2″). The 2-methoxycarbonylphenyl moiety connected with the nitrogen atom of acetamide was clearly represented by discrete four signals in aromatic region in the form of two doublet of triplet (dt), one doublet of doublet (dd) and one doublet (d). These signals appeared at δ 8.31 (d, J = 8.34, 1H, H-6⁗), 7.93 (dd, J = 1.56, 7.92, 1H, H-3⁗), 7.63 (dt, J = 1.56, 7.44, 1H, H-5⁗) and 7.22 (dt, J = 1.14, 7.92, 1H, H-4⁗). The methoxy group attached to a carbonyl was easily identified by singlet at δ 3.83 (s, 3H, 2⁗—CO—O$\underline{CH_3}$). The NH proton of acetamide unit resonated as a singlet at 11.16 (s, 1H,—CO—NH-1⁗) while its methylene group attached to sulfur atom was corroborated by another singlet at δ 4.17 (s, 2H, CH_2-2‴). Two singlets at δ 6.93 (br.s, 2H, H_2N-2) and 6.27 (s, 1H, H-5) were rational for the 2-amino-1,3-thiazol-4-yl moiety, while a singlet at δ 3.95 (s, 2H, CH_2-6) was assignable to the methylene group linking the two heterocycles in a molecule. Fig. S1(a) showed the ^{1}H NMR spectrum of this molecule. The expanded aromatic region of this spectrum has been shown in Fig. S1(b) and the expanded aliphatic region has been depicted in Fig. S1(c).

The ^{13}C NMR spectrum (Fig. S2) also helped to substantiate all the eighteen carbon resonances exhibited by the molecule. The 2-amino-1,3-thiazol-4-yl moiety was clearly indicated by two quaternary signals at δ 168.57 (C-2), and 146.17 (C-4), beside with a methine signal at δ 102.36 (C-5). Similarly, the other heterocycle i.e. (1,2,4-triazol-3-yl) sulfanyl was also designated by two quaternary signals at δ 153.34 (C-5′) and 147.90 (C-3′). Methylene joining the two heterocycles (4-position of the former heterocycle with 5′-position of the latter heterocycle) was obvious at δ 27.43 (C-6). The 2-methoxycarbonylphenyl moiety was also apparent with two quaternary signals at δ 139.15 (C-1⁗), 117.57 (C-4⁗) along with four methine signals at δ 134.01 (C-5⁗), 130.55 (C-3⁗), 123.52 (C-4⁗) and 120.89 (C-6⁗). The 2-methoxycarbonyl group was characterized by a downfield quaternary signal at δ 167.25 (2⁗—$\underline{CO}$—OCH_3) and a methoxy signal at δ 52.40 (2⁗—CO—O$\underline{CH_3}$). Similarly, the acetamide unit was also corroborated by a quaternary carbonyl signal at δ 166.23 (C-1‴) along with a methylene signal at δ 36.80 (C-2″"). The ethyl group attached to the nitrogen (4′) atom of 1,2,4-triazole ring was distinctive by two signals at δ 38.70 (C-1″) and 14.58 (C-2″). The C—H connectivities in the carbon skeleton were thoroughly affirmed by its HMBC spectrum and the important correlations are portrayed on this spectrum in Fig. S3. So, on the basis of above-mentioned accumulative evidences, the structure of **9k** was confirmed and it was named as 2-({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)-*N*-(2-methoxycarbonylphenyl)acetamide. The structures of all other synthesized derivatives were also characterized (Figs. S4–S17) by similar protocols.

2.2. Elastase inhibition and structure-activity relationship

The synthesized ethylated bi-heterocyclic acetamides (**9a–p**) were evaluated against elastase enzyme for their inhibitory potentials and the obtained results are presented in Table 2. All these compounds exhibited very potent inhibitory activities against this enzyme, as it was evident from their lower IC_{50} (µM) values, relative to oleanolic acid (standard), having IC_{50} value of 13,450.5 ± 13.6 nM. Though the exposed activity is an outcome of the whole molecule, yet a limited structure-activity relationship (SAR) was predicted by discerning the effect of different aryl entities on the inhibitory potential, as it was the only varying part and all other parts were similar in all molecules. The general structural parts of the studied acetamides are accentuated in Fig. 1.

The comparison of inhibitory potential of following dimethylated regio-isomers indicated that the compound **9a** having 2,3-dimethylphenyl ring possessed better activity (IC_{50} = 175.9 ± 75.2 nM), relative to other analogues. The compound **9d** (IC_{50} = 1123.8 ± 171.2 nM), with 2,6-dimethylphenyl ring was second in this row. These isomers can be arranged in the following row according to their inhibitory activity: **9a** > **9d** > **9b** > **9c**. It can be guessed from structure-activity relationship that those molecules in which the two methyl groups were close in space to the acetamide unit,



2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)-*N*-(aryl)acetamides

Scheme 1. Outline for the synthesis of 2-({5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)-*N*-(aryl)acetamides. **Reagents & Conditions**: (I) MeOH/$N_2H_4 \cdot H_2O$/refluxing for 2 hrs. (II) MeOH/**3**/Refluxing for 1 hr. (III) The ppt. of **4** dissolved in 10% NaOH/filtration/acidification of filtrate in cold state to get ppt. of **5**. (IV) Aq. Na_2CO_3 soln./pH 9–10/vigorous manual shaking at RT for 2–3 hrs. (V) DMF/LiH/stirring for 12–24 hrs.

Table 1
Different groups (-R_1 & -R_2) in scheme 1.

Compd.	-R_1	-R_2	Compd.	-R_1	-R_2
6a, 8a, 9a	2-CH_3	3-CH_3	**6i, 8i, 9i**	3-CH_3	-H
6b, 8b, 9b	2-CH_3	4-CH_3	**6j, 8j, 9j**	-H	4-CH_3
6c, 8c, 9c	2-CH_3	5-CH_3	**6k, 8k, 9k**	2-CO-OCH_3	-H
6d, 8d, 9d	2-CH_3	6-CH_3	**6l, 8l, 9l**	2-CH_2-CH_3	-H
6e, 8e, 9e	3-CH_3	4-CH_3	**6m, 8m, 9m**	-H	4-CH_2-CH_3
6f, 8f, 9f	3-CH_3	5-CH_3	**6n, 8n, 9n**	2-CH_2-CH_3	6-CH_3
6g, 8g, 9g	-H	-H	**6o, 8o, 9o**	3-O-CH_2-CH_3	-H
6h, 8h, 9h	2-CH_3	-H	**6p, 8p, 9p**	-H	4-O-CH_2-CH_3

generally posed superb activity as compared to other isomers (Fig. 2).

However, both the compounds **9e** (IC_{50} = 296.8 ± 11.6 nM), and **9f** (IC_{50} = 251.6 ± 11.5 nM) exhibited very comparable inhibitory activities (Fig. 3). It means, these molecules bearing 3,4-dimethylphenyl and 3,5-dimethylphenyl groups respectively, made almost resembling interactions with the active site of the enzyme.

When the inhibitory potentials of un-substituted and mono-methylated compounds were compared, it was observed that **9h** (IC_{50} = 9.4 ± 2.3 nM) having 2-methylphenyl ring and **9i** (IC_{50} = 88.7 ± 19.5 nM) with 3-methylphenyl group possessed superb activities. The molecule **9j** (IC_{50} = 205.1 ± 12.8 nM) in which the same methyl group was little away in space from the acetamide unit even possessed slightly milder activity as compared to the un-substituted **9g** (IC_{50} = 146.5 ± 45.1 nM). It means, only those compounds, in which a small sized electron donating methyl group is present in the aryl part and it is also in close proximity of the acetamide unit, are better prone to make suitable interactions with the enzyme. Indeed, the **9h** was the most active while **9i** was the second most active compound in the synthetic series (Fig. 4).

The compound **9k** had a medium polar group at *ortho*-position in aryl part while **9l** was having a medium sized non-polar ethyl group at the same position. In **9m**, the same ethyl group was present at *para*-position. The molecule **9n** was credited with di-*ortho* substitutions, one ethyl and other of methyl group. Among these molecules a decreasing order of inhibitory activity was observed as **9k** > **9l** > **9m** > **9n** and this order was also consistent to some extent with the aforementioned observations. In compounds **9k** and **9l** the inherited groups were closer to the acetamide moiety and thus rendering the interactions of this moiety in a suitable manner with the active site of the enzyme. The decreased activity of **9n** in this case can be attributed to the increased steric crowding in this molecule (Fig. 5).

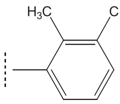
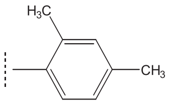
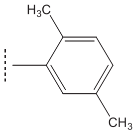
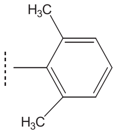
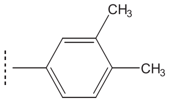
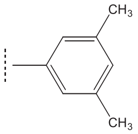
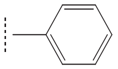
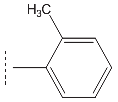
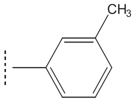
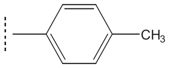
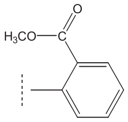
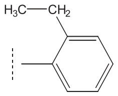
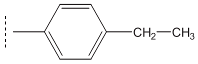
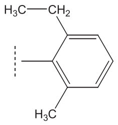
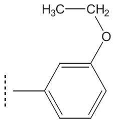
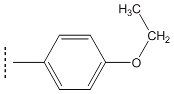
On comparison of inhibitory potential of **9o** (IC_{50} = 230.7 ± 5.6 nM) and **9p** (IC_{50} = 350.9 ± 67.8 nM), it was observed that the shifting of medium sized medium-polar group from *meta* (in **9o**) to *para* position (in **9p**) resulted in a slightly decreased inhibitory potential (Fig. 6). It was again endorsed that presence a group away from the acetamide unit was not a creditable option for enhanced activity among these molecules.

So, it was inferred from the structure-activity relationships of such ethylated bi-heterocyclic acetamides that if the small sized electron donating groups in aryl part are lying closer in space to the acetamide entity, generally result in superb interactions of the molecule, however, the increased steric crowding reversed this trend.

2.3. *Kinetic mechanism*

To understand the inhibitory mode of synthetic compounds against elastase, kinetic study was performed. Based upon the IC_{50} results, the most potent compound **9h** to was selected to determine the inhibition type and inhibition constant. The kinetics results of the enzyme by the Lineweaver-Burk plot of 1/V versus substrate *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide 1/[S] in the presence of different inhibitor concentrations gave a series of straight lines. The result of Lineweaver-Burk plot of compound **9h** showed that V_{max} remains the same without significantly affecting the slopes. K_m increases with increasing concentration while V_{max} remains the same with insignificant difference. This behavior

Table 2
Elastase inhibitory activity of ethylated bi-heterocyclic acetamides, **9a–p**.

Compounds	Aryl part	Elastase activity IC_{50} ± SEM (nM)
9a		175.9 ± 75.2
9b		1992.8 ± 116.2
9c		2749.1 ± 345.1
9d		1123.8 ± 171.2
9e		296.8 ± 11.6
9f		251.6 ± 11.5
9g		146.5 ± 45.1
9h		9.4 ± 2.3
9i		88.7 ± 19.5
9j		205.1 ± 12.8
9k		181.6 ± 75.8
9l		235.4 ± 19.4
9m		621.9 ± 98.9
9n		2296.6 ± 2077.1
9o		230.7 ± 5.6
9p		350.9 ± 67.8
Oleanolic Acid		13,450.5 ± 13.6

SEM = Standard error of the mean; values are expressed in mean ± SEM.

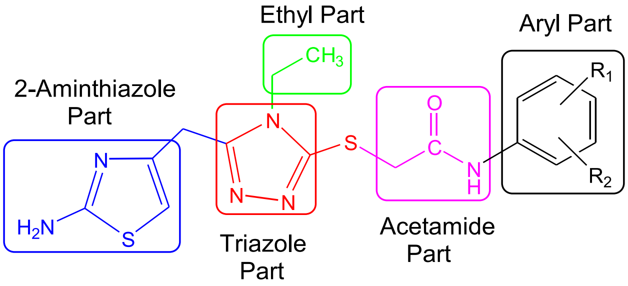


Fig. 1. General structural features of compounds **9a–p**.

indicated that **9h** compound inhibited the enzyme in competitive manner (Fig. 7**a**). Second plot (Fig. 7**b**) of slope against concentration of **9h** showed EI dissociation constant. K_i was calculated from inhibitor concentration of **9h** versus the slope and K_i was found to be 0.9 µM.

2.4. Computational structural assessment of elastase

Porcine pancreatic elastase consists of 240 amino acids having calcium atom embedded in the protein structure. The X-ray diffraction study showed its resolution: 1.8 Å having unit cell coordinates a = 52.530, b = 57.470 and c = 75.260 with angles α = 90.00, β = 90.00 and γ = 90.00. The Ramachandran graph values also shows 88.70% of residues were present in favored region while 98.70% residues were lie in allowed region. The 3D protein structure and Ramachandran graph s mentioned in Fig. 8(A and B).

2.5. Molecular docking analysis

2.5.1. Glide energy evaluation of synthesized compounds

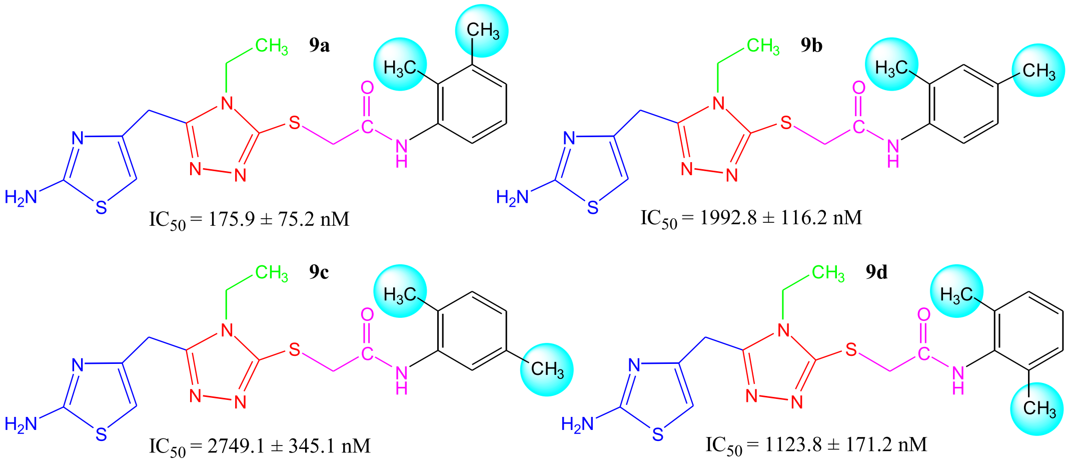
Molecular docking approach is good computational technique to study the binding conformational behavior of ligands against target proteins [52,53]. All the synthesized ligands (**9a–9p)** were docked against elastase to predict their binding conformational behavior within the active region of target protein. The generated docked complexes were evaluated on the basis of glide docking energy values (kcal/mol) and binding interaction (hydrogen/hydrophobic) pattern. The docking results showed that all the ligands were binds within the active region of target protein with different conformational poses (Fig. 9). The glide docking energy values fluctuated among all ligands and **9d** and **9l** possessed highest energy values −5.31 and −5.36 kcal/mol, respectively. Moreover, all others compounds also exhibited good docking energy values −4.33, −4.58, −4.83, −4.88, −4.4, −4.46, −4.71, −4.74, −4.57, −4.6, −4.81, −5.26, −4.62 and −4.2 kcal/mol, respectively. The basic skeleton of ligands was similar in all synthetic compounds therefore, no big energy values differences were observed in all docking results. All docking energy values of synthesized ligands are mentioned in Fig. 10.

2.5.2. Ligand-binding analysis of elastase docked complexes

Based on *in vitro* results (IC_{50}) **9h** was selected to check the binding interaction behavior among all other synthesized derivatives. The docking results showed that single hydrogen bond was observed in **9h**-docking complex. The nitrogen atom of amino group forms a hydrogen with Val216 having a bond length 2.20 Å. Literature data also ensured the importance of these residues in bonding with other elastase inhibitors which strengthen our docking results [54]. The 3D graphical representations of **9h** docking complex is mentioned in Fig. 11. The other docking complexes are mentioned in supplementary data (Figs. S18–S33).

3. Conclusion

A structurally unique series of bi-heterocyclic acetamides was



9a IC_{50} = 175.9 ± 75.2 nM

9b IC_{50} = 1992.8 ± 116.2 nM

9c IC_{50} = 2749.1 ± 345.1 nM

9d IC_{50} = 1123.8 ± 171.2 nM

Fig. 2. Structure-activity relationships of compounds **9a**, **9b**, **9c**, and **9d**.

synthesized as promising elastase inhibitor. Particularly, the compound **9h** (IC_{50} = 0.0094 ± 0.0023 μM) bearing 2-methylphenyl ring and **9i** (IC_{50} = 0.0887 ± 0.0195 μM) having 3-methylphenyl group possessed very excellent activities. Moreover, molecular docking results also envisaged their binding within the active region of elastase with good docking energy values. Taken together, it was concluded generally that these bi-heterocyclic acetamides might be utilized as leading medicinal scaffolds for the treatment of elastase related disorders.

4. Experimental

Chemicals were purchased from Sigma Aldrich & Alfa Aesar (Germany) and solvents of analytical grades were supplied by local suppliers. By using open capillary tube method, melting points were taken on Griffin and George apparatus and were uncorrected. By using thin layer chromatography (with ethyl acetate and *n*-hexane (30:70) as mobile phase), initial purity of compounds was detected at 254 nm. Elemental analyses were performed on a Foss Heraeus CHN-O-Rapid instrument and were within ± 0.4% of the theoretical values. IR peaks were recorded on a Jasco-320-A spectrometer by using KBr pellet method. EI-MS spectra were measured on a JEOL JMS-600H instrument with data processing system. ^{1}H NMR signals were recorded at 600 MHz and ^{13}C NMR at 150 MHz in DMSO-d_6 using Bruker spectrometers. The abbreviations used in interpretation of ^{1}H NMR spectra are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br.t, broad triplet; q, quartet; quin, quintet; m, multiplet; dist. distorted.

4.1. *Synthesis of 2-(2-amino-1,3-thiazol-4-yl)acetohydrazide (2)*

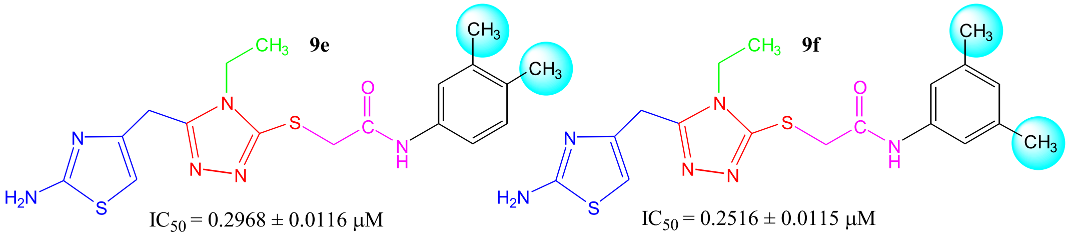
Ethyl 2-(2-amino-1,3-thiazol-4-yl)acetate (0.15 mol.; **1**) was dissolved in 60 mL methanol in 500 mL round bottom flask. 80%; 20 mL hydrazine monohydrate was added gradually in the reaction mixture and was refluxed for about 2 h. TLC was carried out for the determination of completion of reaction. At the end of reaction, excess solvent was evaporated to get white crystalline product, 2-(2-amino-1,3-thiazol-4-yl)acetohydrazide (**2**), which was further purified by washing with cold *n*-hexane and dried.

White crystalline solid; Yield: 90%; m.p. 170–171 °C; Mol. Formula: $C_5H_8N_4OS$; Mol. Mass.: 172 g mol^{-1}; IR (KBr, υ, cm^{-1}): 3358 (NH_2 str.), 3351 (N–H str.), 3032 (C–H str.), 2950 (–CH_2– str.), 1566 (C=C str.), 1587 (C=N str.), 1162 (C–N–C str.), 648 (C–S str.); ^{1}H NMR (DMSO-d_6, 600 MHz, δ, ppm): 9.02 (br.s, 1H, $1'{-}CO{-}\underline{NH}{-}NH_2$), 6.85 (br.s, 2H, 2-$NH_2$), 6.23 (s, 1H, H-5), 4.19 (br.s, 2H, $1'{-}CO{-}NH{-}\underline{NH_2}$), 3.19 (s, 2H, CH_2-2′); ^{13}C NMR (DMSO-d_6, 150 MHz, δ, ppm): 168.97 (C-1′), 168.51 (C-2), 146.43 (C-4), 102.76 (C-5), 37.32 (C-2′). Anal. Calc. for $C_5H_8N_4OS_2$ (172.21): C, 34.87; H, 4.68; N, 32.53. Found: C, 34.98; H, 4.84; N, 32.69; EI-MS: *m/z* 172 $[M]^+$, 130 $[C_4H_6N_2OS]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.2. *Synthesis of 2-[2-(2-amino-1,3-thiazol-4-yl)acetyl]-N-ethyl-1-hydrazinecarbothioamide (4)*

2-(2-Amino-1,3-thiazol-4-yl)acetohydrazide (0.13 mol.; **2**) was taken in 500 mL round bottom flask containing 50 mL methanol and was dissolved by heating. Ethyl isothiocyanate (0.13 mol.; **3**) was added in it and reaction mixture was refluxed for about one hour. After the completion of reaction, the resulting precipitates of 2-[2-(2-amino-1,3-thiazol-4-yl)acetyl]-*N*-ethyl-1-hydrazinecarbothioamide **(4)** were obtained by using filtration process and dried for further use.

White amorphous solid; Yield: 86%; m.p. 176–177 °C; Mol. Formula: $C_8H_{13}N_5OS_2$; Mol. Mass.: 259 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3372 (N–H str.), 3052 (C–H str.), 2922 (–CH_2– str.), 1585 (C=C str.), 1533 (C=N str.), 1158 (C–N–C bond str.), 622 (C–S str.); ^{1}H NMR (DMSO-d_6, 600 MHz, δ, ppm): 9.87 (br.s, 1H, $-CO{-}\underline{NH}{-}NH-$), 9.21 (br.s, 1H, $-CO{-}NH{-}\underline{NH}-$), 7.87 (br.s, 1H, $-CS{-}\underline{NH}-$), 6.88 (br.s, 2H, H_2N-2), 6.31 (s, 1H, H-5), 3.50–3.48 (m, 4H, CH_2-2′ & CH_2-1‴), 1.03 (br.t,



9e IC_{50} = 0.2968 ± 0.0116 μM

9f IC_{50} = 0.2516 ± 0.0115 μM

Fig. 3. Structure-activity relationships of **9e** and **9f**.

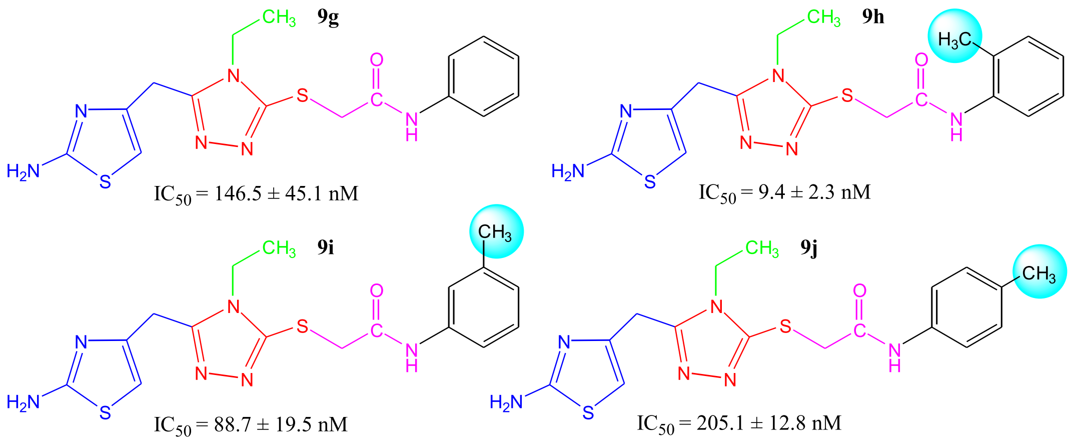


Fig. 4. Structure-activity relationships of **9g**, **9h**, **9i**, and **9j**.

J = 8.5 Hz, 3H, CH_3-2‴); ^{13}C NMR (DMSO-d_6, 150 MHz, δ, ppm): 180.25 (C-1″; HH—CS—NH), 169.05 (C-2), 168.93 (C-1′; CO—NH), 145.60 (C-4), 103.18 (C-5), 38.83 (C-1‴), 37.36 (C-2′), 14.96 (C-2‴). Anal. Calc. for $C_8H_{13}N_5OS_2$ (259.06): C, 37.05; H, 5.05; N, 27.00. Found: C, 37.18; H, 5.12; N, 27.13; EI-MS: m/z 259 [M$^+$], 215 $[C_6H_7N_4OS_2]^+$, 141 $[C_5H_5N_2OS]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.3. Synthesis of 5-[(2-amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazole-3-thiol (5)

Dissolved 2-[2-(2-amino-1,3-thiazol-4-yl)acetyl]-*N*-ethyl-1-hydrazinecarbothioamide (0.13 mol.; **4)** intermediate in 10% NaOH (100 mL). Filtered the solution to remove any insoluble impurity and then acidifying the filtrate with conc. HCl (cold state) upto 5–6 pH to obtain the desired precipitates of cyclized product **5**, in purified form.

Light brown solid; Yield: 94%; m.p. 206–207 °C; Mol. Formula: $C_8H_{11}N_5S_2$; Mol. Mass.: 241 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3341 (N—H str.), 3062 (C—H str.), 2912 (—CH_2— str.), 1580 (C=C str.), 1533 (C=N str.),1158 (C—N—C bond str.), 628 (C—S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 13.51 (s, 1H, HS-3′), 6.99 (s, 2H, H_2N-2), 6.40 (s, 1H, H-5), 3.93 (m, 4H, CH_2-6, CH_2-1″), 1.04 (t, J = 8.52, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 169.22 (C-2), 166.69 (C-3′), 150.46 (C-5′), 145.55 (C-4), 103.46 (C-5), 38.92 (C-1″), 28.26 (C-6), 13.36 (C-2″). Anal. Calc. for $C_8H_{11}N_5S_2$ (241.34): C, 39.81; H, 4.59; N, 29.02. Found: C, 39.76; H, 4.65; N, 29.15; EI-MS: m/z 241 [M]$^+$, 208 $[C_8H_{10}N_5S]^+$, 199 $[C_7H_9N_3S_2]^+$, 139 $[C_5H_5N_3S]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.4. General synthesis of 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(un/substituted-phenyl)acetamides (9a–p)

N,N-Dimethyl formamide (DMF, 3 mL) was taken in 100 mL round bottom flask and 5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4*H*-1,2,4-triazole-3-thiol (0.2 g, **5**) was dissolved in it. Added one pinch of LiH in this solution and mixture was stirred for half an hour at room temperature. After that equimolar amount of different electrophiles, *N*-substituted-2-bromoacetamide (**8a–l,** one in each reaction), were added in each respective reaction and stirred for 12–24 h. The completion of reaction was monitored by TLC. *n*-Hexane and ethyl acetate were used as solvent system in the ratio of 70:30. Single spot of product was the sign of completion of reaction. The product was precipitated out by adding excess ice cold distilled water. The targeted products, **9a–p**, were obtain through filtration, excess washing with distilled water and dried respectively for further use.

4.4.1. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2,3-dimethylphenyl)acetamide (9a)

Light yellow amorphous solid; Yield: 92%; m.p. 118–119 °C; Mol. Formula: $C_{18}H_{22}N_6OS_2$; Mol. Mass.: 402 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3354 (N—H str.), 3024 (C—H str.), 2938 (—CH_2— str.), 1680 (C=O str.), 1554 (C=C str.), 1535 (C=N str.), 1182 (C—N—C bond str.), 624 (C—S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.75 (s, 1H, —CO—NH-1⁗), 7.14 (br.d, J = 7.74, 1H, H-6⁗), 7.03 (br.t, J = 7.56, 1H, H-5⁗), 7.00 (br.d, J = 7.32, 1H, H-4⁗), 6.94 (br.s, 2H, H_2N-2), 6.29 (s, 1H, H-5),

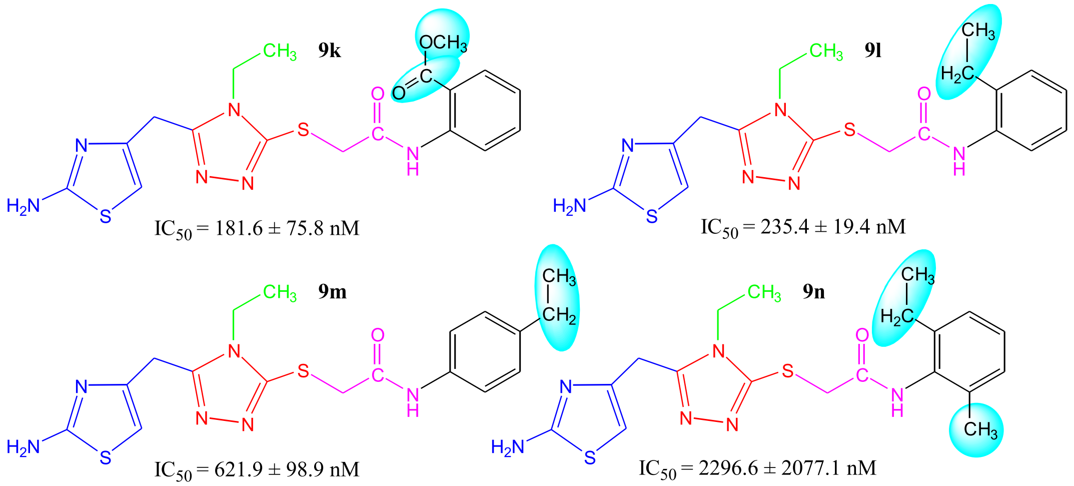


Fig. 5. Structure-activity relationships of **9k**, **9l**, **9m**, and **9n**.

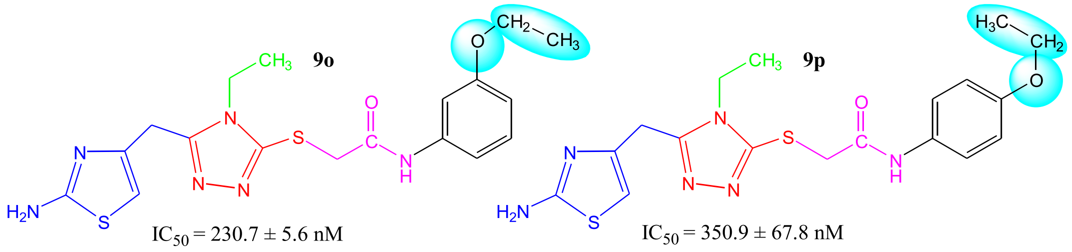


Fig. 6. Structure-activity relationships of **9o**, and **9p**.

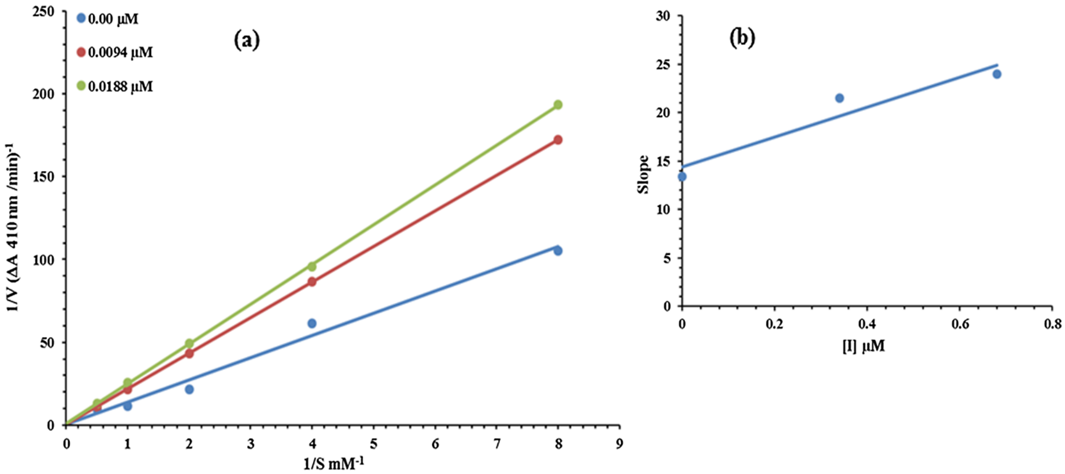


Fig. 7. Lineweaver-Burk plots for inhibition of elastase from porcine pancreas in the presence of Compound **9h.** (a) Concentrations of **9h** were 0.00, 0.0094 and 0.0188 µM, while substrate *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide concentrations were 2, 1, 0.5, 0.25, and 0.125 mM. (b) The insets represented the plot of the slope.

4.11 (s, 2H, CH_2-2‴), 3.95 (br.s, 4H, CH_2-6 & CH_2-1″), 2.22 (s, 3H, CH_3-3⁗), 2.02 (s, 3H, CH_3-2⁗), 1.10 (br.t, *J* = 6.9, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, *δ*, ppm): 168.12 (C-2), 165.96 (C-1‴), 153.20 (C-5′), 148.46 (C-3′), 146.19 (C-4), 136.94 (C-3⁗), 135.69 (C-1⁗), 130.75 (C-2⁗), 126.94 (C-4⁗), 125.15 (C-5⁗), 122.96 (C-6⁗), 102.09 (C-5), 38.73 (C-1″), 36.87 (C-2‴), 27.48 (C-6), 20.09 (CH_3-3⁗), 14.71 (C-2″), 13.79 (CH_3-2⁗). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.84; H, 5.97; N, 20.72; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 148 $[C_9H_{10}NO]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

*4.4.2. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2,4-dimethylphenyl)acetamide (**9b**)*

Drak brown gummy liquid; Mol. Formula: $C_{18}H_{22}N_6OS_2$; Mol. Mass.: 402 $gmol^{-1}$; IR (KBr, *υ*, cm^{-1}): 3332 (N—H str.), 3063 (C—H str.), 2935 (—CH_2— str.), 1654 (C=O str.), 1547 (C=C str.), 1535 (C=N str.), 1148 (C—N—C bond str.), 609 (C—S str.); ^{1}H NMR (600 MHz, DMSO-d_6, *δ*, ppm): 9.65 (s, 1H, —CO—NH-1⁗), 7.31 (br.d, *J* = 8.2, 1H, H-6⁗), 7.03 (br.s, 1H, H-3⁗), 6.92 (br.d, *J* = 8.2, 1H, H-5⁗), 6.92 (br.s, 2H, H_2N-2), 6.27 (s, 1H, H-5), 4.09 (s, 2H, CH_2-2‴), 3.92 (br.s, 4H, CH_2-6 & CH_2-1″), 2.26 (s, 3H, CH_3-4⁗), 2.12 (s, 3H, CH_3-2⁗), 1.10 (br.t, *J* = 7.02, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, *δ*, ppm): 168.52

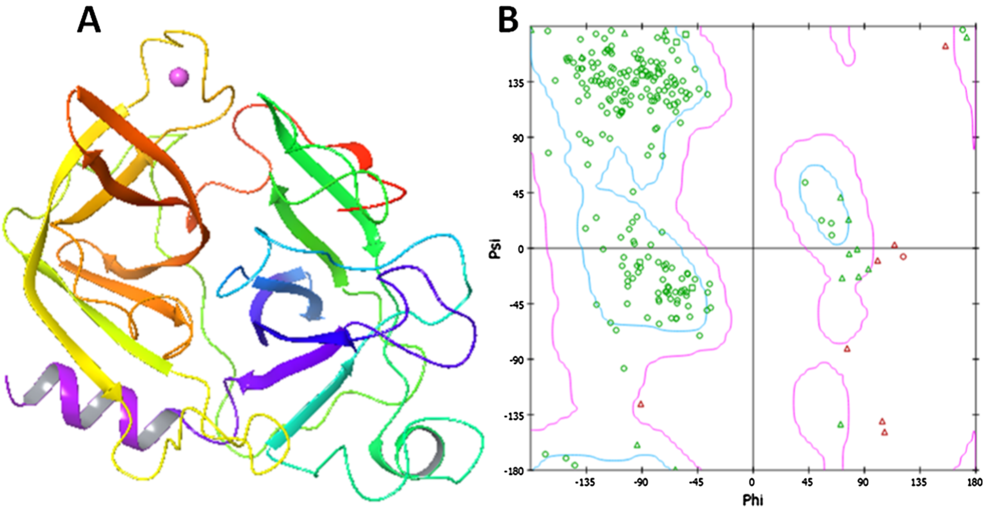


Fig. 8. (A & B) The overall protein structure elastase and Ramachandran graph.

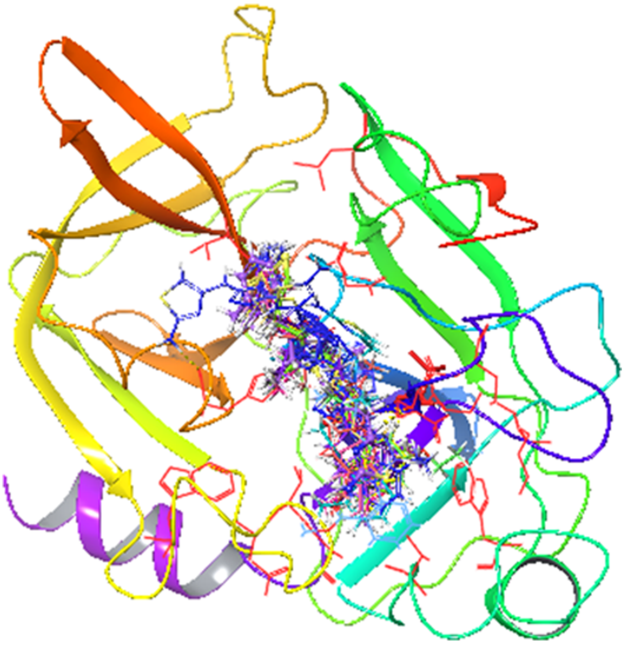


Fig. 9. All the docking complexes and ligand conformations.

(C-2), 165.92 (C-1‴), 153.02 (C-5′), 148.43 (C-3′), 146.21 (C-4), 134.21 (C-1⁗), 133.32 (C-2⁗), 131.05 (C-4⁗), 130.85 (C-5⁗), 126.32 (C-3⁗), 124.42 (C-6⁗), 102.32 (C-5), 38.84 (C-1″), 36.86 (C-2‴), 27.56 (C-6), 20.31 (CH_3-4⁗), 17.63 (CH_3-2⁗), 14.67 (C-2″). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.88; H, 5.99; N, 20.78; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 148 $[C_9H_{10}NO]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

*4.4.3. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2,5-dimethylphenyl)acetamide (**9c**)*

Drak brown amorphous solid; Yield: 90%; m.p. 104–105 °C; Mol. Formula: $C_{18}H_{22}N_6OS_2$; Mol. Mass.: 402 $gmol^{-1}$; IR (KBr, *υ*, cm^{-1}): 3385 (N—H str.), 3095 (C—H str.), 2968 (—CH_2— str.), 1642 (C═O str.), 1532 (C═C str.), 1562 (C═N str.),1134 (C—N—C bond str.), 642 (C—S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.55 (s, 1H, —CO—NH-1⁗), 7.32 (br.s, 1H, H-6⁗), 7.07 (br.d, J = 7.68, 1H, H-3⁗), 6.90–6.88 (m, 1H, H-4⁗), 6.88 (br.s, 2H, H_2N-2), 6.28 (s, 1H, H-5), 3.97 (s, 2H, CH_2-2‴), 3.89 (m, 4H, CH_2-6 & CH_2-1″), 2.23 (br.s, 3H, CH_3-2⁗), 2.03 (br.s, 3H, CH_3-5⁗), 1.12 (br.t, J = 7.05, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.63 (C-2), 165.92 (C-1‴), 153.44 (C-5′), 148.67 (C-3′), 145.47 (C-4), 135.73 (C-1⁗), 135.44 (C-5⁗), 135.00 (C-2⁗), 130.03 (C-3⁗), 129.67 (C-4⁗), 124.53 (C-6⁗), 102.42 (C-5), 38.65 (C-1″), 36.82 (C-2‴), 27.48 (C-6), 21.92 (CH_3-5⁗), 16.89 (CH_3-2⁗), 14.77 (C-2″). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.92; H, 5.68; N, 20.94; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 148 $[C_9H_{10}NO]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

*4.4.4. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2,6-dimethylphenyl)acetamide (**9d**)*

Light orange amorphous solid; Yield: 92%; m.p. 138–139 °C; Mol. Formula: $C_{18}H_{22}N_6OS_2$; Mol. Mass.: 402 $gmol^{-1}$; IR (KBr, *υ*, cm^{-1}): 3378 (N—H str.), 3043 (C—H str.), 2962 (—CH_2— str.), 1642 (C═O str.), 1538 (C═C str.), 1541 (C═N str.),1184 (C—N—C bond str.), 637 (C—S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.63 (s, 1H, —CO—NH-1⁗), 7.08–7.03 (m, 3H, H-3⁗, H-4⁗ & H-5⁗), 6.93 (br.s, 2H, H_2N-2), 6.30 (s, 1H, H-5), 4.14 (s, 2H, CH_2-2‴), 3.99–3.91 (m, 4H, CH_2-6 & CH_2-1″)), 2.08 (s, 6H, CH_3-2⁗ & CH_3-6⁗), 1.11 (t, J = 7.14, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.54 (C-2), 165.49 (C-1‴), 153.15 (C-5′), 148.42 (C-3′), 146.21 (C-4), 135.27 (C-2⁗ & C-6⁗), 134.62 (C-1⁗), 127.59 (C-3⁗ & C-5⁗), 126.49 (C-4⁗), 102.41 (C-5), 38.71 (C-1″), 36.36 (C-2‴), 27.46 (C-6), 17.94 (CH_3-2⁗ & CH_3-6⁗), 14.71 (C-2″). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.78; H, 5.77; N, 20.80; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 148 $[C_9H_{10}NO]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

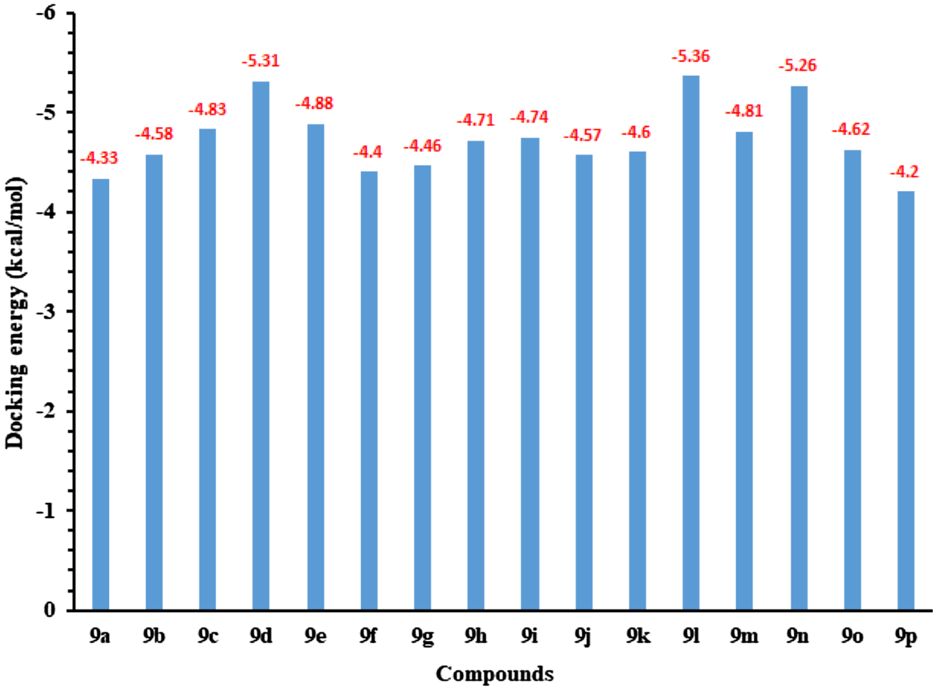


Fig. 10. All the docking complexes energy values.

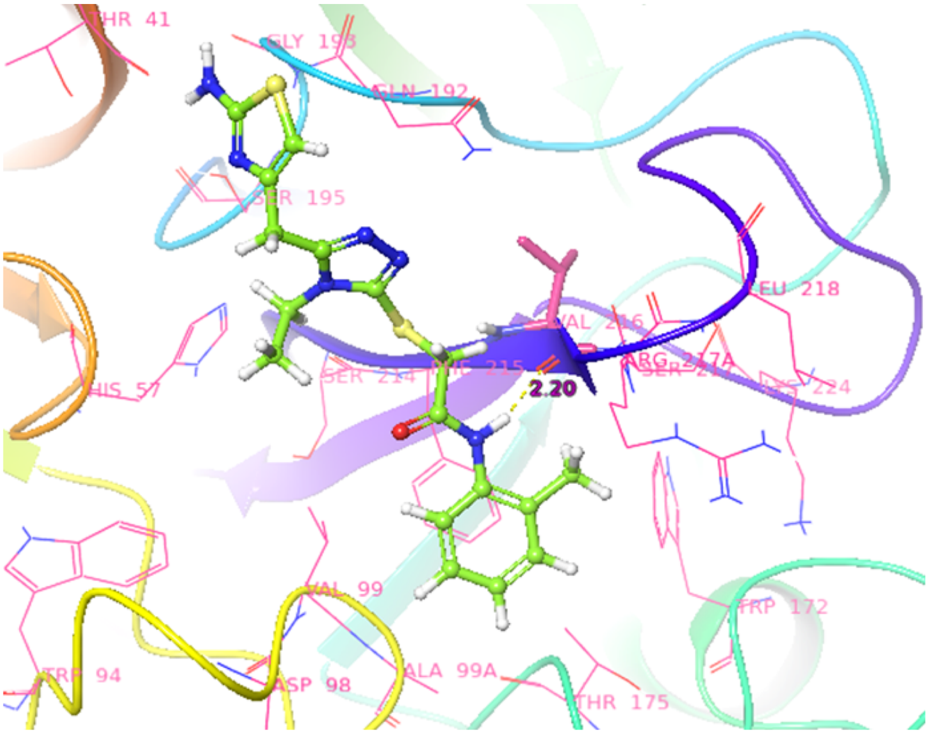


Fig. 11. Binding interactions of **9h** against elastase protein.

4.4.5. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(3,4-dimethylphenyl)acetamide (*9e*)

Drak brown gummy liquid; Mol. Formula: $C_{18}H_{22}N_6OS_2$; Mol. Mass.: 402 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3322 (N−H str.), 3033 (C−H str.), 2964 (−CH₂− str.), 1685 (C═O str.), 1592 (C═C str.), 1581 (C═N str.), 1132 (C−N−C bond str.), 648 (C−S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.17 (s, 1H, −CO−NH-1⁗), 7.33 (d, J = 1.6, 1H, H-2⁗), 7.28–7.26 (dd, J = 1.96, 8.22, 1H, H-6⁗), 7.06 (br.d, J = 8.22, 1H, H-5⁗), 6.94 (br.s, 2H, H_2N-2), 6.26 (s, 1H, H-5), 4.08 (s, 2H, CH_2-2‴), 3.96–3.93 (m, 4H, CH_2-6 & CH_2-1″)), 2.17 (s, 3H, CH_3-4⁗), 2.15 (s, 3H, CH_3-3⁗), 1.10 (t, J = 7.2, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.58 (C-2), 165.37 (C-1‴), 153.17 (C-5′), 148.39 (C-3′), 146.19 (C-4), 136.46 (C-3⁗), 136.33 (C-1⁗), 131.24 (C-4⁗), 120.26 (C-5⁗), 116.60 (C-2⁗ & C-6⁗) 102.34 (C-5), 38.71 (C-1″), 37.44 (C-2‴), 27.49 (C-6), 19.58 (CH_3-3⁗) 18.72 (CH_3-4⁗), 14.69 (C-2″). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.88; H, 5.68; N, 20.82; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 148 $[C_9H_{10}NO]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.4.6. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(3,5-dimethylphenyl)acetamide (*9f*)

Bright orange amorphous solid; Yield: 88%; m.p. 98–99 °C; Mol. Formula: $C_{18}H_{22}N_6OS_2$; Mol. Mass.: 402 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3348 (N−H str.), 30,488 (C−H str.), 2963 (−CH₂− str.), 1643 (C═O str.), 1535 (C═C str.), 1568 (C═N str.), 1148 (C−N−C bond str.), 605 (C−S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.18 (s, 1H, −CO−NH-1⁗), 7.18 (s, 2H, H-2⁗ & H-6⁗), 6.94 (br.s, 2H, H_2N-2), 6.71 (s, 1H, H-4⁗), 6.26 (s, 1H, H-5), 4.08 (s, 2H, CH_2-2‴), 3.96–3.94 (m, 4H, CH_2-6 & CH_2-1″), 2.23 (s, 6H, CH_3-3⁗ & CH_3-5⁗), 1.10 (t, J = 7.2, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.58 (C-2), 165.56 (C-1‴), 153.17 (C-5′), 148.39 (C-3′), 146.19 (C-4), 138.58 (C-1⁗), 137.64 (C-3⁗ & C-5⁗), 125.03 (C-4⁗), 116.87 (C-2⁗ & C-6⁗) 102.35 (C-5), 38.72 (C-1″), 37.43 (C-2‴), 27.49 (C-6), 20.05 (CH_3-3⁗ & CH_3-5⁗), 14.69 (C-2″). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.95; H, 5.81; N, 21.02; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 148 $[C_9H_{10}NO]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.4.7. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-phenylacetamide (*9g*)

Light yellow amorphous solid; Yield: 90%; m.p.112–113 °C; Mol. Formula: $C_{16}H_{18}N_6OS_2$; Mol. Mass.: 374 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3378 (N−H str.), 3074 (C−H str.), 2953 (−CH₂− str.), 1687 (C═O str.), 1512 (C═C str.), 1538 (C═N str.),1181 (C−N−C bond str.), 622 (C−S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.33 (s, 1H, −CO−NH-1⁗), 7.55 (br.d, J = 7.62, 2H, H-2⁗ & H-6⁗), 7.31 (br.t, J = 1.74, 7.56, 2H, H-3⁗ & H-5⁗), 7.05 (br.t, J = 7.38, 1H, H-4⁗), 6.93 (br.s, 2H, H_2N-2), 6.26 (s, 1H, H-5), 4.11 (s, 2H, CH_2-2‴), 3.96–3.92 (dist. q, J = 7.56, 4H, CH_2-6 & CH_2-1″), 1.09 (t, J = 7.2, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.58 (C-2), 165.70 (C-1‴), 153.19 (C-5′), 148.36 (C-3′), 146.18 (C-4), 138.72 (C-1⁗), 128.76 (C-3⁗ & C-5⁗), 123.48 (C-4⁗), 119.06 (C-2⁗ & C-6⁗) 102.36 (C-5), 38.72 (C-1″), 37.42 (C-2‴), 27.48 (C-6), 14.68 (C-2″). Anal. Calc. for $C_{16}H_{18}N_6OS_2$ (374.48): C, 51.32; H, 4.84; N, 22.44. Found: C, 51.48; H, 4.95; N, 22.52; EI-MS: *m/z* 374 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$, 92 $[C_6H_6N]^+$, 77 $[C_6H_5]$,

4.4.8. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2-methylphenyl)acetamide (*9h*)

Light yellow amorphous solid; Yield: 88%; m.p.: 120–121 °C; Mol. Formula: $C_{17}H_{20}N_6OS_2$; Mol. Mass.: 388 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3351 (N−H str.), 3035 (C−H str.), 2944 (−CH₂− str.), 1681 (C═O str.), 1532 (C═C str.), 1548 (C═N str.),1192 (C−N−C bond str.), 648 (C−S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.72 (s, 1H, −CO−NH-1⁗), 7.44 (br.d, J = 7.8, 1H, H-6⁗), 7.20 (br.d, J = 7.38, 1H, H-3⁗), 7.16 (br.t, J = 7.44, 1H, H-5⁗), 7.08 (br.t, J = 7.44, 1H, H-4⁗), 6.94 (br.s, 2H, H_2N-2), 6.30 (s, 1H, H-5), 4.13 (s, 2H, CH_2-2‴), 3.96 (m, 4H, CH_2-6 & CH_2-1″), 2.18 (s, 3H, CH_3-2⁗), 1.10 (t, J = 7.26, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.58 (C-2), 165.96 (C-1‴), 153.24 (C-5′), 148.51 (C-3′), 146.18 (C-4), 135.95 (C-1⁗), 131.16 (C-2⁗), 130.28 (C-3⁗), 125.94 (C-5⁗), 125.16 (C-4⁗), 124.27 (C-6⁗) 102.40 (C-5), 38.74 (C-1″), 36.86 (C-2‴), 27.44 (C-6), 17.68 (CH_3-2⁗), 14.68 (C-2″). Anal. Calc. for $C_{18}H_{20}N_6OS_2$ (388.51): C, 52.55; H, 5.19; N, 21.63. Found: C, 52.68; H, 5.27; N, 21.81; EI-MS: *m/z* 388 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 222 $[C_9H_{10}N_4OS]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 113 $[C_4H_5N_2S]^+$, 106 $[C_7H_8N]^+$, 99 $[C_3H_3N_2S]^+$, 91 $[C_7H_7]$.

*4.4.9. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(3-methylphenyl)acetamide (**9i**)*

Light grey amorphous solid; Yield: 88%; m.p.: 112–113 °C; Mol. Formula: $C_{17}H_{20}N_6OS_2$; Mol. Mass.: 388.51 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3393 (N–H str.), 3084 (C–H str.), 2962 (–CH_2– str.), 1632 (C=O str.), 1512 (C=C str.), 1571 (C=N str.), 1142 (C–N–C bond str.), 619 (C–S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.26 (s, 1H, –CO–NH-1⁗), 7.40 (s, 1H, H-2⁗), 7.34 (d, *J* = 8.22, 1H, H-6⁗), 7.19 (t, *J* = 7.74, 1H, H-5⁗), 6.94 (br.s, 2H, H_2N-2), 6.88 (d, *J* = 7.5, 1H, H-4⁗), 6.27 (s, 1H, H-5), 4.10 (s, 2H, CH_2-2‴), 3.96–3.93 (m, 4H, CH_2-6 & CH_2-1″), 2.28 (s, 3H, CH_3-3⁗), 1.10 (t, *J* = 7.2, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.58 (C-2), 165.63 (C-1‴), 153.18 (C-5′), 148.37 (C-3′), 146.18 (C-4), 138.66 (C-1⁗), 137.95 (C-3⁗), 128.59 (C-5⁗), 124.19 (C-4⁗), 119.26 (C-2⁗), 116.26 (C-6⁗), 102.34 (C-5), 38.72 (C-1″), 37.43 (C-2‴), 27.48 (C-6), 21.14 (CH_3-3⁗), 14.69 (C-2″). Anal. Calc. for $C_{18}H_{20}N_6OS_2$ (388.51): C, 52.55; H, 5.19; N, 21.63. Found: C, 52.74; H, 5.33; N, 21.87; EI-MS: *m/z* 388 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 222 $[C_9H_{10}N_4OS]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 113 $[C_4H_5N_2S]^+$, 106 $[C_7H_8N]^+$, 99 $[C_3H_3N_2S]^+$, 91 $[C_7H_7]$.

*4.4.10. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(4-methylphenyl)acetamide (**9j**)*

Drak brown gummy liquid; Mol. Formula: $C_{17}H_{20}N_6OS_2$; Mol. Mass.: 388 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3387 (N–H str.), 3022 (C–H str.), 2952 (–CH_2– str.), 1696 (C=O str.), 1571 (C=C str.), 1551 (C=N str.), 1177 (C–N–C bond str.), 637 (C–S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.25 (s, 1H, –CO–NH-1⁗), 7.45 (br.d, *J* = 8.2, 2H, H-2⁗ & H-6⁗), 7.12 (br.d, *J* = 8.2, 2H, H-3⁗ & H-5⁗), 6.94 (br.s, 2H, H_2N-2), 6.26 (s, 1H, H-5), 4.09 (s, 2H, CH_2-2‴), 3.97–3.93 (m, 4H, CH_2-6 & CH_2-1″), 2.25 (s, 3H, CH_3-4⁗), 1.10 (br.t, *J* = 7.2, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.58 (C-2), 165.44 (C-1‴), 153.18 (C-5′), 148.38 (C-3′), 146.18 (C-4), 136.22 (C-1⁗), 132.43 (C-4⁗), 129.08 (C-3⁗ & C-5⁗), 119.06 (C-2⁗ & C-6⁗), 102.35 (C-5), 38.71 (C-1″), 37.44 (C-2‴), 27.48 (C-6), 20.40 (CH_3-4⁗), 14.68 (C-2″). Anal. Calc. for $C_{18}H_{20}N_6OS_2$ (388.51): C, 52.55; H, 5.19; N, 21.63. Found: C, 52.62; H, 5.24; N, 21.72; EI-MS: *m/z* 388 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 222 $[C_9H_{10}N_4OS]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 113 $[C_4H_5N_2S]^+$, 106 $[C_7H_8N]^+$, 99 $[C_3H_3N_2S]^+$, 91 $[C_7H_7]$.

*4.4.11. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2-methoxycarbonylphenyl)acetamide (**9k**)*

Light pink amorphous solid; Yield: 90%; m.p.: 108–109 °C; Mol. Formula: $C_{18}H_{20}N_6O_3S_2$; Mol. Mass.: 432 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3334 (N–H str.), 3022 (C–H str.), 2948 (–CH_2– str.), 1667 (C=O str.), 1587 (C=C str.), 1535 (C=N str.), 1162 (C–N–C bond str.), 622 (C–S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 11.16 (s, 1H, –CO–NH-1⁗), 8.31 (d, *J* = 8.34, 1H, H-6⁗), 7.93 (dd, *J* = 1.56,7.92, 1H, H-3⁗), 7.63 (dt, *J* = 1.56, 7.44, 1H, H-5⁗), 7.22 (dt, *J* = 1.14, 7.92, 1H, H-4⁗), 6.93 (br.s, 2H, H_2N-2), 6.27 (s, 1H, H-5), 4.17 (s, 2H, CH_2-2‴), 3.98 (q, *J* = 7.2, 2H, CH_2-1″), 3.95 (s, 2H, CH_2-6), 3.83 (s, 3H, 2⁗–CO–O$\underline{CH_3}$), 1.13 (t, *J* = 7.2, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.57 (C-2), 167.25 (2⁗–CO–O$\underline{CH_3}$), 166.23 (C-1‴), 153.34 (C-5′), 147.90 (C-3′), 146.17 (C-4), 139.15 (C-1⁗), 134.01 (C-5⁗), 130.55 (C-3⁗), 123.52 (C-4⁗), 120.89 (C-6⁗), 117.57.12 (C-2⁗) 102.36 (C-5), 52.40 (2⁗–CO–O$\underline{CH_3}$), 38.70 (C-1″), 36.80 (C-2‴), 27.43 (C-6), 14.58 (C-2″). Anal. Calc. for $C_{18}H_{20}N_6O_3S_2$ (432.52): C, 49.98; H, 4.66; N, 19.43. Found: C, 50.16; H, 4.82; N, 19.52; EI-MS: *m/z* 432 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 135 $[C_8H_7O_2]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

*4.4.12. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2-ethylphenyl)acetamide (**9l**)*

Drak brown gummy liquid; Mol. Formula: $C_{18}H_{22}N_6O_2S_2$; Mol. Mass.: 402 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3348 (N–H str.), 3065 (C–H str.), 2945 (–CH_2– str.), 1688 (C=O str.), 1565 (C=C str.), 1562 (C=N str.),1184 (C–N–C bond str.), 611 (C–S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.72 (s, 1H, –CO–NH-1⁗), 7.37 (m, 1H, H-6⁗), 7.21 (br.d, *J* = 7.38, 1H, H-3⁗), 7.17–7.13 (m, 2H, H-4⁗ & H-5⁗), 6.94 (br.s, 2H, H_2N-2), 6.30 (s, 1H, H-5), 4.14 (s, 2H, CH_2-2‴), 3.99–3.95 (m, 4H, CH_2-6 & CH_2-1″), 2.56 (q, *J* = 7.5, 2H, CH_3–$\underline{CH_2}$-2⁗), 1.19 (t, *J* = 7.56, 3H, $\underline{CH_3}$–CH_2-2⁗), 1.10 (t, *J* = 7.02, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.61 (C-2), 165.28 (C-1‴), 153.26 (C-5′), 148.61 (C-3′), 146.15 (C-4), 137.32 (C-1⁗), 135.20 (C-2⁗), 128.53 (C-3⁗), 125.89 (C-5⁗), 125.62 (C-4⁗), 125.15 (C-6⁗) 102.43 (C-5), 38.76 (C-1″), 36.78 (C-2‴), 27.44 (C-6), 23.67 (CH_3–$\underline{CH_2}$-2⁗), 14.62 (C-2″), 14.23 ($\underline{CH_3}$–CH_2-2⁗). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.85; H, 5.67; N, 20.92; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 105 $[C_8H_9]^+$, 99 $(C_3H_3N_2S)^+$.

*4.4.13. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(4-ethylphenyl)acetamide (**9m**)*

Bright yellow amorphous solid; Yield: 92%; m.p.:102–103 °C; Mol. Formula: $C_{18}H_{22}N_6O_2S_2$; Mol. Mass.: 402 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3381 (N–H str.), 3063 (C–H str.), 2934 (–CH_2– str.), 1665 (C=O str.), 1574 (C=C str.), 1562 (C=N str.), 1175 (C–N–C bond str.), 642 (C–S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.25 (s, 1H, –CO–NH-1⁗), 7.44 (br.d, *J* = 8.46, 2H, H-2⁗ & H-6⁗), 7.13 (br.d, *J* = 8.46, 2H, H-3⁗ & H-5⁗), 6.93 (br.s, 2H, H_2N-2), 6.26 (s, 1H, H-5), 4.09 (s, 2H, CH_2-2‴), 3.95 (m, 4H, CH_2-6 & CH_2-1″), 2.55 (q, *J* = 7.56, 2H, CH_3–$\underline{CH_2}$-4⁗), 1.14 (t, *J* = 7.62, 3H, $\underline{CH_3}$–CH_2-4⁗), 1.09 (t, *J* = 7.2, 3H, CH_3-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.57 (C-2), 165.45 (C-1‴), 153.18 (C-5′), 148.37 (C-3′), 146.18 (C-4), 138.90 (C-1⁗), 136.41 (C-4⁗), 127.93 (C-3⁗ & C-5⁗), 119.15 (C-2⁗& 6⁗), 102.35 (C-5), 38.71 (C-1″), 37.43 (C-2‴), 27.48 (C-6), 27.45 (CH_3–$\underline{CH_2}$-4⁗), 15.62 ($\underline{CH_3}$–CH_2-4⁗), 14.69 (C-2″). Anal. Calc. for $C_{18}H_{22}N_6OS_2$ (402.54): C, 53.71; H, 5.51; N, 20.88. Found: C, 53.90; H, 5.72; N, 20.99; EI-MS: *m/z* 402 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 120 $[C_8H_{10}N]^+$, 113 $[C_4H_5N_2S]^+$, 105 $[C_8H_9]^+$, 99 $[C_3H_3N_2S]^+$.

*4.4.14. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(2-ethyl-6-methyphenyl)acetamide (**9n**)*

Off-white amorphous solid; Yield: 87%; m.p.: 124–125 °C; Mol. Formula: $C_{19}H_{24}N_6OS_2$; Mol. Mass.: 416 gmol^{-1}; IR (KBr, υ, cm^{-1}): 3362 (N–H str.), 3085 (C–H str.), 2934 (–CH_2– str.), 1634 (C=O str.), 1522 (C=C str.), 1545 (C=N str.), 1164 (C–N–C bond str.), 620 (C–S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.63 (s, 1H, –CO–NH-1⁗), 7.11 (br.t, *J* = 7.5, 1H, H-4⁗), 7.05 (dis.d, *J* = 7.8, 2H, H-3⁗ & H-5⁗), 6.94 (br.s, 2H, H_2N-2), 6.31 (s, 1H, H-5), 4.14 (s, 2H, CH_2-2‴), 3.97 (m, 4H, CH_2-6 & CH_2-1″), 2.44 (q, *J* = 7.56, 2H, CH_3–$\underline{CH_2}$-6⁗), 2.07 (s, 3H, CH_3-2⁗), 1.10 (t, *J* = 7.2, 3H, CH_3-2″), 1.03 (t, *J* = 7.53, 3H, $\underline{CH_3}$–CH_2-6⁗); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.59 (C-2), 165.90 (C-1‴), 153.15 (C-5′), 148.45 (C-3′), 146.22 (C-4), 140.99 (C-1⁗), 135.55 (C-6⁗), 134.00 (C-2⁗), 127.60 (C-4⁗), 126.86 (C-3⁗), 125.93 (C-5⁗), 102.41 (C-5), 38.71 (C-1″), 36.34 (C-2‴), 27.46 (C-6), 24.15 (CH_3–$\underline{CH_2}$-6⁗), 17.91 ($\underline{CH_3}$–CH_2-6⁗), 14.71 (C-2″), 14.55 (CH_3-2⁗). Anal. Calc. for $C_{19}H_{24}N_6OS_2$ (416.56): C, 54.78; H, 5.81; N, 20.17.

Found: C, 54.87; H, 5.92; N, 20.22; EI-MS: *m/z* 416 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 134 $[C_9H_{12}N]^+$, 119 $[C_9H_{11}]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.4.15. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(3-ethoxyphenyl)acetamide (**9o**)

White amorphous solid; Yield: 88%; m.p.:178–179 °C; Mol. Formula: $C_{18}H_{22}N_6O_2S_2$; Mol. Mass.: 418 gmol^{-1}; IR (KBr, *υ*, cm^{-1}): 3351 (N—H str.), 3043 (C—H str.), 2958 (—CH$_2$— str.), 1627 (C═O str.), 1582 (C═C str.), 1542 (C═N str.), 1182 (C—N—C bond str.), 619 (C—S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.30 (s, 1H, —CO—NH-1⁗), 7.25 (br.d, J = 2.1, 1H, H-2⁗), 7.19 (br.t, J = 8.1, 1H, H-5⁗), 7.05 (dd, J = 0.96, 8.04, 1H, H-6⁗), 6.93 (br.s, 2H, H$_2$N-2), 6.62 (dd, J = 2.04, 8.16, 1H, H4⁗), 6.26 (s, 1H, H-5), 4.10 (s, 2H, CH$_2$-2‴), 3.99–3.92 (m, 6H, CH$_2$-6, CH$_2$-1″ & CH$_3$—$\underline{CH_2}$—O-3⁗), 1.31 (t, J = 6.96, 3H, $\underline{CH_3}$—CH$_2$—O-3⁗), 1.09 (t, J = 7.14, 3H, CH$_3$-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.58 (C-2), 165.73 (C-1‴), 158.74 (C-3⁗), 153.19 (C-5′), 148.35 (C-3′), 146.18 (C-4), 139.36 (C-1⁗), 129.55 (C-5⁗), 111.22 (C-6⁗), 109.44 (C-4⁗), 105.35 (2⁗), 102.34 (C-5), 62.88 (CH$_3$—$\underline{CH_2}$—O-3⁗), 38.71 (C-1″), 37.48 (C-2‴), 27.48 (C-6), 14.68 (C-2″), 14.60 ($\underline{CH_3}$—CH$_2$—O-3⁗). Anal. Calc. for $C_{18}H_{22}N_6O_2S_2$ (418.54): C, 51.65; H, 5.30; N, 20.08. Found: C, 51.81; H, 5.42; N, 20.17; EI-MS: *m/z* 418 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 136 $[C_8H_{10}NO]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.4.16. 2-({5-[(2-Amino-1,3-thiazol-4-yl)methyl]-4-ethyl-4H-1,2,4-triazol-3-yl}sulfanyl)-N-(4-ethoxyphenyl)acetamide (**9p**)

Light pink amorphous solid; Yield: 92%; m.p.:188–189 °C; Mol. Formula: $C_{18}H_{22}N_6O_2S_2$; Mol. Mass.: 418 gmol^{-1}; IR (KBr, *υ*, cm^{-1}): 3355 (N—H str.), 3021 (C—H str.), 2938 (—CH$_2$— str.), 1662 (C═O str.), 1582 (C═C str.), 1542 (C═N str.), 1166 (C—N—C bond str.), 632 (C—S str.); ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.18 (s, 1H, —CO—NH-1⁗), 7.45 (br.d, J = 6.9, 2H, H-2⁗ & H-6⁗), 6.96 (br.s, 2H, H$_2$N-2), 6.87 (br.d, J = 6.9, 2H, H-3⁗ & H-5⁗), 6.27 (s, 1H, H-5), 4.07 (s, 2H, CH$_2$-2‴), 4.00–3.93 (m, 6H, CH$_2$-6, CH$_2$-1″ & CH$_3$—$\underline{CH_2}$—O-4⁗), 1.31 (t, J = 6.96, 3H, $\underline{CH_3}$—CH$_2$—O-4⁗), 1.12 (t, J = 7.2, 3H, CH$_3$-2″); ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 168.57 (C-2), 166.14 (C-1‴), 154.61 (C-4⁗), 153.17 (C-5′), 148.37 (C-3′), 146.17 (C-4), 131.77 (C-1⁗), 120.59 (C-2⁗ & C-6⁗), 114.41 (C-3⁗& 5⁗), 102.35 (C-5), 63.05 (CH$_3$—$\underline{CH_2}$—O-4⁗), 38.71 (C-1″), 37.38 (C-2‴), 27.48 (C-6), 14.69 ($\underline{CH_3}$—CH$_2$—O-4⁗), 14.64 (C-2″). Anal. Calc. for $C_{18}H_{22}N_6O_2S_2$ (418.54): C, 51.65; H, 5.30; N, 20.08. Found: C, 51.88; H, 5.48; N, 20.15; EI-MS: *m/z* 418 $[M]^+$, 282 $[C_{10}H_{12}N_5OS_2]^+$, 255 $[C_9H_{11}N_4OS_2]^+$, 240 $[C_8H_{10}N_5S_2]^+$, 208 $[C_8H_{10}N_5S]^+$, 139 $[C_5H_5N_3S]^+$, 136 $[C_8H_{10}NO]^+$, 113 $[C_4H_5N_2S]^+$, 99 $[C_3H_3N_2S]^+$.

4.5. Elastase inhibition assay

The elastase (Elastase from porcine pancreas) inhibition activity was performed by following already reported method [46,55–57] with few modifications. The amount of released *p*-nitroaniline, which was hydrolyzed from the substrate (*N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide) by elastase, was determined by measuring the absorbance at 410 nm. In detail, 0.8 mM solution of *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide was prepared in a 0.2 M Tris-HCl buffer (pH 8.0) and this buffer (130 µL) was added to the test sample (10 µL) in a 96 well microplate. The microplate was pre-incubated for 10 min at 25 °C before an elastase (0.0375 Unit/mL) stock solution (10 µL) was added. After enzyme addition, the microplate was kept at 25 °C for 30 min, and the absorbance was measured at 410 nm using microplate reader. All experiments were carried out in triplicate. The elastase inhibition activities were calculated according to the following formula:

$$Elastase\ inhibition\ activity\ (\%) = (OD_{control} - OD_{sample} \times 100)/OD_{control}$$

where OD$_{\text{control}}$ and OD$_{\text{sample}}$ represents the optical densities in the absence and presence of sample, respectively. Oleanolic acid was used as the standard inhibitor for elastase.

4.6. Protocol for kinetics

Kinetic analysis was carried out to determine the mode of inhibition. The most potent compound **9h** was selected on the basis of lowest IC$_{50}$ value. Kinetics was carried out by varying the concentration of *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide in the presence of different concentrations of compound **9h** (0.00, 0.0094 and 0.0188 µM). Briefly, the *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide concentration was changed from 2, 1, 0.5, 0.25 and 0.125 mM for its kinetics studies and remaining procedure was same for all kinetic studies as describes in elastase inhibition assay. Maximal initial velocities were determined from initial linear portion of absorbances up to 10 min after addition of enzyme at per minute interval. The inhibition type on the enzyme was assayed by Lineweaver-Burk plot of inverse of velocities (1/V) versus inverse of substrate concentration 1/ [S] mM^{-1}. The EI dissociation constant K$_i$ was determined by secondary plot of 1/V versus inhibitor concentration. The results were processed by using SoftMaxPro.

4.7. Molecular docking process

4.7.1. Retrieval of porcine pancreatic elastase in protein preparation wizard

The porcine pancreatic elastase structure was retrieved from Protein Data Bank (PDB) with PDBID 7EST (https://www.rcsb.org/structure/7EST) in protein preparation wizard. The selected protein structure of was preprocessed and minimized using default parameters adjusted in Maestro interface.

4.7.2. Grid generation and molecular docking

Prior to molecular docking, the optimized elastase structure was prepared using the "Protein Preparation Wizard" workflow in Schrödinger Suite. Bond orders were assigned and hydrogen atoms were added to the protein. The structure was then minimized to reach the converged root mean square deviation (RMSD) of 0.30 Å with the OPLS_2005 force field. The active site of the enzyme is defined from the co-crystallized ligands from Protein Data Bank and literature data [54,58]. Furthermore, the docking approach was performed against all the synthesized ligands (**9a–9p**) sketched by 2D sketcher in Maestro and target protein by using Glide docking protocol [59]. The predicted binding energies (docking scores) and conformational positions of ligands within active region of protein were also performed using Glide experiment. Throughout the docking simulations, both partial flexibility and full flexibility around the active site residues are performed by Glide/SP/XP and induced fit docking (IFD) approaches [60,61].

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Conflict of interest

Authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.040.

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