Benzimidazole Tethered Thioureas as a New Entry to Elastase inhibition and Free Radical Scavenging: Synthesis, Molecular docking, and Enzyme Inhibitory Kinetics

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Graphical Abstract

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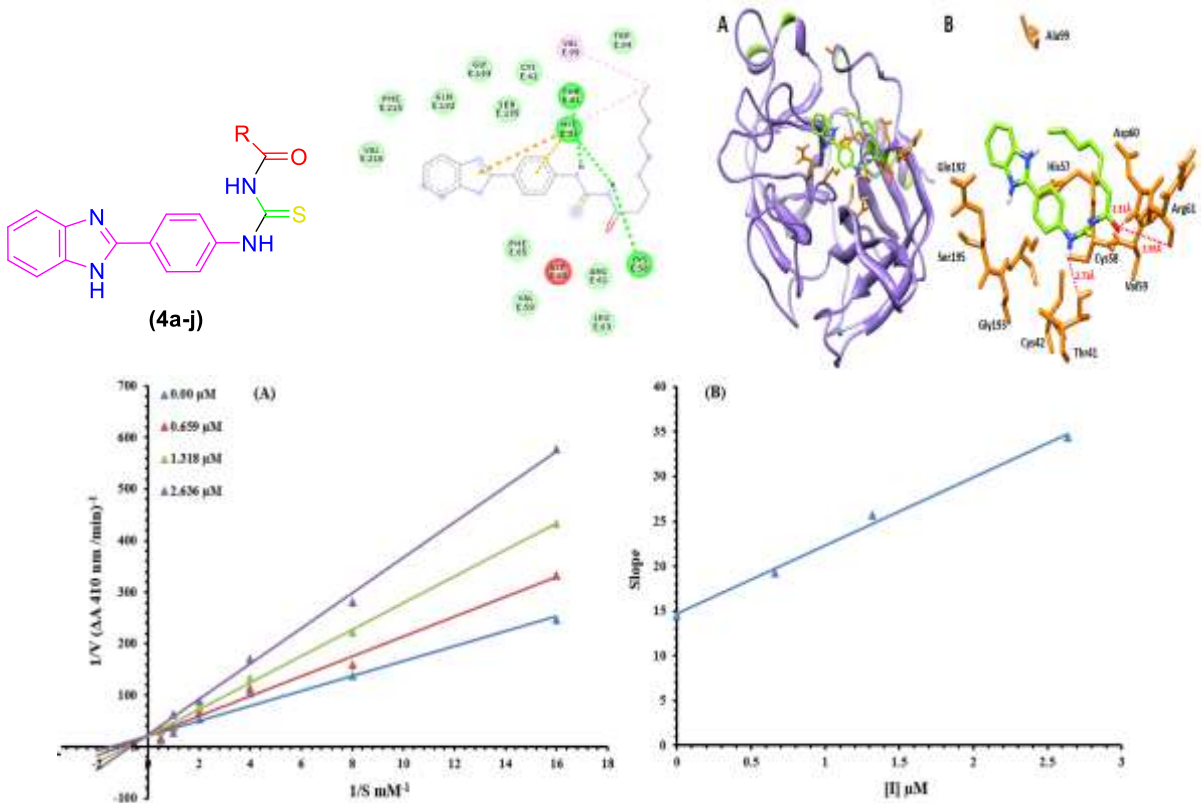

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Abstract

The porcine pancreatic elastase inhibition and free-radical scavenging play a crucial role in age progression. All the series of ten newly synthesized benzimidazole thioureas (**4a-j**) were assessed for elastase inhibition and radical scavenging activity to identify the suitable anti-aging ingredient for cosmetics products. The compounds **4e, 4f, 4g**, and **4h** showed inhibition better than the standard whilst compound **4f** showed the most significant elastase inhibition with the IC_{50} value of 1.318 ± 0.025 µM compared with oleanic acid IC_{50} 13.451 ± 0.014 used ± 1.989 and 41.563 ± 0.824, respectively.as standard. Molecular docking studies were performed and the compound **4f** showed binding energy of 7.2 Kcal/mol. Kinetics studies revealed inhibition of the pancreatic elastase in a competitive manner. The relative binding energy and SAR identified compound **4f** as an effective inhibitor of porcine pancreatic elastase. Compounds **4e** and **4i** showed remarkable free-radical scavenging activity with SC_{50} values of 26.421

Keywords: Elastase inhibition; benzimidazole thioureas; radical scavenging activity; structure-property relationship.

1. Introduction

Skin aging is a common occurrence witnessed with the increase in human age. It is reflected by wrinkling, loss of elasticity, laxity, rough-textured look together with phenotypic changes. Various cosmetic products are used to break this process. Elastin is a major part of the elastic fibers in animal tissues primarily responsible for the skin elasticity. The skin elasticity is reduced by the inhibition of elastin fibers by the enzyme Elastase. Therefore Inhibition of this enzyme can assist to avoid the skin aging process [1]. On the contrary, Exposure of skin to sunlight and UV radiations can cause free radicals to react with the skin epidermal cells resulting in oxidative damage [2,3]. Therefore, elastase inhibition and free-radical scavenging processes are vital to the skin health [4].

Amongst the most widely employed for both inhibition of porcine pancreatic elastase as well as free radical scavenging are natural polyphenolic compounds such as phenolic acids, flavonoids, stilbenes and lignans [5-7]. Peel and seed extract of *Passiflora edulis* were tested against porcine pancreatic elastase and found to be potent anti-aging ingredients for cosmetics [8]. Similarly, flavonoids Scutellarin and apigenin and other phytochemicals have also been employed [9].

Over and above natural products, several synthetic molecules have also been used for both purposes. We have already identified densely substituted piperidines and 1,4-dihydropyridines as innovative skin shielding and anti-aging agents [10-11]. Alternatively, quinolyl based acyl thioureas, and valeroyl-3-arylthioureas have been confirmed as efficient free radical scavengers. [12-14]. Benzimidazole is a bicyclic heterocyclic motif having a range of beneficial uses cover antitumor, antifungal, antiparasitic, analgesics, antiviral, antihistamine, along with cardiovascular, neurological, and ophthalmological diseases [15].

Keeping in view the importance of elastase inhibitors and free-radical scavengers in the anti-aging treatment of skin on one hand and the biological significance of benzimidazoles and thioureas on the other, we designed and synthesized benzimidazole based thioureas as a novel entry to elastase inhibitors and free-radical scavengers. These compounds contain benzimidazole as main core along with thiourea linker and the aryl or alkyl group for structural variation in a single structural unit to obtain compounds as dual inhibitors of elastase as well as free radicals (Figure1).

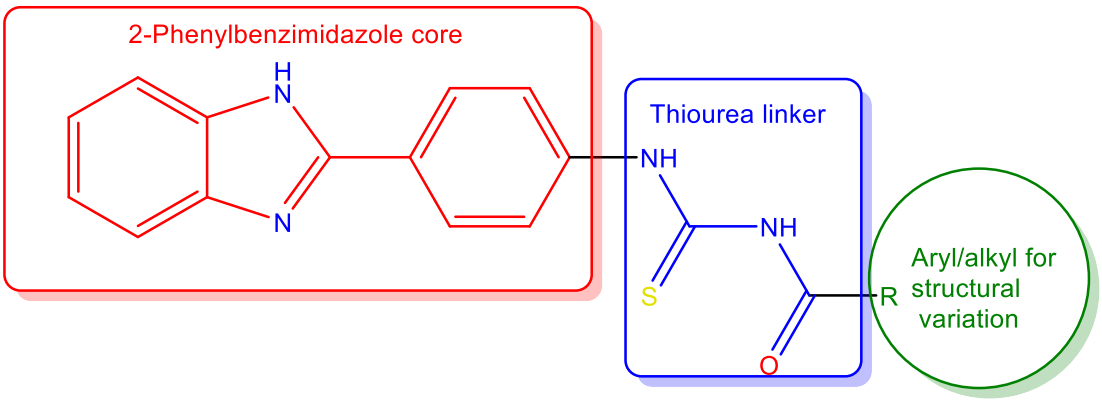


Figure 1 Molecular architecture of target compounds

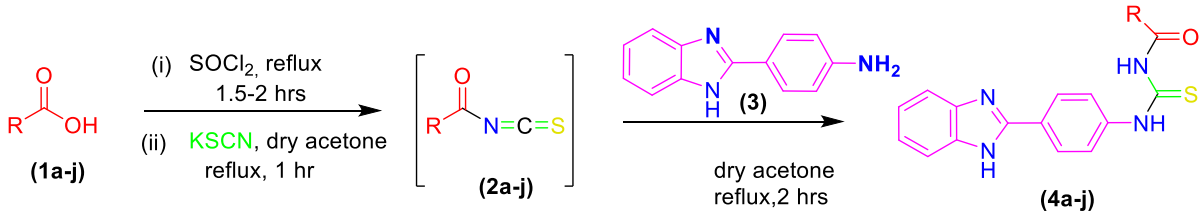
Newly prepared benzimidazole fused thioureas were obtained in good yields, purified by recrystallization, and characterized by spectroscopic techniques and evaluated for the aforesaid biological potential.

2. Results and discussion

2.1 Synthesis

Scheme1, indicates the synthetic pathway adopted for the preparation of benzimidazole linked thioureas. Accordingly, freshly prepared acid chlorides were added to a solution of potassium thiocyanate in anhydrous acetone to yield isothiocyanates intermediates (**2a-j**). After cooling, a solution of benzimidazolyl amine (**3)** in dry acetone was added dropwise and

the mixture under reflux to afford benzimidazole-thiourea derivatives **(4a-j)** in good yields. FTIR analysis revealed that distinctive absorption bands for N-H at 3215-3350 cm^{-1}, C-H aromatic at 3033-3080 cm^{-1}, C=O of carbonyl at 1680-1748 cm^{-1}, C=N of imine at 1542-1694 cm^{-1} and C=C (Ar) 1532-1575. The ^{1}H NMR spectrum of a specific compound **4j** signifies three distinctive broad singlets for N-H protons at δ 12.79, 11.66 ppm, 10.98 ppm, and multiplets for aromatic protons at δ 8.02-7.93 and δ 7.68-7.21 ppm. ^{13}C NMR spectrum of compound **4j** revealed signals for thiocarbonyl at δ 179.34, and carbonyl carbon at δ 168.75 ppm, while the imino carbon resonated at δ 151.15 ppm.



Scheme 1: Synthetic route of benzimidazole fused thioureas **(4a-j)**

The molecular structures of benzimidazole-thioureas **(4a-j)** are shown in Figure 2.

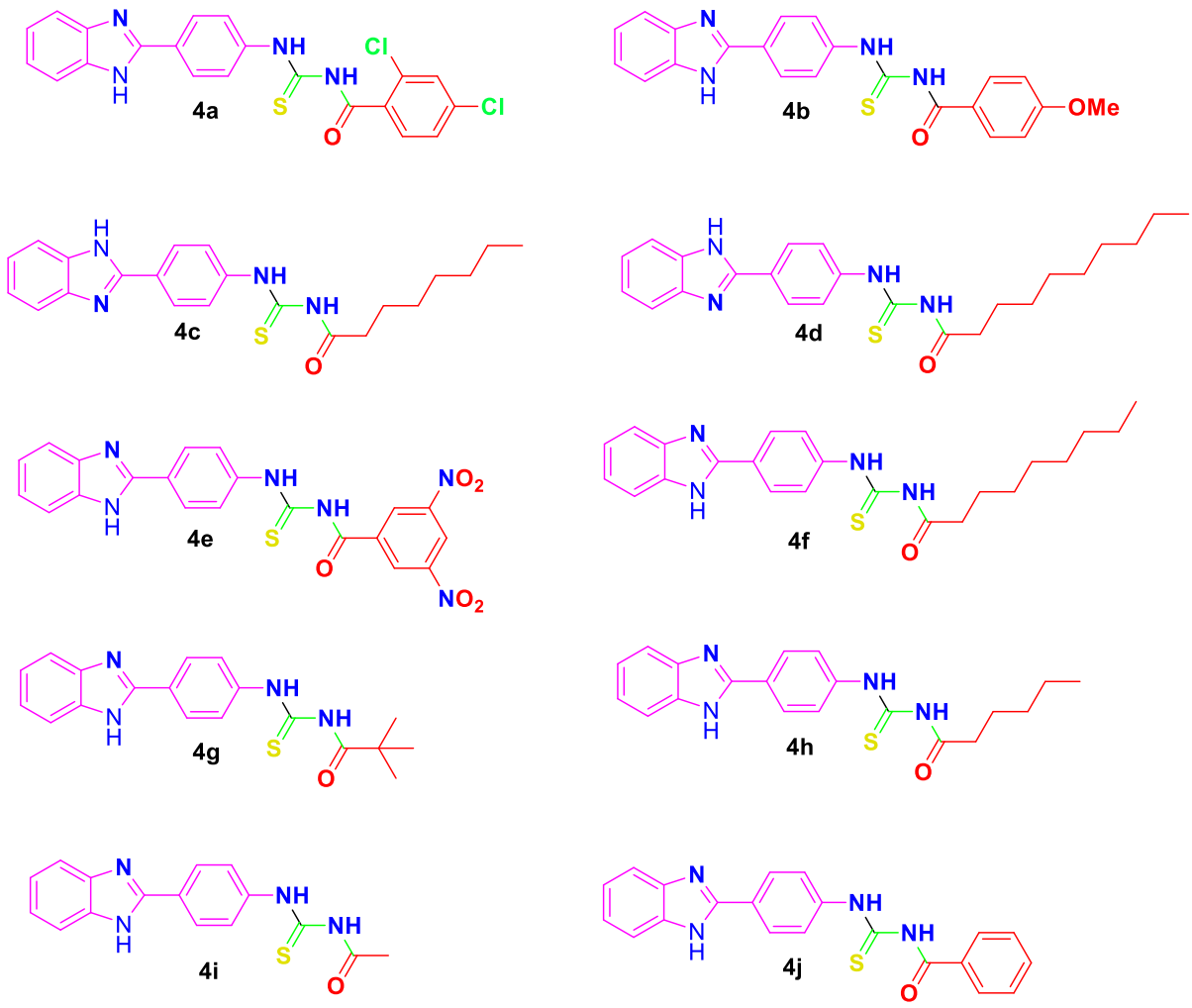


Figure 2: Molecular structures of benzimidazole-thioureas **(4a-j)**

2.2 Porcine pancreatic elastase inhibition

Table.1 lists the Elastase and Free radical scavenging inhibitory activity of the synthesized derivatives (**4a-j**). Compounds **4e 4f, 4g** and **4h** indicated improved inhibition than the standard whilst compound **4f** having a C-8 alkyl side chain showed considerable elastase inhibition with IC_{50} value of 1.318 ± 0.025 µM compared to the Oleanolic Acid, with IC_{50} value 13.451 ± 0.014 µM. The presence of a C-8 alkyl side chain adopts a conformation suitable for binding to active site of enzyme. Thus, varying the alkyl chain length on the hydrophobic tail can selectivity toward inhibition of elastase. Increasing or decreasing the

length of the hydrophobic tail away from eight carbons leads to a decrease enzyme inhibition. It can be seen that C-8 chain interacts with histidine and valine residues in the docking complex (Fig S6). The relative binding energy and SAR identified compound **4f** as a potent inhibitor of elastase.

2.3 Free radical scavenging

All the synthesized **(4a-j)** compounds were evaluated for DPPH free radical scavenging ability. The compounds **4e** and **4h** showed some activity, however rest of the compounds did not show significant radical scavenging activity even at the higher concentration (100µg/mL) (Table 1). These molecules **4e** having a phenyl ring substituted at 3 and 5 positions with electron withdrawing NO_2 and **4h** possessing a C-5 alkyl side chain showed noteworthy free-radical scavenging activity with SC_{50} value of 26.421 ± 1.989 and 41.563 ± 0.824 µM respectively compared to Ascorbic acid. It indicates that presence of strong electron withdrawing NO_2 group helps in free radical scavenging potential.a C-5 alkyl side chain adopts a conformation suitable for DPPH free radical scavenging. Similarly, presence of electron withdrawing NO_2 at 3 and 5 position of the phenyl ring can enhance the potency of synthesized derivatives.

Table.1 Porcine pancreas elastase and Free radical scavenging activity of derivatives (**4a-j**)

Compound	**Elastase IC_{50}± SEM (µM)**	**Free radical % scavenging (100 µg/mL)**
4a	30.159 ± 0.112	9.428 ± 0.745
4b	39.005 ±0.841	16.136 ± 0.991
4c	4.502 ± 0.095	12.737 ± 1.558
4d	14.988 ± 0.967	6.032 ± 0.887

4e	54.307 ± 1.719	26.421 ± 1.989
4f	1.318 ± 0.025	4.303 ± 0.259
4g	3.769 ± 0.068	8.386 ± 0.554
4h	8.355 ± 0.081	41.563 ± 0.824
4i	67.806 ± 2.81	2.656 ± 0.985
4j	10.532 ± 1.083	16.915 ± 0.519
Oleanolic Acid	13.451 ± 0.014	----------
Vitamin C	----------	94.823 ± 0.046

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

2.4 Kinetic Mechanism

To understand the inhibitory style of the synthetic compounds versus elastase inhibition, kinetic study was carried out. On the basis of IC_{50} the most potent compound **4 f** was chosen to determine the type of inhibition and inhibition constant. The kinetic 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. Lineweaver-Burk plot of compound **4f** indicated that Vmax remains the equal without much effecting the slopes. K_m increases with increasing concentration while V_{max} remains the same with insignificant difference. This behavior indicates that **4f** compound inhibits the enzymes in a competitive manner **(Figure. 3a),** second plot **(Figure. 3b)** of slope against concentration of **4f** showed EI dissociation constant. *K*i was calculated from inhibitor concentration of 4f versus the slope and *K*i was 1.9 µM.

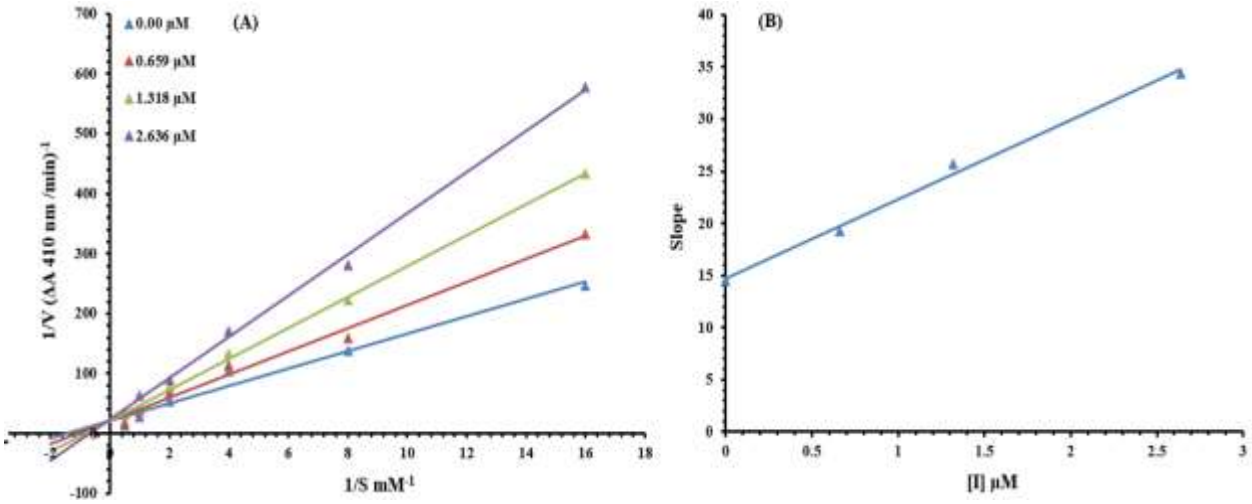


Figure 3. Lineweaver–Burk plots for inhibition of elastase from porcine pancreas in the presence of Compound 4f (A) Concentrations of 4f were 0.00, 0.659, 1.318 and 2.636 µM, Substrate N-succinyl-Ala-Ala-Ala-p-nitroanilide concentrations were 2, 1, 0.5, 0.25, 0.125 and 0.0625 mM. (B) The insets represent the plot of the slope.

4.0 Molecular docking analysis

4.1 Docking energy analyses of synthesized compounds

To envisage the best tailored conformation of compounds (**4a-j**) within the active zone of protein molecular docking analysis was executed. The docked complexes were evaluated based on minimum energy values (Kcal/mol) as H- and hydrophobic interactions. Docking results indicated that all compounds have good energy values (**Fig 3)**. Nonetheless, all compounds revealed good binding energy values (Kcal/mol) against receptor with slight variations. The standard error mean of docking energy for Autodock is 2.5 Kcal/mol.

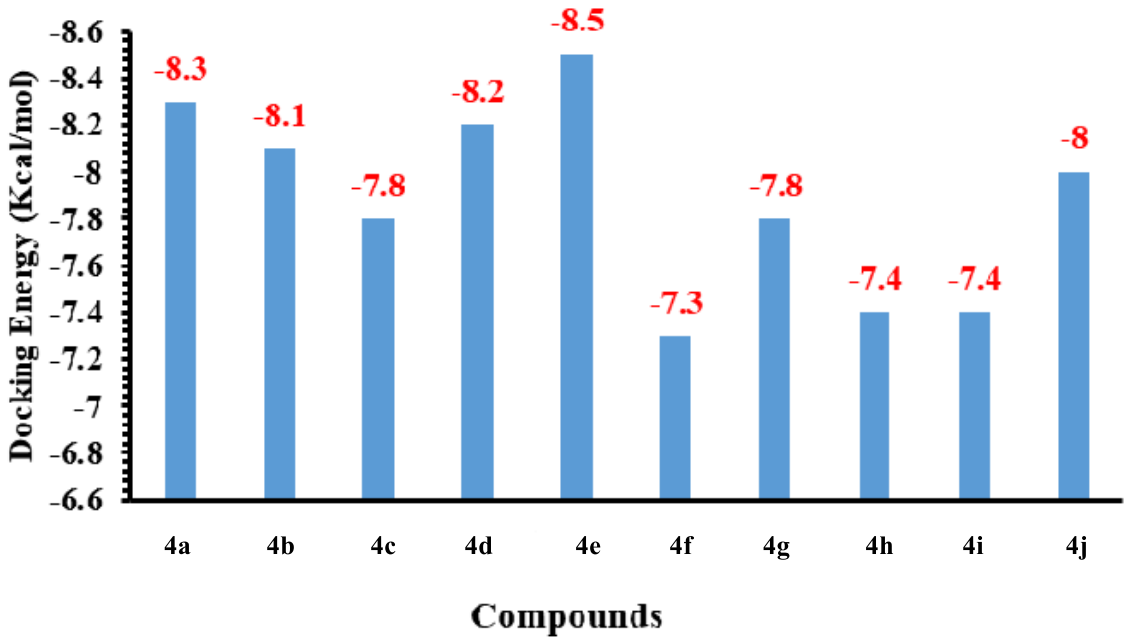


Fig 3. The graphical depiction of docking energy values.

5. Binding pocket and ligand conformational analysis

The docked complexes were analyzed on the basis of hydrogen and hydrophobic bonding interactions. The best *in vitro* result compound was designated to check best conformational position inside active site of the protein. The binding pocket analysis showed that **4f** binds within the active site of protein (**Fig 4**).

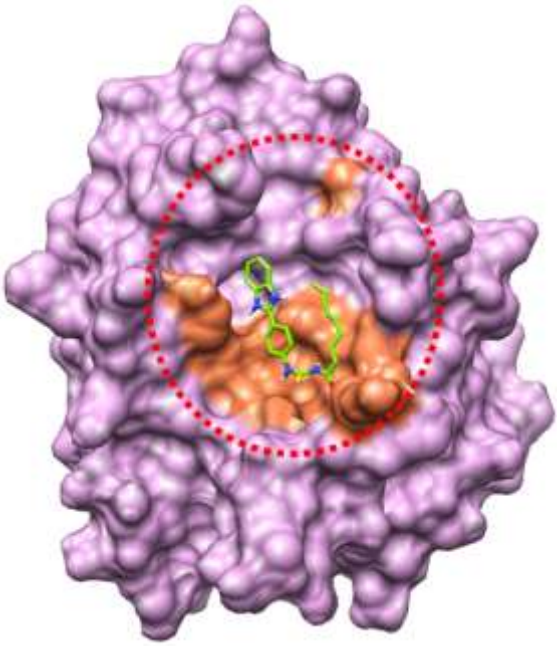


Figure 4. Binding pocket depiction of target protein

The comprehensive docking analysis showed that in **4f** docking complex three H-bonds in the active region of protein. The amino group made good interactive bond against Thr41 with bond distances 2.73 Å. Even though, oxygen atom of **4f** formed couple of hydrogen bonds Asp60 and Arg61 with bond distances 2.31 and 2.95 Å, respectively (**Figure 5**). Literature data also confirmed the value of these residues in bonding with other elastase inhibitors which strengthens the docking results [25]. The relative binding energy and SAR showed **4f** to be considered as potent inhibitor. The other docking complexes are mentioned in the supplementary data (Fig S1-S10).

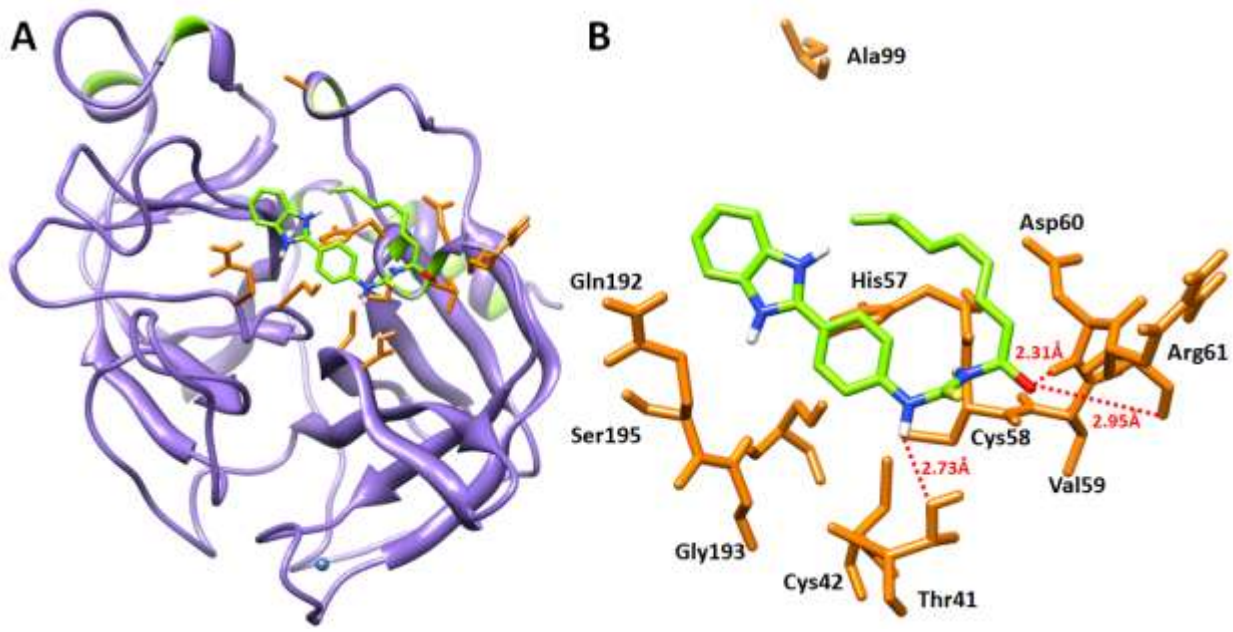


Figure 5. Molecular docking interaction of **4f**. **A**) The general overview of docking depiction. **B**) The closer view of docking complex. The residues involve in binding interactions are shown in brown color. Red dotted lines with distance stated in angstrom (Å) are justified for H-bond distances.

In-silico Methodology

3.1 Retrieval of porcine pancreatic elastase structure

The retrieval of porcine pancreatic elastase structure was in line with procedure reported earlier [21-23].

3.2 Molecular docking of synthesized compounds

The synthesized chemical structures (**4a-j**) were sketched in ACD/ChemSketch and minimized by UCSF Chimera 1.6rc tool. PyRx docking tool [24] was employed to perform molecular docking experiment. All the synthesized ligands (**4a-j**) were docked separately against elastase. Docking analysis was done based on lowest binding energy (Kcal/mol) values and structure activity relationship (SAR) analyses. The three dimensional (3D)

graphical depictions of all the docked complexes were accomplished by Discovery Studio (2.1.0).

In-vitro Methodology

3.3 Elastase inhibition assay

The inhibition of Elastase from porcine pancreas was achieved following reported method [16-17]. The elastase inhibition activities were calculated according to the following formula:

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

Where $OD_{control}$ and OD_{sample} represents the optical densities in the absence and presence of sample, respectively using oleanolic acid as the standard.

3.6 Protocol for Kinetics

Kinetic analysis was done to determine the mode of inhibition using reported method [18]. The compound **4f** was selected because of most potent IC_{50} values. Kinetics were carried out by varying the concentration of N-succinyl-Ala-Ala-Ala-p-nitroanilide the presence of different concentrations of compound 4f (0.00, 0.659, 1.318 and 2.636 µM). The inhibition mode of enzyme was evaluated by Lineweaver-Burk plot of inverse of velocities (1/V) versus inverse of substrate concentration 1/ [S] mM^{-1}. The EI dissociation constant Ki was determined by secondary plot of 1/V versus inhibitor concentration and processed using SoftMaxPro.

3.7 Free radical scavenging assay

Radical scavenging activity was determined by reported method [19-20] by 2, 2-diphenyl-1 picryl hydrazyl (DPPH) assay.

4. Conclusions

A new series of the benzimidazole-thioureas (**4a-j**) were prepared, characterized, and appraised for elastase inhibition and antioxidant activities. Compound **4f** having a C-8 hydrophobic alkyl chain showed maximum elastase inhibition (IC_{50} = 1.318 ± 0.025 µM) compared to other compounds of the series. Kinetic analysis revealed the competitive mode of inhibition of the elastase enzyme with the Ki value of 1.9 µM. The molecular docking of the compound **4f** showed good binding energy value of 7.3 Kcal/mol against receptor. The presence of benzimidazole and alkyl chain with the acyl thiourea play crucial role in elastase inhibition. Compound **4f** was recognized as potent elastase inhibitor that could be used a potential anti-aging ingredient in cosmetics.

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