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ARTICLE

Convergent synthesis, free radical scavenging, Lineweaver-Burk plot exploration, hemolysis and in silico study of novel indole-phenyltriazole hybrid bearing acetamides as potent urease inhibitors

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Abstract

In the current paper, through a convergent multi-step approach, a library of novel indole-phenyltriazole hybrids containing an amide moiety (**9a-k**) was synthesized. The structural verification of all synthesized molecules was accomplished by CHN and spectral analyses data. These synthesized bi-heterocyclic derivatives (**9a-k**) were evaluated for their anti-ulcer potential by inhibitory action against Jack bean urease enzyme and subsequently their structure-activity relationship was perceived. Moreover, these compounds were inspected for cytotoxic profile by hemolytic activity and it was professed that nearly all the synthesized compounds showed low cytotoxicity. In addition, free radical scavenging activity and kinetic analysis were also carried out for these compounds to understand their mode of inhibition. So, it was summated that these derivatives might lead to further research gateways for obtaining better and safe anti-ulcer agents.

1 | INTRODUCTION

Urea has exclusive role in the history of organic and medicinal chemistry. It was the first organic compound that was synthesized from an inorganic compound by Friedrich Wohler in 1928.[1] It is an exogenous product of amino acids and proteins dissimilation, human body excretes about 20 to 30 g of urea in human urine in a single day. Urea is also used as important fertilizer in agriculture. Ureases (urea aminohydrolase E.C.3.5.1.5) are the members of phosphotriesterases and aminohydrolases family which exhibit active metals in the active sites of these enzymes. Ureases contain two Ni^{+2} ions in their active sites[2–6] that catalyze the hydrolysis of urea up to 1014 times compared to uncatalyzed reaction.[7] Basically, this enzyme works by catalyzing the hydrolysis of urea into NH_3 and carbamate (which yields second molecule of NH_3 on decomposition). Release of these two NH_3 molecules causes considerable increase in pH which imparts negative effects on human body and agriculture.[8] One of the most common urease associated gram-negative bacterial specie is *Helicobacter pylori* which is capable of living in acidic environment like stomach (pH 1-2) and is the cause of a chief public health problem.[9] Its infections stimulate stomach inflammation and enhance the possibility of emergent gastric and duodenal ulcers, gastric lymphoma, gastric adenocarcinoma,[10–13]

stomach cancer and peptic ulcer.[14,15] Around 50% of worldwide population is septic to *H. pylori* because this specie can survive in stomach all through the life of infected people without any indication of disease. This high occurrence of *H. pylori* in humans points to the development of bacterial immunity against human host immunity. A broad range of urease enzymes have been isolated from fungi, algae, bacteria and plants[16] and all the urease enzymes persue the similar catalysis pattern. This is due to the analogous sequence of amino acids and the active sites of enzyme containing two nickel ions, showing common ancestry. Therefore, urease inhibitors have been considered as target for novel antiulcer drugs.[17] Keeping in view the association of ureases in several pathological conditions, the exploration of safe and potent urease enzyme inhibitors has become a significant topic of research in pharmaceutical industry.[18] Imidazole, phosphorodiamidates, hydroxamic acid derivatives and many other urease inhibitors have been examined in past decades, but majority of these compounds are unstable or too toxic to be allowed for their use in living organisms. Therefore, more research is needed for the synthesis of new urease inhibitors with predicting levels of activities.[19]

Ascorbic acid (Vitamin C) is a urease inhibitor, an antioxidant and also a free radical scavenger. High doses of antimicrobials can enhance the risk of side effects but ascorbate is a safe natural compound present in many edibles in large quantities. One of the remarkable characteristic of Vitamin C is that its activities are pH dependent. It is unstable at neutral pH and degrades at pH 6 to 7.[20] Other urease inhibitors used are benzoquinones,[21] hydroxyurea,[22] barbituric and thiobarbituric acid,[18] and triazoles.[23] Thus, the novel and safe treatment of gastric ulcers with lesser side effects is an attractive field for pharmaceutical researchers.

Indole is a naturally occurring heterocyclic molecule found in indole alkaloids, marine natural products and fungal metabolites. Structurally, it is a benzopyrrole ring which is composed of benzene and pyrrole rings fused with each other at positions 2- and 3- of pyrrole ring. Here C-2 and C-3 bonds often react like alkenes. The *N*-substitution reactions are always favored by ionic salts (Li^+, Na^+, K^+, etc.) while softer counter ions prefer C-3 substitution. This helps in binding of indole with different compounds.[24] Indole and its derivatives containing thiazole and pyrazole moieties are of great importance as antioxidant,[25,26] anti-cancer,[27] and anti-proliferative agents.[28,29] Moreover, indole derivatives are also found to have numerous biological activities like analgesic, anti-inflammatory, anti-malarial, anti-convulsant, anti-ulcer,[24] anti-depressant,[30–32] and anti-hypertensive.[33] Similarly, 1,2,4-triazole nuclei and their derivatives have also been accounted for their wide range of

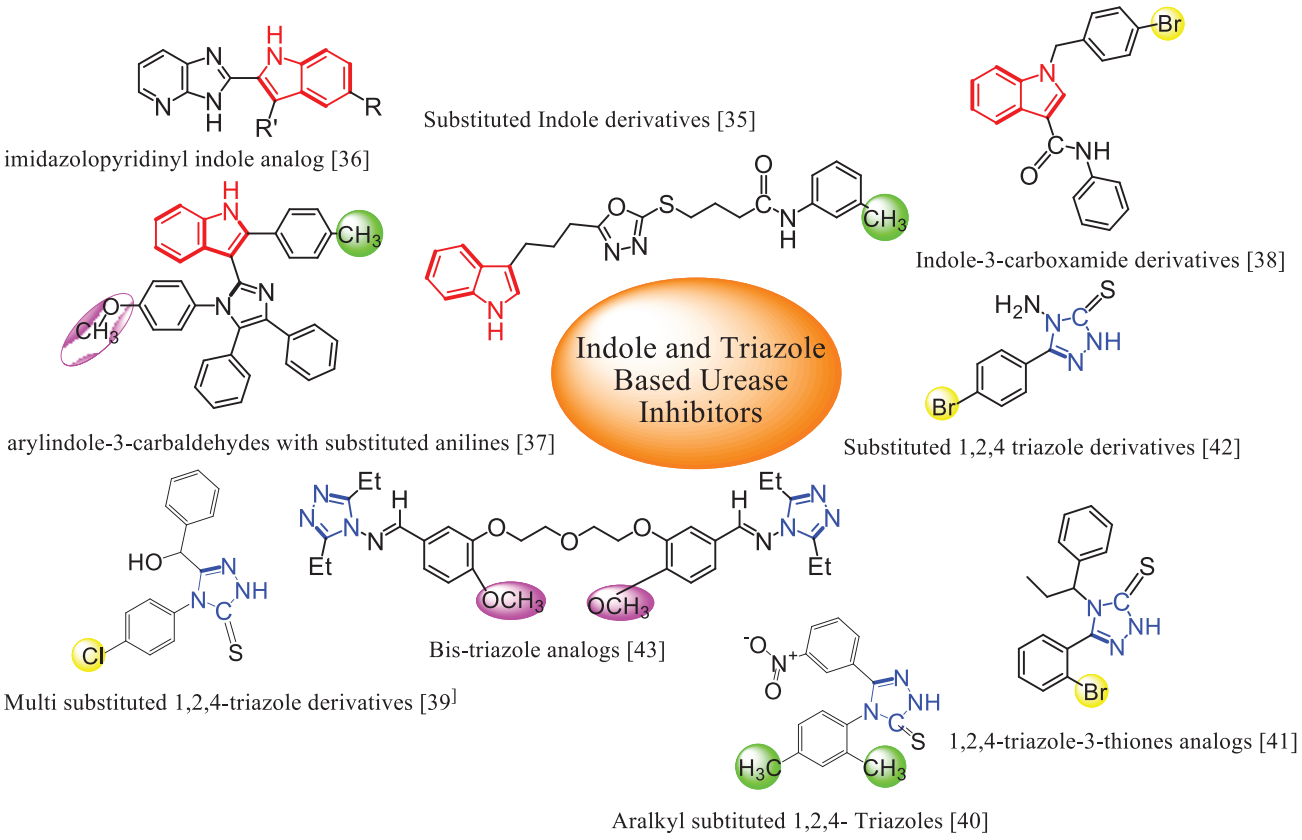


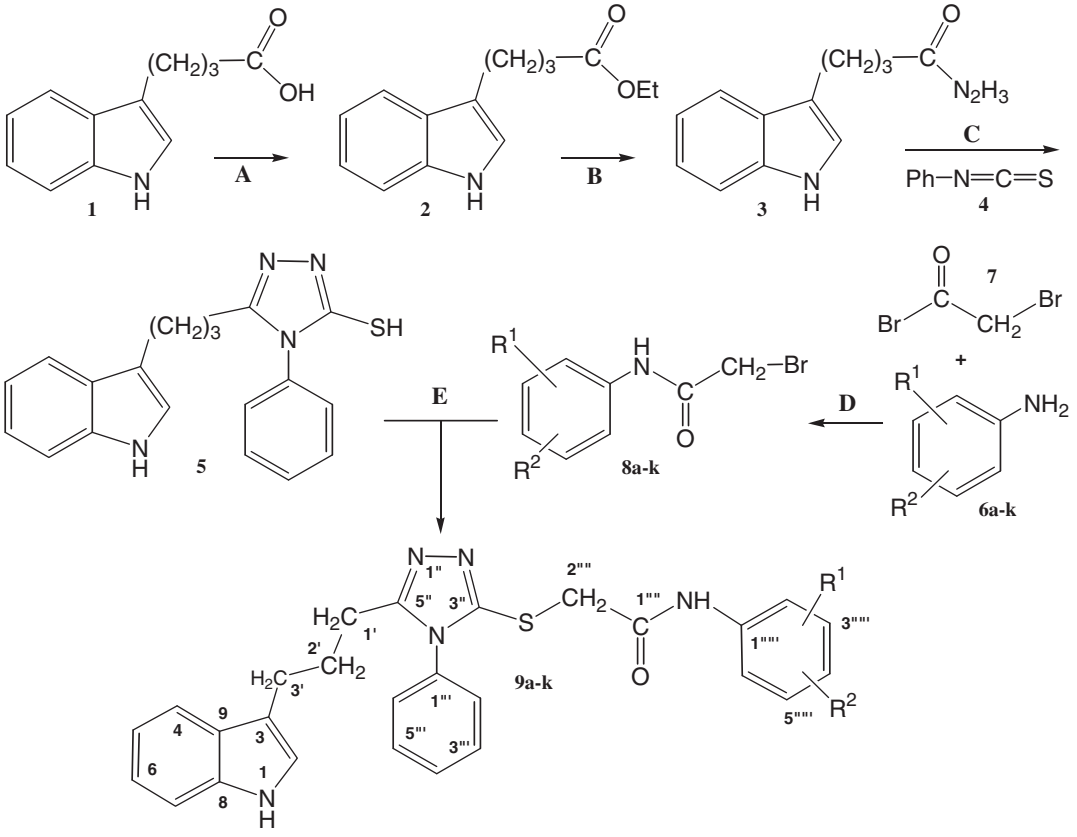
FIGURE 1 Rationale of current study

pharmacological and medicinal properties like anti-inflammatory, anti-tumor, anti-tubercular, anti-convulsant, antimicrobial, and anti-cancer activities.[8]

From the already reported literature,[34–42] the bioactivity of triazole and indole cores encouraged us to produce some hybrid compounds having both indole and 1,2,4-triazole moieties altogether (Figure 1). Based on our preceding efforts, in this current research, the designed bi-heterocyclic molecules were examined for inhibitory potential against urease enzyme in addition to assess the kinetic mechanism. Moreover, their molecular docking study was also done to find the interactions of synthesized compounds with the target enzyme.

2 | RESULTS AND DISCUSSION

The convergent synthesis of targeted bi-heterocyclic hybrids converged with an amide moiety was carried out in ample yields through a number of steps. In first step, the acid (**1**) was esterified by using EtOH and conc. H_2SO_4 (catalytic amount). Here, EtOH was utilized both as reactant and as solvent to avoid reversible reaction. Weak base with excess of water was added in workup followed by solvent extraction. The purpose of adding base here was to neutralize the unreacted amount of conc. H_2SO_4 and acid (**1**). The resulting ester, ethyl 4-(1*H*-indol-3-yl)butanoate (**2**) moved to organic layer and remaining salts of acids partitioned to aqueous layer in solvent extraction. Ester **2** obtained was a brownish liquid in appearance. Next step was the conversion of this ester into respective hydrazide **3**, which was done by refluxing it with hydrazine hydrate in methanol for 14 hours. The hydrazide, 4-(1*H*-indol-3-yl)butanohydrazide (**3**) was recovered as light brown colored solid. The third step involved the cyclization into 5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazole-3-thiol (**5**) by treating hydrazide **3** with phenyl isothiocyanate (**4**). The triazole (**5**) was having —SH group at C-3 position, which was a lone nucleophilic site for attachment with electrophiles. In a sidewise set of reactions, different electrophiles, **8a-k**, were synthesized by reacting substituted/unsubstituted anilines (**6a-k**) with 2-bromoethanoyl bromide (**7**). In the final step, the nucleophilic thiol (**5**) was amalgamated with different acetamides (**8a-k**) using LiH as catalyst in polar aprotic medium, resulting in the formation of designed derivatives, **9a-k**, as illustrated in Scheme 1 and Table 1.



SCHEME 1 Outline for the synthesis of **9a-k**. Reagents and conditions: (A) EtOH/H_2SO_4/8 hours reflux. (B) MeOH/$N_2H_4{\cdot}H_2O$/14 hours reflux. (C) MeOH/**4**/16 hours reflux/dissolved precipitate in 10% NaOH followed by filtration and acidification of filtrate in cold state. (D) Aq. Na_2CO_3 solution/pH 9-10/vigorous shaking for 20-30 minutes at RT. (E) DMF/LiH/60-70 hours stirring

TABLE 1 Different groups (—R_1 and —R_2) in Scheme 1

Compound	—R_1	—R_2
6a, **8a**, **9a**	—H	—H
6b, **8b**, **9b**	2-CH_3	—H
6c, **8c**, **9c**	—H	4-C_2H_5
6d, **8d**, **9d**	—H	4-OC_2H_5
6e, **8e**, **9e**	2-CH_3	3-CH_3
6f, **8f**, **9f**	2-CH_3	4-CH_3
6g, **8g**, **9g**	2-CH_3	5-CH_3
6h, **8h**, **9h**	2-CH_3	6-CH_3
6i, **8i**, **9i**	3-CH_3	4-CH_3
6j, **8j**, **9j**	3-CH_3	5-CH_3
6k, **8k**, **9k**	2-C_2H_5	6-CH_3

2.1 | Urease inhibitory activity

Urease enzyme was used to test the inhibitory potential of the synthesized compounds **(9a-k)** and it was evident from the resultant IC_{50} (μM) values that all the synthesized compounds were having remarkable inhibitory potential against urease enzyme relative to that of thiourea (standard) (Table 2). The IC_{50} values of compounds **9a-k** were lower than the standard thiourea ($IC_{50} = 4.721 \pm 1.374$ μM). Keeping in view the inhibitory effects of these compounds **(9a-k)** with different alkyl (R) groups on them, a confined structure-activity relationship (SAR) was streamlined.

2.2 | Structure-activity relationship

Compound **9a** ($IC_{50} = 1.448 \pm 0.992$ μM) with unsubstituted phenyl group depicted remarkable inhibiton comparatively to that of thiourea ($IC_{50} = 4.721 \pm 1.374$ μM). Compound **9b** ($IC_{50} = 2.272 \pm 0.997$ μM) is the only single methyl-substituted compound in the series. It showed higher IC_{50} value as compared to unsubstituted **9a**, owing to the positive inductive effect of electron-donating methyl group substituted at *ortho* position on the ring, however the enzyme inhibitory effect of **9b** was lower than the standard used (Figure 2).

A significant increase in the inhibition ($IC_{50} = 0.139 \pm 0.006$ μM) was observed in compound **9c**, when an ethyl group was present at *para* position of the aromatic ring. It can be explained by the fact that ethyl group is more electron donating as compared to methyl group (in **9b**), therefore the molecule with an ethyl group will interact with urease enzyme more competently than that having a methyl group. Compound **9d** ($IC_{50} = 0.594 \pm 0.743$ μM) also showed potent inhibitory activity due to presence of relatively bulky but polar ethoxy group at *para*-position in the aromatic ring (Figure 3).

While discussing dimethl substituted compounds, compound **9i** ($IC_{50} = 0.029 \pm 0.028$ μM) was the most potent compound of this series with one methyl at *meta* and other at *para* position of the ring. However, when these dimethyl substituents were repositioned at different *ortho*, *meta* and *para* positions on the ring, a slight change was observed in inhibitory activities of those compounds as **9e** ($IC_{50} = 0.667 \pm 0.099$ μM), **9f** ($IC_{50} = 0.158 \pm 0.215$ μM), **9g** ($IC_{50} = 0.246 \pm 0.113$ μM), **9h** ($IC_{50} = 0.224 \pm 0.072$ μM) and **9j** ($IC_{50} = 0.378 \pm 0.224$ μM) (Figures 4 and 5).

Compound **9k** ($IC_{50} = 0.497 \pm 0.049$ μM) with one ethyl group and one methyl group at two *ortho* positions on the ring also showed efficient inhibitory effect due to presence of two electron donating groups (Figure 6). The activity of this molecule was also closely analogous to those of some other di-substituted molecules like **9g**, **9h**, and **9j**.

2.3 | Hemolytic activity

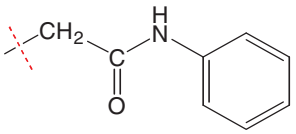
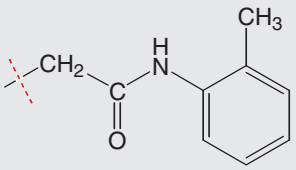
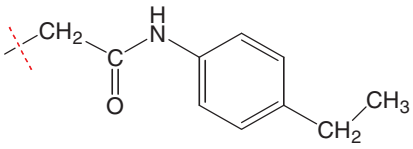
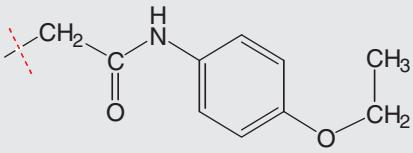
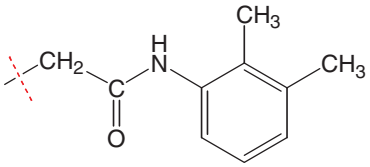
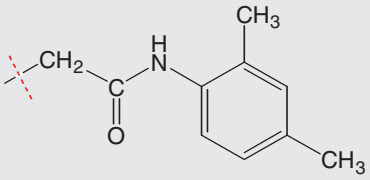
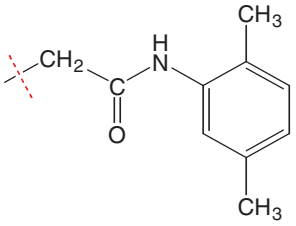
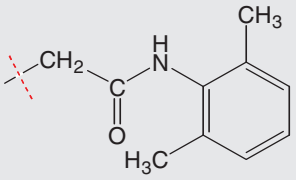
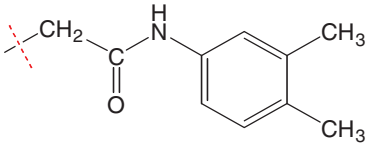
To determine the cytotoxicity of the synthesized derivatives, **9a-k**, these were subjected to hemolytic assay. Percentage hemolysis (%) results are given in Table 2. According to the results, almost all the derivatives of this series showed mild cytotoxicity towards RBC plasma membrane. Compound **9d** (23.52%) showed maximum membrane cytotoxicity while **9f** (1.63%) gave minimum membrane toxicity as compared to the standard Triton-X (89%). Rest of the compounds depicted relatively low cytotoxicity values of **9a** (4.93%), **9b** (4.41%), **9c** (3.54%), **9e** (3.24%), **9g** (4.45%), **9h** (2.45%), **9i** (5.31%), **9j** (2.48%), and **9k** (3.02%).

2.4 | Kinetic analysis

Based on the IC_{50} results of synthesized compounds, it was pertinent to select the most potent compound **9i** to find its inhibition type and constant on Jack bean urease. The inhibition potential of this compound was checked in terms of enzyme substrate inhibitor and enzyme inhibitor constants. A number of straight lines graphs were obtained showing kinetic studies of urease enzyme (Lineweaver Burk $1/[S]$ vs $1/V$) (Figure 7A).

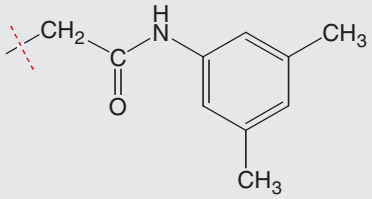
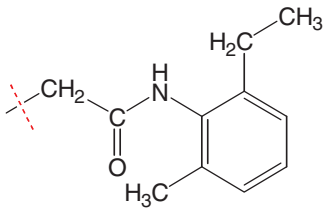
According to results, **9i** intersected in the second quadrant, K_m (constant) remained same while in V_{max} (reaction velocity) a decrease was noticed with increasing dose of **9i**, showing non-competitive behavior of the said compound. K_i (EI dissociation constant) is shown in second plot of slope vs **9i** concentration (Table 3).

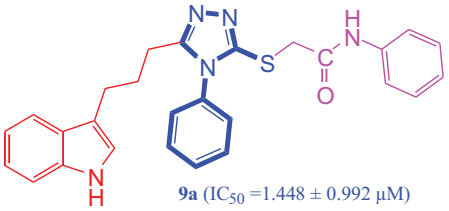
TABLE 2 Free radical scavenging, urease inhibition activity and hemolytic activity of manufactured derivatives (**9a-k**). Mean ± SEM, n = 3

Compound	—R	Free radical % scavenging (100 µg/mL)	Urease activity, IC_{50} (µM) ± SEM	Hemolysis (%) (mean ± SEM)
9a		15.765 ± 0.854	1.448 ± 0.992	4.93 ± 0.04
9b		3.163 ± 0.144	2.272 ± 0.997	4.41 ± 0.035
9c		8.467 ± 0.691	0.139 ± 0.006	3.54 ± 0.023
9d		4.567 ± 0.874	0.594 ± 0.743	23.52 ± 0.08
9e		3.602 ± 0.541	0.667 ± 0.099	3.24 ± 0.021
9f		6.665 ± 1.257	0.158 ± 0.215	1.63 ± 0.11
9g		6.787 ± 2.015	0.246 ± 0.113	4.45 ± 0.03
9h		5.135 ± 0.988	0.224 ± 0.072	2.45 ± 0.02
9i		28.374 ± 3.335	0.029 ± 0.028	5.31 ± 0.04

(Continues)

TABLE 2 (Continued)

Compound	—R	Free radical % scavenging (100 µg/mL)	Urease activity, IC_{50} (µM) ± SEM	Hemolysis (%) (mean ± SEM)
9j		4.142 ± 0.886	0.378 ± 0.224	2.48 ± 0.019
9k		2.623 ± 0.774	0.497 ± 0.049	3.02 ± 0.02
Standards		Vitamin C: 95.562 ± 7.40	Thiourea: 4.721 ± 1.374	Triton X: 89.00 ± 0.67; PBS hemolysis: (%) = 2.93 ± 0.018



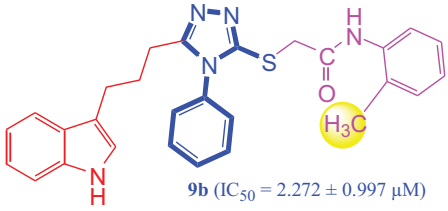
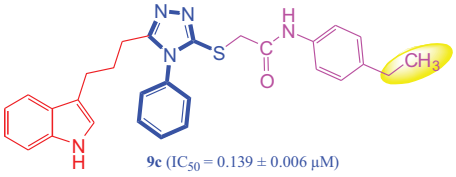


FIGURE 2 Struture-activity relationship of compounds **9a** and **9b**



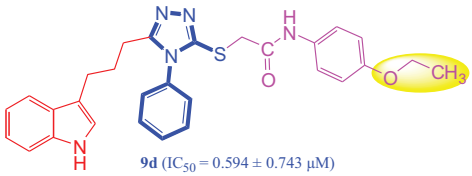


FIGURE 3 Struture-activity relationship of compounds **9c** and **9d**

2.5 | Free radical scavenging

Synthesized bi-heterocyclic amides (**9a-k**) were assessed for 2,2-diphenyl-1-picrylhydrazyl free radical scavenging potential. Some derivatives did not show considerable scavenging potential even in increased concentrations (100 µg/mL) (Table 2). Maximum radical scavenging potential was showed by compound **9i** (IC_{50} = 28.374 ± 3.335 µg/mL) while minimum scavenging potential was showed by compound **9k** (IC_{50} = 2.623 ± 0.774 µg/mL) against standard ascorbic acid (Vitamin C) (IC_{50} = 95.562 ± 7.40 µg/mL). However, low values of scavenging potential was seen for compound **9a** (IC_{50} = 15.765 ± 0.854 µg/mL), **9b** (IC_{50} = 3.163 ± 0.144 µg/mL), **9c** (IC_{50} = 8.467 ± 0.691 µg/mL), **9d** (IC_{50} = 4.567 ± 0.874 µg/mL), **9e** (IC_{50} = 3.602 ± 0.541 µg/mL), **9f** (IC_{50} = 6.665 ± 1.257 µg/mL), **9g** (IC_{50} = 6.787 ± 2.015 µg/mL), **9h** (IC_{50} = 5.135 ± 0.988 µg/mL), and **9j** (IC_{50} = 4.142 ± 0.886 µg/mL).

2.6 | Binding energy estimation of synthesized derivatives

All the synthesized compounds, **9a-k**, were docked to check their conformational arrangements, in relation to their bonding interaction pattern and minimum energy value (kcal/mol). Results showed that all the

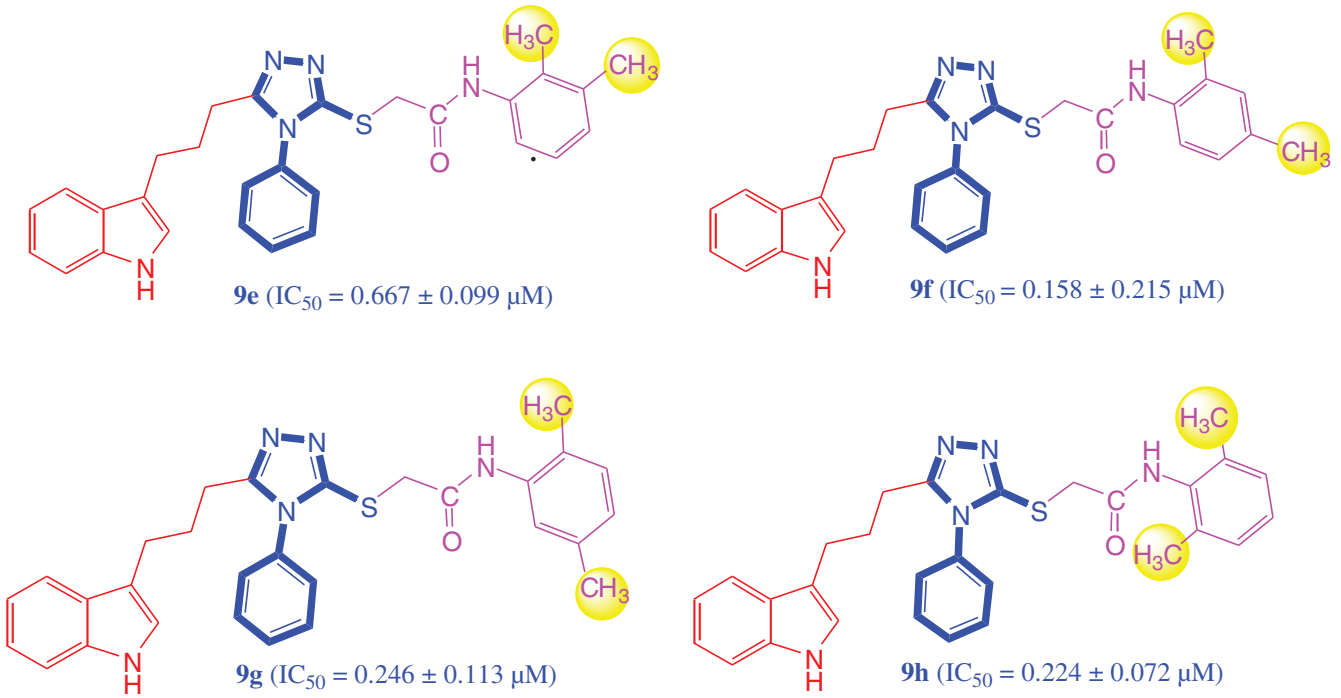


FIGURE 4 Struture-activity relationship of compounds **9e**, **9f**, **9g**, and **9h**

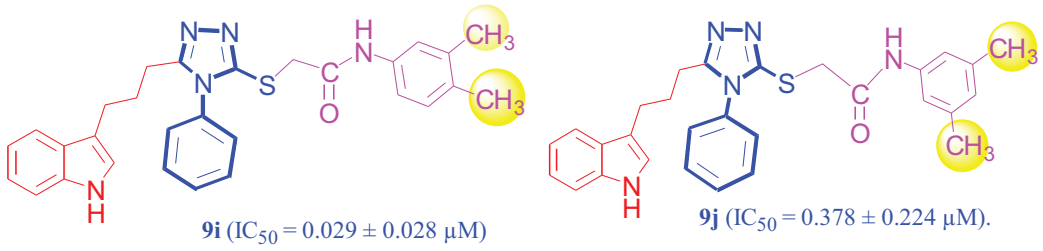


FIGURE 5 Struture-activity relationship of compounds **9i** and **9j**

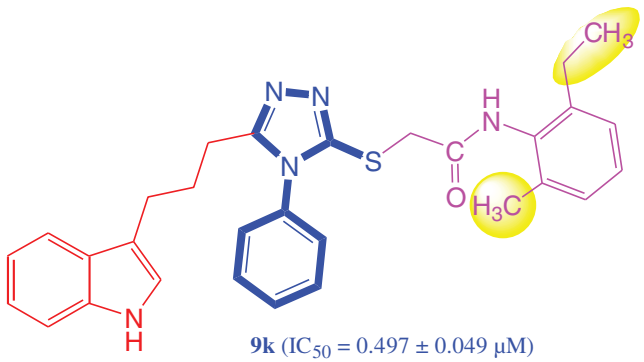


FIGURE 6 Struture-activity relationship of compound **9k**

compounds presented significant values of binding energy as compared to standard values (Figure 8). Following equation was used to evaluate energy values:

$$\Delta G_{\text{binding}} = \Delta G_{\text{Gauss}} + \Delta G_{\text{repulsion}} + \Delta G_{\text{hbond}} + \Delta G_{\text{hydrophobic}} + \Delta G_{\text{tors}}$$

where ΔG_{Gauss} is the scattering of two Gaussian functions, $\Delta G_{\text{repulsion}}$ is the square of distance (if closer than entrance value), ΔG_{hbond} is the used metal ions interactions, $\Delta G_{\text{hydrophobic}}$ is the ramp function, and ΔG_{tors} is the relative to the no. of rotatable bonds.

2.7 | Binding pocket evaluation of urease docked compounds

Based on in vitro and in silico results, **9i** complex was further assessed for binding interactions. Although all the compounds showed different conformational arrangements with active site of binding receptor but most excellent

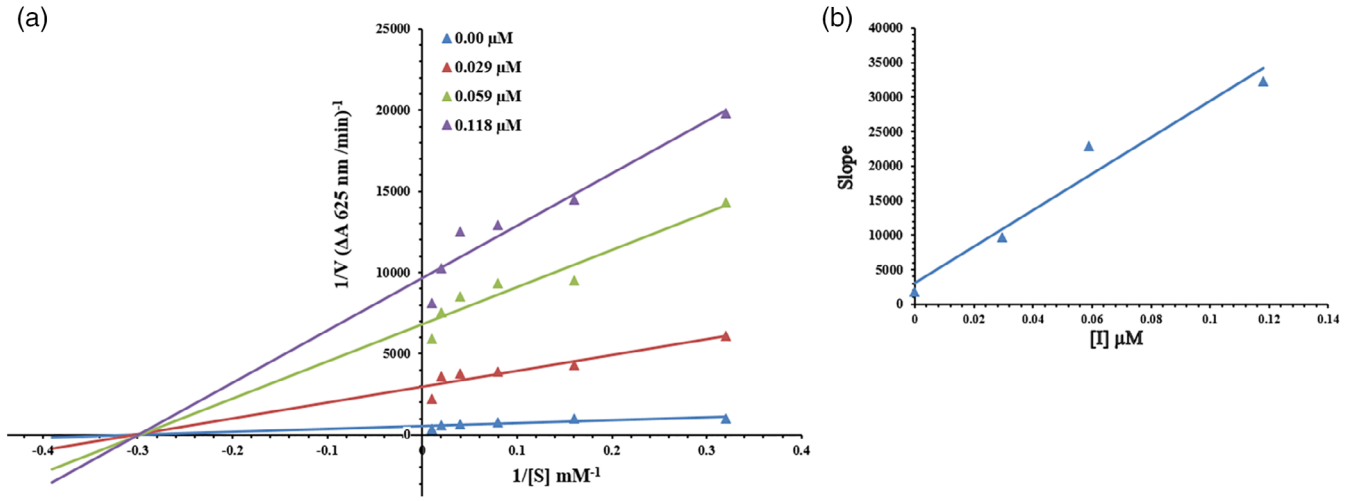


FIGURE 7 Lineweaver-Burk plots for urease inhibition using **9i**. (**A**) Concentrations of **9i** were 0.00, 0.029, 0.059, and 0.0118 µM. (**B**) The intersect shows the plot of slope or the vertical against **9i** concentrations

Dose (µM)	V_{max} (Δ*A*/Min)	K_m (mM)	Inhibition type	K_i (µM)
0.00	0.00286	3.333	Non-competitive	—
0.029	0.0004512	3.333		0.012
0.059	0.000169	3.333		—
0.118	0.000123	3.333		—

TABLE 3 Kinetic parameters of Jack bean urease with some different concentrations of **9i**

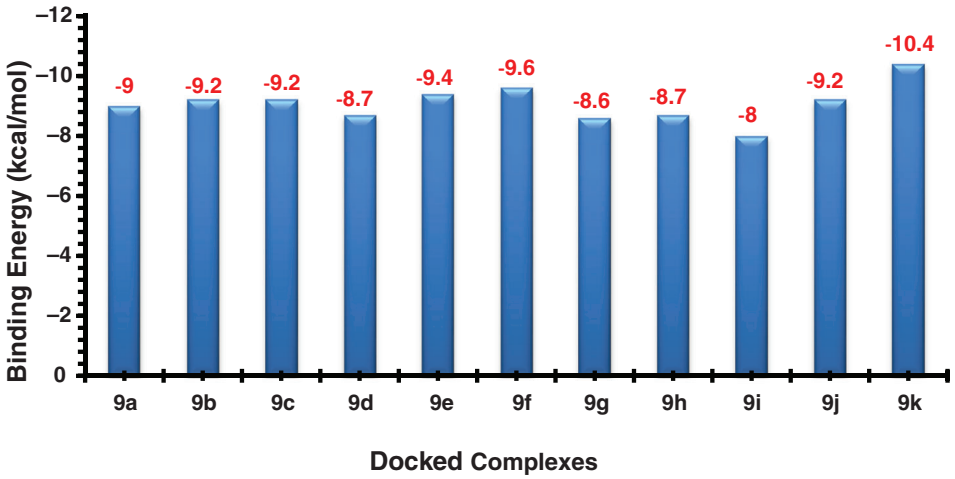


FIGURE 8 Binding energy of derivatives **9a-k**

energy value of docked compound **9i** is given in Figure 9. Rest of docked compounds is mentioned in Figures S23-S32. In SAR analysis, three hydrogen and two hydrophobic connections were studied in **9i** docked compounds. Amino group of carbonyl pentene ring forms H-interactions with Ala636 containing bond distances 2.84 and 3.19 Å. Moreover, another amino group of carbonyl interacts to Gln635 having bond length 2.71 Å. The methoxy benzene form couple of hydrophobic interactions against Pro434 and Ile411 having bond distances 4.89 and 4.95 Å, respectively. Already reported literature on significance of these residues supported our docked results.[43–47]

3 | EXPERIMENTAL

We purchased all the chemicals from Alfa Aesar and Sigma Aldrich (Germany). Solvents bought from local

dealers were of analytical grades. Open capillary tube method was implemented to find the melting points on Griffin and George apparatus. TLC (mobile phase: EtOAc and *n*-hexane in 30:70 ratio) was used to detect initial purity of synthesized compounds at 254 nm. KBr pellet method and Jasco-320-A spectrometer were used to record IR peaks. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) signals were recorded in DMSO-d_6 using Bruker spectrometers.

Ethyl 4-(1*H*-indol-3-yl) butanoate (2). Carboxylic acid (**1**, 0.2 mol) was dissolved in EtOH (70 mL) in 500 mL round bottom flask and conc. H_2SO_4 (20 mL) was added in catalytic amount. It was refluxed for around 8 hours under the supervision of TLC to check maximum reaction completion. Ten percent aq. Na_2CO_3 (40 mL) was added in workup for neutralization of reaction mixture followed by solvent extraction with CH_3Cl (50 mL × 3). Ester (**2**) formed was recovered from organic solvent layer as reddish brown liquid. Yield: 98%.[34,48]

4-(1*H*-indol-3-yl)butanohydrazide (3). Ester (**2**, 0.15 mol) was dissolved in 60 mL CH_3OH and $N_2H_4{\cdot}H_2O$ (25 mL, 80%) in round bottom flask (500 mL). After 14 hours stirring at room temperature, acid hydrazide **3** was formed. Workup was done by filtration of precipitate followed by washing with cold *n*-hexane and air drying. Yield: 80%.[34,48]

5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazole-3-thiol (5). Hydrazide (**3**, 0.13 mol) was mixed with KOH (0.13 mol) and EtOH (30 mL) in flask (500 mL RB), after that phenyl isothiocyanate (**4**) was added. Refluxing the reaction mixture for 16 hours resulted in the formation of uncyclized product, which was further cyclized by adding excess of chilled water with dilute HCl to adjust pH at 5 to 6. The resulting precipitate was washed after filtration and air dried to get final cyclized product **5**. Yield: 70%; ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.75 (1H, s, NH-1), 7.52 (3H, m, H-3‴, H-4‴ & H-5‴), 7.36 (3H, dist.d, J = 8.8 Hz, H-2‴, H-6‴ & H-7), 7.04 (1H, br.t, J = 9.1 Hz, H-6), 6.97 (1H, s, H-2), 6.93 (1H, br.t, J = 8.9 Hz, H-5), 2.64 (2H, t, J = 8.7 Hz, CH_2-3′), 2.47 (2H, t, J = 8.8 Hz, CH_2-1′), 1.84 (2H, quintet, J = 8.7 Hz, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 168.10 (C-3″), 152.63 (C-5″), 136.75 (C-8), 134.26 (C-1‴), 129.82 (C-3‴, C-4‴ & C-5‴), 128.65 (C-2‴ & C-6‴), 127.48 (C-9), 122.69 (C-2), 121.30 (C-6), 118.63 (C-7), 118.58 (C-5), 113.87 (C-3), 111.75 (C-4), 26.48 (C-2′), 25.45 (C-1′), 24.28 (C-3′).

2-Bromo-*N*-(un/substituted-phenyl)acetamides (8a-k). Various un/substituted anilines (**6a-k**) were reacted with 2-bromoacetyl bromide (**7**) in equimolar amount (0.001 mol) and manual shaking in sodium carbonate 10% solution. After 20 to 30 minutes precipitates will start forming, filtration followed by washing using cold water will result in separation of desired electrophiles (**8a-k**).[48]

2-({5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)-*N*(un/substituted-phenyl) acetamides (9a-k). Triazole (**5**, 0.2 g), DMF (5 mL) and pinch of catalyst LiH were stirred for half hour in 250 mL RB (room temperature). Equimolar concentration of different electrophiles **(8a-k)** was mixed in reaction mixture with 60 to 70 hours stirring. TLC was used for monitoring of reaction completion. After that reaction contents were poured in 100 mL chilled water and precipitates of derivatives (**9a-k**) were filtered or extracted by solvent according to the physical state of product formed.

2-({5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)-*N*-phenyl acetamide (9a). Light brown sticky liquid; Yield: 72%; Mol. Formula: $C_{27}H_{25}N_5SO$; Mol. Weight: 467 g/mol; IR (KBr, cm^{-1}): υ 3310 (N—H str.), 2955 (C—H aromatic str.), 1654 (C═O str.), 1595 (C═C aromatic str.), 1525, 1481, 1445 (str. for

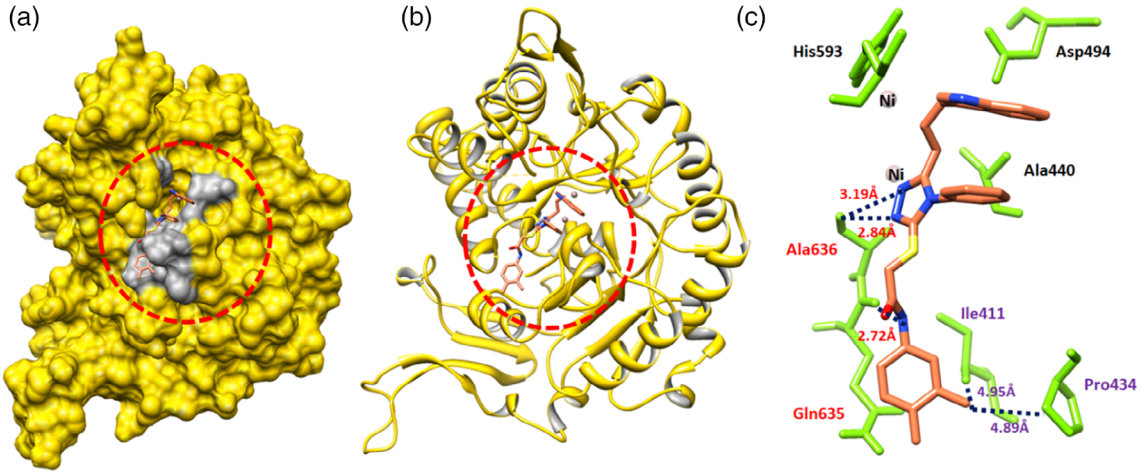


FIGURE 9 Binding pockets of urease with active binding position of **9i** docked compound

triazole), 1151 (C—O—C str.), 1100 (C═N str.), 685 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.71 (1H, s, NH-1), 10.34 (1H, s, CONH), 7.57-7.53 (5H, m, H-3‴, H-4‴, H-5‴, H-2⁗ & H-6⁗), 7.41 (2H, br.dd, J = 1.2, 7.6 Hz, H-2‴ & H-6‴), 7.36 (1H, br.d, J = 7.8 Hz, H-7), 7.32-7.29 (3H, m, H-4,H-3⁗ & H-5⁗), 7.07-7.02 (1H, m, H-6 & H-4⁗), 6.97 (1H, dist.d, J = 2.04 Hz, H-2), 6.92 (1H, br.dt, J = 0.7, 7.8 Hz, H-5), 4.09 (2H, s, CH_2-2⁗), 2.64 (2H, t, J = 7.4 Hz, CH_2-3′), 2.59 (2H, t, J = 7.5 Hz, CH_2-1′), 1.89 (2H, quintet, J = 7.3 Hz, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 165.56 (C-1⁗), 155.57 (C-5″), 149.38 (C-3″), 138.72 (C-1⁗), 136.21 (C-8), 132.96 (C-1‴), 129.93 (C-4‴), 129.90 (C-3‴ & C-5‴), 128.75 (C-3⁗ & C-5⁗), 127.14 (C-2‴ & C-6‴), 126.99 (C-9), 123.48 (C-4⁗), 122.17 (C-2), 120.77 (C-6), 119.07 (C-2⁗ & C-6⁗), 118.18 (C-7), 118.06 (C-5), 113.50 (C-3), 111.26 (C-4), 36.96 (C-2⁗), 26.93 (C-2′), 24.27 (C-1′), 23.91 (C-3′); Anal. Calc. for $C_{27}H_{25}N_5SO$ (467.18): C, 69.35; H, 5.39; N, 14.98. Found: C, 69.31; H, 5.36; N, 14.91. EI-MS *m/z*: 467, 334, 324, 190, 158, 143, 130, 120, 91, 78.

2-({5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)-*N*-(2 methylphenyl) acetamide (9b). Light brown sticky liquid; Yield: 91%; Mol. Formula: $C_{28}H_{27}N_5SO$; Mol. Weight: 481 g/mol; IR (KBr, cm^{-1}): υ 3286 (N—H str.), 2945 (C—H aromatic str.), 1652 (C═O str.), 1599 (C═C aromatic str.), 1517, 1478, 1441 (str. for triazole), 1159 (C—O—C str.), 1115 (C═N str.), 689 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.72 (1H, s, NH-1), 9.71 (1H, s, CONH), 7.55-7.54 (1H, m, H-3‴, H-4‴ & H-5‴), 7.46 (1H, br.d, J = 7.7 Hz, H-6⁗), 7.41 (2H, dist.d, J = 5.9 Hz, H-2‴ & H-6‴), 7.37 (1H, br.d, J = 7.7 Hz, H-7), 7.31 (1H, br.d, J = 7.8 Hz, H-4), 7.19 (1H, br.d, J = 7.5 Hz, H-3⁗), 7.14 (1H, br.t, J = 7.4 Hz, H-5⁗), 7.08-7.03 (2H, m, H-6, & H-4⁗), 6.97 (1H, s, H-2), 6.93 (1H, br.t, J = 7.2 Hz, H-5), 4.12 (2H, s, CH_2-2⁗), 2.65 (2H, t, J = 7.2 Hz, CH_2-3′), 2.60 (2H, t, J = 7.2 Hz, CH_2-1′), 2.19 (3H, s, CH_3-2⁗), 1.88 (2H, quintet, J = 7.1 Hz, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 165.83 (C-1⁗), 155.61 (C-5″), 149.52 (C-3″), 136.24 (C-8), 135.98 (C-1⁗), 132.97 (C-1‴), 131.13 (C-2⁗), 130.28 (C-3⁗), 129.91 (C-4‴), 129.86 (C-3‴ & C-5‴), 127.14 (C-2‴ & C-6‴), 127.01 (C-9), 125.94 (C-6⁗), 125.14 (C-4⁗), 124.24 (C-5⁗), 122.19 (C-2), 120.78 (C-6), 118.19 (C-7), 118.06 (C-5), 113.51 (C-3), 111.26 (C-4), 36.43 (C-2⁗), 26.98 (C-2′), 24.29 (C-1′), 23.92 (C-3′), 17.72 (CH_3-2⁗); Anal. Calc. for $C_{28}H_{27}N_5SO$ (481.19): C, 69.83; H, 5.65; N, 14.54. Found: C, 69.79; H, 5.60; N, 14.48. EI-MS (*m/z*): 481, 338, 334, 190, 158, 143, 130, 120, 91, 78.

***N*-(4-Ethylphenyl)-2-({5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9c)**. Dark brown sticky liquid; Yield: 86%; Mol. Formula: $C_{29}H_{29}N_5SO$; Mol. Weight: 495 g/mol; IR (KBr, cm^{-1}): υ 3298 (N—H str.), 2961 (C—H aromatic str.), 1653 (C═O str.), 1581 (C═C aromatic str.), 1527, 1483, 1441 (str. for triazole), 1157 (C—O—C str.), 1121 (C═N str.), 689 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.74 (1H, s, NH-1), 10.29 (1H, s, CONH), 7.54-7.51 (3H, m, H-3‴, H-4‴ & H-5‴), 7.48 (2H, br.d, J = 7.5 Hz, H-2⁗ & H-6⁗), 7.41 (2H, dist.d, J = 6.9 Hz, H-2‴ & H-6‴), 7.38 (1H, br.d, J = 7.5 Hz, H-7), 7.31 (1H, br.d, J = 7.9 Hz, H-4), 7.14 (2H, br.d, J = 7.6 Hz, H-3⁗ & H-5⁗), 7.04 (1H, br.t, J = 7.5 Hz, H-6), 6.97 (1H, s, H-2), 6.93 (1H, br.t, J = 7.4 Hz, H-5), 4.10 (2H, s, CH_2-2⁗), 2.64 (2H, t, J = 7.1 Hz, CH_2-3′), 2.60 (2H, t, J = 7.2 Hz, CH_2-1′), 2.54 (2H, q, J = 7.3 Hz, CH_3-$\underline{CH_2}$-4⁗), 1.14 (3H, t, J = 7.2 Hz, $\underline{CH_3}CH_2$-4⁗), 1.90-1.86 (2H, m, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 165.32 (C-1⁗), 155.56 (C-5″), 149.43 (C-3″), 138.91 (C-1⁗), 136.43 (C-4⁗), 136.24 (C-8), 132.97 (C-1‴), 129.88 (C-4‴), 129.84 (C-3‴ & C-5‴), 127.91 (C-3⁗ & C-5⁗), 127.13 (C-2‴ & C-6‴), 127.01 (C-9), 122.17 (C-2), 120.78 (C-6), 119.18 (C-2⁗ & C-6⁗), 118.19 (C-7), 118.06 (C-5), 113.52 (C-3), 111.26 (C-4), 36.97 (C-2⁗), 27.56 ($CH_3\underline{CH_2}$-4⁗), 26.95 (C-2′), 24.29 (C-1′), 23.93 (C-3′), 15.62 ($\underline{CH_3}CH_2$-4⁗); Anal. Calc. for $C_{29}H_{29}N_5SO$ (495.21): C, 70.27; H, 5.90; N, 14.13. Found: C, 70.21; H, 5.86; N, 14.08. EI-MS (*m/z*): 495, 352, 334, 190, 158, 143, 130, 120, 91, 78.

***N*-(4-Ethoxyphenyl)-2-({5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9d)**. Brown sticky liquid; Yield: 82%; Mol. Formula: $C_{29}H_{29}N_5SO_2$; Mol. Weight: 511 g/mol; IR (KBr, cm^{-1}): υ 3292 (N—H str.), 2949 (C—H aromatic str.), 1666 (C═O str.), 1599 (C═C aromatic str.), 1528, 1487, 1449 (str. for triazole), 1159 (C—O—C str.), 1109 (C═N str.), 688 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.72 (1H, s, NH-1), 10.18 (1H, s, CONH), 7.57-7.51 (3H, m, H-3‴, H-4‴ & H-5‴), 7.45 (2H, br.d, J = 8.8 Hz, H-2⁗ & H-6⁗), 7.41 (2H, dist.d, J = 6.4 Hz, H-2‴ & H-6‴), 7.36 (1H, br.d, J = 7.8 Hz, H-7), 7.30 (1H, br.d, J = 8.0 Hz, H-4), 7.04 (1H, br.t, J = 7.5 Hz, H-6), 6.97 (1H, s, H-2), 6.92 (1H, br.t, J = 7.4 Hz, H-5), 6.86 (2H, br.d, J = 8.8 Hz, H-3⁗ & H-5⁗), 4.06 (2H, s, CH_2-2⁗), 3.96 (2H, q, J = 6.9 Hz, $CH_3\underline{CH_2}$O-4⁗), 2.64 (2H, t, J = 7.3 Hz, CH_2-3′), 2.59 (2H, t, J = 7.3 Hz, CH_2-1′), 1.88 (2H, quintet, J = 7.3 Hz, CH_2-2′), 1.29 (3H, t, J = 6.9 Hz, $\underline{CH_3}CH_2O$-4⁗). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 164.99 (C-1⁗), 155.53 (C-4⁗), 154.60 (C-5″), 149.41 (C-3″), 136.23 (C-8), 132.99 (C-1‴), 131.79 (C-1⁗), 129.88 (C-4‴), 129.84 (C-3‴ & C-5‴), 127.16 (C-2‴ & C-6‴), 127.00 (C-9), 122.18 (C-2), 120.77 (C-6), 120.61 (C-2⁗ & C-6⁗), 118.19 (C-7), 118.05 (C-5), 114.39 (C-3⁗ & C-5⁗), 113.51 (C-3), 111.25 (C-4), 63.04 ($CH_3\underline{CH_2}$O-4⁗), 36.91 (C-2⁗), 26.95 (C-2′), 24.30 (C-1′), 23.93 (C-3′), 14.63 ($\underline{CH_3}CH_2O$-4⁗); Anal. Calc. for $C_{29}H_{29}N_5SO_2$ (511.20): C, 68.08; H, 5.71; N, 13.69. Found: C, 68.05; H,

5.66; N, 13.66. EI-MS (*m/z*): 511, 369, 368, 353, 334, 190, 158, 143, 130, 91, 78.

***N*-(2,3-Dimethylphenyl)-2-({5-[3-(1*H*-indol-3-yl) propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9e)**. Brown sticky liquid; Yield: 78%; Mol. Formula: $C_{29}H_{29}N_5SO$; Mol. Weight: 495 g/mol; IR (KBr, cm^{-1}): υ 3296 (N—H str.), 2958 (C—H aromatic str.), 1652 (C═O str.), 1591 (C═C aromatic str.), 1531, 1489, 1444 (str. for triazole), 1155 (C—O—C str.), 1122 (C═N str.), 687 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.75 (1H, s, NH-1), 9.66 (1H, s, CONH), 7.56-7.55 (3H, m, H-3‴, H-4‴ & H-5‴), 7.42 (2H, dist.d, J = 5.8 Hz, H-2‴ & H-6‴), 7.38 (1H, br.d, J = 7.7 Hz, H-7), 7.32 (2H, br. t, J = 8.28 Hz, H-4 & H-6⁗), 7.05 (1H, br.t, J = 7.4 Hz, H-6), 6.99 (1H, s, H-2), 6.96-6.93 (3H, m, H-5,H-4⁗ & H-5⁗), 4.11 (2H, s, CH_2-2⁗), 2.67 (2H, t, J = 7.2 Hz, CH_2-3′), 2.61 (2H, t, J = 7.3 Hz, CH_2-1′), 2.23 (3H, s, CH_3-3⁗), 2.16 (3H, s, CH_3-2⁗), 1.90 (2H, quintet, J = 7.3 Hz, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 166.24 (C-1⁗), 156.10 (C-5″), 150.03 (C-3″), 136.76 (C-8), 134.75 (C-3⁗), 133.90 (C-1⁗), 133.49 (C-1‴), 131.65 (C-2⁗), 131.24 (C-4⁗), 130.39 (C-4‴), 130.34 (C-3‴ & C-5‴), 127.58 (C-2‴ & C-6‴), 127.53 (C-9), 126.86 (C-5⁗), 124.84 (C-6⁗), 122.68 (C-2), 121.27 (C-6), 118.69 (C-7), 118.56 (C-5), 114.04 (C-3), 111.77 (C-4), 36.95 (C-2⁗), 27.49 (C-2′), 24.80 (C-1′), 24.42 (C-3′), 20.91 (CH_3-3⁗), 18.13 (CH_3-2⁗); Anal. Calc. for $C_{29}H_{29}N_5SO$ (495.21): C, 70.27; H, 5.90; N, 14.13. Found: C, 70.22; H, 5.85; N, 14.08. EI-MS (*m/z*): 495, 352, 334, 190, 158, 143, 130, 120, 91, 78.

***N*-(2,4-Dimethylphenyl)-2-({5-[3-(1*H*-indol-3-yl) propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9f)**. Brown sticky liquid; Yield: 71%; Mol. Formula: $C_{29}H_{29}N_5SO$; Mol. Weight: 495 g/mol; IR (KBr, cm^{-1}): υ 3274 (N—H str.), 2953 (C—H aromatic str.), 1703 (C═O str.), 1590 (C═C aromatic str.), 1531, 1489, 1452 (str. for triazole), 1159 (C—O—C str.), 1100 (C═N str.), 666 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.74 (1H, s, NH-1), 9.77 (1H, s, CONH), 7.55 (3H, br.s, H-3‴, H-4‴ & H-5‴), 7.42 (2H, br.s, H-2‴ & H-6‴), 7.37 (1H, dist.d, J = 5.3 Hz, H-7), 7.31 (1H, dist.d, J = 5.8 Hz, H-4), 7.16 (1H, br.s, H-6⁗), 7.03 (2H, br.s, H-6 & H-3⁗), 6.99 (1H, s, H-2), 6.98 (1H, s, H-5⁗), 6.93 (1H, s, H-5), 4.11 (2H, s, CH_2-2⁗), 2.65 (2H, s, CH_2-3′), 2.60 (2H, s, CH_2-1′), 2.22 (3H, s, CH_3-4⁗), 2.05 (3H, s, CH_3-7⁗), 1.89 (2H, s, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 165.82 (C-1⁗), 155.56 (C-5″), 149.49 (C-3″), 136.92 (C-1⁗), 136.24 (C-8), 135.72 (C-2⁗), 132.99 (C-1‴), 130.74 (C-4⁗), 129.89 (C-4‴), 129.85 (C-3‴ & 5‴), 127.15 (C-2‴ & 6‴), 127.01 (C-9), 126.93 (C-3⁗), 125.15 (C-5⁗), 122.96 (C-6⁗), 122.18 (C-2), 120.77 (C-6), 118.19 (C-7), 118.05 (C-5), 113.51 (C-3), 111.27 (C-4), 36.39 (C-2⁗), 26.97 (C-2′), 24.30 (C-1′), 23.93 (C-3′), 20.09 (CH_3-4⁗), 13.84 (CH_3-2⁗); Anal. Calc. for $C_{29}H_{29}N_5SO$ (495.21): C, 70.27; H, 5.90; N, 14.13. Found: C, 70.23; H, 5.85; N, 14.07. EI-MS (*m/z*): 495, 352, 334, 190, 158, 143, 130, 120, 91, 77.

***N*-(2,5-Dimethylphenyl)-2-({5-[3-(1*H*-indol-3-yl) propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9g)**. Brown sticky liquid; Yield: 93%; Mol. Formula: $C_{29}H_{29}N_5SO$; Mol. Weight: 495 g/mol; IR (KBr, cm^{-1}): υ 3317 (N—H str.), 2955 (C—H aromatic str.), 1654 (C═O str.), 1599 (C═C aromatic str.), 1535, 1484, 1449 (str. for triazole), 1155 (C—O—C str.), 1103 (C═N str.), 681 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.74 (1H, s, NH-1), 9.68 (1H, s, CONH), 7.56-7.53 (3H, m, H-3‴, H-4‴ & H-5‴), 7.44-7.41 (2H, m, H-2‴ & H-6‴), 7.37 (1H, br.d, J = 7.8 Hz, H-7), 7.31 (1H, br.d, J = 8.1 Hz, H-4), 7.29 (1H, s, H-6⁗), 7.07-7.03 (2H, m, H-6 & H-3⁗), 7.00 (1H, dist.d, J = 1.86 Hz, H-2), 6.89-6.86 (1H, m, H-4⁗), 6.93 (1H, br.t, J = 7.6 Hz, H-5), 4.12 (2H, s, H-2⁗), 2.75 (2H, t, J = 7.5 Hz, CH_2-3′), 2.61 (2H, t, J = 7.4 Hz, CH_2-1′), 2.22 (3H, s, CH_3-5⁗), 2.15 (3H, s, CH_3-2⁗), 1.91 (2H, s, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 165.77 (C-1⁗), 155.60 (C-5″), 149.57 (C-3″), 136.25 (C-8), 135.77 (C-1⁗), 134.95 (C-5⁗), 132.97 (C-1‴), 130.08 (C-3⁗), 129.90 (C-4‴), 129.86 (C-3‴ & 5‴), 128.99 (C-2⁗), 127.14 (C-2‴ & 6‴), 127.03 (C-9), 125.61 (C-4⁗), 125.61 (C-6⁗), 122.19 (C-2), 120.78 (C-6), 118.20 (C-7), 118.06 (C-5), 113.52 (C-3), 111.28 (C-4), 36.44 (C-2⁗), 26.99 (C-2′), 24.29 (C-1′), 23.93 (C-3′), 20.57 (CH_3-5⁗), 14.04 (CH_3-2⁗); Anal. Calc. for $C_{29}H_{29}N_5SO$ (495.21): C, 70.27; H, 5.90; N, 14.13. Found: C, 70.25; H, 5.86; N, 14.10. EI-MS (*m/z*): 495, 352, 334, 190, 158, 143, 130, 120, 91, 77.

***N*-(2,6-Dimethylphenyl)-2-({5-[3-(1*H*-indol-3-yl) propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9h)**. White colored amorphous powder; Yield: 79%; Melting Point 93°C; Mol. Formula: $C_{29}H_{29}N_5SO$; Mol. Weight: 495 g/mol; IR (KBr, cm^{-1}): υ 3286 (N—H str.), 2957 (C—H aromatic str.), 1656 (C═O str.), 1599 (C═C aromatic str.), 1527, 1481, 1441 (str. for triazole), 1141 (C–O–C str.), 1101 (C═N str.), 677 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, δ/ppm): 10.72 (1H, s, NH-1), 9.62 (1H, s, CONH), 7.56-7.55 (3H, m, H-3‴, H-4‴ & H-5‴), 7.42 (2H, m, H-2‴ & H-6‴), 7.37 (1H, br.d, J = 7.8 Hz, H-7), 7.31 (1H, br.d, J = 7.8 Hz, H-4), 7.03-7.02 (4H, m, H-6,H-3⁗, H-4⁗ & H-5⁗), 6.97 (1H, s, H-2), 6.93 (1H, br.t, J = 7.1 Hz, H-5), 4.12 (2H, s, CH_2-2⁗), 2.65 (2H, t, J = 7.2 Hz, CH_2-3′), 2.60 (2H, t, J = 7.2 Hz, CH_2-1′), 2.09 (6H, s, CH_3-2⁗ & CH_3-6⁗), 1.87 (2H, quintet, J = 7.2 Hz, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, δ/ppm): 165.34 (C-1⁗), 155.49 (C-5″), 149.38 (C-3″), 136.23 (C-8), 135.13 (C-2⁗ & C-6⁗), 134.64 (C-1⁗), 133.01 (C-1‴), 129.86 (C-4‴), 129.82 (C-3‴ & C-5‴), 127.59 (C-3⁗ & C-5⁗), 127.13 (C-2‴ & C-6⁗), 127.00

(C-9), 126.50 (C-4‴″), 122.18 (C-2), 120.78 (C-6), 118.19 (C-7), 118.05 (C-5), 113.51 (C-3), 111.26 (C-4), 35.84 (C-2‴″), 27.00 (C-2′), 24.28 (C-1″), 23.91 (C-3′), 17.95 (CH_3-2‴″ & CH_3-6‴″); Anal. Calc. for $C_{29}H_{29}N_5SO$ (495.21): C, 70.27; H, 5.90; N, 14.13. Found: C, 70.23; H, 5.85; N, 14.09. EI-MS (*m/z*): 495, 352, 334, 190, 158, 143, 130, 120, 91, 77.

***N*-(3,4-Dimethylphenyl)-2-({5-[3-(1*H*-indol-3-yl) propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9i)**. Brown colored sticky liquid; Yield: 69%; Mol. Formula: $C_{29}H_{29}N_5SO$; Mol. Weight: 495 g/mol; IR (KBr, cm^{-1}): *υ* 3293 (N—H str.), 2966 (C—H aromatic str.), 1642 (C═O str.), 1598 (C═C aromatic str.), 1529, 1488, 1447 (str. for triazole), 1153 (C—O—C str.), 1126 (C═N str.), 687 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, *δ*/ppm): 10.72 (1H, s, NH-1), 10.19 (1H, s, CONH), 7.55-7.51 (3H, m, H-3‴, H-4‴ & H-5‴), 7.41 (2H, br.d, *J* = 7.6 Hz, H-2‴ & H-6‴), 7.37 (1H, br.d, *J* = 7.6 Hz, H-7), 7.33 (1H, s, H-2‴″), 7.31 (1H, br.d, *J* = 8.0 Hz, H-4), 7.27 (1H, br.d, *J* = 7.9 Hz, H-6‴″), 7.04 (2H, dist.t, *J* = 7.6 Hz, H-6 & H-5‴″), 6.99 (1H, s, H-2), 6.92 (1H, br.t, *J* = 7.5 Hz, H-5), 4.07 (2H, s, CH_2-2‴″), 2.66 (2H, t, *J* = 7.3 Hz, CH_2-3′), 2.59 (2H, t, *J* = 7.4 Hz, CH_2-1′), 2.17 (3H, s, CH_3-4‴″), 2.15 (3H, s, CH_3-3‴″), 1.89 (2H, quintet, *J* = 7.3 Hz, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, *δ*/ppm): 165.21 (C-1‴″), 155.53 (C-5″), 149.42 (C-3″), 136.48 (C-3‴″), 136.31 (C-1‴″), 136.23 (C-8), 132.99 (C-1‴), 131.21 (C-4‴″), 129.84 (C-4‴), 129.80 (C-3‴ & C-5‴), 129.56 (C-5‴″), 127.15 (C-2‴ & C-6‴), 127.00 (C-9), 122.18 (C-2), 120.77 (C-6), 120.29 (C-2‴″), 118.19 (C-7), 118.05 (C-5), 116.63 (C-6‴″), 113.51 (C-3), 111.26 (C-4), 36.96 (C-2‴″), 26.95 (C-2′), 24.29 (C-1′), 23.93 (C-3′), 19.56 (CH_3-3‴″), 18.74 (CH_3-4‴″); Anal. Calc. for $C_{29}H_{29}N_5SO$ (495.21): C, 70.27; H, 5.90; N, 14.13. Found: C, 70.25; H, 5.87; N, 14.10. EI-MS (*m/z*): 495, 352, 334, 190, 158, 143, 130, 120, 91, 77.

***N*-(3,5-Dimethylphenyl)-2-({5-[3-(1*H*-indol-3-yl) propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetamide (9j)**. Red colored amorphous powder; Yield: 84%; Melting Point 91°C; Mol. Formula: $C_{29}H_{29}N_5SO$; Mol. Weight: 495 g/mol; IR (KBr, cm^{-1}): *υ* 3282 (N—H str.), 2945 (C—H aromatic str.), 1664 (C═O str.), 1599 (C═C aromatic str.), 1535, 1473, 1456 (str. for triazole), 1155 (C—O—C str.), 1116 (C═N str.), 689 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, *δ*/ppm): 10.72 (1H, s, NH-1), 10.17 (1H, s, CONH), 7.56-7.52 (3H, m, H-3‴, H-4‴ & H-5‴), 7.40 (2H, dist.d, *J* = 7.7 Hz, H-2‴ & H-6‴), 7.36 (1H, br.d, *J* = 7.8 Hz, H-7), 7.31 (1H, br.d, *J* = 8.0 Hz, H-4), 7.17 (2H, br.s, H-2‴″ & H-6‴″), 7.04 (1H, br.t, *J* = 7.8 Hz, H-6), 6.97 (1H, dist.d, *J* = 1.4 Hz, H-2), 6.92 (1H, br.t, *J* = 7.4 Hz, H-5), 6.69 (1H, br.s, H-4‴″), 4.07 (2H, s, CH_2-2‴″), 2.65 (2H, t, *J* = 7.4 Hz, CH_2-3′), 2.59 (2H, t, *J* = 7.4 Hz, CH_2-1′), 2.21 (6H, s, CH_3-3‴″ & CH_3-5‴″), 1.88 (2H, quintet, *J* = 7.4 Hz, CH_2-2′). ^{13}C-NMR (150 MHz, DMSO-d_6, *δ*/ppm): 165.40 (C-1‴″), 155.53 (C-5″), 149.40 (C-3″), 138.59 (C-1‴″), 137.70 (C-3‴″ & C-5‴″), 136.23 (C-8), 132.98 (C-1‴), 129.88 (C-4‴), 129.84 (C-3‴ & C-5‴), 127.14 (C-2‴ & C-6‴), 127.00 (C-9), 125.00 (C-4‴″), 122.18 (C-2), 120.77 (C-6), 118.19 (C-7), 118.05 (C-5), 116.85 (C-2‴″ & C-6‴″), 113.51 (C-3), 111.25 (C-4), 36.96 (C-2‴″), 26.96 (C-2′), 24.29 (C-1′), 23.93 (C-3′), 21.04 (CH_3-3‴″ & CH_3-5‴″); Anal. Calc. for $C_{29}H_{29}N_5SO$ (495.21): C, 70.27; H, 5.90; N, 14.13. Found: C, 70.23; H, 5.85; N, 14.07. EI-MS (*m/z*): 495, 352, 334, 190, 158, 143, 130, 120, 91, 77.

***N*-(2-Ethyl-6-methylphenyl)-2-({5-[3-(1*H*-indol-3-yl)propyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl) acetamide (9k)**. Light brown colored sticky liquid; Yield: 76%; Mol. Formula: $C_{30}H_{31}N_5SO$; Mol. Weight: 509 g/mol; IR (KBr, cm^{-1}): *υ* 3250 (N—H str.), 2956 (C—H aromatic str.), 1684 (C═O str.), 1591 (C═C aromatic str.), 1508, 1471, 1433 (str. for triazole), 1157 (C—O—C str.), 1126 (C═N str.), 696 (C—S str.); ^{1}H-NMR (600 MHz, DMSO-d_6, *δ*/ppm): 10.72 (1H, s, NH-1), 9.61 (1H, s, CONH), 7.56-7.55 (3H, m, H-3‴, H-4‴ & H-5‴), 7.42-7.41 (2H, m, H-2‴ & H-6‴), 7.37 (1H, br.d, *J* = 7.9 Hz, H-7), 7.31 (1H, br.d, *J* = 7.5 Hz, H-4), 7.11 (1H, dist.d, *J* = 7.7 Hz, H-4‴″), 7.05-7.02 (3H, m, H-6,H-3‴″ & H-5‴″), 6.97 (1H, s, H-2), 6.93 (1H, br.t, *J* = 7.4 Hz, H-5), 4.12 (2H, s, CH_2-2‴″), 2.65 (2H, t, *J* = 7.1 Hz, CH_2-3′), 2.60 (2H, t, *J* = 6.7 Hz, CH_2-1′), 2.46 (2H, q, *J* = 7.3 Hz, $CH_3\underline{CH_2}$-2‴″), 2.07 (3H, s, CH_3-6‴″), 1.88 (2H, quintet, *J* = 7.2 Hz, CH_2-2′) 1.03 (3H, t, *J* = 7.3 Hz, $\underline{CH_3}CH_2$-2‴″). ^{13}C-NMR (150 MHz, DMSO-d_6, *δ*/ppm): 165.76 (C-1‴″), 155.47 (C-5″), 149.41 (C-3″), 141.04 (C-1‴″), 136.23 (C-8), 135.58 (C-6‴″), 134.01 (C-2‴″), 133.00 (C-1‴), 129.86 (C-4‴), 129.81 (C-3‴ & C-5‴), 127.59 (C-4‴″), 127.11 (C-2‴ & C-6‴), 126.87 (C-3‴″), 127.00 (C-9), 125.94 (C-5‴″), 122.17 (C-2), 120.77 (C-6), 118.18 (C-7), 118.04 (C-5), 113.51 (C-3), 111.26 (C-4), 35.79 (C-2‴″), 27.00 (C-2′), 24.28 (C-1′), 24.17 ($CH_3\underline{CH_2}$-2‴″), 23.91 (C-3′), 17.96 ($\underline{CH_3}CH_2$-2‴″), 14.58 (CH_3-6‴″); Anal. Calc. for $C_{30}H_{31}N_5SO$ (509.22): C, 70.70; H, 6.13; N, 13.74. Found: C, 70.63; H, 6.08; N, 13.68. EI-MS (*m/z*): 509, 366, 334, 190, 158, 143, 130, 120, 91, 78.

In vitro urease inhibition assay. Jack bean urease activity was done by determining the quantity of NH_3 formed with indo-phenols method as described in literature.[49–51] Equimolar concentrations (20 μL) of compound and urease enzyme with KH_2PO_4 buffer (pH 8.2) were left in 96 well plate for half hour incubation at 37°C. Equal amount (50 μL) of phenol and base were poured into each well. A microplate reader (SpectraMax ABS) was used to calculate the absorbance (at 625 nm) after 10 minutes, all the readings were taken

in triplicate. Inhibition activity was calculated by following formula:

$$\text{Urease inhibition activity } (\%) = \left(OD_{control}{}^{-} OD_{sample} \times 100\right)/OD_{control},$$

where $OD_{control}$ is the optical density in the absence of sample and OD_{sample} is the optical density in the presence of sample. Standard enzyme inhibitor used for urease is thiourea.

Hemolytic assay. Bovine blood samples were taken for the formation of RBCs suspension following already reported method.[52,53] Solution (10 mg/mL, 20 μL) of synthesized compound was incubated with 180 μL of red blood cells suspension at room temperature. Triton 100-X and PBS was used as positive and negative control, respectively. Percentage of hemolysis was calculated by following formula:

$$\%\text{ of hemolysis} = \frac{\text{Absorbance of sample} - \text{Absorbance of negative control}}{\text{Absorbance of positive control}} \times 100.$$

Kinetic assay. Mode of inhibition was followed through kinetic analysis. For this purpose, **9i** derivative with best IC_{50} value was selected. Kinetic study was performed by changing the amount of urea with varying concentrations of selected compound. Procedure is same for all kinetic activities as discussed in urease inhibition assay.[54]

Free radical scavenging assay. Previously reported method was utilized after a slight change, to measure the radical scavenging potential of synthesized compounds.[55,56] Vitamin C (ascorbic acid) was the standard inhibitor. Microplate reader (SpectraMax ABS) at 517 nm was used to measure scavenging assay. Rate of reactions were equated and percentage inhibition by test inhibitors was measured. Each experiment was done in triplicate.

Selection of Jack bean urease structure. Protein Data Bank (PDB) (www.rcsb.org) with PDBID 4H9M was used to retrieve Jack bean urease enzyme structure. This structure was reduced by applying UCSF Chimera 1.10.1 tool.[57] Further Molprobity server[58] and Protparam[59] were used to find stereochemical characteristics. Graph showing hydrophobic characteristic of target protein was employed by "Discovery Studio 4.1 Client tool".[60] The structural conformations of protein were taken from online VADAR 1.8 server.[61]

Molecular docking simulation. The structures of synthesized derivatives (**9a-k**) were drawn in ACD/ChemSketch drawing tool. Molecular docking was performed by PyRx docking tool.[62] All the synthesized ligands were docked separately against urease. SAR analysis and low binding energy values were used to evaluate expected docked compounds. Discovery Studio (2.1.0) helped in three-dimensional graphical interpretations of the entire docked compounds.

4 | CONCLUSION

Aimed synthesis of indole-triazole hybrids bearing *N*-substituted acetamides (**9a-k**) was accomplished in excellent yields. All the molecules exposed an efficient potential against urease enzyme and depicted lower IC_{50} values relative to standard used. Moreover, all the compounds were attributed with mild cytotoxicity and suitable free radical scavenging potential. Consequently, it was concluded that these chemical entities might be complemented as valuable additions to the already existing anti-ulcer therapeutic agents.

Statistical analysis. Microsoft Excel 2010 was used to perform statistical analysis. The results are displayed as mean ± SEM with CL 96%.

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