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Synthesis, biological evaluation, and in silico study of some unique multifunctional 1,2,4-triazole acetamides

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Abstract: The imperative demand for antibacterial agents and enzyme inhibitors prompted us to synthesize some new compounds, **6a**–**6k**, bearing multifunctional moieties. The target acetamides were derived from 4-phenyl-5-(1-tosylpiperidin-4-yl)-4*H*-1,2,4-triazole-3-thiol (**3**). The structural analysis was carried out using modern spectroscopic techniques including IR, NMR, and EIMS spectral analysis. The antibacterial activity was screened against five bacterial strains including three gram-negative and two gram-positive ones. Enzyme inhibition was carried out against lipoxygenase enzyme and results were supported by in silico study. The synthesized compounds were proved to be potent antibacterial agents and enzyme inhibitors.

Key words: 1,2,4-Triazole, acetamides, antibacterial activity, lipoxygenase enzyme inhibition, in silico study

1. Introduction

The use of antibiotics is increasing to combat different infectious diseases. This excessive use of antibiotics makes bacterial strains more resistant to presently available antibiotic drugs. These circumstances demand an imperative supply of novel antibiotics.[1,2] Enhanced expression and activity of lipoxygenase (LOX) in the body of humans and other mammals is the cause of many diseases including asthma,[3] cardiovascular diseases,[4] rheumatoid arthritis,[5] inflammatory bowel,[6] and cancer.[7]

Amides, RCONHR', are effective antibacterial[8,9] and LOX inhibitors.[10–12] In conjunction with antibacterial and enzyme inhibition activity, amide derivatives are anti-HIV,[13] antitumor,[14] antipsychotic,[15] analgesic,[16] antiulcer,[17] anticonvulsant,[18] cardiotonic,[19] and antiinflammatory[20] agents. Keeping in view the importance of amide functionality, it has been merged with 4-phenyl-5-(1-tosylpiperidin-4-yl)-4*H*-1,2,4-triazole-3-thiol (**3**), which also bears other bioactive moieties including a piperidine core,[21–23] sulfonamide linkage,[24,25] and 1,2,4-triazole heterocycle.[26–29] The different bioactive 1,2,4-triazole derivatives include 6-aryl-3-{(4-substitutedphenoxy)methyl}-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles[26] and 3,6-disubstituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles.[27–29]

Five bacterial strains were considered for antibacterial study. *Bacillus subtilis* is believed to cause dermal allergy and hyper sensitivity,[30] *Staphylococcus aureus* is a pathogenic bacterial strain,[31] *Salmonella typhi* is a

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cause of enteric fever and some other diseases,[32] *Escherichia coli* can cause food poisoning,[33] and *Pseudomonas aeruginosa* is a cause of chronic infections.[34] All these bacterial strains cause different diseases and so are used as model bacterial strains for antibacterial screening.

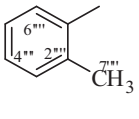
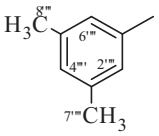
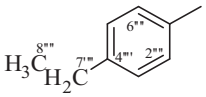
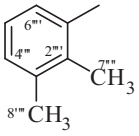
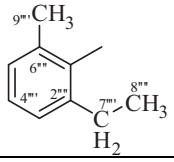
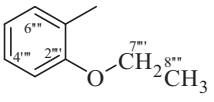
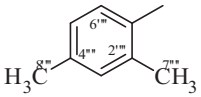
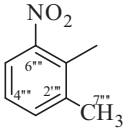
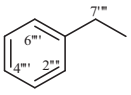
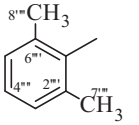
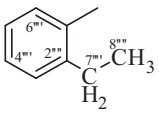
The advancement in crystallography, nuclear magnetic resonance spectroscopy, and other structural elucidation techniques proves very important in three-dimensional structural determinations of biomolecules and drugs. The three-dimensional structures of proteins such as enzymes are used to study possible interactions of drugs with enzymes using computational studies.[35] The three-dimensional structure of enzyme active sites interact with drug molecules such as ligands with the help of docking software. The software finds different possible interactions between ligand and enzyme; such studies are regarded as virtual screening, in silico studies, or molecular docking.[36–39]

The resistance to existing antibiotics[1,2] and bioactive moieties[21–29] prompted us to synthesize some new bioactive compounds bearing different functionalities altogether in a single molecule. The hypothesis was that these functionalities may boost the bioactivity potential of each other. A series of *S*-substituted derivatives of 4-phenyl-5-(1-tosylpiperidin-4-yl)-4*H*-1,2,4-triazole-3-thiol (**3**) has been synthesized and most of them were found to be potent antibacterial agents along with having notable LOX inhibition.

2. Results and discussion

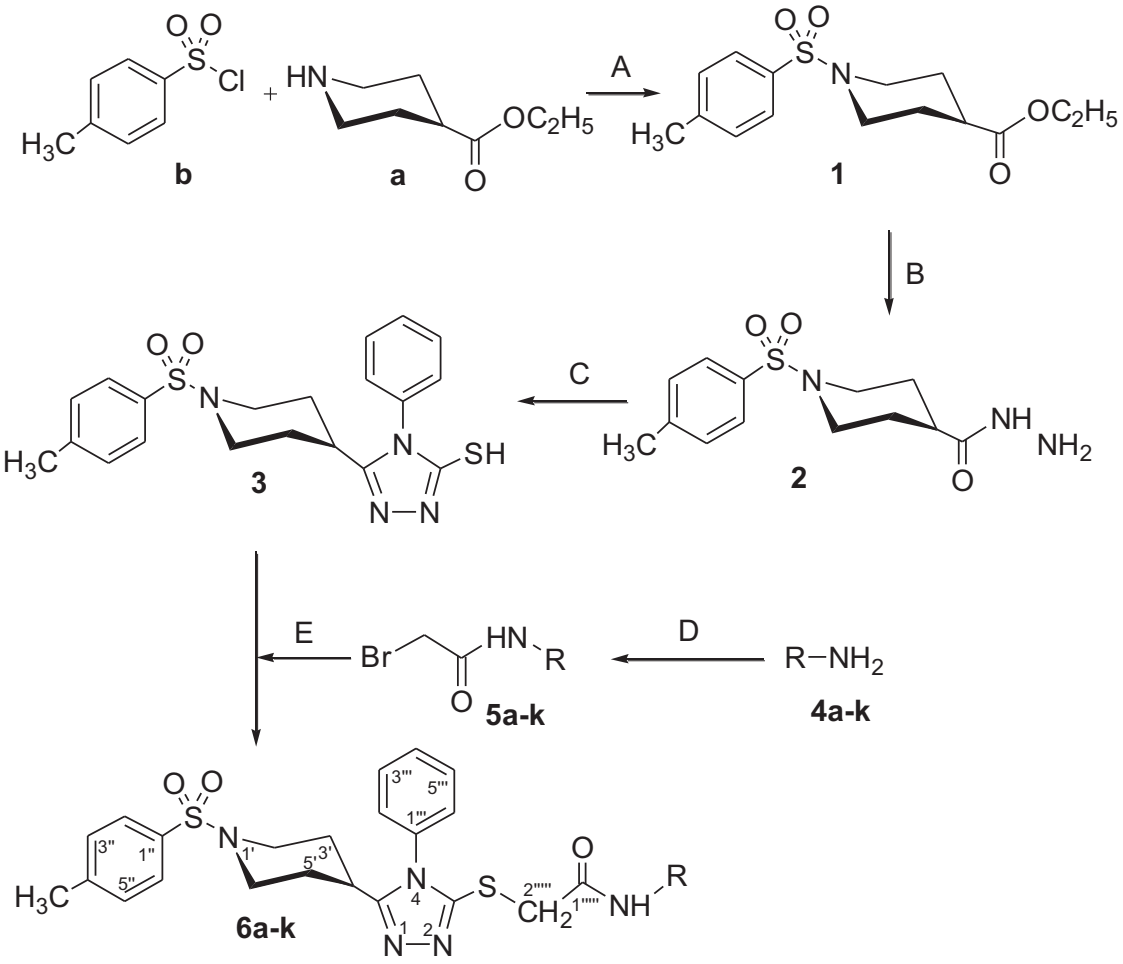
The derivatives of 4-phenyl-5-(1-tosylpiperidin-4-yl)-4*H*−1,2,4-triazole-3-thiol (**3**) were synthesized according to the Scheme and evaluated for antibacterial activity and LOX inhibition potentials. The different aryl/aralkyl groups of the synthesized compounds are given in Table 1.

Table 1. Different *N*-substituted aryl/aralkyl groups.

Comp.	R	Comp.	R	Comp.	R
6a		**6e**		**6i**	
6b		**6f**		**6j**	
6c		**6g**		**6k**	
6d		**6h**			

2.1. Chemistry

Compound **6a** was obtained as a white amorphous solid with percentage yield of 79%. The molecular ion peak was observed at 561 (m/z) in EIMS and the ^{1}H NMR data established the formula of **6a** as $C_{29}H_{31}N_5O_3S_2$.



Scheme. Outline for the synthesis of derivatives of 4-phenyl-5-(1-tosylpiperidin-4-yl)-4*H*-1,2,4-triazole-3-thiol (**6a**–**6k**). Reagents and conditions: A) 5% Na_2CO_3 solution, H_2O, stir for 4 h. B) $N_2H_4.H_2O$, EtOH, reflux for 3 h. C) i- Phenyl isothiocyanate, EtOH, reflux for 2 h. ii- 10% NaOH, reflux for 5 h. D) $BrCH_2COBr$, 5% Na_2CO_3 solution, H_2O, vigorously stir 30 min. E) DMF, NaH, stir for 4–5 h.

The peaks in the IR spectrum appeared at 3447, 3071, 1738, 1629, 1536, and 1341, corresponding to N-H stretching, C-H stretching of the aromatic ring, C=O stretching, C=N stretching, C=C aromatic stretching, and -SO_2- stretching, respectively. The IR spectral peaks confirmed all the intended moieties and functional groups in compound **6a**. The EIMS peak at m/z420 represented the removal of *p*-toluene sulfonyl moiety from compound **6a**. The peak at m/z264 confirmed the removal of the phenyl group present at position 4 of the 1,2,4-triazole ring and *N*-(4-ethylphenyl)-2-sulfanylacetamide moiety along with partial cleavage of triazole ring. The peak at m/z 109 represented the presence of piperidine moiety and the peak at m/z 91 represented the presence of toluene moiety. Other prominent fragments are given in Figure 1. In the ^{1}H NMR spectrum, the $p-$toluenesulfonyl group was supported by two doublets and one singlet at δ (ppm) 7.59 (d, J = 8.4 Hz, 2H, H-2″ & H-6″), 7.27 (d, J = 7.8 Hz, 2H, H-3″ & H-5″), and 2.39 (s, 3H, CH_3-7″). The phenyl group directly attached to the nitrogen of the triazole ring resonated at δ (ppm) 7.54–7.50 (m, 3H, H-3‴, H-4‴ & H-5‴) and 7.18–7.17 (m, 2H, H-2‴, H-6‴). The 2-methylphenyl group was confirmed by δ (ppm) 7.94 (d, J = 8.4 Hz, 1H,

H-6′′′′), 7.18–7.17 (m, 1H, H-5′′′′), 7.15 (d, J = 7.2 Hz, 1H, H-3′′′′), 7.02 (dt, J = 1.2, 7.8 Hz, 1H, H-4′′′′), and 2.27 (s, 3H, CH_3-7′′′′). The acetamoyl moiety presented two signals at δ (ppm) 9.68 (s, 1H, N-H) and 3.92 (s, 2H, H-2′′′′′. Spectral study corroborated compound **6a** as 2-(4-phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4H-1,2,4-triazol-3-ylthio)-N-(2-methylphenyl) acetamide. The structural elucidation of remaining compounds under study was also performed through IR, EIMS, and ^{1}H NMR analysis.

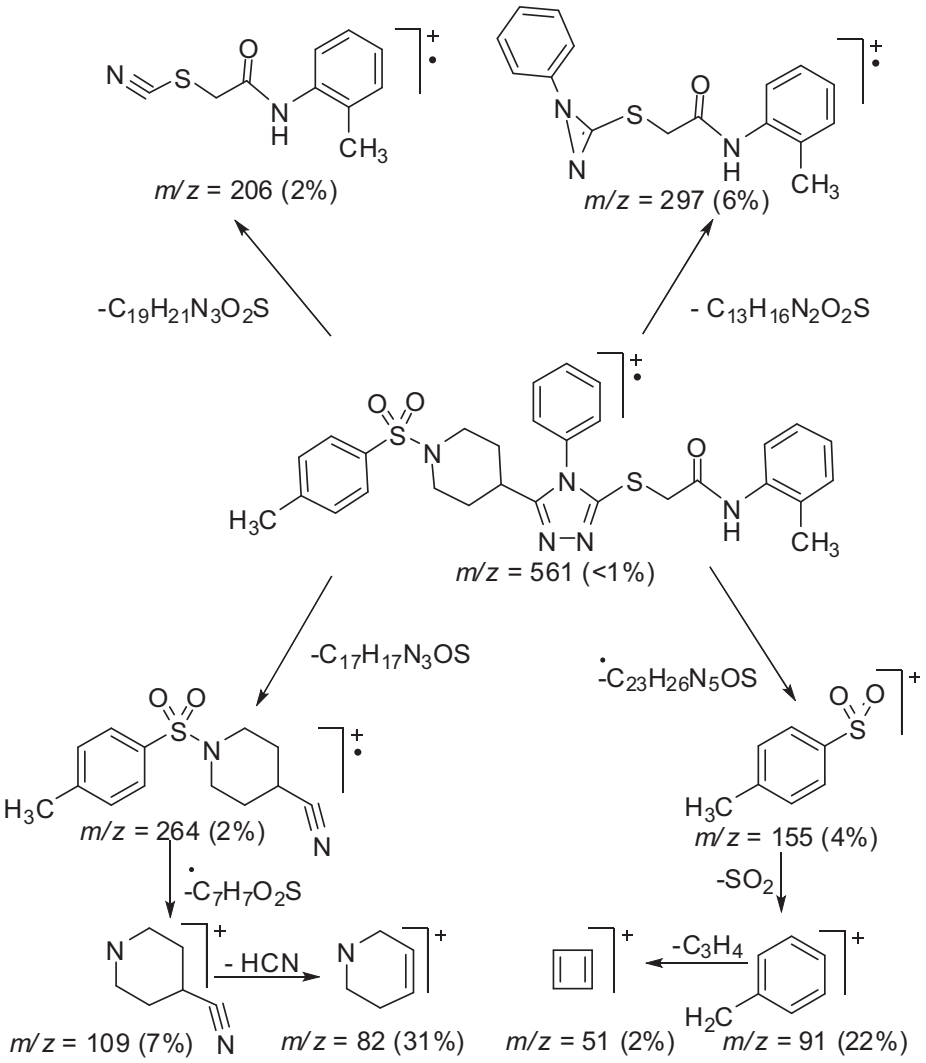


Figure 1. Mass fragmentation pattern of **6a**.

2.2. Antibacterial activity

The antibacterial study of synthetic compounds **6a**–**6k** was performed against five bacterial strains. The bacterial strains under consideration included three gram-negative strains (*S. typhi*, *E. coli*, and *P. aeruginosa*) and two gram-positive strains (*B. subtilis* and *S. aureus*). The standard antibacterial agent used was ciprofloxacin. The results in the form of % inhibition and minimum inhibitory concentration (MIC) values are given in Tables 2 and 3. All compounds except **6g** and **6h** were active against all the bacterial strains under study. Compounds

6g and **6h** were inactive only against *E. coli* and *S. aureus*, respectively, while for the remaining bacterial strains compounds **6g** and **6h** showed good antibacterial activity. Compounds **6b**, **6d**, and **6k** proved to be proficient antibacterial agents for all microbes under study. The good activity of compounds **6b**, **6d**, and **6k** may be attributed to the varying moieties as 2,3-dimethylphenyl, 2,5-dimethylphenyl. and benzyl, respectively. Compounds **6b, 6d**, and **6k** were proved to be the most active antibacterial agents in the following order, **6d >6b >6k**. Compounds **6d** and **6k** were the most active against *S. aureus* with MIC values of 8.15 ± 0.85 and 8.21 ± 0.43 μ M, respectively, with a reference of 7.57 ± 0.65 μ M, the MIC value of ciprofloxacin. Compound **6b** was the most active against *S. typhi* with MIC value of 8.11 ± 0.87 with a reference of 7.47 ± 0.68 μ M. The previously synthesized acetamide analogs bearing oxadiazole as 2-(5-(1-(phenylsulfonyl)piperidin-4-yl)-1,3,4-oxadiazol-2-ylthio)acetamide by Khalid et al.[40] were moderately active against the bacterial strains under study. The current results showed that minor changes in structure led to an enhanced antibacterial activity potential.

Table 2. The % inhibition of bacterial activity of synthesized compounds.

Compounds	% Inhibition				
	S. typhi (-)	*E. coli* (-)	*P. aeruginosa* (-)	*B. subtilis* (+)	*S. aureus* (+)
6a	76.68 ± 0.51	65.25 ± 0.34	74.24 ± 0.17	75.24 ± 0.57	59.00 ± 0.53
6b	86.17 ± 0.32	73.45 ± 1.13	74.38 ± 0.46	75.88 ± 0.23	77.91 ± 0.85
6c	77.08 ± 0.46	72.32 ± 0.48	71.00 ± 0.81	73.36 ± 0.75	60.17 ± 0.56
6d	86.81 ± 0.46	88.89 ± 0.76	74.11 ± 0.19	79.71 ± 0.67	87.54 ± 0.71
6e	70.14 ± 0.87	83.23 ± 0.37	63.45 ± 0.70	74.13 ± 0.65	70.20 ± 0.18
6f	62.56 ± 0.48	52.45 ± 0.97	66.18 ± 0.23	62.57 ± 0.28	78.07 ± 0.65
6g	70.58 ± 0.91	47.18 ± 0.24	76.78 ± 0.67	80.78 ± 0.31	81.58 ± 0.67
6h	60.41 ± 0.48	79.67 ± 0.26	72.21 ± 0.97	73.37 ± 0.52	10.31 ± 0.27
6i	63.49 ± 0.53	76.67 ± 0.67	57.33 ± 1.13	67.35 ± 0.27	62.24 ± 0.43
6j	75.00 ± 0.67	72.03 ± 0.37	71.97 ± 1.25	77.61 ± 0.56	72.18 ± 0.97
6k	86.45 ± 0.67	73.53 ± 0.09	72.27 ± 0.97	80.65 ± 0.23	90.18 ± 1.56
Ciprofloxacin	91.55 ± 0.78	92.65 ± 0.32	92.81 ± 0.56	91.82 ± 063	91.89 ± 0.39

2.3. LOX inhibition activity

All the synthesized compounds, **6a**–**6k**, were proved to be excellently to moderately active, except **6g**. Compounds **6b**, **6c**, **6h**, **6i**, and **6k** were proved to be excellent LOX inhibitors. Table 4 demonstrates the % inhibition and IC_{50} values. The most active compound was **6k**; the enhanced activity of this compound could be attributed to the unsubstituted benzyl moiety, which allowed compound **6k** to fit into the active site of the LOX enzyme more compatibly and inhibit the enzyme strongly. The overall inhibition order of the synthesized compounds may be listed as **6k >6i >6h >6b >6c >6d >6e >6f >6j >6a >6g**.

2.4. Molecular docking

The most active LOX inhibitors under study, **6b**, **6c**, **6h**, **6i**, and **6k**, were docked to find the probable moieties of the synthetic compounds that were the cause of LOX inhibition. The molecular docking interactions of the most active compounds are presented in Table 5. Figure 2 shows that compound **6b** inhibited LOX due to

Table 3. The MIC for antibacterial activity of synthesized compounds.

Compounds	MIC				
	S. typhi (-)	*E. coli* (-)	*P. aeruginosa* (-)	*B. subtilis* (+)	*S. aureus* (+)
6a	9.21 ± 0.87	11.18 ± 0.24	9.46 ± 0.12	9.16 ± 0.96	15.63 ± 0.48
6b	8.11 ± 0.51	9.19 ± 0.53	9.51 ± 0.71	9.14 ± 0.71	8.37 ± 0.65
6c	8.81 ± 0.43	10.72 ± 0.20	10.71 ± 0.21	9.19 ± 0.93	15.88 ± 0.39
6d	8.22 ± 0.43	8.43 ± 0.65	9.67 ± 0.23	8.73 ± 0.51	8.15 ± 0.85
6e	10.14 ± 0.27	8.56 ± 0.97	14.61 ± 0.65	9.35 ± 0.77	10.72 ± 0.45
6f	13.83 ± 0.28	19.54 ± 0.56	12.76 ± 0.41	15.32 ± 0.34	8.63 ± 0.72
6g	10.86 ± 0.23	-	8.72 ± 0.43	8.71 ± 0.27	8.67 ± 0.54
6h	15.61 ± 0.53	8.48 ± 0.35	9.61 ± 0.68	10.47 ± 0.75	-
6i	12.51 ± 0.23	8.57 ± 0.97	16.37 ± 0.23	12.43 ± 0.76	14.48 ± 0.25
6j	8.46 ± 0.23	10.09 ± 0.24	9.47 ± 0.36	8.61 ± 0.48	10.53 ± 0.44
6k	8.78 ± 0.35	9.65 ± 0.48	9.56 ± 0.25	8.67 ± 0.54	8.21 ± 0.43
Ciprofloxacin	7.47 ± 0.68	7.61 ± 0.52	7.23 ± 0.68	7.43 ± 0.87	7.57 ± 0.65

Table 4. Lipoxygenase inhibition activity of synthesized compounds.

Compounds	LOX inhibition (%) at 0.5 mM	IC_{50} (μ M)
6a	53.44 ± 0.76	441.87 ± 0.14
6b	89.54 ± 0.65	86.65 ± 0.36
6c	87.47 ± 1.07	91.07 ± 0.86
6d	79.43 ± 0.55	114.25 ± 0.49
6e	74.27 ± 0.12	156.67 ± 0.36
6f	58.27 ± 0.43	305.35 ± 0.11
6g	35.64 ± 0.83	>500
6h	87.67 ± 0.67	85.61 ± 0.56
6i	89.41 ± 1.17	61.56 ± 0.87
6j	55.54 ± 0.74	435.87 ± 0.41
6k	81.12 ± 0.83	53.70 ± 0.98
Baicalein	93.79 ± 1.27	22.41 ± 0.30

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc., Amherst, NH, USA).

an acidic interaction with His518 through the carbonyl moiety having bond length of 2.26 Å. Compound **6c** made acidic and arene-cation interactions because of the sulfonyl oxygen and toluenyl ring with Thr274 and His266 with bond length of 2.29 Å and on average 4.25 Å, respectively (Figure 3). Compound **6h** made a metallic interaction with Fe^{+3} by virtue of sulfonyl oxygen with bond length 2.58 Å and π-π interaction with His518 through the terminal phenyl ring with bond length of 4.02 Å (Figure 4). Compound **6i** showed three interactions, acidic and arene-arene with His518 and strong metallic with Fe^{+3}, with bond lengths of 2.85 Å,

3.14 Å, and 2.54 Å, respectively (Figure 5). The most active compound, **6k**, interacted with the enzyme active site because of the sulfonyl oxygen and toluenyl ring, which made acidic and arene-cation interactions with Thr274 and His266 with bond lengths of 2.75 Å and 4.25 Å, respectively (Figure 6).

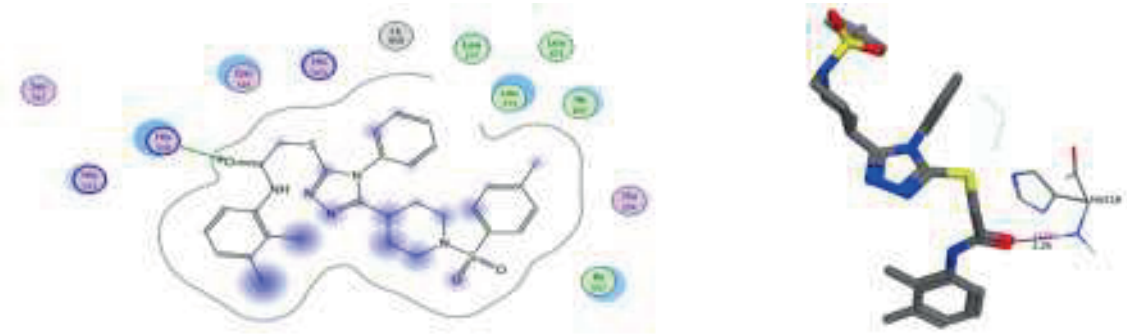


Figure 2. 2D and 3D binding models of compound **6b**.

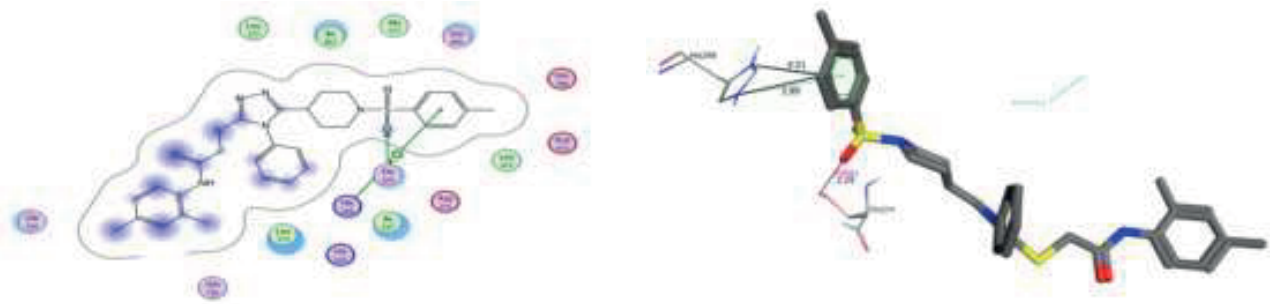


Figure 3. 2D and 3D binding models of compound **6c**.

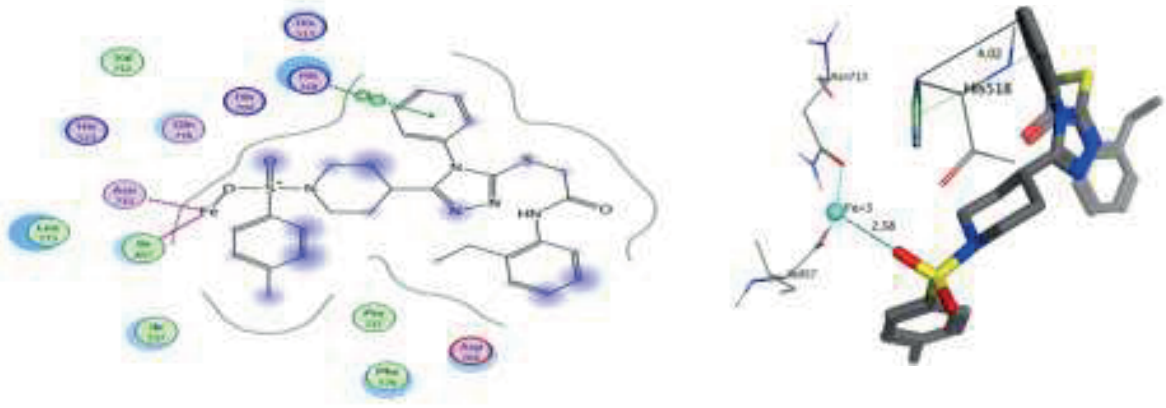


Figure 4. 2D and 3D binding models of compound **6h**.

2.5. Conclusions

The synthesized compounds were strong to moderate antibacterial agents, especially **6b**, **6d**, and **6k**. Compounds **6b**, **6c**, **6i**, and **6k** were potent LOX inhibitors. LOX inhibition was also well supported by docking

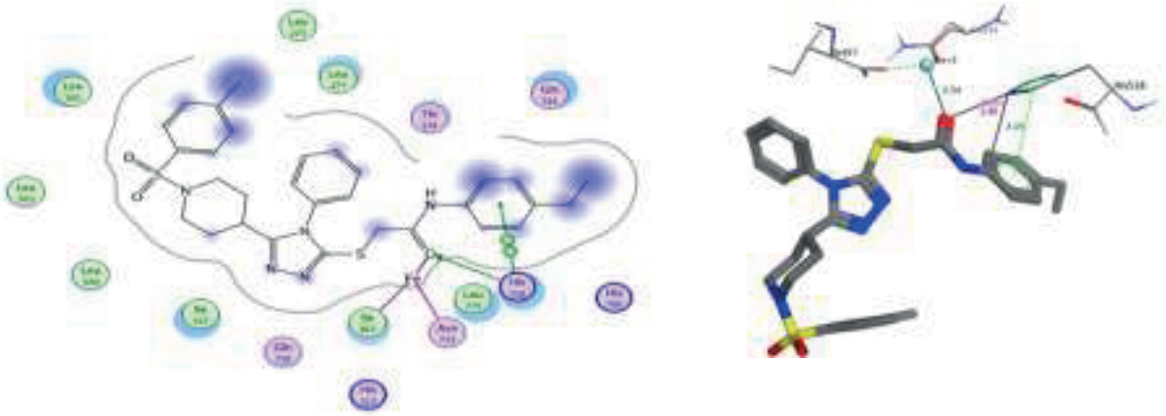


Figure 5. 2D and 3D binding models of compound **6i**.

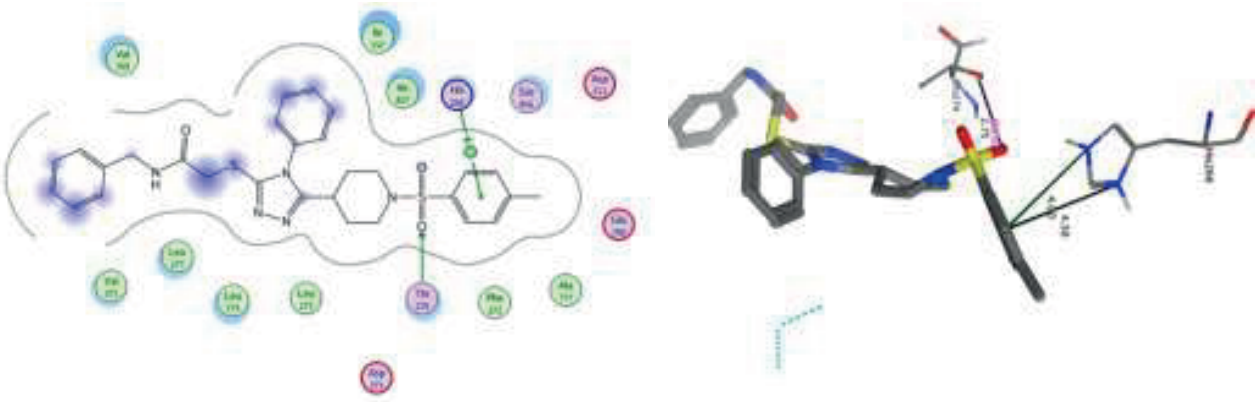


Figure 6. 2D and 3D binding models of compound **6k**.

Table 5. Molecular docking interactions of the most active compounds.

Compound	$\alpha-$Glucosidase		
	Interactions	Functionality	Bond distance, Å
6b	Acidic	His518 with carbonyl oxygen	2.26
6c	Acidic	Thr274 with sulfonyl oxygen	2.29
	Arene-cation	His266 with toluenyl ring	4.25
6h	Metallic	Fe^{+3} with sulfonyl oxygen	2.58
	$\pi - \pi$	His518 with phenyl	4.02
6i	Acidic	His518 with carbonyl oxygen	2.85
	Arene-arene	His518 with phenyl ethyl moiety	3.14
	Metallic	Fe^{+3} with carbonyl oxygen	2.54
6k	Acidic	Thr274 sulfonyl oxygen	2.75
	Arene-cation	Toluenyl ring	4.25

results. The synthesized compounds were proved to be more potent antibacterial agents as compared to previously reported counterparts. The results and comparison with previous studies indicated that slight variation

in structure can affect biological activity tremendously. The most active synthesized compounds may be considered as new drug candidates for diseases caused by bacterial strains and LOX enzyme. Further slight structural modifications may also result in highly bioactive compounds as suitable drug candidates for certain other diseases.

3. Experimental

3.1. General

The chemicals employed for synthesis were purchased from local suppliers from Merck, Sigma-Aldrich, and Alfa Aesar. The analytical-grade solvents were employed without further purification. An open capillary method was used to determine the melting points of the synthesized compounds and the determined melting points were uncorrected. TLC was employed to evaluate the purity of the synthesized compounds. TLC was performed using precoated silica gel G-25-UV_{254} aluminum plates. The mobile system used for the development of TLC was composed of different ratios of n-hexane and ethyl acetate. The components of the reaction mixture and references were illuminated by a 254-nm UV lamp. A JASCO-320-A spectrometer was employed to obtain IR spectra using the KBr pellet method. A Bruker spectrometer working at 400 and 600 MHz was used to obtain ^{1}H NMR spectra and 150 MHz for ^{13}C NMR spectra. A JMS-HX-110 spectrometer was used to develop EIMS spectra.

3.2. Preparation of 4-ethoxycarbonyl-1-(4-methylphenylsulfonyl)piperidine (1)

Ethyl isonipecotate (**a**; 30.0 mmol) was dispersed in distilled water (40 mL), taken in a round-bottomed (RB) flask (250 mL). p-Toluenesulfonyl chloride (**b**; 30.0 mmol) was added to the above reaction mixture pinch by pinch in about 30 min. Na_2CO_3 (5%) was used to maintain the pH around 9.0. The reaction mixture was stirred at room temperature for 4 h. The reaction progress was monitored by TLC. On the completion of the reaction, concentrated HCl (11 M, 4 mL) was added to the reaction contents and pH was adjusted to about 6.0; the mixture was allowed to stand for 15–20 min. White solid precipitates of compound **1** were obtained after filtration, washed with distilled water, and dried in a desiccator.

3.3. Preparation of 1-(4-methylphenylsulfonyl)-4-piperidinecarbohydrazide (2)

4-Ethoxycarbonyl-1-(4-methylphenylsulfonyl)piperidine (**1**; 26.0 mmol) was dissolved in ethanol (40 mL) in a RB flask (250 mL). Hydrazine hydrate (80%, 20 mL) was added steadily to the above reaction mixture and refluxed for 3 h. Completion of the reaction was confirmed through TLC. On completion of the reaction, excess solvent was evaporated to yield a white crystalline product, compound **2**, and the product was washed with cold distilled water and dried.

3.4. Preparation of 4-phenyl-5-(1-tosylpiperidin-4-yl)-4*H*-1,2,4-triazole-3-thiol (3)

The compound, 1-(4-methylphenylsulfonyl)-4-piperidinecarbohydrazide (**2**; 22.0 mmol), was dissolved in ethanol (40 mL), followed by gradual addition of phenyl isothiocyanate (22.0 mmol). The reaction was refluxed for 2 h. The completion of the reaction was confirmed via TLC. Excess solvent was evaporated to acquire the precipitates. The dried precipitates were dispersed in 10% NaOH solution (40 mL) and refluxed for 5 h. The completion of the reaction was also established through TLC. After it, dilute HCl was added to adjust the pH to be slightly acidic (pH 6). White solid crystalline compound **3** was filtered, washed with distilled water, and dried.

3.5. General procedure for the synthesis of compounds (5a–5k)

Aryl/aralkyl amines (**4a**–**4k**; 5.7 mmol) were added in distilled water (10 mL) in a flat-bottomed flask and 5% Na_2CO_3 solution was used to adjust the pH at 8.0 to 9.0. The reaction contents were stirred for 20 min at room temperature. 2-Bromoacetyl bromide (0.50 mL; 5.7 mmol) was added to the reaction mixture in about 10 min along with vigorous shaking. The reaction flask was further stirred until the formation of solid precipitates. The reaction completion was established by TLC. The solid products were filtered, washed with cold distilled water, and dried to produce the title electrophiles, **5a**–**5k**.

3.6. General procedure for the synthesis derivatives of 3 (6a–6k)

$N,N-$Dimethylformamide (DMF) (15 mL) and sodium hydride (0.1 mmol) were taken in a RB flask (50 mL) and mixed by stirring. 4-Phenyl-5-(1-tosylpiperidin-4-yl)-4H-1,2,4-triazole-3-thiol (**3**; 0.1 mmol) was dissolved in the above reaction mixture and stirred at room temperature for 30 min. After that, electrophiles **5a**–**5k** were added in an equimolar ratio to the reaction mixture and further stirred for 4–5 h. The progress and completion of the reaction were assessed by TLC. Distilled water was added in excess to the reaction contents to obtain products **6a**–**6k**. The products thus obtained were filtered, washed, and dried.

3.7. Spectral characterization of synthesized compounds (1–3, 6a–6k)

3.7.1. 4-Ethoxycarbonyl-1-(4-methylphenylsulfonyl)piperidine (1)

White amorphous solid; yield: 89%; mp: 70–72 °C; molecular formula: $C_{15}H_{21}NO_4S$; molecular weight: 311; IR (KBr, cm^{-1}) υ_{max}: 3067 (C-H stretching of aromatic ring), 1732 (C=O stretching), 1531 (C=C aromatic stretching), 1335 (-SO_2- stretching), 1079 (C-O bond stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ / ppm): 7.62 (d, J = 8.0 Hz, 2H, H-2″ & H-6″), 7.32 (d, J = 8.0 Hz, 2H, H-3″ & H-5″), 3.98 (q, J = 7.2 Hz, 2H, O-CH_2), 3.71–3.68 (m, 2H, H_e-2′ & H_e-6′), 2.73–2.62 (m, 1H, H-4′), 2.54–2.48 (m, 2H, H_a-2′ & H_a-6′), 2.42 (s, 3H, CH_3-7″), 2.10–2.08 (m, 2H, H_e-3′ & H_e-5′), 1.60–1.86 (m, 2H, H_a-3′ & H_a-5′), 1.15 (t, J = 7.2 Hz, CH_3); EIMS (m/z): 311 $[M]^+$, 266 $[C_{13}H_{16}NO_3S]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 184 $[C_8H_{10}NO_2S]^+$, 170 $[C_7H_8NO_2S]^+$, 155 $[C_7H_7O_2S]^+$, 91 $[C_7H_7]^+$.

3.7.2. 1-(4-Methylphenylsulfonyl)-4-piperidinecarbohydrazide (2)

White crystalline solid; yield: 91%; mp: 128 °C; molecular formula: $C_{13}H_{19}N_3O_3S$; molecular weight: 297; IR (KBr, cm^{-1}) υ_{max}: 3348 (N-H stretching), 3063 (C-H stretching of aromatic ring), 1682 (C=O stretching), 1534 (C=C aromatic stretching), 1339 (-SO_2- stretching); ^{1}H NMR (400 MHz, $CDCl_3$, δ / ppm): 7.61 (d, J = 8.0 Hz, 2H, H-2″ & H-6″), 7.33 (d, J = 8.0 Hz, 2H, H-3″ & H-5″), 3.72–3.69 (m, 2H, H_e-2′ & H_e-6′), 2.73–2.62 (m, 1H, H-4′), 2.53–2.49 (m, 2H, H_a-2′ & H_a-6′), 2.42 (s, 3H, CH_3-7″), 2.12–2.10 (m, 2H, H_e-3′ & H_e-5′), 1.58–1.84 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 297 $[M]^+$, 266 $[C_{13}H_{16}NO_3S]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 184 $[C_8H_{10}NO_2S]^+$, 170 $[C_7H_8NO_2S]^+$, 155 $[C_7H_7O_2S]^+$, 91 $[C_7H_7]^+$.

3.7.3. 4-Phenyl-5-(1-tosylpiperidin-4-yl)-4H-1,2,4-triazole-3-thiol (3)

White crystalline solid; yield: 95 %; mp: 155 °C; molecular formula: $C_{20}H_{22}N_4O_2S_2$:molecular weight: 414; IR (KBr, cm^{-1}) υ_{max}: 3050 (C-H stretching of aromatic ring), 2617 (S-H), 1613 (aromatic C-C stretching), 1574 (C=N stretching of triazole ring), 1469 (C=C aromatic stretching), 1347 (-SO_2- stretching), 668 (C-S

stretching); ^{1}H NMR (600 MHz, $CDCl_3$,δ / ppm): 11.25 (s, 1H, S-H), 7.57 (d,J = 8.4 Hz, 2H, H-2″ & H-6″), 7.52–7.51 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.27 (d,J = 7.8 Hz, 2H, H-3″ & H-5″), 7.27–7.23 (m, 2H, H-2‴ & H-6‴), 3.68–3.65 (m, 2H, H_e-2′ & H_e-6′), 2.39 (s, 3H, CH_3-7″), 2.37–2.33 (m, 1H, H-4′), 2.25–2.20 (m, 2H, H_a-2′ & H_a-6′), 1.89–1.86 (m, 2H, H_e-3′ & H_e-5′), 1.79–1.60 (m, 2H, H_a-3′ & H_a-5′); ^{13}C NMR (150 MHz, $CDCl_3$, δ/ ppm): 156.8 (C-3), 153.3 (C-5), 143.9 (C-1‴), 132.8 (C-1″), 132.1 (C-4″), 130.5 (C-3″ & C-5″), 129.6 (C-2‴ & C-6‴), 127.5 (C-3‴ & C-5‴), 127.3 (C-2″ & C-6″), 126.1 (C-4‴), 45.3 (C-2′ & C-6′), 32.1 (C-4′), 29.2 (C-3′ & C-5′), 21.4 (C-7″); EIMS (m/z): 414 $[M]^+$, 259 $[C_{13}H_{15}N_4S]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 150 $[C_7H_6N_2S]^+$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 59 $[CHNS]^+$, 51 $[C_4H_3]^+$.

3.7.4. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2-methylphenyl)acetamide (6a)

White amorphous solid; yield: 79%; mp: 181–183 °C; molecular formula: $C_{29}H_{31}N_5O_3S_2$; molecular weight: 561; IR (KBr, cm^{-1}) υ_{max}: 3447 (N-H stretching), 3071 (C-H stretching of aromatic ring), 2887 (CH_2 stretching), 1738 (C=O stretching), 1629 (C=N stretching), 1536 (C=C aromatic stretching), 1341 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.68 (s, 1H, N-H), 7.94 (d,J = 8.4 Hz, 1H, H-6⁗), 7.59 (d,J = 8.4 Hz, 2H, H-2″ & H-6″), 7.54–7.50 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.27 (d, J = 7.8 Hz, 2H, H-3″ & H-5″), 7.18–7.17 (m, 3H, H-2⁗, H-6‴ & H-5⁗), 7.15 (d,J = 7.2 Hz, 1H, H-3⁗), 7.02 (dt,J = 1.2, 7.8 Hz, 1H, H-4⁗), 3.92 (s, 2H, H-2⁗′, 3.71–3.67 (m, 2H, H_e-2′ & H_e-6′), 2.51–2.46 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7″), 2.34–2.30 (m, 2H, H_a-2′ & H_a-6′), 2.27 (s, 3H, CH_3-7⁗), 2.00–1.94 (m, 2H, H_e-3′ & H_e-5′), 1.82–1.80 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 561 $[M]^+$, 406 $[C_{22}H_{24}N_5OS]^+$, 297 $[C_{16}H_{15}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 206 $[C_{10}H_{10}N_2OS]^{\cdot+}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.5. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2,3-dimethylphenyl)acetamide (6b)

White amorphous solid; yield: 85%; mp: 185–187 °C; molecular formula: $C_{30}H_{33}N_5O_3S_2$; molecular weight: 575; IR (KBr, cm^{-1}) υ_{max}: 3436 (N-H stretching), 3065 (C-H stretching of aromatic ring), 2883 (CH_2 stretching), 1730 (C=O stretching), 1623 (C=N stretching), 1531 (C=C aromatic stretching), 1333 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.60 (s, 1H, N-H), 7.60 (d,J = 8.4 Hz, 1H, H-6⁗), 7.58 (d,J = 8.4 Hz, 2H, H-2″ & H-6″), 7.55–7.51 (m, 3H, H-3‴, H-4‴& H-5‴), 7.26 (d,J = 8.4 Hz, 2H, H-3″ & H-5″), 7.17 (dd,J = 1.2, 7.8 Hz, 2H, H-2‴ & H-6‴), 7.06 (t,J = 7.8 Hz, 1H, H-5⁗), 6.96 (d,J = 7.8 Hz, 1H, H-4⁗), 3.93 (s, 2H, H-2⁗′, 3.74–3.70 (m, 2H, H_e-2′ & H_e-6′), 2.48–2.45 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7″), 2.30–2.26 (m, 2H, H_a-2′ & H_a-6′), 2.28 (s, 3H, CH_3-8⁗), 2.16 (s, 3H, CH_3-7⁗), 2.01–1.94 (m, 2H, H_e-3′ & H_e-5′), 1.83–1.80 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 575 $[M]^+$, 420 $[C_{23}H_{26}N_5OS]^+$, 311 $[C_{17}H_{17}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 220 $[C_{11}H_{12}N_2OS]^{\cdot+}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.6. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2,4-dimethylphenyl)acetamide (6c)

White amorphous solid; yield: 87%; mp: 181–183 °C; molecular formula: $C_{30}H_{33}N_5O_3S_2$; molecular weight: 575; IR (KBr, cm^{-1}) υ_{max}: 3439 (N-H stretching), 3067 (C-H stretching of aromatic ring), 2882 (CH_2 stretching), 1731 (C=O stretching), 1628 (C=N stretching), 1533 (C=C aromatic stretching), 1337 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.55 (s, 1H, N-H), 7.74 (d, J = 8.4 Hz, 1H, H-6′′′′), 7.58 (d, J = 7.8 Hz, 2H, H-2′′ & H-6′′), 7.55–7.51 (m, 3H, H-3′′′, H-4′′′ & H-5′′′), 7.27 (d, J = 7.8 Hz, 2H, H-3′′ & H-5′′), 7.18–7.16 (m, 2H, H-2′′′ & H-6′′′), 6.97 (d, J = 7.8 Hz, 1H, H-5′′′′), 6.96 (s, 1H, H-3′′′′), 3.91 (s, 2H, H-2′′′′′, 3.71–3.68 (m, 2H, H_e-2′ & H_e-6′), 2.50–2.46 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7′′), 2.33–2.30 (m, 2H, H_a-2′ & H_a-6′), 2.26 (s, 3H, CH_3-8′′′′), 2.21 (s, 3H, CH_3-7′′′′), 2.00–1.93 (m, 2H, H_e-3′ & H_e-5′), 1.83–1.80 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 575 $[M]^+$, 420 $[C_{23}H_{26}N_5OS]^+$, 311 $[C_{17}H_{17}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 220 $[C_{11}H_{12}N_2OS]^{\cdot+}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.7. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2,6-dimethylphenyl)acetamide (6d)

White amorphous solid; yield: 81%; mp: 189–191 °C; molecular formula: $C_{30}H_{33}N_5O_3S_2$; molecular weight: 575; IR (KBr, cm^{-1}) υ_{max}: 3431 (N-H stretching), 3060 (C-H stretching of aromatic ring), 2878 (CH_2 stretching), 1725 (C=O stretching), 1618 (C=N stretching), 1525 (C=C aromatic stretching), 1329 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.27 (s, 1H, N-H), 7.58 (d, J = 8.4 Hz, 2H, H-2′′ & H-6′′), 7.55–7.53 (m, 3H, H-3′′′, H-4′′′ & H-5′′′), 7.26 (d, J = 8.4 Hz, 2H, H-3′′ & H-5′′), 7.17 (dd, J = 1.2, 7.8 Hz, 2H, H-2′′′ & H-6′′′), 7.07–704 (m, 1H, H-4′′′′), 7.02 (d, J = 7.2 Hz, 2H, H-3′′′′ & H-5′′′′), 3.94 (s, 2H, H-2′′′′′, 3.70–3.68 (m, 2H, H_e-2′ & H_e-6′), 2.52–2.47 (m, 1H, H-4′), 2.38 (s, 3H, CH_3-7′′), 2.34–2.30 (m, 2H, H_a-2′ & H_a-6′), 2.13 (s, 6H, CH_3-7′′′′ & CH_3-8′′′′), 1.99–1.93 (m, 2H, H_e-3′ & H_e-5′), 1.83–1.80 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 575 $[M]^+$, 420 $[C_{23}H_{26}N_5OS]^+$, 311 $[C_{17}H_{17}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 220 $[C_{11}H_{12}N_2OS]^{\cdot+}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.8. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(3,5-dimethylphenyl)acetamide (6e)

White amorphous solid; yield: 84%; mp: 184–186 °C; molecular formula: $C_{30}H_{33}N_5O_3S_2$; molecular weight: 575; IR (KBr, cm^{-1}) υ_{max}: 3439 (N-H stretching), 3067 (C-H stretching of aromatic ring), 2885 (CH_2 stretching), 1734 (C=O stretching), 1628 (C=N stretching), 1533 (C=C aromatic stretching), 1343 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.97 (s, 1H, N-H), 7.59 (d, J = 8.4 Hz, 2H, H-2′′ & H-6′′), 7.55–7.50 (m, 3H, H-3′′′, H-4′′′ & H-5′′′), 7.27 (d, J = 8.4 Hz, 2H, H-3′′ & H-5′′), 7.21 (s, 2H, H-2′′′′ & H-6′′′′), 7.18–7.17 (m, 2H, H-2′′′ & H-6′′′), 6.72 (s, 1H, H-4′′′′), 3.83 (s, 2H, H-2′′′′′, 3.76–3.73 (m, 2H, H_e-2′ & H_e-6′), 2.49–2.44 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7′′), 2.27 (s, 6H, CH_3-7′′′′ & CH_3-8′′′′), 2.26–2.24 (m, 2H, H_a-2′ & H_a-6′), 2.03–1.97 (m, 2H, H_e-3′ & H_e-5′), 1.84–1.81 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 575 $[M]^+$, 420 $[C_{23}H_{26}N_5OS]^+$, 311 $[C_{17}H_{17}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 220 $[C_{11}H_{12}N_2OS]^{\cdot+}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.9. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2-ethyl-6-methylphenyl)acetamide (6f)

Light pink amorphous solid; yield: 85%; mp: 187–189 °C; molecular formula: $C_{31}H_{35}N_5O_3S_2$; molecular weight: 589; IR (KBr, cm^{-1}) υ_{max}: 3438 (N-H stretching), 3063 (C-H stretching of aromatic ring), 2881 (CH_2 stretching), 1735 (C=O stretching), 1627 (C=N stretching), 1537 (C=C aromatic stretching), 1342 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.28 (s, 1H, N-H), 7.58 (d, J = 8.4 Hz, 2H, H-2″ & H-6″), 7.57–7.54 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.26 (d, J = 7.8 Hz, 2H, H-3″ & H-5″), 7.18–7.16 (m, 2H, H-2‴ & H-6‴), 7.11 (t, J = 7.8 Hz, 1H, H-4⁗), 7.05–703 (m, 2H, H-3⁗ & H-5⁗), 3.95 (s, 2H, H-2⁗, 3.69–3.67 (m, 2H, H_e-2′ & H_e-6′), 2.51–2.47 (m, 3H, H-4′ & CH_2-7⁗, 2.38 (s, 3H, CH_3-7″), 2.35–2.31 (m, 2H, H_a-2′ & H_a-6′), 2.14 (s, 3H, CH_3-9⁗), 1.99–1.92 (m, 2H, H_e-3′ & H_e-5′), 1.83–1.80 (m, 2H, H_a-3′ & H_a-5′), 1.06 (t, J = 7.8 Hz, 3H, CH_3-8⁗); EIMS (m/z): 589 $[M]^+$, 434 $[C_{24}H_{28}N_5OS]^+$, 325 $[C_{18}H_{19}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 234 $[C_{12}H_{14}N_2OS]^{\cdot+}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.10. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2-methyl-6-nitrophenyl)acetamide (6g)

Light yellow amorphous solid; yield: 88%; mp: 215–217 °C; molecular formula: $C_{29}H_{30}N_6O_5S_2$; molecular weight: 606; IR (KBr, cm^{-1}) υ_{max}: 3435 (N-H stretching), 3064 (C-H stretching of aromatic ring), 2882 (CH_2 stretching), 1731 (C=O stretching), 1624 (C=N stretching), 1556 (-NO_2 stretching), 1532 (C=C aromatic stretching), 1345 (-NO_2 stretching), 1335 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.97 (s, 1H, N-H), 7.70 (dd, J = 1.2, 8.4 Hz, 1H, H-5⁗), 7.58 (d, J = 8.4 Hz, 2H, H-2″ & H-6″), 7.55–7.54 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.45 (dd, J = 1.2, 8.4 Hz, 1H, H-3⁗), 7.29–7.23 (m, 5H, H-3″, H-5″, H-2‴, H-6‴ & H-4⁗), 3.97 (s, 2H, H-2⁗, 3.71–3.68 (m, 2H, H_e-2′ & H_e-6′), 2.54–2.49 (m, 1H, H-4′), 2.38 (s, 3H, CH_3-7″), 2.35–2.32 (m, 2H, H_a-2′ & H_a-6′), 2.31 (s, 3H, CH_3-7⁗), 2.02–1.96 (m, 2H, H_e-3′ & H_e-5′), 1.86–1.83 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 606 $[M]^+$, 451 $[C_{22}H_{23}N_6O_3S]^+$, 342 $[C_{16}H_{14}N_4O_3S]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 251 $[C_{10}H_9N_3O_3S]^{\cdot+}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.11. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2-ethylphenyl)acetamide (6h)

Light pink amorphous solid; yield: 89%; mp: 194–196 °C; molecular formula: $C_{30}H_{33}N_5O_3S_2$; molecular weight: 575; IR (KBr, cm^{-1}) υ_{max}: 3446 (N-H stretching), 3071 (C-H stretching of aromatic ring), 2879 (CH_2 stretching), 1728 (C=O stretching), 1626 (C=N stretching), 1537 (C=C aromatic stretching), 1345 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.62 (s, 1H, N-H), 7.86 (d, J = 8.4 Hz, 1H, H-6⁗), 7.59 (d, J = 8.4 Hz, 2H, H-2″ & H-6″), 7.55–7.51 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.28 (d, J = 8.4 Hz, 2H, H-3″ & H-5″), 7.18–7.16 (m, 4H, H-2‴, H-6‴, H-3⁗ & H-5⁗), 7.08 (dt, J = 1.2, 7.8 Hz, 1H, H-4⁗), 3.94 (s, 2H, H-2⁗, 3.71–3.68 (m, 2H, H_e-2′ & H_e-6′), 2.58 (q, J = 7.8 Hz, 2H, H-7⁗), 2.52–2.48 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7″), 2.36–2.32 (m, 2H, H_a-2′ & H_a-6′), 2.01–1.94 (m, 2H, H_e-3′ & H_e-5′), 1.83–1.80 (m, 2H, H_a-3′ & H_a-5′), 1.10 (t, J = 7.8 Hz, 3H, CH_3-8⁗); EIMS (m/z): 575 $[M]^+$, 420 $[C_{23}H_{26}N_5OS]^+$,

311 $[C_{17}H_{17}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 220 $[C_{11}H_{12}N_2OS]^{\cdot +}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.12. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-ethylphenyl)acetamide (6i)

White amorphous solid; yield: 86%; mp: 193–195 °C; molecular formula: $C_{30}H_{33}N_5O_3S_2$; molecular weight: 575; IR (KBr, cm^{-1}) υ_{max}: 3428 (N-H stretching), 3071 (C-H stretching of aromatic ring), 2874 (CH_2 stretching), 1746 (C=O stretching), 1632 (C=N stretching), 1526 (C=C aromatic stretching), 1345 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 10.09 (s, 1H, N-H), 7.59 (d, J = 8.4 Hz, 2H, H-2″ & H-6″), 7.54–7.49 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.49 (d, J = 8.4 Hz, 2H, H-2⁗ & H-6⁗), 7.28 (d, J = 8.4 Hz, 2H, H-3″ & H-5″), 7.18–7.16 (m, 2H, H-2‴ & H-6‴), 7.12 (d, J = 8.4 Hz, 2H, H-3⁗ & H-5⁗), 3.83 (s, 2H, H-2⁗⁗, 3.74–3.72 (m, 2H, H_e-2′ & H_e-6′), 2.57 (q, J = 7.8 Hz, 2H, H-7⁗), 2.48–2.44 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7″), 2.29–2.25 (m, 2H, H_a-2′ & H_a-6′), 2.02–1.95 (m, 2H, H_e-3′ & H_e-5′), 1.83–1.81 (m, 2H, H_a-3′ & H_a-5′), 1.18 (t, J = 7.8 Hz, 3H, CH_3-8⁗); EIMS (m/z): 575 $[M]^+$, 420 $[C_{23}H_{26}N_5OS]^+$, 311 $[C_{17}H_{17}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 220 $[C_{11}H_{12}N_2OS]^{\cdot +}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.13. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(2-ethoxyphenyl)acetamide (6j)

Light pink solid; yield: 77%; mp: 211–213 °C; molecular formula: $C_{30}H_{33}N_5O_4S_2$; molecular weight: 591; IR (KBr, cm^{-1}) υ_{max}: 3443 (N-H stretching), 3072 (C-H stretching of aromatic ring), 2890 (CH_2 stretching), 1742 (C=O stretching), 1633 (C=N stretching), 1532 (C=C aromatic stretching), 1337 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 9.66 (s, 1H, N-H), 8.31 (d, J = 7.8 Hz, 1H, H-6⁗), 7.58 (d, J = 7.8 Hz, 2H, H-2″ & H-6″), 7.52–7.48 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.27 (d, J = 7.8 Hz, 2H, H-3″ & H-5″), 7.17–7.16 (m, 2H, H-2‴ & H-6‴), 6.99 (dt, J = 1.2, 7.8 Hz, 1H, H-5⁗), 6.89 (dt, J = 1.2, 7.2 Hz, 1H, H-4⁗), 6.84 (dd, J = 1.2, 8.4 Hz, 1H, H-3⁗), 4.09 (q, J = 7.2 Hz, 2H, H-7⁗), 3.98 (s, 2H, H-2⁗⁗, 3.72–3.69 (m, 2H, H_e-2′ & H_e-6′), 2.48–2.44 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7″), 2.32–2.28 (m, 2H, H_a-2′ & H_a-6′), 2.01–1.95 (m, 2H, H_e-3′ & H_e-5′), 1.83–1.79 (m, 2H, H_a-3′ & H_a-5′), 1.49 (t, J = 7.2 Hz, 3H, CH_3-8⁗); EIMS (m/z): 591 $[M]^+$, 436 $[C_{23}H_{26}N_5O_2S]^+$, 327 $[C_{17}H_{17}N_3O_2S]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 236 $[C_{11}H_{12}N_2O_2S]^{\cdot +}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.7.14. 2-(4-Phenyl-5-(4-methylphenylsulfonylpiperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-benzylacetamide (6k)

White amorphous solid; yield: 76%; mp: 179–181 °C; molecular formula: $C_{29}H_{31}N_5O_3S_2$; molecular weight: 561; IR (KBr, cm^{-1}) υ_{max}: 3439 (N-H stretching), 3068 (C-H stretching of aromatic ring), 2886 (CH_2 stretching), 1733 (C=O stretching), 1626 (C=N stretching), 1534 (C=C aromatic stretching), 1337 (-SO_2- stretching); ^{1}H NMR (600 MHz, $CDCl_3$, δ / ppm): 8.06 (s, 1H, N-H), 7.58 (d, J = 8.4 Hz, 2H, H-2″ & H-6″), 7.53–7.49 (m, 3H, H-3‴, H-4‴ & H-5‴), 7.28–7.25 (m, 4H, H-3″, H-5″, H-2‴ & H-6‴), 7.23–7.21 (m, 3H, H-3⁗, H-4⁗ & H-5⁗), 7.13–7.11 (m, 2H, H-2⁗ & H-6⁗), 4.42 (d, J = 6.0 Hz, 2H, H-7⁗), 3.77 (s, 2H, H-2⁗⁗, 3.71–3.68 (m, 2H, H_e-2′ & H_e-6′), 2.46–2.43 (m, 1H, H-4′), 2.39 (s, 3H, CH_3-7″), 2.31–2.27 (m,

2H, H_a-2′ & H_a-6′), 1.97–1.91 (m, 2H, H_e-3′ & H_e-5′), 1.81–1.78 (m, 2H, H_a-3′ & H_a-5′); EIMS (m/z): 561 $[M]^+$, 406 $[C_{22}H_{24}N_5OS]^+$, 297 $[C_{16}H_{15}N_3OS]^+$, 264 $[C_{13}H_{16}N_2O_2S]^+$, 206 $[C_{10}H_{10}N_2OS]^{\cdot +}$, 155 $[C_7H_7O_2S]^+$, 109 $[C_6H_9N_2]^+$, 91 $[C_7H_7]^+$, 82 $[C_5H_8N]^+$, 77 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

3.8. Biological screening and docking studies

3.8.1. Antibacterial activity assay

Synthetic compounds **6a**–**6k** were screened for antibacterial activity using 96-well microplates under aseptic conditions. There is a proportional increase in the absorbance and increase in the bacterial growth. This relationship was used as a principle in this method.[41] Five bacteria were included in the study, three gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). An appropriate dilution of each was added to the well (20 μ L well^{-1}). Fresh bacterial culture, maintained overnight and diluted with nutrient broth, was added to the wells (180 μ L well^{-1}). The volume of each well was maintained at 200 μ L well^{-1}. The initial absorbance of culture was maintained in the range of 0.12–0.19. The incubation was processed at 37 °C for 24 h. The lids of microplates were kept closed during the incubation period. A microplate reader was used to take the initial and final absorbances. Percent inhibition was calculated using the formula given below.

$$\text{Inhibition } (\%) = \frac{Control - Test}{Control} \times 100$$

'Control' is the absorbance of only the bacterial culture and 'Test' is the absorbance of the bacterial culture containing the test sample. MIC was computed with suitable dilutions (5–30 μ g/well) for each sample. Ciprofloxacin was used as a reference standard.

3.8.2. LOX inhibition assay

Lipoxygenase inhibition activity of synthesized compounds was evaluated using a previously reported assay.[42] The change in absorbance at 234 nm was taken as the index of lipoxygenase inhibition. The positive control and reference standard used was baicalein.

3.8.3. Molecular docking protocol

For the bioactive conformations, five compounds with the highest anti-LOX potential, **6b**, **6c**, **6h**, **6i**, and **6k**, were docked into the active pocket of LOX using the default parameters of the MOE-Dock program. Before docking of these ligands, ChemDraw Ultra 12.0 was used to draw the structures of synthesized compounds. These were saved in .mol files and reopened in MOE 2009–2010 software. Energy minimization was preceded up to 0.05 gradients by using the MMFF94x force field through the default parameter of the MOE energy minimization algorithm. A database was created in which all the compounds were saved in their 3D structures in the .mdb file format. The protein molecule of LOX (PDB code: 1IK3) was retrieved from the Protein Data Bank. All the water molecules were released from the receptor protein and 3D protonation was carried out using the Protonate 3D option. The energy of the protein molecule was minimized by the default parameters of the MOE 2009–2010 energy minimization algorithm (gradient: 0.05, force field: MMFF94x). Finally, all the compounds were docked into the binding pocket of the enzyme. A redocking procedure was also applied to confirm the validity of the docking protocol.[43] After docking with 30 conformations of each compound, the

best 2D images were selected for specific types of interactions and their 3D images were drawn along with their bond lengths.

3.8.4. Statistical analysis

The results are presented as mean ± SEM after three independent experiments and statistical analysis was performed with Microsoft Excel 2010. The MIC and IC_{50} values were calculated using EZ-Fit software (Perrella Scientific Inc., Amherst, NH, USA).

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