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Synthesis, antimicrobial evaluation, and molecular modeling studies of thiazole-based derivatives

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Abstract: Different series of thiazole-based derivatives were prepared in this work including thiazolidinone, thiourea, amide, sulfonamide, imidazothiazole, and thiazolopyrimidine derivatives. They were tested for their antimicrobial activity against the G+ve bacteria *Staphylococcus aureas*, and the G-ve bacteria *Escherichia coli* and *Klebsiella pneumoniae* utilizing ampicillin and gentamicin as reference antibacterial drugs. In addition, their antifungal activity was assessed against *Candida albicans* utilizing fluconazole as a standard antifungal drug. The most active compounds in the antimicrobial evaluation were further subjected to enzyme assay and the results revealed that the thiourea derivative **35**, and the 4-nitrobenzamide **38** were the most potent inhibitors to DNA gyrase and topoisomerase IV. They exhibited IC_{50} values of 25.7 and 30.4 µM respectively against DNA gyrase and topoisomerase IV in comparison with 24.5 and 24.4 µM for ciprofloxacin as a standard drug. Molecular modeling studies were also carried out for the prepared compounds, including docking into the studied enzymes active site. Results explained the superior binding of compounds **35** and **38** with the corresponding enzymes.

keywords: Thiazole; Synthesis; Antibacterial; Antifungal; Molecular modeling

1.Introduction

Antimicrobial resistance is continuing to be one of the major health problems that can threat the fate of mankind [1]. The urgency of the problem has led to an impressive progress in designing antimicrobial drugs that have broader spectra and safer therapeutic profiles than the currently used ones.

In the context of identifying various chemical substances that can serve as a lead for designing new antimicrobial agents, the present study was particularly interested in thiazole derivatives. Thiazoles have long been an attractive and inspiring source for medicinal chemists. They are incorporated into the β-lactam ring system of penicillins, the first discovered antibiotics which paved the way to fight bacterial diseases [1]. Thiazoles are also found in many clinically useful antimicrobial drugs, such as sulfathiazole and abafungin as antibacterial and antifungal drugs respectively [2].

The present work objective was to synthesize a novel series of *N*-(substituted) phenylthiazol-4-amine derivatives bearing thiazolidinone, thiourea, amide or sulfonamide motifs, in addition to new hybrid compounds of *N*-phenylthiazol-4-amine/ imidazole or *N*-phenylthiazol-4-amine/ pyrimidin-5-one that might work by different or dual mechanisms to lessen the possibility of microbial resistance. The newly synthesized compounds were tested *in vitro* for their antimicrobial activity, and the most active compounds were further tested for their DNA gyrase, topoisomerase IV and dihydrofolate reductase inhibition activity. Molecular modeling studies were also

conducted to explain the mode of binding of the most active antibacterial agents with the studied enzymes.

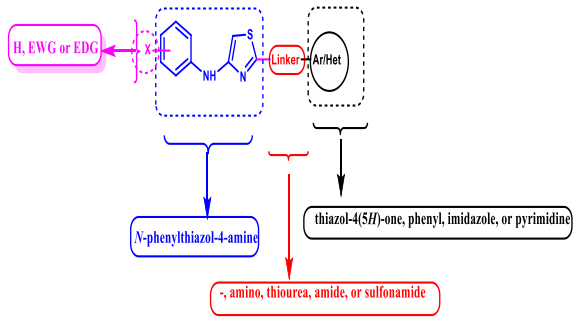


Fig. 1: Design of hybrid target compounds.

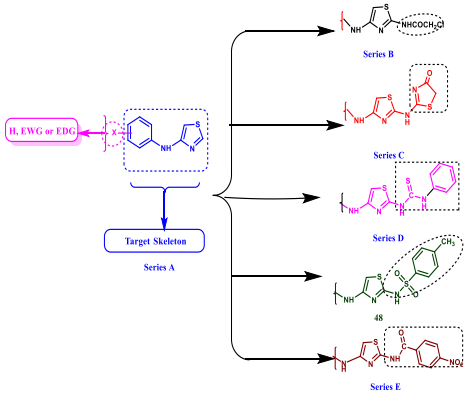


Fig. 2: Design of hybrid compounds of *N*-(substituted) phenylthiazole-4-amine with other functional groups or heterocyclic rings.

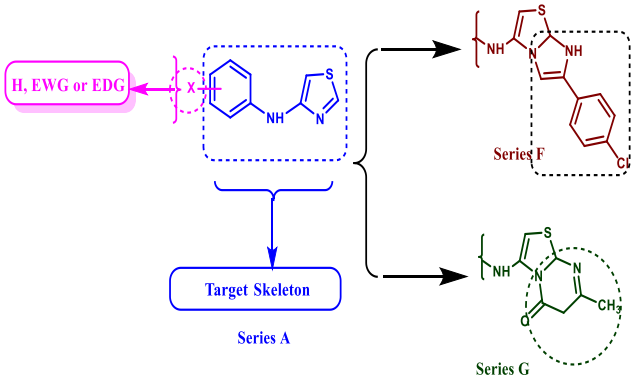


Fig. 3: Design of *N*-(substituted) phenylthiazole-4-amine fused with other heterocyclic rings.

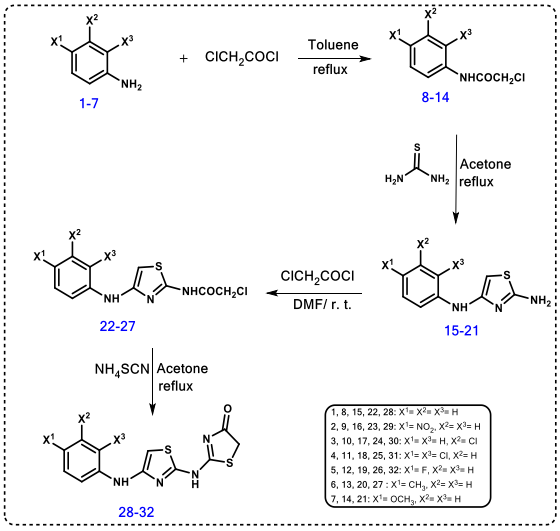
Results and discussion

2.1. Chemistry

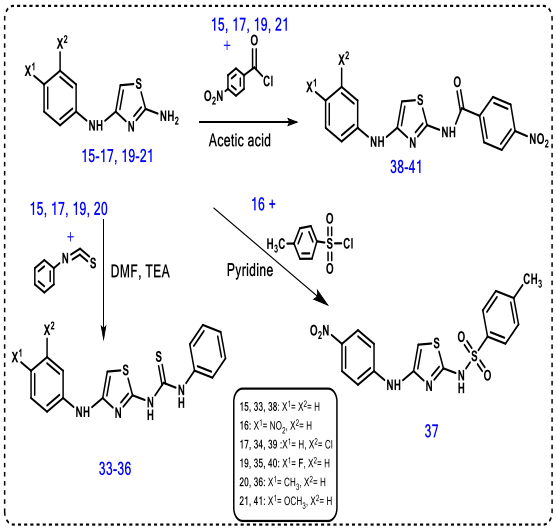
The designed target compounds were synthesized as outlined in schemes **1-3**. In scheme **1**, the appropriate aniline derivative (**1-7**) reacted with chloroacetyl chloride in toluene to afford the corresponding chloroacetamide derivatives **8-14**. Cyclization of the latter compounds with thiourea was achieved in refluxing acetone yielding the corresponding 2-aminothiazole compounds **15-21**. The ^{1}H-NMR spectra of the newly synthesized N^{4}-phenylthiazole-2,4-diamine derivatives confirmed the presence of aminothiazole functionality through two singlet peaks at δ 7.89-11.59 and 7.33-9.40 ppm representing one proton of NH and the two protons of NH_2 respectively. Acylation of the 2-aminothiazoles with chloroacetyl chloride was conducted in DMF at room temperature. The appearance of singlet signals at δ 3.47-4.29 ppm representing the two aliphatic protons of CH_2 and the C=O signal at δ 167.9-173.3 ppm in their ^{1}H-NMR and ^{13}C-NMR spectra respectively proved the acylation reaction. In addition, reacting the chloroacetamides **22-27** with ammonium thiocyanate in refluxing acetone yielded the corresponding 2-aminothiazol-4-ones **28-32**.

Scheme **2** outlines the synthesis of groups of derivatives utilizing N^{4}-(substituted) phenylthiazole-2,4-diamine as a key compound. The thiourea derivatives **33-36** were obtained through reacting **15, 17, 19, 20** with phenylisothiocyanate in DMF using triethylamine as a catalyst. The NH included between the two rings did not get involved in the reaction according to the obtained ^{1}H-NMR data which showed a singlet proton at δ 8.7-8.9 ppm. This may be due to the steric hindrance around NH group of these compounds, and the use of 1:1 molar ratios of both the starting material and the reagents. Other spectral data were also consistent with the assigned structures. IR spectra showed bands at 1220-1273 cm^{-1} representing the C=S groups. ^{1}H-NMR spectra revealed two singlet protons of chemical shift δ 9.09-10.61 ppm corresponding to the two NH protons of the thiourea moiety (NHCSNH). ^{13}C-NMR spectra displayed the presence of C=S signal at chemical shift δ 180.0-187.5 ppm. The sulfonamide derivative **37** was obtained through the interaction of compound **16** with tosyl chloride in pyridine. The ^{1}H-NMR and ^{13}C-NMR spectra were supportive for the assigned structure and confirmed the presence of CH_3 group where it appeared as singlet protons of chemical shift δ 2.52 ppm and at δ 39.3 ppm, respectively. Nucleophilic substitution of the aminothiazoles **15, 17, 19, 21** with *p*-nitrobenzoyl chloride in refluxing glacial acetic acid using anhydrous sodium acetate as a catalyst yielded the

corresponding benzamides **38-41**. The spectral data of the new compounds agreed with their assigned structures, where the notable feature was the appearance of a characteristic signal at δ 166.2-188.2 ppm corresponding to the CONH carbon in their ^{13}C-NMR spectra.

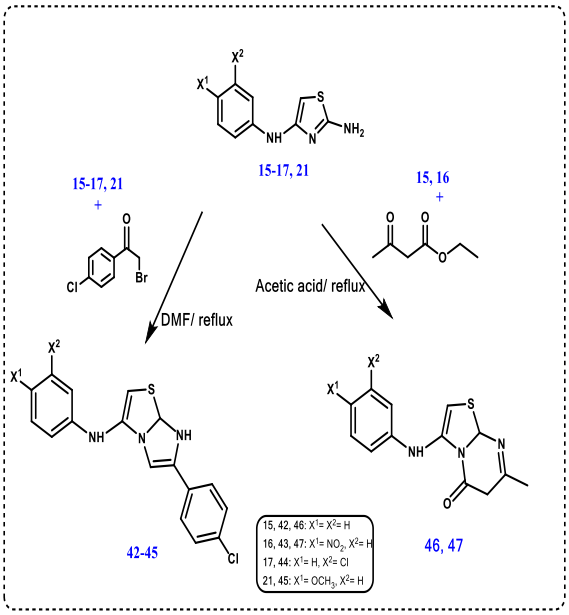


Scheme 1: Synthesis of compounds **15-32**.



Scheme2: Synthesis of compounds **33-41**.

Scheme **3** illustrates the synthesis of imidazo[2,1-*b*]thiazoles **42-45** through the interaction of *N*^{4}-phenylthiazole-2, 4-diamine **15-17, 21** in DMF with 2-bromo-1-(4-chlorophenyl)ethan-1-one in presence of TEA. The ^{1}H-NMR spectra proved the suggested structure and confirmed the presence of imidazole moiety, where the imidazole CH appeared as a singlet signal at δ 3.54-4.79 ppm. Finally, the thiazolo[3,2-*d*]primidin-5-ones **46** and **47** were obtained through refluxing a mixture of ethyl acetoacetate and *N*^{4}-phenylthiazole-2,4-diamine **15**, **16** in glacial acetic acid in presence of sodium acetate. The CH_3 group appeared as singlet signals at δ 1.93-2.50 and 24.7 ppm in the ^{1}H-NMR and ^{13}C-NMR spectra respectively. Meanwhile, the carbonyl carbon atom in the pyrimidin-5-one ring appeared as a characteristic signal at δ 183.3 ppm in the ^{13}C-NMR spectra.



Scheme3: Synthesis of compounds **42-47**.

2.2. Biological evaluation

2.2.1. In vitro antimicrobial activity

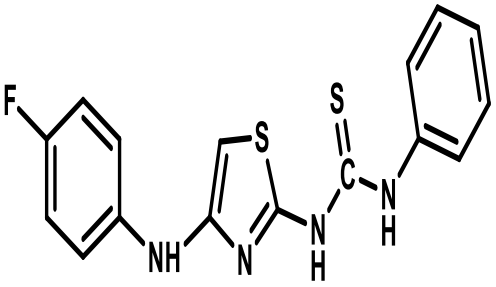
Thirty-three newly synthesized aminothiazole derivatives were determined for *in vitro* antimicrobial efficacy toward G+ve bacteria *Staphylococcus aureus* Newman, and G-ve bacteria; the Egyptian clinical isolates of *Escherichia coli* and *Klebsiella pneumonia*, using ampicillin and gentamicin as reference antibacterial drugs. Antifungal assay against *Candida albicans* Sc5314 was also performed using fluconazole as a standard drug. Inhibition zone diameters (mm) and minimal inhibitory concentrations (MICs, µg/mL) of the active compounds against the selected bacterial and fungal strains were determined and provided in Tables 1 and 2.

The obtained results demonstrated different degrees of activity against the tested microbial strains. Compounds **23, 24** and **27** exhibited antibacterial activity against the G+ve strains**,** while compounds **38-41** showed antibacterial activity against G-ve bacterial strains. None of the tested compounds showed antifungal activity against *Candida albicans* except compounds **23** and **24**.

Table 1. Susceptibility of microbial strains to the tested compounds.

Compound No.	Diameter of inhibition zone (mm)			
	S. aureus Newman	*K. pneumonia*	*E. coli*	*C. albicans* Sc5314
15	*6.5*	*6*	-	-
16	*6*	-	*7*	*6*
17	-	-	-	-
18	-	-	-	-
19	*7*	*6*	*8*	*7*
20	-	*6*	-	-
21	-	-	-	-
22	*7*	*6*	-	*8*
23	*16*	-	-	*18*
24	*17*	*6*	*7*	*17*
25	-	*6*	-	-
26	*6*	-	*7*	*8*
27	*14*	-	*7*	*10*
28	-	-	-	-
29	-	-	-	-
30	-	-	-	-
31	-	-	-	-
32	-	-	-	-
33	*11*	-	-	-
34	-	-	-	-
35	*8,5*	-	-	-
36	*7*	-	-	-
37	*8*	*9*	*11*	-
38	*7*	*12*	*14*	*7*
39	*7*	*12*	*14*	*8*
40	*7*	*12*	*15*	*7*
41	-	*12*	*14*	*8*
42	*9*	-	-	-
43	*11*	-	-	-
44	-	-	-	-
45	*8*	-	-	-
46	-	-	-	-
47	-	-	*7*	-
Amipcillin	21	18	25	ND [(a)]
Gentamicin	31	26	25	ND [(a)]
Fluconazole	ND [(a)]	ND [(a)]	ND [(a)]	23
DMSO	-	-	-	-

[a] ND, not done.



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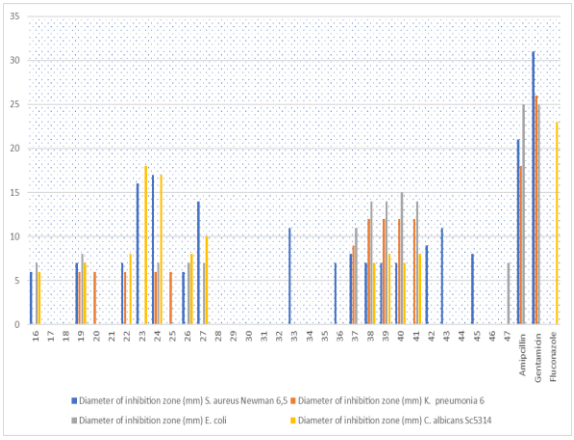


Fig. 4: Antimicrobial screening results of the tested compounds.

Table 2. MICs of the most active compounds in µg/ml.

Compound No.	Minimum inhibitory concentartion (MIC, µg/ml)			
	S. aureus Newman	*K. pneumonia*	*E. coli*	*C. albicans Sc5314*
23	555	ND [(a)]	ND [(a)]	185
24	185	ND [(a)]	ND [(a)]	185
27	1666	ND [(a)]	ND [(a)]	ND [(a)]
38	ND [(a)]	1500	1500	ND [(a)]
39	ND [(a)]	>5000	5000	ND [(a)]
40	ND [(a)]	5000	1500	ND [(a)]
41	ND [(a)]	>5000	5000	ND [(a)]
Amipicillin	555	166	3	ND [(a)]
Gentamicin	0.13	0.5	0.5	ND [(a)]
Fluconazole	ND [(a)]	ND [(a)]	ND [(a)]	5

[a] ND, not done.

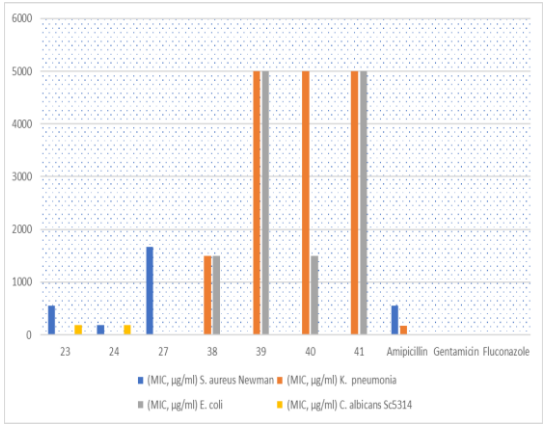


Fig. 5: MICs of the most active compounds in µg/ml.

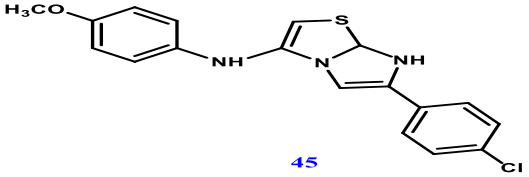
2.2.2. DNA gyrase and topoisomerase IV activity assay

DNA gyrase and topoisomerase IV are type II topoisomerases found in most bacteria. They play an important role in DNA replication,

repair and decatenation. Gyrase plays four roles in chromosome function. It maintains a particular level of negative supercoiling in DNA in front of the replication fork and thereby relieves torsional strain during replication, provides the genetic apparatus with a way to sense some types of environmental change (as the level of supercoiling depends on the alterations in the extracellular environment), removes knots from DNA, and helps in the process of bending and folding of DNA. Topoisomerase IV is a homolog of gyrase discovered by Kato *et al.* in 1990 which is involved in the control of DNA supercoiling and in the decatenation of daughter chromosomes after DNA replication. Although there is a potential for bacterial resistance development, DNA gyrase and topoisomerase IV will stay as attractive targets in antibacterial agents discovery as a result of their dual targeting potential [3,4].

Ciprofloxacin (used as a reference drug in the *in vitro* enzyme inhibition antimicrobial assay of the newly synthesized compounds) is a fluoroquinolone antibacterial drug that inhibits both DNA gyrase and topoisomerase IV. In an attempt to determine the mode of antibacterial action of the newly synthesized compounds, four compounds; **24**, **35**, **38**, **45**, from different chemical classes, were chosen and evaluated for their inhibition of DNA gyrase and topoisomerase IV. IC_{50} (µM) and percentages of enzyme inhibition were determined at serial dilutions of the examined compounds and provided in Table 3. Results showed that the examined compounds had a dual inhibitory activity against DNA gyrase and topoisomerase IV with IC_{50} values ranging from 25.7-45.4 µM and 30.43-45.31 µM, respectively.

Concerning the activity against DNA gyrase of *E. coli*, both compounds **35** and **45** showed higher percentage inhibition of 83 and 81 (at log conc. 2), respectively than that of reference drug ciprofloxacin (80%). The IC_{50} values of compounds **35** and **45** were 25.7 and 27.7 µM, respectively which were comparable with that of the reference drug; 24.5 µM.



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Regarding the activity against topoisomerase IV of *S. aureus*, compound **38** proved to be the most potent inhibitor showing % inhibition= 84.5 and IC_{50} = 30.43 µM which were close to the activity of the standard drug ciprofloxacin (% inhibition= 84.5 and IC_{50}: 24.46 µM). These results agreed with the obtained *in vitro* antimicrobial results and revealed the importance of 4-nitrobenzamide ring in increasing the antibacterial activity and spectrum

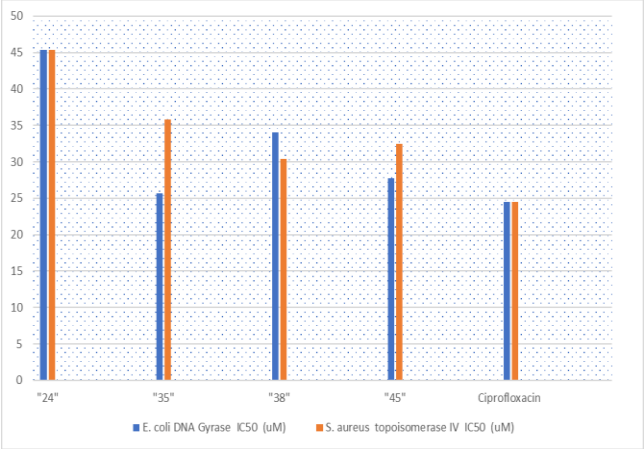


Fig. 6. IC_{50} of the tested compounds and ciprofloxacin against *E. coli* DNA gyrase and topoisomerase IV

2.3. Molecular docking studies

Molecular docking is used to assess the binding affinities of small to medium-sized ligands to a given macromolecular target, probably a protein. In the present study, molecular docking was performed for compounds **24, 35, 38** and **45** along with ciprofloxacin (positive control in boliogical study) to explore their binding affinity to the ATP binding sites in GyrB/ParA catalytic subunits of the two bacterial enzymes, *E. coli* DNA gyrase and *S. aureus* topoisomerase IV, respectively. Validation of the docking methodology is accomplished through re-docking of the original ligand co-crystallized in the active site of *E. coli* DNA gyrase (Gyr B) enzyme (reference ligand). Ciprofloxacin tends to bind with docking score = -6.86 Kcal/mol and RMSD= 0.81 *via* H-bonding with Arg136, Gly101 and HOH 541, 702, ionic interaction with Arg76, and Arg136, and lipophilic interaction with the amino acid residues lining the active site.

2.3.1. Docking into DNA gyrase

The binding affinity of the synthesized compounds resembled that of ciprofloxacin.

The interacting amino acid residues and docking scores were detailed in Table 4. Compound **35** showed the highest binding affinity to DNA gyrase *via* H-acceptor with Gly101 with distance 3.63 and 3.59 Å and energy -0.6 Kcal/mol and pi-H with Lys103 with distance 4.44 and 4.39 Å and energy -0.6 and -0.9 Kcal/mol, while compound **38** was the lowest in its binding affinity (Figures 7-9).

Table 3. Percentages of enzyme inhibition of serial dilutions of the examined compounds against DNA gyrase and topoisomerase IV and their IC_{50}.

Cpd. No.	*E. coli* DNA gyrase			*S. aureus* topoisomerase IV		
	Log conc.	% Inhibition	IC50 (uM)	Log conc.	% Inhibition	IC50 (uM)
24	2	73	45.4	2	72.3	45.31
	1.7	49		1.7	50.8	
	1.4	33		1.4	31.6	
	1.1	19		1.1	20.1	
35	2	83	25.7	2	76.3	35.76
	1.7	69		1.7	58.7	
	1.4	49		1.4	36.7	
	1.1	32		1.1	27.2	
38	2	75	34.0	2	84.5	30.43
	1.7	61		1.7	61.7	
	1.4	42		1.4	39.9	
	1.1	26		1.1	30.9	
45	2	81	27.7	2	82.5	32.43
	1.7	67		1.7	59.7	
	1.4	47		1.4	37.9	
	1.1	30		1.1	28.9	
Ciprofloxacin	2	80	24.5	2	84.5	24.46
	1.7	61		1.7	71.9	
	1.4	42		1.4	52.6	
	1.1	26		1.1	30	

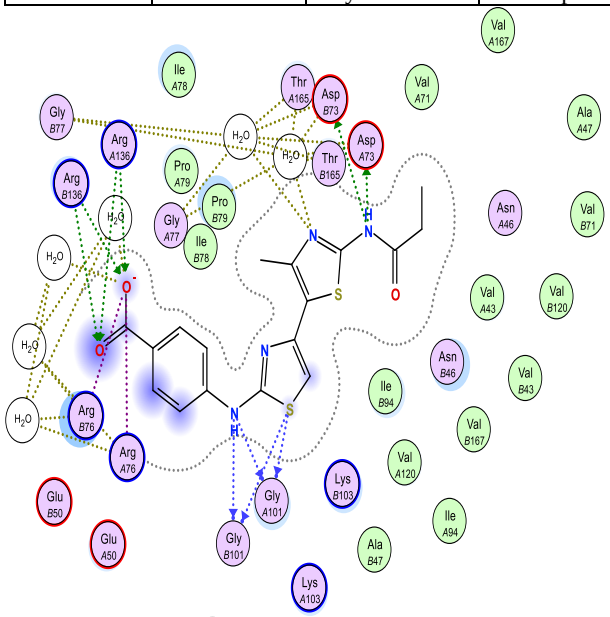
Table 4. The mode of binding of tested compounds within the ATP binding site of *E. coli* DNA gyrase enzyme (GyrB).[a]

Compound	Fragment	Target residue	Interaction	Distance (Å)	Binding energy Kcal/mol	Docking score Kcal/mol
24	N	Asp73	H-donor	2.72	-10.7	-7.65
	N	Asp73	H-donor	2.78	-9.1	
	C	Asp73	H-donor	3.32	-0.5	
	6-ring	Lys103	pi-H	3.56	-0.7	
	6-ring	Lys103	pi-H	3.56	-0.7	
35	S	Gly101	H-acceptor	3.63	-0.6	- 7.90
	S	Gly101	H-acceptor	3.59	-0.6	
	6-ring	Lys103	pi-H	4.44	-0.9	
	6-ring	Lys103	pi-H	4.39	-0.6	
38	N	Gly101	H-donor	3.12	-1.6	-7.28
	N	Gly101	H-donor	3.05	-2.1	
	C	Asp73	H-donor	3.00	-1.1	
	6-ring	Asn46	pi-H	3.77	-0.6	
	5-ring	Pro79	pi-H	3.74	-0.6	
	6-ring	HOH614	pi-H	4.71	-0.5	
	6-ring	Asn46	pi-H	3.72	-0.7	
45	N	Gly101	H-donor	3.14	-0.5	-7.69
	N	Gly101	H-donor	3.14	-0.5	
	5-ring	Pro79	pi-H	4.04	-0.6	
	5-ring	Pro79	pi-H	4.04	-0.6	
Ciprofl-oxacin	C	Gly101	H-donor	3.28	-0.5	-7.44
	C	HOH541	H-donor	2.93	-0.7	
	N	HOH541	H-acceptor	2.66	-3.2	
	O	Arg136	H-acceptor	2.93	-7.1	
	O	Arg136	H-acceptor	3.46	-1.5	

[a]Data presented in the table were obtained from MOE program illustrating the amino acids residues in the enzyme pocket, corresponding fragments of ligands, interaction distances, types of interaction, and their binding energy of tested compounds.

Table 5. The mode of binding of tested compounds within the ATP binding site of *S. aureus* topoisomerase IV (Par E).[a]

Compound	Fragment	Targetresidue	Interaction	Distance(Å)	Binding energyKcal/mol	Docking scoreKcal/mol
24	C	Asp76	H-donor	3.13	-0.5	-6.35
35	6-ring	Asp52	pi-H	3.43	-0.5	-6.52
	6-ring	Asp52	pi-H	3.43	-0.5	
38	C	Asp76	H-donor	3.47	-1.0	-6.52
	C	Asp76	H-donor	3.55	-0.8	
45	S- thiazole	Gly103	H-donor	3.58	-0.7	-6.68
	S-thiazole	Gly103	H-donor	3.58	-0.7	
	6-ring	Asp52	pi-H	3.58	-0.5	
Ciprof-loxacin	O	Ala122	H-acceptor	3.12	-4.9	-5.43
	O	Ala122	H-acceptor	3.12	-4.9	
	O	Asn49	H-acceptor	3.04	-1.8	
	O	Gly121	H-acceptor	3.35	-1.4	
	O	Asn49	H-acceptor	3.08	-1.2	
	O	Gly121	H-acceptor	3.35	-1.4	



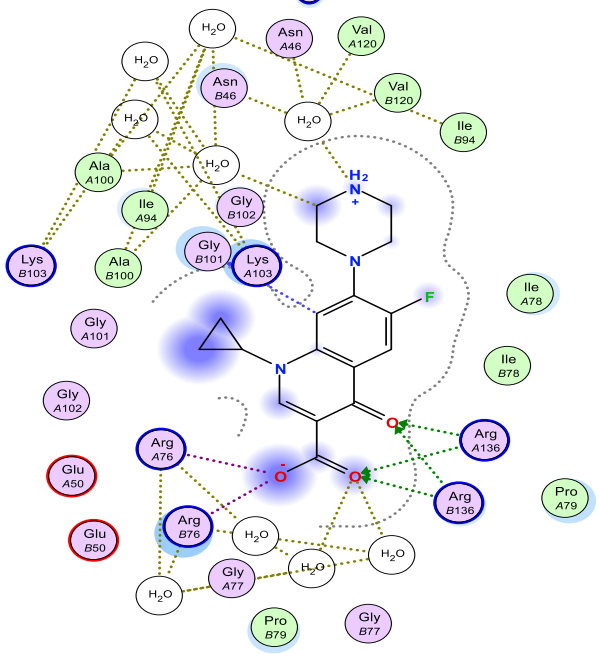


Fig. 7: Ciprofloxacin and reference ligand (v docking score = -6.86 Kcal/mol, RMSD = 0. docked inside the active site of *E. coli* DNA gyr (Gyr B) enzyme.

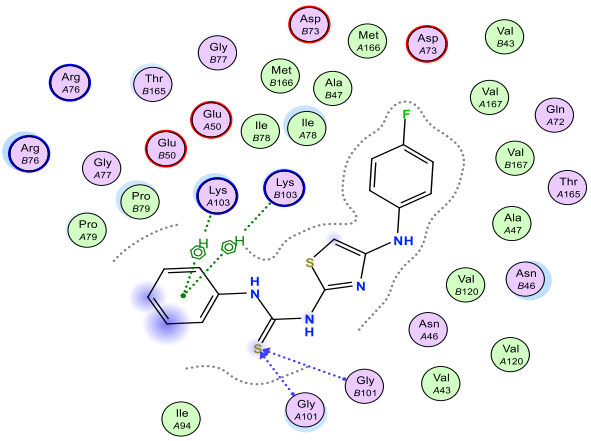


Fig. 8: The most active compound **35** docked inside the active site of *E. coli* DNA gyrase (Gyr B) enzyme.

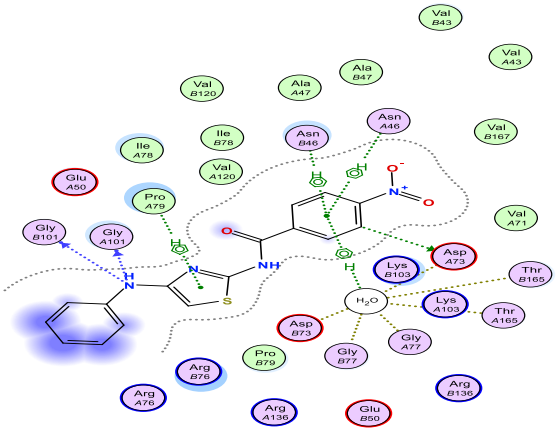


Fig. 9: The least active compound **38** docked ins the active site of *E. coli* DNA gyras (Gyr B) enzy

2.3.2. Docking into topoisomerase IV

Docking was also accomplished into the ATP binding site of *S. aureus* topoisomerase IV (Par E). The sort and pattern of interaction alongside the interacting amino acid residues are shown in Table 5 and Figures 10-12.

[a]Data presented in the table were obtained from MOE program illustrating the amino acids residues in the enzyme pocket, corresponding fragments of ligands, interaction distances, types of interaction, and their binding energy of tested compounds.

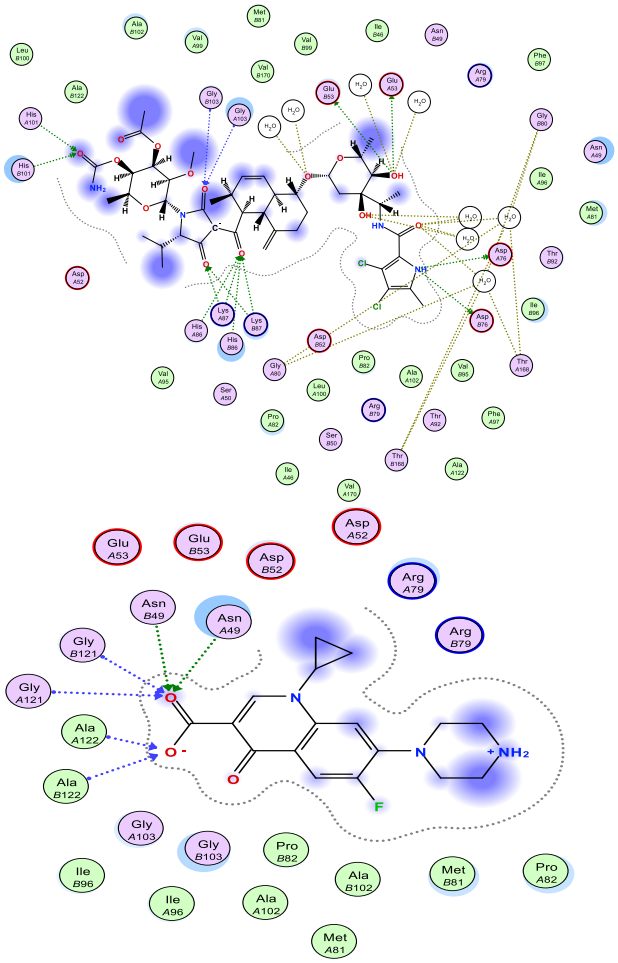


Fig. 10. Ciprofloxacin and reference ligand (with docking score = -10.5 Kcal/mol, RMSD = 1.97) docked inside the active site of *S. aureus* topoisomerase IV (ParE) enzyme.

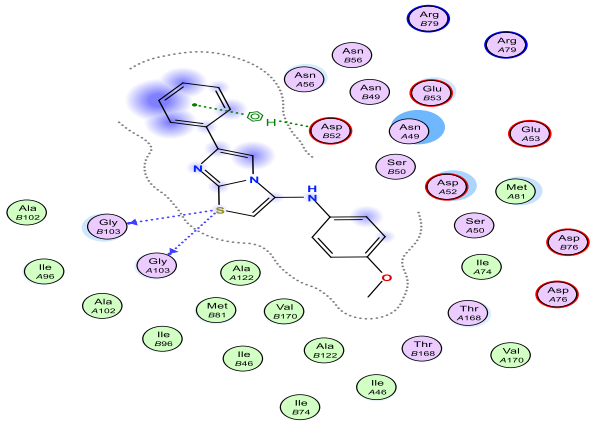


Fig. 11. The most active compound **45** docked inside the active site of *S. aureus* topoisomerase IV (ParE) enzyme.

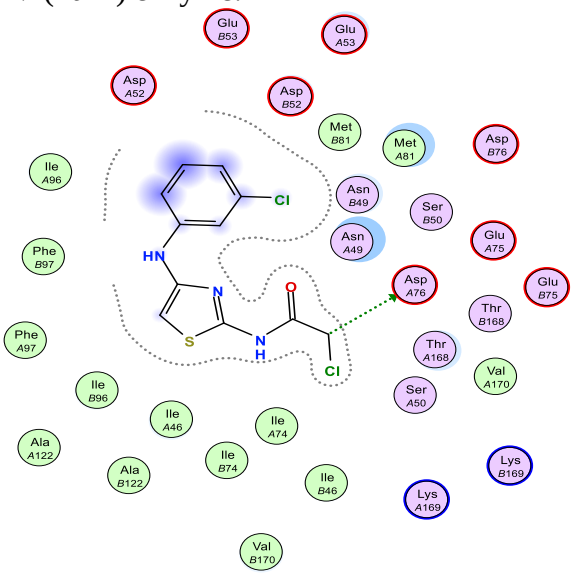


Fig 12. The least active compound **24** docked inside the active site of *S. aureus* topoisomerase IV (ParE) enzyme**.**

2.4. In silico studies

2.4.1. Lipinski's rule of five and Veber's norms:

Lipinski's rule of five is a useful tool for the prediction of the oral absorption of drugs. For a compound to be an orally active drug, it should have a molecular weight not higher than 500 Da, a partition coefficient value not higher than 5, hydrogen bond donors not more than 5, and hydrogen bond acceptors less than 10. A compound that satisfies at least three out of these four criteria is said to obey 'Lipinski's Rule of Five' [5]. Other valuable parameters for drug absorption and transport through biological membranes are topological polar surface area and number of rotatable bonds (Veber's norms). Compounds are predicted to have good oral bioavailability when they have polar surface area not greater than 140 $Å^2$ and 10 or fewer rotatable bonds [6].

In the current work, the synthesized compounds were inspected for the calculation of their molecular properties *via* Molinspiration server [7]. Table 6 shows the calculated Lipinski's rule parameters, TPSA, Nrotb, along with the number of violations for rule of five.

All the tested compounds matched Lipinski's rule of five together with Veber's norms and are expected to display good oral bioavailability.

Table 6. TPSA, Nrotb and calculated Lipinski's rule for the active compounds.

Compd No.	TPSA[a]	Nrotb[b]	miLog P[c]	nHBD[d]	nHBA[e]	M.wt[f]	No. ofviolations
23	99.84	5	2.85	2	7	312.74	0
24	54.02	4	2.9	2	4	302.19	0
27	54.02	4	3.34	2	4	281.77	0
35	48.97	6	4.38	3	4	344.44	0
37	116.91	6	4.28	2	8	390.45	0
38	99.84	5	3.96	2	7	340.36	0
39	99.84	5	4.61	2	7	377.81	0
40	99.84	5	4.12	2	7	358.35	0
41	99.84	5	4.41	2	7	354.39	0
45	41.47	4	5.57	2	4	356.86	1

[a] Topological Polar Surface Area ($A^{0\,2}$). [b] Number of rotatable bonds. [c] The parameter of lipophilicity (Logarithm of octanol-water partition coefficient developed by Molinspiration). [d] Number of hydrogen bond donors. [e] Number of hydrogen bond acceptors. [f] Molecular weight.

3.4.2. Assessment of toxicities, druglikeness, and drug score profiles

The existence of a predetermined set of structural fragments in one structure alerts for the probable toxicity of such compound. Osiris Property Explorer [8] was used for estimating the toxicity of compounds **23, 24**, **27**, **35**, **37-41** and **45** which includes mutagenicity, tumorigenicity, irritancy and the effects on the reproductive system. In addition, it was used for determining the druglikeness of such compounds where a positive value shows that the investigated molecule contains fragments often present in commercial drugs [9].

All examined compounds exhibited no possible *in silico* toxicity risks except **35, 37** and **45**. Compounds **24**, **27**, **45** displayed a positive acceptable drug-likeness value. Compound **45** was found to possess a good drug score value (=0.42) providing it as a safe promising drug (Table 7).

Table 7. Toxicity risks, druglikeness and drug scores of the synthesized active compounds.

Compd No.	Toxicity risks				Druglikeness	Drugscore
	Mutagenicity	Tumorigenicity	Irritancy	Reproductiveeffects		
23	+	++	+	+	- 8.85	0.08
24	+	+	++	+	**2.46**	0.13
27	++	+	++	+	**0.92**	0.13
35	+++	+	+	++	- 1.57	0.27
37	+	+	++	+++	-14.82	0.29
38	+	+	+	+	-11.03	0.30
39	++	++	+	+	-9.32	0.25
40	+	+	+	+	-10.75	0.28
41	++	+++	+	++	-10.95	0.28
45	+	++	+	+	**1.55**	**0.42**

+ low risk; ++ moderate risk; +++ high risk. Bold values represent the good results.

3. Conclusion

In the current work, different series of thiazole derivatives were prepared. The most active antibacterial compounds were the thiourea and benzamide derivatives **35** and **38,** also representing the most potent inhibitors to DNA gyrase and topoisomerase IV with IC_{50} values of 25.7 and 30.4 µM respectively, in comparison with 24.5 and 24.4 µM for ciprofloxacin as a reference drug.

Docking studies into the studied enzymes active site displayed that compound **35** has the highest binding affinity to DNA gyrase and **39** exhibited the lowest binding affinity with docking scores -7.90 and -7.28, respectively. Meanwhile, compound **45** has the highest binding affinity to topoisomerase IV *via* H-donor with Gly103 with distance 3.58 Å and energy -0.7 Kcal/mol and pi-H with Asp52 with distance 3.58 Å and energy -0.5 Kcal/mol and

24 showed the lowest binding affinity with docking scores -6.68 and -6.35, respectively.

4. Experimental protocols

Syntheses of the designed compounds were conducted in the Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. The antimicrobial activity assay was performed in the Department of Microbiology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. Enzyme assay was carried out at the confirmatory diagnostic unit, VACSERA, Egypt. Melting points (°C) were performed on Stuart melting point apparatus and are uncorrected. ^{1}H, ^{13}C-NMR were determined on a Joel 500 MHz FT spectrometer and Brucker 400 MHz spectrometer; chemical shifts are expressed in δ ppm with reference to TMS. Mass spectral (MS) data were recorded on direct inlet part to mass analyzer in thermo scientific GCMS model ISQ at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Thin layer chromatography was done on precoated (0.25 mm) silica gel GF_{254} plates (E. Merck, Germany), compounds were visualized with 254 nm UV lamp. All the fine chemicals and reagents used were bought from Aldrich Chemicals Co, USA. Compounds **8-14** [10], **15** [11**]**, **16** [12], **19, 20** [11**]** were previously reported.

4.1. Chemistry

4.1.1. Synthesis of *N*^{4}-((substituted) phenyl)thiazole-2,4-diamines (15-21)

Thiourea (0.78g, 100 mmol) was added to a solution of the acetamide compounds **8-14** (100 mmol) in acetone (7 mL). The reaction mixture was heated under reflux overnight. The separated solid was filtered, dried, then crystallized from aqueous ethanol to give the aminothiazole compounds **15-21**.

15: Yield: 69%, m.p. 230-232 °C (Lit 228-230) [11], M.wt: 191. IR (KBr, υ_{max}, cm^{-1}); 3360 (N-H), 3240, 3167 (NH_2), 3009 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.15 (s, 1H, C**H** of thiazole), 7.34-7.60 (m, 5H, Ar-**H**), 9.12 (s, 2H, N**H**$_2$), 11.01 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 34.6 (**C**-S), 127.6 (2**C**), 128.2 (2**C**), 129.5 (1**C**), 133.9 (**C**-N), 166.5 (N-**C**-N of thiazole), 170.1 (**C**-NH2).

16: Yield: 71%, m.p. 160-162 °C (Lit 158-160) [12], M.wt: 236. IR (KBr, υ_{max}, cm^{-1}); 3386 (N-H), 3212, 3147 (NH_2), 3019 (Ar-CH), 1514, 1398 (NO_2). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.37 (s, 1H, C**H** of thiazole), 6.35-8.17 (m, 4H, Ar-**H**), 9.35 (s, 2H, N**H**$_2$), 11.58 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 36.4 (**C**-S), 118.9 (2**C**), 126.0 (2**C**), 135.8 (**C**-NO2), 144.5 (**C**-N), 169.6 (N-**C**-N of thiazole), 175.9 (**C**-NH2).

17: Yield: 56%, m.p. 213-215 °C, M.wt: 225. IR (KBr, υ_{max}, cm^{-1}); 3367 (N-H), 3240, 3157 (NH_2), 3011 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.75 (s, 1H, C**H** of thiazole), 6.93-7.78 (m, 4H, Ar-**H**), 9.26 (s, 2H, N**H**$_2$), 10.27 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 34.1 (**C**-S), 117.5 (1**C**), 119.0 (1**C**), 121.2 (1**C**), 126.5 (1**C**), 133.2 (**C**-Cl), 141.5 (**C**-N), 166.2 (**N-C**-N of thiazole), 176.7 (**C**-NH2).

18: Yield: 62%, m.p. 158-160 °C, M.wt: 260. IR (KBr, υ_{max}, cm^{-1}); 3380 (N-H), 3260, 3170 (NH_2), 3019 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.77 (s, 1H, C**H** of thiazole), 7.04-7.27 (m, 3H, Ar-**H**), 7.33 (s, 2H, N**H**$_2$), 7.89 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 35.4 (**C**-S), 119.4 (2**C**), 125.5 (2**C**), 133.9 (**C**-Cl), 145.2 (**C**-N), 166.5 (N-**C**-N of thiazole), 170.1 (**C**-NH$_2$). MS m/z (%); 261 (M^{+} +1, 28.16), 260 (M^{+}, 26.21), 161.0 (100).

19: Yield: 64%, m.p. 220-222 °C (Li t217-219) [11], M.wt: 209. IR (KBr, υ_{max}, cm^{-1}); 3356 (N-H), 3212, 3147 (NH_2), 3037 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.38 (s, 1H, C**H** of thiazole), 7.88-8.23 (m, 4H, Ar-**H**), 9.32 (s, 2H, N**H**$_2$), 11.59 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 35.4 **C**-S), 119.4 (2**C**), 125.5 (2**C**), 142.9 (**C**-F), 145.2 (**C**-N), 166.5 (**N-C**-N of thiazole), 170.1 (**C**-NH2).

20: Yield: 68%, m.p. 235-237 °C (Lit 232-234) [11], M.wt: 205. IR (KBr, υ_{max}, cm^{-1}); 3386 (N-H), 3212, 3147 (NH_2), 3019 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 2.51(s, 3H, C**H**$_3$), 6.44 (s, 1H, C**H** of thiazole), 7.43-7.75 (m, 4H, Ar-**H**), 9.40 (s, 2H, N**H**$_2$), 10.35 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 34.7 (**C**H$_3$), 127.6 (2**C**), 128.2 (2**C**), 129.5 (1**C**), 130.5 (1**C**), 133.9 (**C**-N), 166.5 (N-**C**-N of thiazole), 170.1 (**C**-NH2).

21: Yield: 66%, m.p. 245-247 °C, M.wt: 221. IR (KBr, υ_{max}, cm^{-1}); 3376 (N-H), 3258, 3187 (NH_2), 3019 (Ar-CH). ^{1}HNMR (400

MHz, DMSO, δ ppm); 3.61 (s, 3H, O-C**H**$_3$), 6.88 (s, 1H, C**H** of thiazole), 7.16-7.45 (m, 4H, Ar-**H**), 9.34 (s, 2H, N**H**$_2$), 9.47 (s,1H,N**H**). ^{13}CNMR δ (ppm); 27.9 (O-**C**H$_3$), 124.4 (2**C**), 125.2 (1C), 128.1 (2**C**), 131.4 (**C**-OCH$_3$), 145.7 (**C**-N), 160.2 (N-**C**-N of thiazole), 172.2 (**C**-NH$_2$).

4.1.2. Synthesis of 2-chloro-*N*-(4-(((substituted) phenyl)amino)thiazol-2-yl)acetamides (22-27)

The amino thiazole compounds **15-20** (5 mmol) were dissolved in DMF (10 mL) and then 2-chloroacetyl chloride (0.7 mL, 10 mmol) was added dropwise in an ice bath. The reaction mixture was stirred at room temperature for 3-5 hours. The solvent was concentrated under reduced pressure to half volume then poured into ice-water mixture. The separated solid was filtered, dried, washed with water and petroleum ether then crystallized from hot ethanol/methanol mixture (8:2).

22: Yield: 62%, m.p. 180-182 °C, M.wt: 267. IR (KBr, υ_{max}, cm^{-1}); 3428, 3200 (N-H), 1770 (C=O), 3010 (Ar-CH). ^{1}HNMR (400 MHz DMSO, δ ppm); 4.03 (s, 2H, C**H**$_2$-Cl), 6.78 (s, 1H, C**H** of thiazole), 7.83-8.24 (m, 5H, Ar-**H**), 10.03 (s, 1H, N**H**-CO), 11.24 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 36.8 (**C**-S), 43.0 (**C**H2Cl), 118.4 (1**C**), 119.3 (2**C**), 125.5 (2**C**), 145.5 (**C**-N), 163.1 (**C**-NH-CO), 170.3 (**C**=O), 172.6 (**C**-N of thiazole).

23: Yield: 64%, m.p. 117-119 °C, M.wt: 312. IR (KBr, υ_{max}, cm^{-1}); 3448, 3202 (N-H), 1719 (C=O), 1534, 1378 (NO$_2$), 3059 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 4.29 (s, 2H, C**H**$_2$-Cl), 6.98 (s, C**H** of thiazole), 7.83-8.24 (m, 4H, Ar-**H**), 10.93 (s, 1H, N**H**-CO), 11.85 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 36.8 (**C**-S), 46.3 (**C**H2Cl), 119.3 (2**C**), 125.5 (2**C**), 142.8 (**C**-NO$_2$), 145.5 (**C**-N), 167.1 (**C**-NH-CO), 169.1 (**C**=O), 175.3 (**C**-N of thiazole).

24: Yield: 60%, m.p. 126-128°C, M.wt: 302. IR (KBr, υ_{max}, cm^{-1}); 3420, 3211 (N-H), 1749 (C=O), 3059 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 3.97 (s, 2H, C**H**$_2$-Cl), 6.44 (s, 1H, C**H** of thiazole), 7.43-7.81 (m, 4H, Ar-**H**), 9.17 (s, 1H, N**H**-CO), 9.99 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 45.9 (**C**H$_2$Cl), 88.5 (**C**-S), 128.9 (1**C**), 130.5 (1**C**), 131.9 (1**C**), 132.9 (1**C**), 135.2 (**C**-Cl), 135.9 (**C**-N), 166.6 (**C**-NH-CO), 166.9 (**C**=O), 170.2 (**C**-N of thiazole).

25: Yield: 59%, m.p. 140-142°C, M.wt: 336. IR (KBr, υ_{max}, cm^{-1}); 3448, 3202 (N-H), 1779 (C=O), 3059 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 3.48 (s, 2H, C**H**$_2$-Cl), 6.50 (s, 1H, C**H** of thiazole), 6.88-7.18 (m, 3H, Ar-**H**), 7.60 (s, 1H, N**H**-CO), 11.01 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 28.5 (**C**H$_2$Cl), 119.6 (2**C**), 120.1 (1**C**), 121.3 (1**C**), 124.8 (1**C**), 129.4(1**C**), 136.3 (**C**-N), 144.4 (**C**-NH-CO), 145.3 (**C**=O), 153.5 (**C**-N of thiazole).

26: Yield: 61%, m.p. 150-152 °C, M.wt: 285. IR (KBr, υ_{max}, cm^{-1}); 3468, 3202 (N-H), 1799 (C=O), 3076 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 4.58(s, C**H**$_2$-Cl), 6.87 (s, 1H, C**H** of thiazole), 7.01-8.32 (m, 4H, Ar-**H**), 12.05 (s, 1H, N**H**-CO), 12.70 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 38.9 (**C**H$_2$Cl), 97.9 (**C**-S), 115.9 (1**C**), 122.1 (1**C**), 123.1 (2C), 145.8 (**C**-F), 148.8 (**C**-N), 166.9 (**C**-NH-CO), 167.9 (**C**=O), 168.6 (**C**-N of thiazole).

27: Yield: 62%, m.p. 217-219 °C, M.wt: 281. IR (KBr, υ_{max}, cm^{-1}); 3448, 3202 (N-H), 1759 (C=O), 3059 (Ar-CH). ^{1}HNMR (400 MHz, DMSO, δ ppm); 2.37 (s, 3H, CH$_3$), 3.47 (s, 2H, C**H**$_2$-Cl), 6.49 (s, 1H, C**H** of thiazole), 6.87-7.19 (m, 4H, Ar-**H**), 7.7 (s, 1H, N**H**-CO), 11.01 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 28.5 (**C**H$_3$), 36.8 (**C**-S), 46.3 (**C**H$_2$Cl), 119.3 (2**C**), 125.5 (2**C**), 131.9(1C), 144.4 (**C**-N), 167.1 (**C**-NH-CO), 173.3 (**C**=O), 175.6 (**C**-N of thiazole).

4.1.3. Synthesis of 2-((4-((substituted) phenyl)amino)thiazol-yl)amino)thiazol-4(5*H*)-ones (28-32)

2-Chloro-*N*-(4-(phenylamino)thiazol-2-yl)acetamide **22-26** (100 mmol) and ammonium thiocyanate (0.89 g, 50 mmol) were dissolved in acetone and the reaction mixture was stirred under reflux overnight and filtered while hot. The solution was filtered on hot and the obtained precipitate was washed with boiling acetone and water. The formed precipitate was filtered, dried and crystallized from aqueous ethanol.

28: Yield: 46%, m.p. 215-217 °C, M.wt: 390. IR (KBr, υ_{max}, cm^{-1}); 3370 (N-H), 2946 (Ar-CH), 1670 (C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 3.38 (s, 2H, CH$_2$ of thiazolidinone), 7.01-7.36 (m, 6H, Ar-**H** and C**H** of thiazole), 9.01 (s, 1H, N**H**) 9.13 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 27.9 (CH2-S) 115.0

(C-S of thiazole), 119.7 (2C), 121.8 (2C), 126.7 (2C), 138.6 (C-N), 142.5 (C=N of thiazolidinone) 173.5 (C=O), 178.9 (C=N of thiazole).

29: Yield: 48%, m.p. 220-222°C, M.wt: 335. IR (KBr, υ_{max}, cm^{-1}); 3399 (N-H), 1634, 1278 (NO2), 2922 (Ar-CH), 1681(C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 3.36 (s, 2H, CH_2 of thiazolidinone), 7.19-7.64 (m, 5H, Ar-**H** and C**H** of thiazole), 11.57 (s, 1H, N**H**) 13.27 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 36.0 (CH_2-S) 115.0 (C-S of thiazole), 119.7 (2C), 124.7 (2C), 126.3 (1C), 129.4 (C-NO_2), 138.6 (C-N), 151.2 (C=N of thiazolidinone) 172.3 (C=O), 175.4 (C=N of thiazole).

30: Yield: 42%, m.p. 290-292°C, M.wt: 325. IR (KBr, υ_{max}, cm^{-1}); 3397 (N-H), 2942 (Ar-CH), 1690(C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 3.37 (s, 2H, CH_2-S of thiazolidinone), 6.60-7.55 (m, 5H, Ar-**H** and C**H** of thiazole), 9.83 (s, 1H, N**H**) 10.47 (s, 1H, N**H**). ^{13}CNMR δ (ppm); 35.8 (CH_2-S) 116.2 (**C**-S of thiazole), 118.2 (1**C**), 123.6 (1**C**), 124.3 (1**C**), 128.2 (1**C**), 128.7 (**C**-Cl), 139.7 (2C, **C**-N), 162.0 (C=N of thiazolidinone) 164.4 (C=O), 164.6 (C=N of thiazole).

31: Yield: 43%, m.p. 294-296 °C, M.wt: 359. IR (KBr, υ_{max}, cm^{-1}); 3409 (N-H), 2920 (Ar-CH), 1690 (C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 4.12 (s, 2H, CH_2 of thiazolidinone), 7.36-7.62 (m, 4H, Ar-**H** and C**H** of thiazole), 11.23 (br s, 2H, N**H**). ^{13}CNMR δ (ppm); 35.4(CH_2-S) 105.3 (C-S of thiazole), 119.7 (2C), 125.5 (2C), 142.9 (2C, **C**-Cl), 139.7 (C-N), 157.1 (C=N of thiazolidinone), 157.8 (C=O), 166.6 (C=N of thiazole).

32: Yield: 40%, m.p. 230-232 °C, M.wt: 308. IR (KBr, υ_{max}, cm^{-1}); 3390 (N-H), 2970 (Ar-CH), 1693 (C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 3.34 (s, 2H, CH_2 of thiazolidinone), 6.90-8.28 (m, 5H, Ar-**H** and C**H** of thiazole), 9.01 (s, 1H, N**H**), 9.30 (s, 1H, N**H**).

4.1.4. Synthesis of 1-(4-(((substituted) phenyl)amino)thiazol-2-yl)-3-phenylthiourea (33-36)

A solution of aminothiazole derivetives **15**, **17**, **19**, and **20** (100 mmol) in DMF (3 mL) was added to an ice-cooled solution of phenylisothiocyante (0.6 mL, 100 mmol) in triethylamine (2 mL). The reaction mixture was stirred for 30 minutes at 0-5 °C, then refluxed for 10 hours. After cooling, DMF was concentrated under reduced pressure to half volume then poured into crushed ice. The obtained colored solid was filtered, washed with diethylether (10 mL), dried and crystallized from chloroform: ethanol mixture (9:1).

33: Yield: 40%, m.p. 168-170 °C, M.wt: 326. IR (KBr, υ_{max}, cm^{-1}); 3479, 3254 (N-H), 3056 (Ar-CH), 1312 (C-N), 1273 (C=S). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.78 (s, 1H, C**H** of thiazole), 7.15 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.19 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.31-7.72 (m, 4H, Ar-**H**), 7.82 (d, 2H Ar-**H**, *J*= 7.6 Hz), 7.76 (d, 2H, Ar-**H**, *J*= 7.2 Hz), 8.80 (s,1H, N**H**), 9.3 (s, 1H, NH-CS-N**H**), 10.12 (s, 1H, thiazole-N**H**-CS-NH).

34: Yield: 43%, m.p. 200-202 °C, M.wt: 360. IR (KBr, υ_{max}, cm^{-1}); 3449, 3208 (N-H), 3034 (Ar-CH), 1343 (C-N), 1243 (C=S). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.88 (s, 1H, C**H** of thiazole), 7.12 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.19 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.28-7.38 (m, 4H, Ar-**H**), 7.47 (d, 2H Ar-**H**, *J*= 7.6 Hz), 7.73 (d, 2H, Ar-**H**, *J*= 7.2 Hz), 8.90 (s,1H, N**H**), 9.09 (s, 1H, NH-CS-N**H**), 10.14 (s, 1H, thiazole-N**H**-CS-NH), ^{13}CNMR δ (ppm); 35.4(**C**-S), 122.9 (2**C**), 123.9 (2**C**), 124.8 (1**C**), 125.9 (2**C**), 128.8 (2**C**), 129.1 (2**C**), 139.5 (2C, **C**-NH), 174.9 (**C**=N of thiazole), 187.5 (**C**=S).

35: Yield: 42%, m.p. 160-162 °C, M.wt: 344. IR (KBr, υ_{max}, cm^{-1}); 3459, 3190 (N-H), 3015 (Ar-CH), 1310 (C-N), 1220 (C=S). ^{1}HNMR (400 MHz, DMSO, δ ppm); 6.36 (s, 1H, C**H** of thiazole), 7.12 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.14 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.16-7.36 (m, 4H, Ar-**H**), 7.46 (d, 2H, Ar-**H**, *J*= 7.6 Hz), 7.52 (d, 2H, Ar-**H**, *J*= 7.2 Hz), 8.70 (s,1H, N**H**), 9.88 (s, 1H, NH-CS-N**H**), 10.61 (s, 1H, thiazole-N**H**-CS-NH).

36: Yield: 45%, m.p. 150-152 °C, M.wt: 356. IR (KBr, υ_{max}, cm^{-1}); 3400, 3211 (N-H), 3017 (Ar-CH), 1310 (C-N), 1263 (C=S). ^{1}HNMR (400 MHz, DMSO, δ ppm); 1.62(s, CH_3), 7.10 (s, 1H, C**H** of thiazole), 7.12 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.13 (d, 1H, Ar-**H**, *J*= 6.8 Hz), 7.28-7.34 (m, 4H, Ar-**H**), 7.49 (d, 2H Ar-**H**, *J*= 7.6 Hz), 7.50 (d, 2H, Ar-**H**, *J*= 7.2 Hz), 8.82 (s,1H, N**H**), 9.82 (s, 1H, NH-CS-N**H**), 10.14 (s, 1H, thiazole-N**H**-CS-NH), ^{13}CNMR δ

(ppm); 23.2 (**C**H_3), 46.2 (**C**-S), 124.1 (2**C**), 124.9 (2**C**), 127.2 (1**C**), 128.3 (2**C**), 128.6 (2**C**), 128.9 (2**C**), 129.3 (2C, **C**-NH), 139.9 (**C**=N of thiazole), 180.0 (**C**=S).

4.1.5. Synthesis of 4-methyl-*N*-(4-((4-nitrophenyl)amino)thiazol-2-yl)benzenesulfonamide (37)

A mixture of N^4-(4-nitrophenyl) thiazole-2, 4-diamine **16** (0.23g, 100 mmol) and the appropriate P- tolylsulfonyl chloride derivative (0.19 g, 100 mmol) in pyridine (10 mL) was heated under reflux for 10 hours. The solvent was evaporated under reduced pressure and the obtained residue was triturated with ice and filtered. The obtained solid was dried and recrystallized from aqueous ethanol to give yellow sulfonamide derivative **37**. Yield: 46%, m.p. 170-172 °C, M.wt: 390. IR (KBr, υ_{max}, cm^{-1}); 3386 (N-H), 3019 (Ar-CH), 1678, 1232 (NO_2), 1430 (SO_2). ^{1}HNMR (400 MHz, DMSO, δ ppm); 2.52 (s, 3H, C**H**$_3$), 6.61-6.74 (m, 8H, Ar-**H** and C**H** of thiazole), 7.95 (s, 1H, N-**H**), 7.97 (s, 1H, N**H**-SO_2). ^{13}CNMR δ (ppm); 39.3 (**C**H3), 87.3 (1C,**C**H-S), 112.8 (4C), 119 (1C), 126.9 (4C), 130.1 (1C, C-**S**O_2), 134.3 (1C, **C**-NO_2), 136.1 (2C, **C**-NH), 156.2 (thiazole **C**-NH-SO_2).

4.1.6. Synthesis of *N*-(4-(((substituted) phenyl)amino)thiazol-2-yl)-4-nitrobenzamides (38-41)

The aminothiazole derivatives **15, 17, 19** and **20** (10 mmol) were allowed to react with *p*-nitrobenzoyl chloride (0.73g, 15 mmol) in refluxing glacial acetic acid (10 ml) for 24 hours using anhydrous sodium acetate (0.5 g) as catalyst. The reaction mixture was cooled and poured into ice with stirring. The separated solids were filtered, washed and crystallized from aqueous ethanol to yield the targeted compounds (**38-41**).

38: Yield: 45%, m.p. 218-220 °C, M.wt: 340. IR (KBr, υ_{max}, cm^{-1}); 3386 (N-H), 3212 (NH-CO), 1634, 1278 (NO_2), 1630(C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 8,18- 8.36 (m, 10H, Ar-**H** , C**H** of thiazole), 13.69 (s, 1H, N**H)**, 13.3 (s, 1H, N**H**CO).

39: Yield: 47%, m.p. 230-232 °C, M.wt: 377. IR (KBr, υ_{max}, cm^{-1}); 3356 (N-H), 3212 (NH-CO), 3039 (Ar-CH), 1650 (C=O), 1634, 1348 (NO_2). ^{1}HNMR (400 MHz, DMSO, δ ppm); 8.18-8.34 (m, 9H, Ar-**H** , C**H** of thiazole) 13.68 (s, 1H, N**H)**, 13.70 (s, 1H, N**H**CO). ^{13}CNMR δ (ppm); 124.0 (4C), 124.3 (4C), 131.1 (2C, **C**-NH), 131.3 (2C, **C**-NH and Ar **C**-C=O), 150.4 (C=N of thiazole and C-NO_2), 166.2 (**C**=O).

40: Yield: 42%, m.p. 190-192 °C, M.wt: 358. IR (KBr, υ_{max}, cm^{-1}); 3376 (N-H), 3423 (NH-CO), 1534, 1378 (NO_2), 1650(C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 8.16-8.79 (m, 9H, Ar-**H**, C**H** of thiazole), 13.39 (s, 1H, N**H)**, 13.53 (s, 1H, N**H**CO). ^{13}CNMR δ (ppm); 124.1 (4C), 131.1 (4C), 136.9 (2C, **C**-NH), 150.4 (2C, **C**-NH and Ar **C**-C=O), 166.3 (2C, **C**-F and **C**-NO_2), 183.3 (**C**=N of thiazole), 188.2 (**C**=O).

41: Yield: 46%, m.p. 214-216 °C, M.wt: 354. IR (KBr, υ_{max}, cm^{-1}); 3386 (N-H), 3212 (NH-CO), 1634, 1278 (NO_2), 1670(C=O). ^{1}HNMR (400 MHz, DMSO, δ ppm); 3.53 (s, 3H, OC**H**$_3$), 7.85-8.31 (m, 9H, Ar-**H** , C**H** of thiazole), 13.03 (s, 1H, N**H)**, 13.13 (s, 1H, N**H**CO). ^{13}CNMR δ (ppm); 24.6 (O**C**H_3), 118.7 (4C), 123.8 (2C), 125.3 (2C), 130.9 (2C, **C**-NH), 142.4 (2C, **C**-NH and Ar **C**-C=O), 145.9 (C-OCH_3 and C-NO_2), 149.8 (C=N of thiazole), 169.8 (**C**=O).

4.1.7. General method for preparation of 6-(4-chlorophenyl)-*N*-((substituted) phenyl)-7,7a-dihydroimidazo[2,1-b]thiazol-3-amines (42-45)

A solution of N^4-phenylthiazole-2,4-diamine derivatives (**15-17, 20**) (30 mmol) in DMF (7 mL) was added to a an ice-cooled solution of *p*-chlorophenacyl bromide (0.43 g, 30 mmol) in triethylamine (2 mL). The reaction mixture was stirred for 30 minutes at 0-5 °C, then heated under reflux overnight. After cooling, the reaction mixture was poured into ice, stirred at room temperature for 2 hours then filtered, washed with water, dried and recrystallized from aqueous ethanol.

42: Yield: 41%, m.p. 118-120 °C, M.wt: 326. IR υ_{max}/ cm^{-1}; 3380 (N-H). ^{1}HNMR δ (ppm); 3.38 (d, 1H, S-C**H-N** of fused system, *J*= 6.8), 4.79 (s, 1H, C**H** of imidazole), 6.31 (s, 1H, CH of thiazole), 7.39-7.72 (9H, Ar-**H**), 8.03 (d, 1H, NH of imidazole, *J*= 6.9), 8.04 (N**H**). ^{13}CNMR δ (ppm); 66.0 (**C**-N of fused system), 99.0 (**C**H-N of imidazole), 109.7 (**C**H-S of thiazole), 126.2, 127.9, 128.9, 129.2,

129.7, 130.4, 130.9, 132.8, 133.4 (Ar-**C**), 162.8 (**C**-NH).

43: Yield: 46%, m.p. 124-126 °C, M.wt: 371. IR υ_{max}/ cm^{-1}; 3358 (N-H), 1534, 1378 (NO_2), ^{1}HNMR δ (ppm); 3.18 (d, 1H, S-C**H-N** of fused system, *J*= 6.7), 3.54 (s, 1H, C**H** of imidazole), 6.01 (s, 1H, CH of thiazole), 6.95-8.26 (9H, Ar-**H**), 8.28 (d, 1H, NH of imidazole, *J*= 6.8), 8.71 (N**H**). ^{13}CNMR δ (ppm); 51.0 (**C**-N of fused system), 99.0 (**C**H-N of imidazole), 112.8 (**C**H-S of thiazole), 117.9, 118.6, 119.1, 120.5, 121.7, 122.2, 125.4, 126.9, 129.3 (Ar-**C**), 140.2(**C**-NO_2), 153.0 (**C**-NH).

44: Yield: 42%, m.p. 102-104 °C, M.wt: 360. IR υ_{max}/ cm^{-1}; 3378 (N-H), ^{1}HNMR δ (ppm); 3.36 (d, 1H, S-C**H-N** of fused system, *J*= 6.9), 4.73 (s, 1H, C**H** of imidazole), 6.43 (s, 1H, CH of thiazole), 7.29-8.13 (9H, Ar-**H**), 8.51(d, 1H, NH of imidazole, *J*= 7.1), 8.53 (N**H**).

45: Yield: 39%, m.p. 110-112 °C, M.wt: 356. IR υ_{max}/ cm^{-1}; 3358 (N-H). ^{1}HNMR δ (ppm); 3.52 (s, 3H, OCH_3) 3.38 (d, 1H, S-C**H-N** of fused system, *J*= 6.8), 4.79 (s, 1H, C**H** of imidazole), 6.54 (s, 1H, CH of thiazole), 7.39-7.72 (9H, Ar-**H**), 8.04 (d, 1H, NH of imidazole, *J*= 6.9), 8.06 (N**H**).

4.1.8. Synthesis of 7-methyl-3-((4-(substituted) phenyl)amino)-6,8a-dihydro-5*H*-thiazolo[3,2-*a*]pyrimidin-5-ones (46, 47)

A mixture of ethyl acetoacetate (0.6mL- 20 mmol) and N^4-phenylthiazole-2,4-diamine derivatives **15, 16** (20 mmol) in glacial acetic acid (10 mL) was refluxed for 30 hours. After cooling, the solvent was evaporated under reduced pressure and the obtained residue was triturated with ice, filtered, washed with water and crystallized from ethanol to furnish the entitled compounds **46, 47.**

46: Yield: 47%, m.p. 210-212°C, M.wt: 259. IR υ_{max}/ cm^{-1}; 3340 (N-H), 1748 (C=O). ^{1}HNMR δ (ppm); 2.50 (s, 3H, C**H$_3$**), 3.36 (s, 2H, C**H$_2$** of pyrimidine), 4.57 (s, 1H, N-CH-S), 6.50 (s, 1H, C**H** of thiazole), 6.62-7.97 (m, 5H, Ar-**H**), 10.00 (s, 1H, N-**H**).

47: Yield: 52%, m.p. 144-146°C, M.wt: 304. IR υ_{max}/ cm^{-1}; 3358 (N-H), 1678 (C=O), 1540, 1370 (NO_2). ^{1}HNMR δ (ppm);1.93 (s, 3H, C**H$_3$**), 3.93 (s, 2H, C**H$_2$** of pyrimidine), 4.8 (s, 1H, N-CH-S), 6.61 (s, 1H, C**H** of thiazole), 7.83-8.22 (m, 4H, Ar-**H**), 10.60 (s, 1H, N-**H**), ^{13}CNMR δ (ppm); 24.7 (**C**H_3), 112.8 (**C**H_2 of pyrimidine), 118.9 (**C**=N of thiazole), 125.4 (2C), 126.8 (2C), 136.1 (CH of fused system), 142.4 (**C**H of thiazole), 145.9 (**C**-NO_2), 156.2, 169.8 (2C, **C**-NH), 183.3 (C=O of pyrimidine), 188.3 (**C**-CH_3 of pyrmidine).

4.2. Biological evaluation

4.2.1. *In vitro* antimicrobial screening

The newly prepared thiazole derivatives were screened *via* agar disc-diffusion assay for their *in vitro* antimicrobial activity against Gram positive bacteria; *Staphylococcus aureus Newman***,** and Gram negative bacteria; *Klebsiella pneumonia* and *Escherichia coli* using Mueller-Hinton agar media and ampicillin and gentamicin as reference antibacterial drugs. They were also screened for their potential antifungal activity against pathogenic fungus; *Candida albicans Sc5314* in YPD agar media using fluconazole as a reference antifungal drug.

4.2.1.1. Antibacterial screening:

The diluted overnight cultures of the different strains (OD600 nm of 0.1 that was equivalent to 8 x10^7 cells/ml) were spread on Mueller Hinton agar plates using sterile swab followed by applying sterile cellulose disc papers (6 mm each) [13]. DMSO as stock solution was used to dissolve the tested compounds to a 10 mg/300 µL concentration. 100 µg from each compound were loaded from the stock solution (3 µl) into the disc papers. The standard antibacterial compounds; ampicillin (100 µg/disc) and gentamicin (100 µg/disc) were used in the antibacterial assay, while DMSO was used as a negative control. The plates were incubated at 37 ^{o}C for 24 hours and the diameter of zone of growth inhibition was measured to the nearest millimeter by means of a caliber.

4.2.1.2. Antifungal screening

The primary screening was carried out *via* YPD agar medium [14], then completed as under the antibacterial screening. Fluconazole (40 µg/disc) was used in the antifungal assay, while DMSO was used as a negative control.

4.2.1.3. Quantitative measurement of Minimum Inhibitory Concentration (MIC) by the broth micro dilution method [15]

The most active examined compounds in disc diffusion method, underwent the broth microdilution assay to determine their MICs. The same media that were adopted in the primary screening were further utilized except for the removal of agar form the media. In brief, dilution of the overnight cultures of the bacterial strains to OD600nm of 0.01 (equivalent to 8 x 10^6 cells/mL) and that of *C. albicans* to OD600 nm of 0.5 was done. Dilution of both the selected compounds and the standard antibiotics by three fold serial dilution was done. Application of the dilutions of the compound in DMSO (5000, 1666, 555, 185, …, 2.2 µg/ml) to the diluted cultures in the microtiter plates as 5 % of the final volume in each well was performed. The plates were incubated at 37 °C for 24 hours. The MIC values were set as the lowest concentration that totally inhibits the visible growth of the microorganism after overnight incubation.

4.2.2. DNA gyrase and topoisomerase IV activity

In the present work, enzyme inhibition assay was performed using *E. coli* DNA gyrase (Catalog No. G1001, Inspiralis, UK), *S. aureus* topoisomerase IV (Catalog No. SAT4001, Inspiralis, UK). Compounds **24**, **35**, **38** and **45** were screened for their ability to inhibit both two enzymes with reference to the antibacterial drug ciprofloxacin as stated in the manufacturer's guidelines and to the reference method [16]. DMSO was used to dissolve and serially dilute the tested compounds into different concentrations. (log conc.=1.1, 1.4, 1.7 and 2) IC_{50} in (µM) and % inhibition for each dilution were recorded.

4.3. Molecular modeling studies

Docking studies were done utilizing MOE 2019 to inspect the binding affinities of all synthesized compounds. These compounds were constructed on MOE in the form of a 3D model. After examining their structures and the formal charges on atoms by the 2D depiction, the tested compounds underwent energy minimization. Calculation of partial charges was automatically performed. In the same database, the co-crystallized ligand and the tested compounds were imported and saved in the form of an MDB file to be used in the docking calculations.): The crystal structure of ligand complexed with its co-crystallized inhibitor was downloaded from the Protein Data Bank. Firstly, the crystal structure was protonated, hydrogen atoms were added, automatic correction to check for any errors in the atom's connection, and the type was applied, and fixation of the potential of the receptor and its atoms were carried out. Site Finder was used for selecting the same active site of the co-crystallized inhibitor utilizing all default items, and dummy atoms of the pocket were created.

The following methodology [17] was generally applied: the prepared enzyme active site file was loaded, and a general docking procedure was applied as the docking tool. The program specifications were adjusted so that the docking site was specified as dummy atoms, triangle matcher as the placement methodology, and London dG as the scoring methodology. Rigid receptor as refinement methodology and GBVI/WSA dG as the scoring methodology for selecting the best 20 poses from 200 different poses for each tested compound. The scoring methods were adjusted to their default values. The MDB file of the ten ligands was loaded and general dock calculations were run automatically. After completing the docking processes, the obtained poses were studied, and the best ones displaying the best ligand–enzyme interactions and the better acceptable RMSD values were chosen and saved for energy calculations. Firstly, a validation process was also performed for the target enzyme by running the docking process for only the co-crystallized ligand and small RMSD values between docked and crystal conformations signifies valid performance [18].

4.4. In silico studies

The molecular properties of the synthesized compounds were investigated using Molinspiration server [7]. Structures of compounds were drawn using online Molinspiration website (www.molinspiration.com). After drawing the structures, “Go for prediction” button was clicked, the molecular properties like

topological polar surface area **(**TPSA**)**, number of rotatable bonds (Nrotb), miLog P, number of hydrogen bond donors (nHBD), number of hydrogen bond acceptors (nHBA) and molecular weight (Mwt), appeared and reported.

On the other hand, Osiris Property Explorer [8] was utilized for the study of compounds' overall toxicity, druglikeness and drug score values. The chemical structures of compounds were drawn and the assigned parameters were predicted and recorded.

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