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Synthesis, *in-vitro* and *in-silico* study of novel thiazoles as potent antibacterial agents and MurB inhibitors

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Abstract

Efficient procedures are herein reported for the synthesis of novel hybrid thiazoles via a one-pot three-component protocol. The protocol involves the reaction of novel aldehyde, thiosemicarbazide and halogen-containing reagents in solvent- and catalyst-free conditions. The structures of the new thiazoles were elucidated by elemental analyses and spectroscopic data. The *in-vitro* antibacterial screening and MurB enzyme inhibition assays were performed for the novel thiazoles. The thiazol-4(5*H*)-one derivative **6d**, with *p*-MeO, exhibits the best antibacterial activities with minimum inhibitory concentration values of 3.9, 3.9, 7.8, and 15.6 μg/ml against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus mutans*, and *Escherichia coli*, respectively, as compared to the reference antibiotic drugs. It also exhibits the highest inhibition of the MurB enzyme with an IC_{50} of 8.1 μM. The structure–activity relationship was studied to determine the effect of the structures of the newly prepared molecules on the strength of the antibacterial activities. Molecular docking was also performed to predict the binding modes of the new thiazoles in the active sites of the *E. coli* MurB enzyme.

KEYWORDS

5-(aryldiazenyl)thiazoles, *in-silico* study, *in-vitro* antibacterial activities, *in-vitro* MurB inhibitory activity, one-pot three-component protocol, structure–activity relationship

1 | INTRODUCTION

Resistance to antibiotic *in-vitro* antibacterial activities arises when bacterial strains change in response to the use of these medicines. This harmful resistance results in more medical costs, hospital stays, and so, more deaths.[1] Recently, attempts to prepare new antibacterial agents to avoid antibiotic resistance have gained more interest.[2]

It had been reported that azo- compounds exhibit interesting biological activities such as *in-vitro* antimicrobial activity.[3] In general, azo- compounds exhibit good antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* but they are inactive against Gram-negative bacteria such as *Pseudomonas aeruginosa*.[4] This behavior is attributed to the structural similarity of azo- compounds with stilbenes, and so, they could share the same mechanism of action. They could inhibit ATP synthase binding at the interface between α and γ subunits.[5] Attempts were made to prepare derivatives containing the azo- moiety with enhanced antibacterial activities.[6]

On the contrary, several publications reported the synthesis of 2-hydrazineylthiazole derivatives[7–9] because they exhibit an important role in medicinal chemistry,[10] such as antibacterial and antifungal agents,[11–13] as well as inhibitors for bacterial DNA gyrase B.[14] Several thiazoles exhibit promising antibacterial activities by their inhibition of the MurB enzyme, which is responsible for the biosynthesis of bacterial cell walls.[15–17] Moreover, thiazoles exhibit anti-inflammatory and analgesic activities.[18–20] Heterocyclic compounds incorporating the thiazole moiety show fascinating antitumor and cytotoxic activities.[21,22] Moreover, it has been reported that the presence of thioether moiety in the tested derivatives increases their bioactivities such as *in-vitro* antimicrobial activity.[23–25]

In connection with the fascinating bioactivities of thiazoles and our efforts regarding the preparation and characterization of heterocyclic derivatives incorporating the sulfur atom,[26–32] the work in this study was aimed to design a facile protocol for the synthesis of hybrid arylazo- 4-methylthiazoles and thiazol-4(5*H*)-ones, bearing thioether moieties, as potent antibacterial agents against both Gram-positive and -negative bacterial strains. The *in-vitro* antibacterial screening, as well as *in-vitro* MurB inhibitory activity, were performed for the novel thiazoles. Molecular docking was elucidated to predict the possible binding interaction modes between the thiazoles and the active binding sites of the MurB enzyme from *Escherichia coli*.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

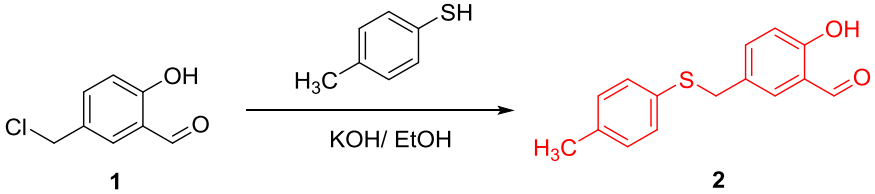
5-(Chloromethyl)-2-hydroxybenzaldehyde **1** was prepared by the reaction of a ternary mixture containing salicylaldehyde, formaldehyde and concentrated HCl.[33] Compound **1** reacted with 4-methylbenzenethiol in ethanol containing an equivalent amount of potassium hydroxide to give the novel 2-hydroxy-5-((*p*-tolylthio)-methyl)benzaldehyde **2** (Scheme 1 and Section 4).

Benzaldehyde derivative **2** was used as a building block for the synthesis of novel thiazoles, bearing both arylazo- and thioether moieties, with potent biological activity via a one-pot three-component protocol. Initially, we investigated the reaction of **2**, thiosemicarbazide and hydrazonyl chloride **3a** under different reaction conditions to prepare the corresponding 4-methyl-5-(phenyldiazenyl)thiazole derivative **4a** with maximum reaction yield and minimum reaction time (Scheme 2 and Table 1).[34]

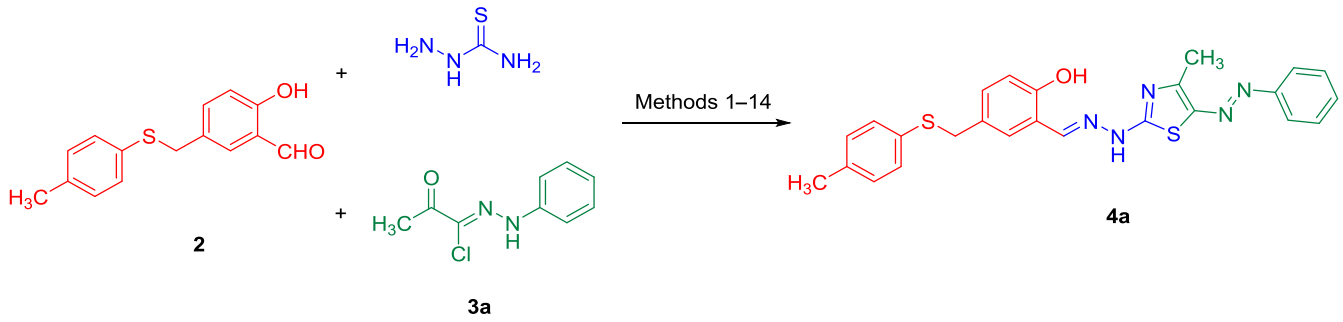
Both piperidine and triethylamine were investigated as reactive basic catalysts in the presence of polar or nonpolar solvents and under either conventional heating or microwave irradiation. Using water as a solvent gave only traces of **4a** as detected by thin-layer chromatography (TLC) analysis (Table 1 and entries 1 and 7), whereas using ethanol or 1,4-dioxane gave 17–45% yields of **4a** (Table 1 and entries 2, 3, 8, and 9). The action of the above catalysts was also investigated in acetonitrile or dichloromethane (as an example of nonpolar solvents) to give **4a** in 25–38% yields (Table 1 and entries 4, 5, 10, and 11). The use of glacial acetic acid as a dual solvent and catalyst was also studied. It gave **4a** in 20–28% yield (Table 1 and entry 13). Moreover, the reaction was carried out using the above catalysts and under solvent-free conditions to give **4a** in 45–54% yields (Table 1 and entries 6 and 12). After examining Table 1, it was clear that the best yield of **4a** was obtained by carrying out the one-pot reaction under both solvent-free as well as catalyst-free conditions (Table 1 and entry 14). Using the above protocol at 120°C, the conventional heating method gave **4a** in 82% yield, whereas the microwave irradiation method gave 86% yield. It is worthy of note that heating of the reaction mixture at 100 or 110°C gave an ununiform melting of the reactants and so gave a mixture of unidentified components. On the contrary, using 120°C as the temperature of the reactants was suitable to obtain a uniform melting of all reactants and so, **4a** was isolated as a sole product. At 140°C or more, a brown mass was obtained of unidentified products. This may be attributed to the charring of some of the reactants.

Using the above optimum reaction conditions, a variety of 4-methyl-5-(aryldiazenyl)thiazole derivatives containing the thioether moiety **4b–e** were prepared by the one-pot reaction of **2**, thiosemicarbazide and hydrazonyl chlorides **4b–e** under either conventional heating or microwave irradiation (Scheme 3). The ^{1}H-NMR spectrum of **4c**, as a representative example of the prepared thiazoles, revealed three singlet signals due to two *p*-Me, thiazole-CH_3 and CH_2 protons at δ = 2.24, 2.58, and 4.13 ppm, respectively, and 11 aromatic protons at δ = 6.87–7.67 ppm, in addition to three singlet signals due to methine-CH, OH, and NH protons at δ = 8.82, 10.70, and 10.78 ppm, respectively (see Section 4). Similarly, the one-pot three-component reaction of **2**, thiosemicarbazide and hydrazonyl chlorides **5a–e** gave the corresponding 5-(2-arylhydrazineylidene)-thiazol-4(5*H*)-one derivatives **6a–e** (Scheme 3). The ^{1}H-NMR spectrum of 5-(2-phenylhydrazineylidene)thiazol-4(5*H*)-one derivative **6a**, as a representative example, revealed six singlet signals due to *p*-Me, CH_2, methine-CH, OH, and two NH protons at δ = 2.25, 4.13, 8.68, 10.54, 10.58, and 12.58 ppm, respectively, in addition to 12 aromatic protons at δ = 6.87–7.64 ppm (see Section 4). The time of reactions and their yields are shown in Table 2. Generally, using microwave irradiation as an energy source gave better yields than conventional heating.

The study also includes the reaction of a ternary mixture of aldehyde derivative **2** with thiosemicarbazide and each of the 1-chloropropan-2-one, 2-bromo-1-phenylethan-1-one, 2-bromo-1-(4-chlorophenyl)ethan-1-one, 3-(2-chloroacetyl)-2*H*-chromen-2-one, or ethyl chloroacetate in both solvent and catalyst-free conditions to give the corresponding 4-substituted thiazole derivatives **7–10** and thiazol-4(5*H*)-one derivative **11** (Scheme 4).[35,36] The time of reactions and their yields are shown in Table 3. The ^{1}H-NMR spectrum of 4-(4-chlorophenyl)thiazole derivative **9**, as a representative example, revealed five singlet signals due to *p*-Me, CH_2, methine-CH, OH, and NH groups at δ = 2.24, 4.12, 8.27, 10.04, and



SCHEME 1 Synthesis of 2-hydroxy-5-((*p*-tolylthio)methyl)benzaldehyde **2**



SCHEME 2 Synthesis of 4-methyl-5-(phenyldiazenyl)thiazole **4a**

12.09 ppm, respectively, in addition to 12 aromatic protons at δ = 6.80–7.88 ppm (see Section 4).

It is noteworthy that **4a** and **6a** could also be prepared by the coupling of 4-methylthiazole derivative **7** and thiazol-4(5*H*)-one derivative **11** with benzene diazonium chloride in pyridine, respectively (Scheme 4).

2.2 | Antibacterial screening

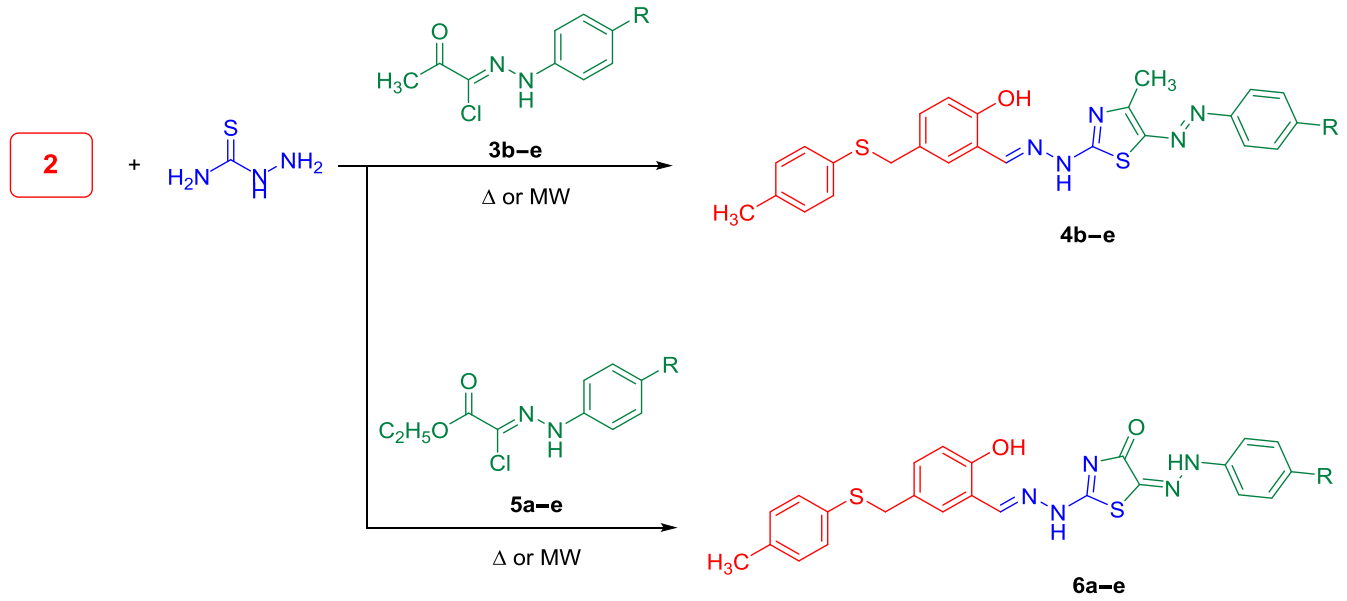
The *in-vitro* antibacterial activities of the newly synthesized thiazoles were evaluated against two Gram-negative (*Klebsiella pneumoniae* and *E. coli*) and two Gram-positive bacterial strains (*Staphylococcus aureus* and *Streptococcus mutans*; see Tables 4 and 5).

5-(2-(4-Methoxyphenyl)hydrazineylidene)thiazol-4(5*H*)-one derivative **6d** was the most potent antibacterial derivative against all the tested bacterial strains. It exhibited the maximum zones of inhibition (63.0 ± 1.0, 45.9 ± 0.6, 62.5 ± 1.0, and 51.2 ± 1.0 mm) against *S. aureus*, *S. mutans*, *K. pneumoniae*, and *E. coli*, respectively, when compared with inhibition zones of the reference antibiotic drugs. Its minimum inhibitory concentration (MIC) values were 3.9 and 7.8 μg/ml against *S. aureus* and *S. mutans*, respectively, when compared with standard ampicillin (62.5 μg/ml). Moreover, its MIC values were 3.9 and 15.6 μg/ml against *K. pneumoniae* and *E. coli*, respectively, when compared with standard gentamicin (62.5 and 31.25 μg/ml, respectively). The analogue **6c**, with the *p*-Me group, was the second in antibacterial strength against the above bacterial strains with MIC values of 7.8, 15.6, 15.6, and 31.25 μg/ml against *S. aureus*, *S. mutans*, *K. pneumoniae*, and *E. coli*, respectively, when compared with reference drugs. The strong activities of both **6c** and **6d** could be attributed to the presence of electron-donating *p*-Me or *p*-MeO groups. The *p*-MeO group is more electron releasing than the *p*-Me group, therefore, compound **6d** showed more enhanced antibacterial activity than **6c**. Compound **2b**, with *p*-Cl, exhibit moderate activities against all the tested bacterial strains. Compound **2a** or **2e**, with *p*-H or *p*-EtOCO, respectively, exhibited weak activities against

TABLE 1 Optimizing the yield of 4-methyl-5-(phenyldiazenyl)thiazole **4a**

Entry	Solvent	Temp. (°C)	Catalyst	Yield (%)[a,b,c]	
				Thermal heating	Microwave irradiation
1	Water	120	Piperidine	Trace	Trace
2	EtOH	100	Piperidine	17	25
3	1,4-Dioxane	120	Piperidine	37	44
4	Acetonitrile	100	Piperidine	32	36
5	Dichloromethane	60	Piperidine	25	30
6	No solvent	120	Piperidine	45	50
7	Water	120	Triethylamine	Trace	Trace
8	EtOH	100	Triethylamine	18	24
9	1,4-Dioxane	120	Triethylamine	40	45
10	Acetonitrile	100	Triethylamine	35	38
11	Dichloromethane	60	Triethylamine	28	33
12	No solvent	120	Triethylamine	48	54
13	Glacial acetic acid	140	Glacial acetic acid	20	28
14	No solvent	120	No catalyst	82	86

[a]The reaction mixture was heated in an oil-bath under constant stirring for 3 hr.
[b]The reaction mixture was irradiated by microwaves under microwave irradiation of power 300 W for 30 min.
[c]The reaction was followed by the thin-layer chromatography analyses.



3, **4b**, R = Cl; **c**, R = CH_3; **d**, R = OCH_3; **e**, R = $CO_2C_2H_5$

5, **6a**, R = H; **b**, R = Cl; **c**, R = CH_3; **d**, R = OCH_3; **e**, R = $CO_2C_2H_5$

SCHEME 3 Synthesis of 4-methyl-5-(aryldiazenyl)thiazole **4b–e** and 5-(2-arylhydrazineylidene)thiazol-4(5*H*)-one derivatives **6a–e**

Gram-positive bacterial strains. Compounds **2a** and **2e** have no activities against Gram-negative bacterial strains.

On the contrary, 5-(aryldiazenyl)-4-methylthiazoles **4** showed a similar pattern of antibacterial activities when compared with compounds **2**. Therefore, the activities of compounds **4** follow the order: **4d** (*p*-MeO) > **4c** (*p*-Me) > **4b** (*p*-Cl) > **4a** (*p*-H) or **4e** (*p*-EtOCO) against all the tested bacteria.

Other thiazole derivatives which lack arylazo- groups showed decreased antibacterial activities when compared with compounds **4** and **6**. Therefore, compounds **7–9** and **11** exhibited weak or zero activities against all the tested bacterial strains. Compound **10** exhibited moderate activities against all the tested bacteria. This may be attributed to the presence of coumarin moiety.

TABLE 2 Synthesis of 4-methyl-5-(aryldiazenyl)thiazole **4b–e** and 5-(2-arylhydrazineylidene)thiazol-4(5*H*)-one derivatives **6a–e** under both microwave irradiation and the conventional method

			Thermal heating[a,c]		Microwave irradiation[b,c]	
Entry	Product	R	Time (hr)	Yield (%)	Time (min)	Yield (%)
1	**4b**	Cl	3	78	30	85
2	**4c**	Me	2.5	74	15	89
3	**4d**	MeO	3	70	30	82
4	**4e**	EtOCO	4	79	45	88
5	**6a**	H	2.5	80	15	89
6	**6b**	Cl	2.5	76	15	90
7	**6c**	Me	3	77	30	92
8	**6d**	MeO	3	74	30	87
9	**6e**	EtOCO	3	81	30	92

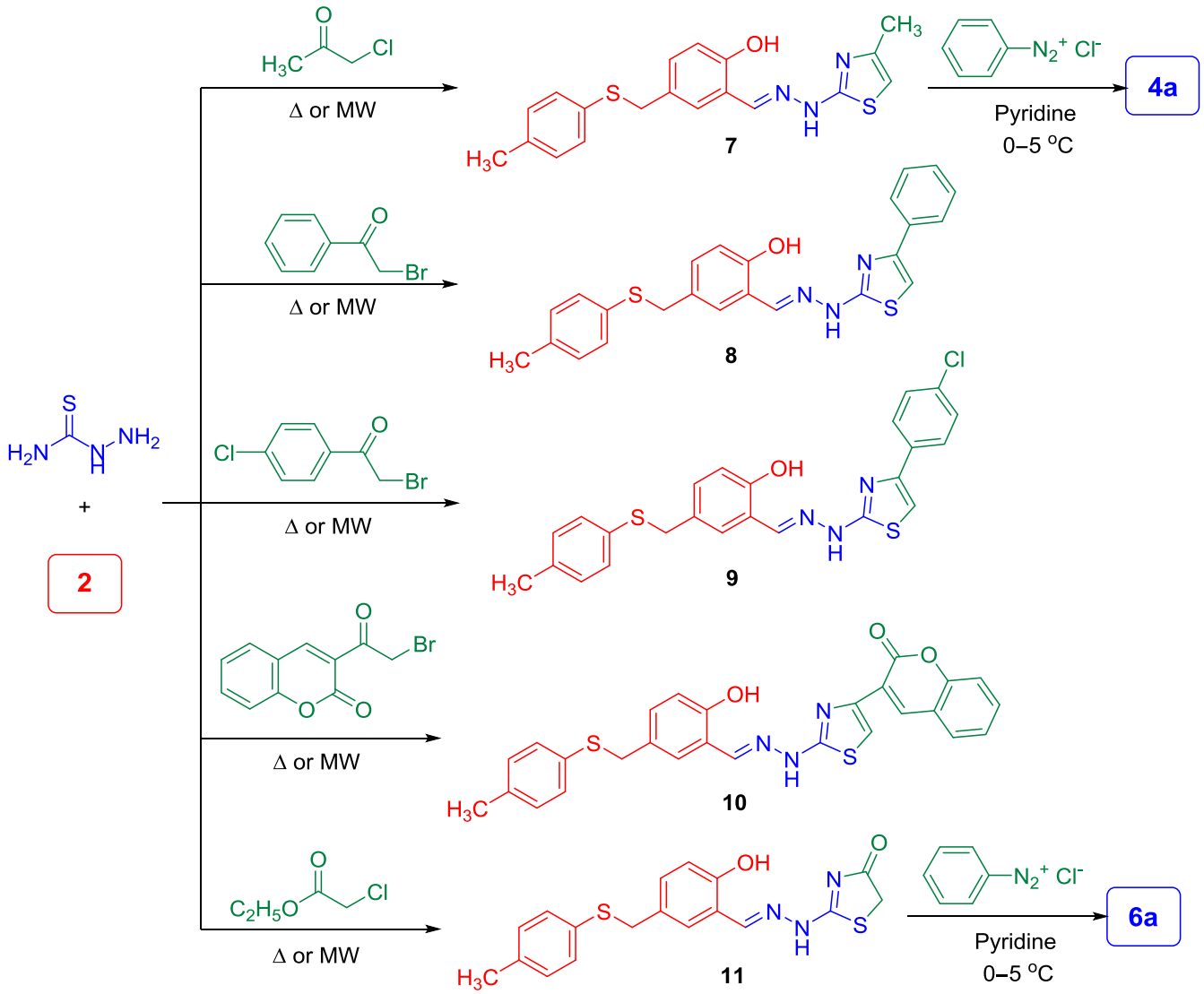
[a]The reaction mixture was heated in an oil-bath at 120°C under constant stirring.
[b]The reaction mixture was irradiated by microwaves at 120°C under microwave irradiation of power 300 W.
[c]The reaction was followed up by thin-layer chromatography analyses.

2.3 | The *in-vitro* enzyme inhibition of MurB

Peptidoglycan is an essential polymer of the cell wall of both Gram-positive and -negative bacterial strains. This polymer consists of repeated units of disaccharide and pentapeptide, which are connected by a lactyl ether bridge.[37] During its biosynthesis, the UDP-*N*-acetylglucosamine-enolpyruvate reductase (MurB) enzyme carries out the reduction of enolpyruvyl uridine diphosphate *N*-acetylglucosamine to uridine diphosphate *N*-acetylmuramic acid.[38] Some antibiotics are able to kill bacteria by inhibiting the role of the MurB enzyme in the biosynthesis of peptidoglycan.[39] It has been reported that 4-thiazolidinones act as inhibitors for MurB enzyme and so they possess promising antibacterial activities.[17]

The enzyme inhibitory activity of the potential antibacterial thiazoles **4c, 4d, 6c,** and **6d** were estimated against the MurB enzyme. Each compound of the tested thiazoles was tested at six different concentrations (5, 10, 25, 50, 75, and 100 μM). 4-Thiazolidinone **I**, as an example of the potent antibacterial agents, was taken as a reference drug[17] (see Figure 1).

IC_{50} values were estimated for all the tested thiazoles. All the tested thiazoles exhibited good to excellent inhibitory activity



SCHEME 4 Synthesis of thiazoles **4a**, **6a**, **7–10**, and **11**

TABLE 3 Synthesis of 4-substituted thiazole derivatives **7–10** and thiazol-4(5*H*)-one derivative **11** under both microwave irradiation and the conventional method

		Thermal heating[a,c]		Microwave irradiation[b,c]	
Entry	Product	Time (hr)	Yield (%)	Time (min)	Yield (%)
1	**7**	3	78	30	89
2	**8**	2.5	74	15	89
3	**9**	2.5	70	15	82
4	**10**	4	79	45	88
5	**11**	3	82	30	92

[a]The reaction mixture was heated in an oil-bath at 120°C under constant stirring.
[b]The reaction mixture was irradiated by microwaves at 120°C under microwave irradiation of power 300 W.
[c]The reaction was followed by thin-layer chromatography analyses.

against the MurB enzyme (see Table 6). Thiazoles **4c, 4d**, and **6c** exhibit good inhibitory activity against MurB enzyme with IC_{50} values in the range of 8.1–18.2 μM. The most potent thiazole in the tested series was **6d**. It exhibited a comparable inhibitory activity, IC_{50} = 8.1 μM, when compared with the reference 4-thiazolidinone **I** (IC_{50} = 7.7 μM).

2.4 | Molecular modeling of novel thiazole derivatives: Docking and structure–activity relationship study

Molecular docking, as one of the *in-silico* study tools, is suitable to obtain an optimized conformation, with minimum free energy, for each of the newly prepared thiazoles as ligand molecules and the target protein.[40,41] The thiazoles **4b**, **4c**, **4d**, **6b**, **6c**, **6d**, and **10** showed different antibacterial activities against *E. coli*. to investigate

TABLE 4 Antibacterial activity of the novel thiazoles against different tested bacterial strains

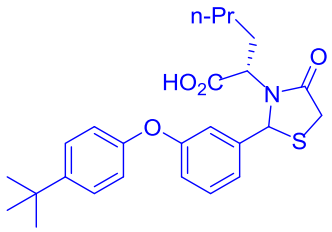
	Zone of inhibition (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
Compound	*Staphylococcus aureus*	*Streptococcus mutans*	*Klebsiella pneumoniae*	*Escherichia coli*
4a	16.5 ± 0.5	14.7 ± 0.5	−ve	−ve
4b	36.3 ± 0.6	22.7 ± 0.5	26.6 ± 0.5	14.0 ± 0.5
4c	51.2 ± 1.0	34.7 ± 0.6	37.5 ± 0.6	30.1 ± 0.6
4d	55.7 ± 1.0	38.4 ± 0.6	47.6 ± 0.6	41.3 ± 0.6
4e	14.8 ± 0.5	−ve	−ve	−ve
6a	18.5 ± 0.5	14.9 ± 0.5	19.0 ± 0.5	−ve
6b	37.1 ± 0.6	25.8 ± 0.5	27.2 ± 0.5	25.0 ± 0.5
6c	59.4 ± 1.0	41.6 ± 0.6	52.5 ± 1.0	46.6 ± 0.6
6d	63.0 ± 1.0	45.9 ± 0.6	62.5 ± 1.0	51.2 ± 1.0
6e	16.7 ± 0.5	15.1 ± 0.5	−ve	−ve
7	15.2 ± 0.5	−ve	17.6 ± 0.5	−ve
8	15.0 ± 0.5	−ve	−ve	−ve
9	−ve	14.2 ± 0.5	−ve	−ve
10	36.8 ± 0.6	25.4 ± 0.5	27.2 ± 0.5	26.3 ± 0.5
11	28.1 ± 0.5	14.6 ± 0.5	14.9 ± 0.5	−ve
Gentamicin	–	–	25 ± 0.5	28 ± 0.5
Ampicillin	22 ± 0.1	28 ± 0.5	–	–

Note: Zone of inhibition is expressed as mm in the form of mean ± standard deviation. Well diameter (6 mm); 100 µl was tested. −ve means inactive (inhibition zone < 8 mm).

TABLE 5 Minimum inhibitory concentration (MIC) of the tested thiazoles

	Minimum inhibitory concentration (µg/ml)			
	Gram-positive bacteria		Gram-negative bacteria	
Compound	*Staphylococcus aureus*	*Streptococcus mutans*	*Klebsiella pneumoniae*	*Escherichia coli*
4a	1,000	1,000	−ve	−ve
4b	250	500	500	1,000
4c	31.25	62.5	125	250
4d	15.6	31.25	31.25	62.5
4e	1,000	−ve	−ve	−ve
6a	1,000	1,000	1,000	−ve
6b	250	250	500	500
6c	7.8	15.6	15.6	31.25
6d	3.9	7.8	3.9	15.6
6e	1,000	1,000	−ve	−ve
7	1,000	−ve	1,000	−ve
8	1,000	−ve	−ve	−ve
9	−ve	1,000	−ve	−ve
10	250	250	500	500
11	500	1,000	1,000	−ve
Gentamicin	–	–	62.5	31.25
Ampicillin	62.5	62.5	–	–

Note: Stock concentration 1 mg/ml; concentrations unit of MIC are represented as µg/ml, MIC was recorded for tested compounds that showed inhibition zone > 10 mm.



I, MurB IC_{50} = 7.7 μM

FIGURE 1 Structure of the potent 4-thiazolidinone MurB inhibitor **I**

TABLE 6 MurB IC_{50} (μM) of the tested thiazoles

Compound	MurB IC_{50} (μM)
4c	18.2
4d	13.8
6c	11.0
6d	8.1
4-Thiazolidinone **I**	7.7

the above thiazoles as potential MurB inhibitors, their calculated structures were docked with the *E. coli* MurB enzyme (PDB ID: 1MBT). The binding energies of the above interactions were calculated using computational docking studies.[42] The docking results are listed in Table 7.

Among the 15 new thiazoles which were prepared in this study, compound **6d**, with the *p*-MeO group, was found to be the most potent antibacterial agent against all the tested bacterial strains. Figure 2 represents the two-dimensional (2D) and 3D ligand interactions of **6d** with *E. coli* MurB. Our docking study showed that compound **6d** had tremendous bioactivity. Its docking pose showed three favorable H-bonding interactions between the S atom in the 4-thiazolone ring and the residue of GLU 128 (3.01 Å, −0.9 kcal/mol), the S atom in the thioether moiety and the residue of ASN 233 (2.57 Å, −2.5 kcal/mol) and the N atom in the azo group with GLU 128 (2.43 Å, −5.9 kcal/mol).

On the contrary, the docking of **4b**, the least active antibacterial agent in the tested thiazoles, with *E. coli* MurB, gave weak H-bonding interaction between the O atom in the hydroxy group and the residue of ASN 233 (3.09 Å, −0.9 kcal/mol) and between the S atom in the thiazole ring and the residue of GLU 325 (4.03 Å, −0.1 kcal/mol; see Figure 3).

The docking poses of the rest tested thiazoles showed H-bonding interactions with residues of different amino acids as follows: between the S atom in the thiazole ring of **4d**, **6b**, and **6c** with the residue of GLU 325, SER 229, and PRO 111; the S atom in the thioether moiety of **4c**, **6c**, and **10** with the residue of GLN 120, SER 229, and ARG 214; and the N atom in the arylazo-group of **4c**, **4d**, and **6c** with GLU 334, ARG 214, PRO 111, and ASN 51. In addition, other H-bonding interactions were present as follows: the O atom in the 4-thiazolone ring of **6b** with TYR 125; N atom in the hydrazinyl group of **6b** with GLY 123; O atom in the chromene-CO group of **10** with LYS 262. Moreover, the thiazoles **4d**, **6b**, and **10** form hydrophobic contacts (π–H or π–π) with the residue of PRO 111, SER 229, TYR 125, and TYR 158 (see Supporting Information).

Experimental results as well as docking studies revealed that compound **10** exhibited moderate activities against all the tested bacteria. This may be attributed to the presence of the coumarin moiety which enhances its antibacterial activity.[43] Other

TABLE 7 Docking results of thiazoles **4b**, **4c**, **4d**, **6b**, **6c**, **6d**, and **10** with the *Escherichia coli* MurB enzyme

Compound	Ligand moiety	Site	Interaction	Distance (Å)	*E* (kcal/mol)
4b	O 28	OD1 ASN 233 (A)	H-donor	3.09	−0.9
	S 37	OE1 GLU 325 (A)	H-acceptor	4.03	−0.1
4c	S 11	NE2 GLN 120 (A)	H-acceptor	2.87	−1.6
	N 45	N GLU 334 (A)	H-acceptor	3.35	−3.0
4d	S 37	OE1 GLU 325 (A)	H-donor	2.98	−1.8
	N 45	NH2 ARG 214 (A)	H-acceptor	3.03	−3.6
	6-ring	CD PRO 111 (A)	π–H	3.78	−0.7
6b	N 33	O GLY 123 (A)	H-donor	3.52	−1.5
	S 37	OG SER 229 (A)	H-donor	3.51	−0.2
	O 39	N TYR 125 (A)	H-acceptor	3.61	−0.4
	6-ring	N SER 229 (A)	π–H	4.39	−0.5
6c	S 11	OG SER 229 (A)	H-donor	2.88	−2.1
	S 37	O PRO 111 (A)	H-donor	3.12	−0.5
	N 42	O PRO 111 (A)	H-donor	2.51	−5.0
	N 41	CB ASN 51 (A)	H-acceptor	3.37	−0.9
6d	S 37	OE1 GLU 128 (A)	H-donor	3.01	−0.9
	N 42	OE1 GLU 128 (A)	H-donor	2.43	−5.9
	S 11	ND2 ASN 233 (A)	H-acceptor	2.57	−2.5
10	S 11	NH2 ARG 214 (A)	H-acceptor	3.32	−1.2
	O 56	NZ LYS 262 (A)	H-acceptor	3.46	−0.8
	5-ring	CE1 TYR 158 (A)	π–H	4.74	−0.8
	6-ring	6-ring TYR 125 (A)	π–π	3.94	−0.0

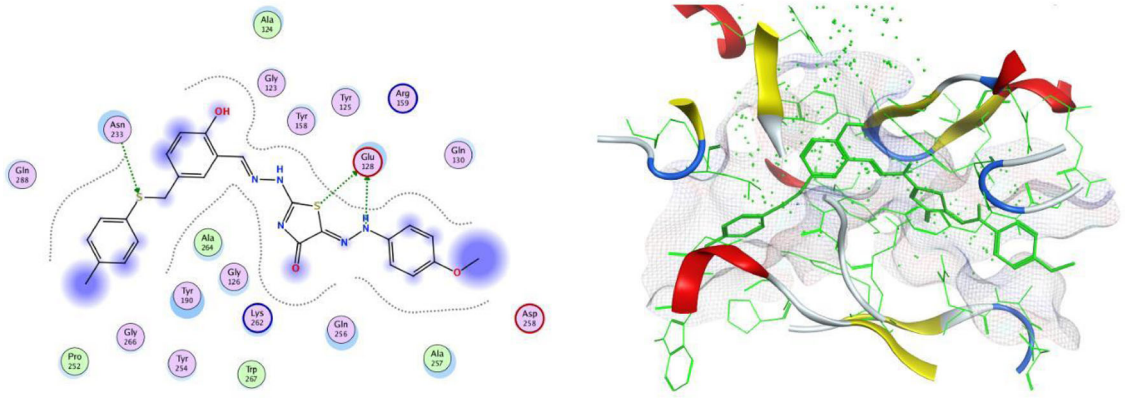


FIGURE 2 Two-dimensional (2D) ligand interaction and 3D ligand interaction **6d** with *Escherichia coli* MurB

thiazole derivatives, which lack arylazo- groups showed decreased antibacterial activities when compared with compounds **4** and **6**. Therefore, compounds **7–9** and **11** exhibited weak or zero activities against all the tested bacterial strains. This suggests that the presence of arylazo- groups enhances the antibacterial activities.

Moreover, both the experimental and docking results revealed differences in antibacterial activity between derivatives with different substituents in the *para*-position of the arylazo- group at position-5 of thiazole derivatives, indicating the effect of substituents on the resulting activity. The results showed that the antibacterial activities of the new thiazoles **4** and **6** were enhanced when a strong electron-donating substituent was attached to the *p*-position of the arylazo- group. This may be attributed to the electron density of these compounds. The presence of the electron-donating group is responsible for increasing the electron density of azo-nitrogen atoms and so enhancing their antibacterial activities by lowering their binding energies with residues of amino acids that are present in the target MurB enzyme.[44–46] These results are another confirmation of the role of arylazo- group in increasing the antibacterial activities of the new thiazoles.

Although we prepared both 5-(aryldiazenyl)-4-methylthiazoles **4** and 5-(2-arylhydrazineylidene)thiazol-4(5*H*)-ones **6** with the same set of substituents attached to *para*-position of arylazo- group, in general, compounds **6** showed stronger antibacterial activities when compared with compounds **4**. This may be attributed to the presence of 4-thiazolone moiety in **6**.[17]

To elucidate the relationship between electronic properties of the target thiazoles and the antibacterial activity, we compare the values of electronic substituent constant (σ_p) [47] with the MIC values against each of *S. aureus* and *E. coli* as examples of Gram-positive and -negative bacterial strains (Table 8). After examining Table 8, a clear relation is observed between the values of σ_p and MIC values. As the substituent becomes more electron-donating, the antibacterial activity increases.[48]

Furthermore, docking studies revealed that most of the tested thiazoles showed strong H-bonding interactions between the S atom in the thioether moieties and residues of amino acids, which are present in the MurB enzyme. Hence, the inhibition ability of the prepared thiazoles toward MurB enzyme could be increased by the incorporation of the thioether moiety in their structure. These results are in agreement with other publications that report the increased *in-vitro* antimicrobial activity of the derivatives containing thioether moieties.[24]

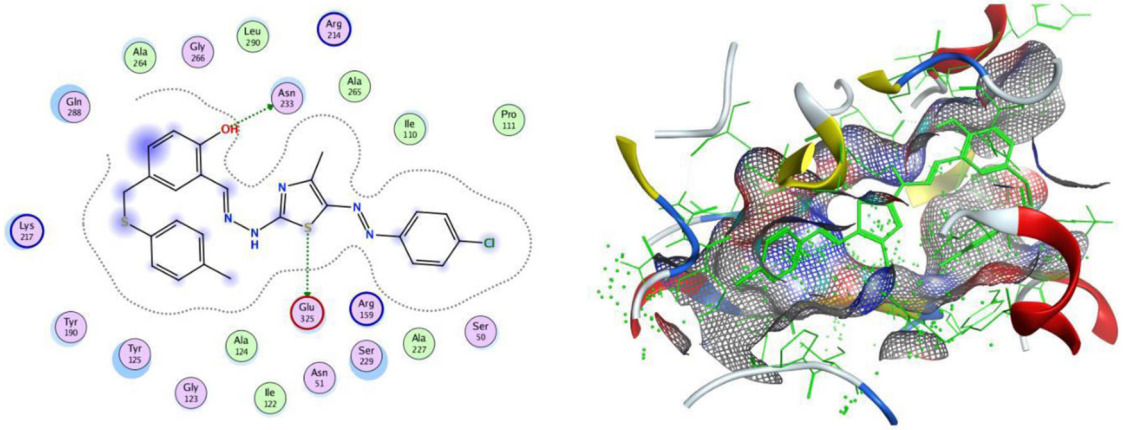


FIGURE 3 2D ligand interaction and 3D ligand interaction of **4b** with *Escherichia coli* MurB

TABLE 8 The relation between electronic substituent constants and minimum inhibitory concentration (MIC) values

Compound	Substituent	σ_p	MIC (μg/ml)	
			Staphylococcus aureus	*Escherichia coli*
4a	H	0.00	1,000	−ve
4b	Cl	0.23	250	1,000
4c	Me	−0.17	31.25	250
4d	MeO	−0.27	15.6	62.5
4e	EtOCO	0.45	1,000	−ve
6a	H	0.00	1,000	−ve
6b	Cl	0.23	250	500
6c	Me	−0.17	7.8	31.25
6d	MeO	−0.27	3.9	15.6
6e	EtOCO	0.45	1,000	−ve

3 | CONCLUSION

This study describes the synthesis of novel thiazoles in various reaction conditions. The optimized protocol involves the three-component reaction of novel aldehyde, thiosemicarbazide and the appropriate halogen-containing reagent in both solvent and catalyst-free conditions. The *in-vitro* antibacterial screening and enzyme inhibition of MurB assays were performed for the novel thiazoles. Molecular docking was also performed to predict the binding modes of the new thiazoles with the active sites of the MurB enzyme. Both *in-vitro* as well as *in-silico* studies show that the thiazol-4(5*H*)-one derivative **6d** with *p*-MeO was the most potent antibacterial derivative against all the tested bacterial strains with the highest inhibition of the MurB enzyme.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All organic solvents were acquired from commercial sources and used as received unless otherwise stated. All other chemicals were acquired from Merck or Aldrich and used without further purification. The melting points (m.p.) were measured on a Stuart melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Smart iTR, which is an ultra-high-performance, versatile, attenuated total reflectance-sampling accessory on the Nicolet iS10 FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian Mercury at 300 MHz and 100 MHz spectrophotometers, respectively, using tetramethylsilane as an internal standard and dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent and chemical shifts were expressed as δ ppm units. Elemental analyses were carried out on a EuroVector instrument C, H, N, S analyzer EA3000 Series. Mass spectra (MS) were recorded on a GC-MS-QP1000EX spectrometer using the inlet type at 70 eV. Microwave experiments were performed using a CEM Discover & Explorer SP microwave apparatus (300 W), utilizing 35-ml capped glass reaction vessels automated power control based on temperature feedback. Compounds **1**,[33] **3a–e**,[49] and **5a–e**[49] are prepared according to the literature procedure.

The original spectra of the investigated compounds are provided as Supporting Information, as are their InChI codes together with some biological data.

4.1.2 | Synthesis of 2-hydroxy-5-((*p*-tolylthio)-methyl)benzaldehyde (2)

A ternary mixture of 5-(chloromethyl)-2-hydroxybenzaldehyde (**1**, 5 mmol), 4-methylbenzenethiol (5 mmol) and potassium hydroxide (5 mmol) in ethanol (20 ml) was heated at reflux for 1 hr. The reaction mixture was cooled, filtered off, washed with cold water and then the product was recrystallized from ethanol as colorless crystals (82%); m.p. 94–96°C; IR (υ cm^{-1}): 3,433, 2,905, 1,656, 1,577, and 1,484; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.90–7.59 (m, 7H, Ar–H), 10.21 (s, 1H, CHO), 10.65 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6): δ 20.2, 36.9, 116.2, 126.3, 128.7, 129.0, 129.5, 129.9, 132.7, 135.9, 137.9, 161.9, and 194.0; MS *m/z* (%): 258 (M^+, 60.2); Anal. for $C_{15}H_{14}O_2S$: C, 69.74; H, 5.46; S, 12.41. Found: C, 69.99; H, 5.61; S, 12.20%.

4.1.3 | Synthesis of thiazoles 4, 7–10 and thiazol-4(5*H*)-ones 6 and 11

Method I: A ternary mixture of aldehyde **2** (5 mmol), thiosemicarbazide (5 mmol) and each of the appropriate hydrazonyl chlorides **3a–e**, **5a–e** or the appropriate halogen-containing reagents (5 mmol) was heated in an oil-bath at 120°C and the reaction was followed by TLC. The reaction mixture was cooled, poured into 100 g of ice-water,

filtered off, washed with cold ethanol and then the product was recrystallized from the proper solvent.

Method II: A ternary mixture of aldehyde **2** (5 mmol), thiosemicarbazide (5 mmol) and each of the appropriate hydrazonyl chlorides **3a–e**, **5a–e** or the appropriate halogen-containing reagents (5 mmol) was prepared. The mixture was placed in process and was irradiated by microwaves with a power of 300 W to reach a reaction temperature of 120°C under autogenerated pressure and the reaction was followed by TLC. The reaction mixture was cooled, poured into 100 g of ice-water, filtered off, washed with cold ethanol, and then, the product was recrystallized from the proper solvent.

Method III: A benzene diazonium chloride solution (5 mmol) was prepared via the addition of sodium nitrite solution (0.5 g into 5 ml of water) to aniline hydrochloride (5 mmol in 5 ml of concentrated HC1) with stirring in an ice bath. The obtained solution was then poured to a solution of each of compound **7** or **11** (5 mmol) in pyridine (15 ml) with stirring for 1 hr at 0–5°C. The reaction mixture was stirred for an additional 3 hr in an ice bath and then left for 12 hr at 4°C in a refrigerator. The solid obtained was filtrated and recrystallized from the appropriate solvent to give the corresponding **4a** and **6a**.

*2-((2-(4-Methyl-5-(phenyldiazenyl)thiazol-2-yl)hydrazineylidene)-methyl)-4-((p-tolylthio)methyl)phenol (**4a**)*

Red crystals (ethanol/1,4-dioxane mixture); m.p. 164–166°C; IR (υ cm^{-1}): 3,411, 3,220, 2,963, 1,600, and 1,488; ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 4.14 (s, 2H, CH_2), 6.89–7.68 (m, 12H, Ar–H), 8.83 (s, 1H, methine-H), 10.74 (s, 1H, OH), and 10.76 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.6, 20.3, 36.8, 116.3, 120.6, 122.7, 128.0, 128.4, 129.3, 129.8, 130.5, 130.9, 132.9, 135.2, 137.7, 138.4, 144.3, 147.5, 151.6, 156.7, and 161.8; MS *m/z* (%): 473 (M^+, 44.5); Anal. for $C_{25}H_{23}N_5OS_2$: C, 63.40; H, 4.90; N, 14.79; S, 13.54. Found: C, 63.62; H, 5.07; N, 14.98; S, 13.36%.

*2-((2-(5-((4-Chlorophenyl)diazenyl)-4-methylthiazol-2-yl)-hydrazineylidene)methyl)-4-((p-tolylthio)methyl)phenol (**4b**)*

Red crystals (1,4-dioxane); m.p. 190–192°C; IR (υ cm^{-1}): 3,411, 3,221, 2,958, 1,601, and 1,484; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.14 (s, 2H, CH_2), 6.88–7.69 (m, 11H, Ar–H), 8.81 (s, 1H, methine-H), 10.70 (s, 1H, OH), and 10.78 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.5, 20.3, 36.7, 116.4, 120.4, 122.8, 128.6, 128.8, 129.3, 129.5, 130.9, 132.6, 134.2, 135.3, 137.2, 138.3, 144.5, 147.6, 150.9, 156.3, and 161.8; Anal. for $C_{25}H_{22}ClN_5OS_2$ (508.06): C, 59.10; H, 4.36; N, 13.78; S, 12.62. Found: C, 58.87; H, 4.11; N, 13.89; S, 12.84%.

*2-((2-(4-Methyl-5-(p-tolyldiazenyl)thiazol-2-yl)hydrazineylidene)-methyl)-4-((p-tolylthio)methyl)phenol (**4c**)*

Red crystals (ethanol/1,4-dioxane mixture); m.p. 186–188°C; IR (υ cm^{-1}): 3,414, 3,220, 2,957, 1,600, and 1,485; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 6H, $2CH_3$), 2.58 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.87–7.67 (m, 11H, Ar–H), 8.82 (s, 1H, methine-H), 10.70 (s, 1H, OH), and 10.78 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.5, 20.2, 20.6, 37.0, 116.6, 120.4, 122.0, 128.1, 129.3, 129.6, 130.7, 131.1, 132.7, 133.9, 134.4, 134.8, 139.2, 144.4, 147.8, 150.0, 156.3, and 161.7; MS *m/z* (%): 487 (M^+, 74.1); Anal. for $C_{26}H_{25}N_5OS_2$: C, 64.04; H, 5.17; N, 14.36; S, 13.15. Found: C, 64.23; H, 5.01; N, 14.14; S, 13.11%.

*2-((2-(5-((4-Methoxyphenyl)diazenyl)-4-methylthiazol-2-yl)-hydrazineylidene)methyl)-4-((p-tolylthio)methyl)phenol (**4d**)*

Red crystals (1,4-dioxane); m.p. 178–180°C; IR (υ cm^{-1}): 3,414, 3,224, 2,961, 1,601, and 1,486; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 4.14 (s, 2H, CH_2), 6.86–7.68 (m, 11H, Ar–H), 8.82 (s, 1H, methine-H), 10.42 (s, 1H, OH), and 10.61 (s, 1H, NH); Anal. for $C_{26}H_{25}N_5O_2S_2$ (503.64): C, 62.01; H, 5.00; N, 13.91; S, 12.73. Found: C, 61.88; H, 4.82; N, 14.05; S, 12.81%.

*Ethyl 4-((2-(2-(2-hydroxy-5-((p-tolylthio)methyl)benzylidene)-hydrazineyl)-4-methylthiazol-5-yl)diazenyl)benzoate (**4e**)*

Red crystals (ethanol/1,4-dioxane mixture); m.p. 168–170°C; IR (υ cm^{-1}): 3,410, 3,220, 2,947, 1,739, 1,607, and 1,488; ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 3H, $CH_2\underline{CH_3}$), 2.24 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 4.14 (s, 2H, CH_2), 4.28 (q, J = 7.2 Hz, 2H, $\underline{CH_2}CH_3$), 6.85–7.91 (m, 11H, Ar–H), 8.69 (s, 1H, methine-H), 10.74 (s, 1H, OH), and 10.99 (s, 1H, NH); Anal. for $C_{28}H_{27}N_5O_3S_2$ (545.68): C, 61.63; H, 4.99; N, 12.83; S, 11.75. Found: C, 61.58; H, 5.14; N, 13.01; S, 11.59%.

*2-(2-(2-Hydroxy-5-((p-tolylthio)methyl)benzylidene)hydrazineyl)-5-(2-phenylhydrazineylidene)thiazol-4(5H)-one (**6a**)*

Yellow crystals (ethanol/1,4-dioxane mixture); m.p. 284–286°C; IR (υ cm^{-1}): 3,434, 3,250, 2,917, 1,703, 1,639, 1,546, and 1,490; ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.87–7.64 (m, 12H, Ar–H), 8.68 (s, 1H, methine-H), 10.54 (s, 1H, OH), 10.58 (s, 1H, NH), and 12.58 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.2, 36.8, 114.9, 116.4, 121.0, 124.8, 128.4, 129.1, 129.4, 129.8, 130.6, 132.3, 135.0, 136.2, 138.2, 143.0, 147.1, 156.0, 157.3, and 163.1; MS *m/z* (%): 475 (M^+, 85.7); Anal. for $C_{24}H_{21}N_5O_2S_2$: C, 60.61; H, 4.45; N, 14.73; S, 13.48. Found: C, 60.65; H, 4.28; N, 14.86; S, 13.30%.

*5-(2-(4-Chlorophenyl)hydrazineylidene)-2-(2-(2-hydroxy-5-((p-tolylthio)methyl)benzylidene)hydrazineyl)thiazol-4(5H)-one (**6b**)*

Yellow crystals (1,4-dioxane); m.p. 280–282°C; IR (υ cm^{-1}): 3,436, 3,243, 2,926, 1,702, 1,644, 1,548, and 1,491; ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.87–7.64 (m, 11H, Ar–H), 8.68 (s, 1H, methine-H), 10.58 (s, 1H, OH), 10.62 (s, 1H, NH), and 12.65 (s, 1H, NH); Anal. for $C_{24}H_{20}ClN_5O_2S_2$ (510.03): C, 56.52; H, 3.95; N, 13.73; S, 12.57. Found: C, 56.74; H, 4.10; N, 13.77; S, 12.49%.

*2-(2-(2-Hydroxy-5-((p-tolylthio)methyl)benzylidene)hydrazineyl)-5-(2-(p-tolyl)hydrazineylidene)thiazol-4(5H)-one (**6c**)*

Yellow crystals (1,4-dioxane); m.p. 290–292°C; IR (υ cm^{-1}): 3,433, 2,918, 1,684, 1,636, 1,534, and 1,489; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 6H, $2CH_3$), 4.14 (s, 2H, CH_2), 6.84–7.70 (m, 11H, Ar–H), 8.69 (s, 1H, methine-H), 10.65 (s, 1H, OH), 10.78 (s, 1H, NH), and 12.64 (s, 1H, NH); MS *m/z* (%): 489 (M^+, 62.9); Anal. for $C_{25}H_{23}N_5O_2S_2$: C, 61.33; H, 4.74; N, 14.30; S, 13.10. Found: C, 61.54; H, 4.87; N, 14.24; S, 13.26%.

*2-(2-(2-Hydroxy-5-((p-tolylthio)methyl)benzylidene)hydrazineyl)-5-(2-(4-methoxyphenyl)hydrazineylidene)thiazol-4(5H)-one (**6d**)*

Yellow crystals (ethanol/1,4-dioxane mixture); m.p. 262–264°C; IR (υ cm^{-1}): 3,434, 3,241, 2,936, 1,688, 1,634, 1,549, and 1,495; ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2), 6.87–7.63 (m, 11H, Ar–H), 8.67 (s, 1H, methine-H), 10.42 (s, 1H, OH), 10.60 (s, 1H, NH), and 12.50 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.2, 36.9, 55.1, 114.2, 116.3, 118.1, 120.7, 128.8, 129.7, 129.8, 131.1, 132.6, 135.1, 136.6, 138.2, 140.0, 147.0, 155.2, 156.1, 157.6, and 163.3; Anal. for $C_{25}H_{23}N_5O_3S_2$ (505.61): C, 59.39; H, 4.59; N, 13.85; S, 12.68. Found: C, 59.31; H, 4.45; N, 13.99; S, 12.74%.

*Ethyl 4-(2-(2-(2-(2-hydroxy-5-((p-tolylthio)methyl)benzylidene)-hydrazineyl)-4-oxothiazol-5(4H)-ylidene)hydrazineyl)benzoate (**6e**)*

Yellow crystals (1,4-dioxane); m.p. 286–288°C; IR (υ cm^{-1}): 3,438, 2,924, 1,738, 1,689, 1,638, 1,552, and 1,494; ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 3H, $CH_2\underline{CH_3}$), 2.24 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 4.27 (q, J = 7.2 Hz, 2H, $\underline{CH_2}CH_3$), 6.87–7.92 (m, 11H, Ar–H), 8.69 (s, 1H, methine-H), 10.55 (s, 1H, OH), 10.87 (s, 1H, NH), and 12.70 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.1, 20.3, 36.8, 61.1, 114.7, 116.6, 121.4, 123.2, 128.5, 129.1, 129.5, 131.0, 131.4, 132.2, 135.4, 136.3, 138.2, 146.3, 146.7, 156.2, 157.6, 163.8, and 166.2; Anal. for $C_{27}H_{25}N_5O_4S_2$ (547.65): C, 59.22; H, 4.60; N, 12.79; S, 11.71. Found: C, 59.07; H, 4.74; N, 12.85; S, 11.57%.

*2-((2-(4-Methylthiazol-2-yl)hydrazineylidene)methyl)-4-((p-tolylthio)-methyl)phenol (**7**)*

Pale beige crystals (ethanol); m.p. 146–148°C; IR (υ cm^{-1}): 3,416, 3,224, 2,944, 1,602, and 1,488; ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.57–7.70 (m, 8H, Ar–H), 8.81 (s, 1H, methine-H), 10.70 (s, 1H, OH), and 10.75 (s, 1H, NH); Anal. for $C_{19}H_{19}N_3OS_2$ (369.50): C, 61.76; H, 5.18; N, 11.37; S, 17.35. Found: C, 61.80; H, 5.07; N, 11.52; S, 17.50%.

*2-((2-(4-Phenylthiazol-2-yl)hydrazineylidene)methyl)-4-((p-tolylthio)-methyl)phenol (**8**)*

Beige crystals (ethanol/1,4-dioxane mixture); m.p. 216–218°C; IR (υ cm^{-1}): 3,433, 3,321, 2,915, 1,618, 1,562, and 1,488; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.78–7.87 (m, 13H, Ar–H), 8.26 (s, 1H, methine-H), 10.02 (s, 1H, OH), and 12.05 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.3, 36.8, 106.4, 116.8, 120.4, 126.0, 128.5, 128.7, 129.0, 129.2, 129.5, 131.2, 132.1, 133.2, 134.6, 138.7, 147.6, 152.2, 156.5, and 167.7; MS *m/z* (%): 431 (M^+, 70.8); Anal. for $C_{24}H_{21}N_3OS_2$: C, 66.79; H, 4.90; N, 9.74; S, 14.86. Found: C, 66.94; H, 5.12; N, 9.85; S, 14.72%.

*2-((2-(4-(4-Chlorophenyl)thiazol-2-yl)hydrazineylidene)methyl)-4-((p-tolylthio)methyl)phenol (**9**)*

Beige crystals (1,4-dioxane); m.p. 232–234°C; IR (υ cm^{-1}): 3,434, 3,312, 2,916, 1,617, 1,564, and 1,488; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 6.80–7.88 (m, 12H, Ar–H), 8.27 (s, 1H, methine-H), 10.04 (s, 1H, OH), and 12.09 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.2, 36.6, 108.6, 116.7, 120.2, 127.6, 128.4, 128.7, 129.6, 129.7, 130.0, 131.9, 132.1, 134.8, 135.1, 138.9, 147.3, 150.1, 156.6, and 167.8; Anal. for $C_{24}H_{20}ClN_3OS_2$ (466.01): C, 61.86; H, 4.33; N, 9.02; S, 13.76. Found: C, 62.04; H, 4.18; N, 9.17; S, 13.69%.

*3-(2-(2-(2-Hydroxy-5-((p-tolylthio)methyl)benzylidene)hydrazineyl)-thiazol-4-yl)-2H-chromen-2-one (**10**)*

Yellow crystals (1,4-dioxane); m.p. 288–290°C; IR (υ cm^{-1}): 3,433, 3,222, 2,916, 1,700, 1,578, and 1,491; ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.80–7.77 (m, 12H, Ar–H), 8.30 (s, 1H, methine-H), 8.54 (s, 1H, coumarin H-4), 10.02 (s, 1H, OH), and 12.11 (s, 1H, NH); MS *m/z* (%): 499 (M^+, 53.4); Anal. for $C_{27}H_{21}N_3O_3S_2$: C, 64.91; H, 4.24; N, 8.41; S, 12.83. Found: C, 65.12; H, 4.14; N, 8.63; S, 13.01%.

*2-(2-(2-Hydroxy-5-((p-tolylthio)methyl)benzylidene)hydrazineyl)-thiazol-4(5H)-one (**11**)*

Beige crystals (ethanol); m.p. 260–262°C; IR (υ cm^{-1}): 3,432, 2,919, 1,718, 1,640, and 1,487; ^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.97 (s, 2H, thiazole-CH_2), 4.13 (s, 2H, CH_2), 6.82–7.66 (m, 7H, Ar–H), 8.66 (s, 1H, methine-H), 10.54 (s, 1H, OH), and 10.54 (s, 1H, NH); MS *m/z* (%): 371 (M^+, 39.9); Anal. for $C_{18}H_{17}N_3O_2S_2$: C, 58.20; H, 4.61; N, 11.31; S, 17.26. Found: C, 58.46; H, 4.73; N, 11.44; S, 17.35%.

4.2 | Biological assays

4.2.1 | The *in-vitro* antibacterial assay

The *in-vitro* antibacterial activity of all tested molecules was achieved using the agar well diffusion procedure.[50,51] The antibacterial activity was evaluated against each of *S. aureus* (ATCC: 6538) and *S. mutans*, (ATCC:25175) as Gram-positive bacterial strains (the reference drug was ampicillin, 10 µg susceptibility disc; Oxford-England) and *Klebsiella pneumoniae* (ATCC:10031) and *E. coli* (ATCC: 9637) as gram-negative bacterial strains (the reference drug was gentamicin, 120 µg susceptibility disc; Oxford-England) using nutrient agar medium. The concentration of the tested molecules was 15 mg/ml against bacterial strains. DMSO was used as the solvent. Negative control was the wells containing only DMSO.

Sterilized media were poured onto Petri dishes, and these then solidified at room temperature. The microbial suspension was prepared in sterilized saline equivalent to McFarland 0.5 standard solution (1.5×10^5 CFU/ml) with a turbidity of OD = 0.13 using a spectrophotometer at 625 nm. After solidification of the media, 6-mm diameter wells were made. To each well, 100 µl of each the tested compounds were added then the plates were incubated at 37°C for 24 hr to observe the formation of inhibition zones. The above procedure was repeated three times and the final reading of the inhibition zones (in mm) was determined as the average.

4.2.2 | Minimum inhibitory concentration

MIC is used to evaluate the minimum concentration of the tested compound which prevents the growth of bacteria after overnight incubation. The twofold serial dilution was used to estimate the MIC values.[51,52]

For each bacterial strain, three to five isolated colonies from a fresh agar plate were transferred into a 3- to 4-ml sterile broth medium. The bacterial suspension was incubated at 35–37°C for 2–6 hr until the turbidity of the bacterial suspension became equal to or greater than the turbidity of a McFarland Standard 0.5. The tested molecules were diluted to 1,000 μg/ml, as a stock solution. Further dilutions for the tested molecules were performed with the broth medium. Then, a fixed volume of the prepared bacterial inoculum was added to each tube of the different concentrations of the tested molecules and incubated for 16–20 hr at 37°C. By turbidity, the growth or a lack of growth in the tested tubes was observed by comparison with the growth control (which represents the original inoculum without any tested compounds). In the same way, both ampicillin and gentamicin were screened under the same conditions for comparison.

4.2.3 | The *in-vitro* MurB inhibition assay

The assay for activity of MurB enzyme, in the presence or absence of an inhibitor, was estimated using Tris-HCl (20 mM, pH 7.4), KCl (20 mM), dithiothreitol (0.5 mM), NADPH (100 μM), and MurB (50 μM).[17] The tested thiazoles were individually dissolved in DMSO at a 100-fold higher concentration than that used in the final assay. The solution of each of the tested thiazoles (3.5 μl) was added to prelabeled flat-bottom 96-well microtiter plates containing a 320-μl MurB assay mixture. For the vehicle controls, 3.5 μl of DMSO was added. The reaction was initiated by adding 30 μl of MurB. Then, the decrease of absorbance at 340 nm was monitored using a Spectra Max 250 microplate spectrophotometer over ten minutes at 37°C. For IC_{50} determination, each of the tested thiazoles was tested at six two-fold serial diluted concentrations (5, 10, 25, 50, 75, and 100 μM). All the experiments were performed in duplicate.

4.3 | Molecular docking

The study of molecular docking was elucidated using Molecular Operating Environment (MOE) version 2015.10 software (https://www.chemcomp.com) and this is a rigid molecular docking software.[53] Studies of molecular docking have a high significance for predicting the probable binding modes of the tested active thiazoles against MurB enzyme from *E. coli* (Protein Data Bank [PDB] ID: 1MBT). MOE is an interactive molecular graphics software that can calculate and show feasible docking modes of the target enzyme and the tested thiazoles. It requires the tested compounds and the target enzyme as input in PDB format. The molecules of water, co-crystallized ligands and other unsupported elements (e.g., Na, Mg, SO_4, etc.) were removed but the amino-acid chain was reserved.[54,55] The ligand's structure in the PDB file format was created by Gaussian 03 software. The structure of the MurB enzyme from *E. coli* was downloaded from PDB (https://www.rcsb.org/).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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