



## Research article

## Design, synthesis, and molecular docking of new phenothiazine incorporated N-Mannich bases as promising antimicrobial agents

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## ABSTRACT

The present work aims to synthesize four series of phenothiazine incorporation Mannich bases. Therefore, 10-methyl-10H-phenothiazine-3-sulfonamide (**4**) which was subjected to react with some secondary amines and formaldehyde to give the Mannich bases **5a-f**, and **6-13**. Compound **13** was then subjected to react with some secondary amines and formaldehyde to give the corresponding Mannich bases **14a-f**. In total, twenty-two new compounds were synthesized and evaluated for *in vitro* growth inhibition activity against *P. aeruginosa*, *E. coli*, and *S. aureus*. Among the tested compounds, compounds **3**, **5a**, **5c**, **6**, **12**, **13**, **14d**, and **14e** exhibited good activity with a MIC value (12.5 µg/mL), compounds **5b**, **10**, **11**, **14a**, and **14c** exhibited strong activity against the growth of *S. aureus* with a MIC value (6.25 µg/mL), and compound **14b** superior against *S. aureus* with a MIC value (3.125 µg/mL) compared to drug reference ciprofloxacin with MIC value (2 µg/mL). The molecular docking investigation revealed the presence of many derivatives with high binding affinities and distinct interaction patterns with the target protein. Derivatives **14a-e** emerged as the most promising possibilities, displaying the greatest binding energies and a varied variety of interaction types, including hydrogen bonding and pi interactions, over different distances, with derivative **14b** exhibiting the highest binding energy at  $S = -8.3093$  kcal/mol. These derivatives displayed superior binding affinities and various interaction mechanisms with the target protein, suggesting that they have great promise as lead compounds for future development into therapeutic medicines.

## 1. Introduction

Formulating and synthesizing compounds with high medical properties is one of the main goals of organic and medicinal chemistry. The scientific community faces a significant dilemma because of the quick emergence of resistance to currently available antimicrobial medications. The synthesis of novel antimicrobial drugs with strong efficacy against drug-resistant microbes is therefore urgently needed [1]. Because the amino group can be readily converted into a variety of other functionalities, the chemistry of the amino

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alkylation of aromatic substrates by the Mannich reaction is of great interest for the synthesis and modification of biologically active compounds having physical [2], chemical importance [3], as well as physiological properties [4,5]. A sensible way to introduce a basic aminoalkyl chain into a variety of medications and substances is by the Mannich reaction [6]. Additionally, a significant degree of pharmacological activity of different Mannich bases for antibacterial, analgesic, anti-inflammatory, and anaesthetic properties as well as intermediates in drug production. Mannich bases have gained importance due to their application in antibacterial activity and other applications are in agrochemicals such as plant growth regulators. Moreover, *N*-bridged heterocyclic derivatives show important antibacterial activity. The amino alkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [2]. Mannich bases have several biological activities such as antimicrobial and anticancer. Morpholine derivatives were reported to possess antimicrobial, anti-inflammatory, and central nervous system activities. Therefore, bearing in mind the above observation, we were led to synthesize and test the antimicrobial activity of a new series of Mannich base derivatives [7–9]. A review of the literature in this area turned up several publications on the antibacterial action of *N*-Mannich bases. The combination of Pregnenolone and Carbamazepine exhibits reduced efficacy against *S. aureus* and *E. coli* [10]. Furthermore, sulphonamide is widely recognized for its antibacterial [11–13], anti-tubercular [14], anti-inflammatory [15], and carbonic inhibitory [16] properties. Strong antibacterial properties and lower toxicity than the parent sulphonamide are reported for the Mannich bases integrated with sulphonamides [17] (Fig. 1).

However, phenothiazine derivatives have long been identified as biologically active therapeutic possibilities and a TB patient has been successfully treated with the phenothiazine drug chlorpromazine (CPZ) [18]. This biological effect is attributed to the presence of chlorine, the phenothiazine ring, and the alkylamine group (Fig. 1).

In addition, phenothiazines were the first drugs to be effectively utilized in the treatment of psychosis; nowadays, they are well-known for their therapeutic applications as antipsychotic, antiemetic, antihypertensive, and antihistaminic drugs [19]. Phenothiazines have demonstrated their ability to function as human cholinesterase inhibitors in recent years [20], and they have frequently been described as multidrug resistance (MDR) reversal medicines [21]. Since they have been utilized for more than 50 years, neuroleptic phenothiazines have been prepared in several ways that have been documented. These methods are primarily found in pharmaceutical company patents, such as Pipothiazine [22] which attributed its biological activity to the presence of phenothiazine, alkyl amine, and SO<sub>2</sub> moieties (Fig. 1).

There are well-known techniques for synthesizing phenothiazines. [2-[(2-Chlorophenyl) thio]phenyl]amine derivatives can be used to synthesize phenothiazines with substituents in positions 2, 3, and 4 [21]. The reaction of 2-aminobenzenethiol with derivatives of 2-chloro-1-nitrobenzene, followed by acetylation and Smiles rearrangement, is a widely utilized technique for the synthesis of 2-substituted phenothiazines [23,24].

The Mannich reaction is a crucial stage in the synthesis of several bioactive compounds and is frequently employed in the production of secondary and tertiary amine derivatives [25–27]. *N*-Mannich bases that are generated from NH-heterocycles and related compounds exhibit a wide range of potential pharmacological effects, including neuroprotection, anticancer, antifungal, anti-HIV, antitubercular, and antibacterial properties [28–34].

## 2. Results and discussion

### 2.1. Characterization of new synthesizes

The intended 10H-phenothiazine-2-sulfonyl chloride was not produced when phenothiazine was sulfonated with chlorosulfonic acid. The literature claims make polysubstitution and easy sulfur atom oxidation make the electrophilic aromatic substitution of the phenothiazine ring challenging. This led us to develop a different strategy that starts with a suitably substituted phenothiazine.

To enhance the poor reactivity of phenothiazine toward chlorosulfonation, compound **3** was prepared to have *N*-methyl group. Finding this alkyl group in the aromatic ring parallel to the sulfur atom was the aim. Keeping this criterion in mind, our synthetic path toward structure **3** resembles that of Warburton et al. [35] in certain ways.

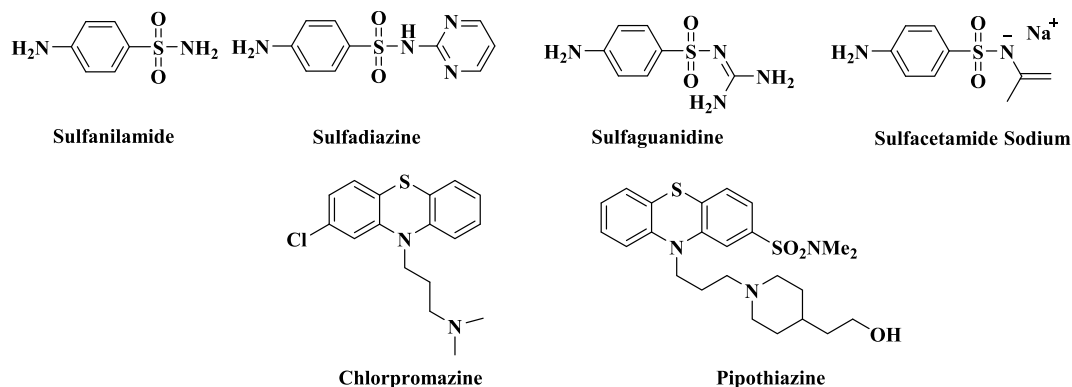


Fig. 1. Some marketed sulfonamide and phenothiazine drugs.

Compound **4** had two singlet signals in its  $^1\text{H}$  NMR spectrum, corresponding to  $\text{NH}_2$  and  $\text{CH}_3$  protons at  $\delta$  3.07 and 6.94 ppm, respectively. The  $\text{NH}_2$  function group was identified in the IR spectra as the source of an absorption band at  $3450\text{ cm}^{-1}$  (Scheme 1).

Following up on the Mannich-bases reported work [36], the current work focuses on attempting to broaden the scope of the Mannich reaction with phenothiazine derivatives. This includes synthesizing new *N*-Mannich bases and bis(Mannich bases), which may have uses in medicine. In the present study, phenothiazine **4** was treated with some secondary amines namely (piperidine, morpholine, diethanol amine, dimethyl amine, diethyl amine, and tetrahydrocarbazole) in the presence of formaldehyde to give Mannich bases **5a-f**. In general,  $^1\text{H}$  NMR of compounds **5a-f** showed a characteristic singlet signal of  $\text{CH}_2$  protons around  $\delta$  4.00–4.50 ppm. The IR spectra, in general, displayed an absorption band around  $3150\text{ cm}^{-1}$  due to the  $\text{NH}$  function group (Scheme 2). (c.f. supplementary file).

In the course of this study, the synthesis of bis(Mannich bases) **6–9** has been achieved by treating 2 mol compound **4** with piperazine/formaldehyde, 4,4'-trimethylenedipiperidine (TMDP)/formaldehyde, glutaraldehyde/dimethylamine, and *p*-phenylenediamine/formaldehyde, respectively. The structure of compound **6** was confirmed based on its analytical and spectral data. The  $^1\text{H}$  NMR spectrum of compound **6** showed an interesting singlet signal at  $\delta$  2.27 ppm due to four  $\text{CH}_2$  protons of the piperazine ring, while its IR spectrum showed an absorption band at  $3160\text{ cm}^{-1}$  for the  $\text{NH}$  group. On the other hand, compound **7** showed an absorption band of  $\text{NH}$  at  $3155\text{ cm}^{-1}$ , and  $^1\text{H}$  NMR showed a multiplet signal at  $\delta$  1.42–1.62 ppm corresponding to seven  $\text{CH}_2 + 2\text{CH}$  protons. Additionally, compound **8** showed in its  $^1\text{H}$  NMR multiplet signal at  $\delta$  1.30 ppm due to  $\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}$  protons, quartet signal at  $\delta$  1.55 ppm due to  $\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}$  protons, and triplet signal at  $\delta$  3.80 ppm due to  $\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}$  protons. Finally, the bis base **9** showed in its IR spectrum absorption band at  $3152\text{ cm}^{-1}$  due to the  $\text{NH}$  absorption band. The  $^1\text{H}$  NMR spectrum showed a characteristic singlet signal at  $\delta$  6.60 ppm corresponding to four aromatic protons of the AB system (Scheme 3).

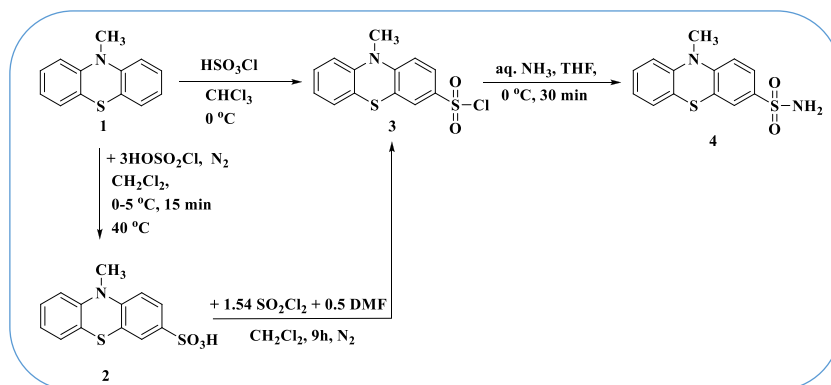
Considerable attention has been devoted to the tetrahydrocarbazole derivatives as anticonvulsant and antihyboxant activity [37, 38]. In view of this and an extension of the presence study, we reported here the reaction of phenothiazine derivatives (**5a**) with tetrahydrocarbazole and formaldehyde to give *N*-((1,2,3,4,4a,9a-hexahydro-9H-carbazol-9-yl)methyl)-10-methyl-*N*-(piperidin-1-ylmethyl)-10H-phenothiazine-3-sulfonamide (**10**). Compound **10** showed a molecular ion peak at  $m/z = 574$  ( $\text{M}^+$ ), corresponding to the molecular weight of the formula  $\text{C}_{32}\text{H}_{38}\text{N}_4\text{O}_2\text{S}_2$ . The  $^1\text{H}$  NMR displayed new signals at  $\delta$  1.20–1.80 ppm as multiplet related to the tetrahydrocarbazole ring. The periodate oxidation of the tetrahydrocarbazole moiety in compound **10** is of particular interest because it provides convenient access to the formation of the functionalized hexahydrobenzo[*b*]azonine system *N*-((2,7-dioxo-2,3,4,5,6,7-hexahydro-1H-benzo[*b*]azonin-1-yl)methyl)-10-methyl-*N*-(piperidin-1-ylmethyl)-10H-phenothiazine-3-sulfonamide (**11**). Synthesis of compound **11** is of particular interest, because of azonine core is present in vinblastine and vincristine alkaloids [39,40] which possess significant antitumor activity and have been widely used clinically. In addition, some azonine and benzoazonine have been studied with interest centred on their potential pharmaceutical activity [41–44]. In continuation compound **11** has been used as a precursor to 10-methyl-*N*-((9-oxo-1,2,3,9-tetrahydro-4H-cyclopenta[*b*]quinolin-4-yl)methyl)-*N*-(piperidin-1-ylmethyl)-10H-phenothiazine-3-sulfonamide (**12**), which was obtained on treating compound **11** with dil. sodium hydroxide solution. The tendency of **11** to cyclize readily under the mild basic condition to give compound **12** is in line with an earlier report by Witkop et al. [45] (Scheme 4).

Moreover, compound **3** reacted with sulfanilamide to give the corresponding 10-methyl-*N*-(4-sulfamoylphenyl)-10H-phenothiazine-2-sulfonamide (**13**). The  $^1\text{H}$  NMR spectrum showed three singlet signals at  $\delta$  2.97, 6.84, and 10.10 ppm corresponding to  $\text{CH}_3$ ,  $\text{NH}_2$ , and  $\text{NH}$  protons, while its mass spectrum displayed  $m/z = 447$  as exact mass. Compound **13** was subjected to react with some secondary amines in the presence of formaldehyde to give *N*-Mannich bases (**14a-f**) (Scheme 5). The structure of compounds **14a-f** was characterized by analytical and spectroscopical data. (c.f. supplementary data)

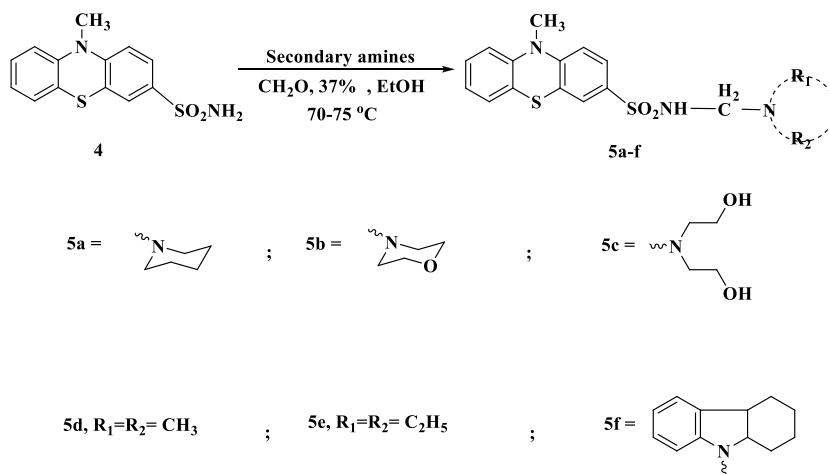
## 2.2. Antimicrobial evaluation

### *In vitro* antibacterial activity and Structure-activity relationship (SAR):

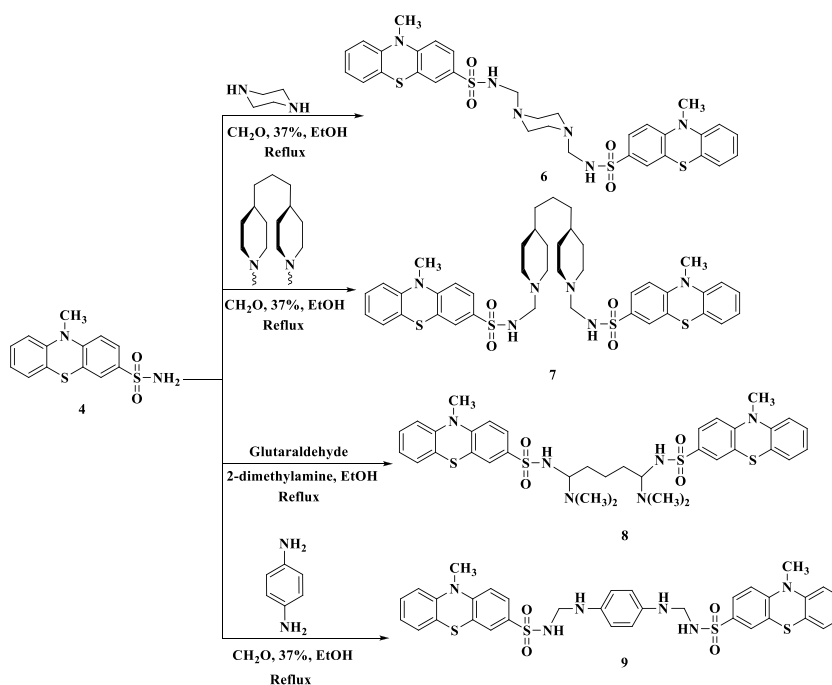
Using the agar dilution method to determine the lowest inhibitory concentration, all the resulting compounds were evaluated for



Scheme 1. Synthesis of 3-chlorosulfonyl and 3-sulfonamide phenothiazine derivatives **3** and **4**.



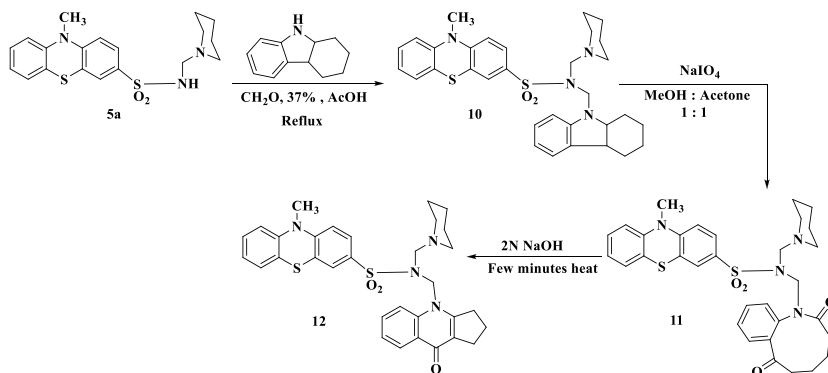
**Scheme 2.** Reaction of phenothiazine sulfonamide **4** with secondary amine and formaldehyde.



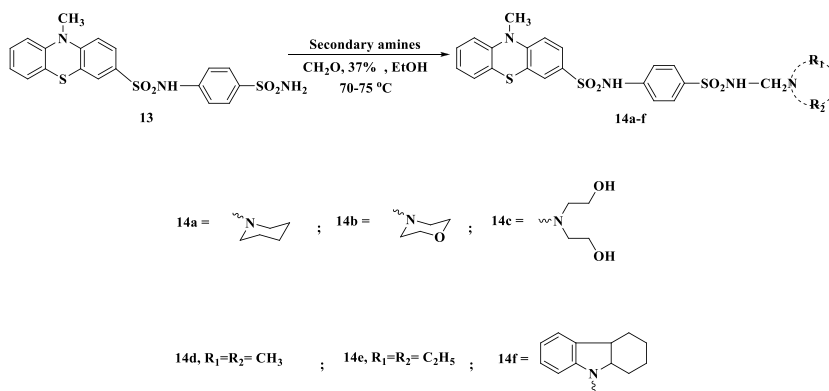
**Scheme 3.** Synthesis of bis-Mannich bases **6–9**.

their *in vitro* antibacterial activity against strains of *P. aeruginosa*, *E. coli*, and *S. aureus* [46]. The commercial antibiotic ciprofloxacin was used as the reference drug for comparison. Compared to the other tested strains, most of the compounds showed superior inhibitory efficacy against the *S. aureus* strain (Table 1).

Interestingly, compounds with an electron-withdrawing group and a chlorine atom exhibited outstanding inhibitory activity, indicating that the chlorine substituent plays a substantial role in the compound's antibacterial action. Compound 3's MIC value, however, indicates that it has a better inhibitory effect against *S. aureus* (MIC 12.5  $\mu\text{g/mL}$ ) than it does against the other examined bacterial strains. When comparing the results of Mannich bases 14a-f with their counterparts Mannich bases 5a-f, it was found that the sulfamoyl phenyl group in Mannich bases 14a-f increased the effectiveness of the compounds as antibacterial agents, as sulfonamides are known to interfere with bacterial folic acid synthesis. However, the specific activity against Gram-positive or Gram-negative bacteria can depend on various factors, including the overall structure, charge, and lipophilicity of the compounds. The results in Table 1 showed antibacterial activity against *S. aureus* due to the presence of morpholinomethyl group (compounds 14b and/or 5b) (MIC 3.125, and/or 6.25  $\mu\text{g/mL}$ ) which enhance antibacterial activity compared to piperidinomethyl group in 14a and/or 5a (6.25, and/or 12.5  $\mu\text{g/mL}$ ).



**Scheme 4.** Reaction of phenothiazine sulfonamide **5a** with tetrahydrocarbazole and formaldehyde: synthesis of benzoazonine derivative **11** and related compound.



**Scheme 5.** Reaction of sulfamoyl phenyl phenothiazine sulfonamide **13** with secondary amines and formaldehyde.

**Table 1**  
Antimicrobial activity of the newly synthesized compounds.

Compound No.	Gram Positive bacteria		Gram Negative bacteria	
	<i>S. aureus</i>		<i>E. coli</i>	<i>P. aeruginosa</i>
3	12.5		50	50
4	25		50	100
5a	12.5		25	50
5b	6.25		12.5	25
5c	12.5		25	50
5d	25		50	50
5e	25		50	50
5f	50		100	100
6	12.5		25	50
7	25		25	50
8	100		100	100
9	50		50	100
10	6.25		25	50
11	6.25		12.5	50
12	12.5		25	100
13	12.5		25	50
14a	6.25		12.5	50
14b	3.125		6.25	25
14c	6.25		12.5	25
14d	12.5		25	50
14e	12.5		25	50
14f	25		50	50
Ciprofloxacin	2		2	4

Compounds 6–9 showed moderate to weak activity against the tested strains (MIC 12.5–100  $\mu\text{g}/\text{mL}$ ). The antibacterial activity of a compound can depend on various factors, including its size, structure, and ability to penetrate bacterial cells. Generally, larger compounds may face challenges in entering bacterial cells, but their antimicrobial activity can still be influenced by other factors. This result is based on the assumption that the more flexible and shorter linkers in compounds 6 and 7 may facilitate better interaction with bacterial cells compared to the longer and less flexible linkers in compounds 8 and 9.

When comparing the antibacterial activity of compounds 10 and 11 with the biological effect of compound 5a as an antibacterial agent against *S. aureus* strain, it was found that the process of replacing NH in compound 5a with methyl tetrahydrocarbazol and methyl benzoazonine increased the biological effect significantly. It is noteworthy that, all compounds carrying electron-withdrawing groups are active. Depending on this fact, compound 11 (MIC 6.25  $\mu\text{g}/\text{mL}$ ) which contains two carbonyl groups is more active than compounds 10 and 12. Compound 11 contains hexahydrobenzoazonine ring, which is more structurally complex and might have a different mode of action compared to the other compounds, potentially contributing to higher activity. Compound 10 has a hexahydrocarbazolylmethyl group, which is also complex but less so than the hexahydrobenzoazonine ring in compound 11.

### 2.3. Molecular docking

In sight the molecular docking stimulation for the synthesized derivatives with good antibacterial activities with the DHPS (dihydropteroate synthase) protein structure (PBD: 3TZF) to establish the most preferred mode of interaction (Table S1) [47]. Derivative 3 displayed binding energy score (S) =  $-6.0013$  kcal/mol, indicating moderate affinity, RMSD = 1.0146, suggesting a reliable docking pose, and hydrogen acceptor bonds with Arg255 and Thr62, crucial for binding through intermolecular distance 3.49 Å and 2.89 Å, respectively, enhancing interaction stability (Fig. 2). Meanwhile, derivative 5a revealed a good energy score (S) =  $-7.6491$  kcal/mol, showing strong affinity, RMSD = 1.6136, indicating a stable conformation upon binding, over N16, engages Lys221 via hydrogen bonding, while the benzene ring forms pi-H interactions with Arg63, suggesting a robust binding mechanism (Fig. 3). Though derivative 5b showed a binding Energy score (S) with  $-6.1538$  kcal/mol, reflecting moderate to high affinity, showcases a mix of hydrogen and both of pi-H and H-pi interactions, indicating versatile binding capabilities (Fig. 4). Likewise, derivative 5c presented binding energy score (S) =  $-6.9959$  kcal/mol, highlighting strong affinity, RMSD = 1.5995, suggesting a stable interaction, over hydrogen bonding and pi-H interactions, indicating a complex interaction pattern with the target (Fig. 5). However, derivative 6 recorded a binding energy score (S) =  $-7.1426$  kcal/mol, indicative of strong affinity, through a singular hydrogen acceptor bond with Arg63, showing specific but potentially narrow interaction over an intermolecular distance of 3.26 Å, within the ideal range for hydrogen bonding, suggesting effective but focused interaction (Fig. 6). Nevertheless, derivative 11 displayed binding energy score (S) =  $-6.6470$  kcal/mol, showing good affinity through RMSD = 1.9820, indicating a stable but slightly variable binding mode, and forms a hydrogen bond with Arg 63, similar to Derivative 6, suggesting a targeted but potentially limited interaction scope (Fig. 7). Derivative 12 exhibited a potent energy score (S) =  $-8.0267$  kcal/mol, one of the highest affinities observed, indicating excellent potential as an inhibitor through RMSD = 1.7753, suggesting a reliable docking pose, and A strong hydrogen bond with Arg 63, emphasizing a potent and specific interaction over intermolecular distance 3.38 Å. Moreover, derivative 13 showed energy score (S) =  $-7.9849$  kcal/mol, showcasing high affinity comes from RMSD = 1.2861, reflecting a precise and stable binding conformation. Features a combination of hydrogen donor/acceptor and pi interactions, suggesting multifaceted binding, through distances ranging from 2.83 Å to 4.46 Å, enabling a strong and diverse set of interactions (Fig. 7). Furthermore, Derivatives 14a-e demonstrated amazing binding energy score (S) ranging from  $-8.2036$  to  $-7.7650$  kcal/mol, all showing very high affinity with RMSD Values indicating stable and reliable docking poses over a rich diversity of interaction types, including hydrogen bonding (donor/acceptor) and pi interactions, indicative of strong, specific, and span intermolecular distances from 2.90 Å to 4.58 Å which are optimal for the interactions, suggesting these derivatives have potent inhibitory capabilities through varied binding modes (Figs. S1–S5; supplementary file).

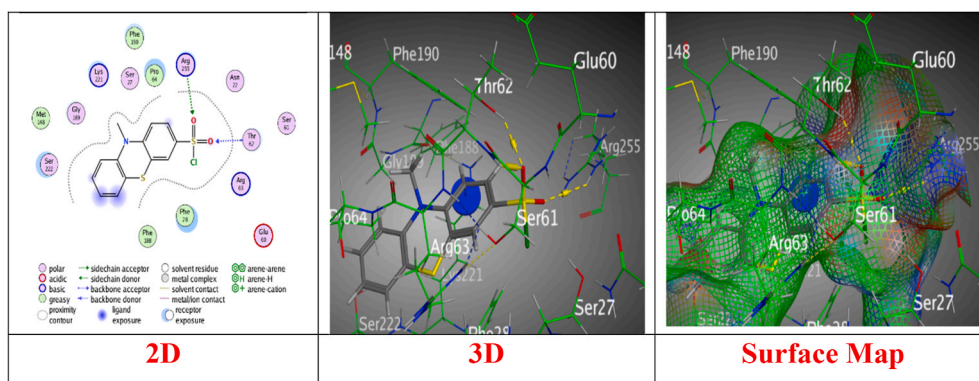


Fig. 2. Interaction images between derivative 3 with (PDB: 3tzf).

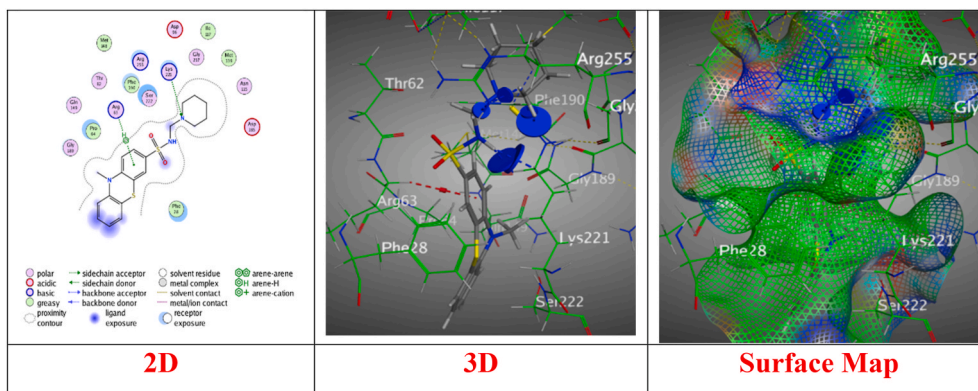


Fig. 3. Interaction images between derivative 5a with (PDB: 3tzf).

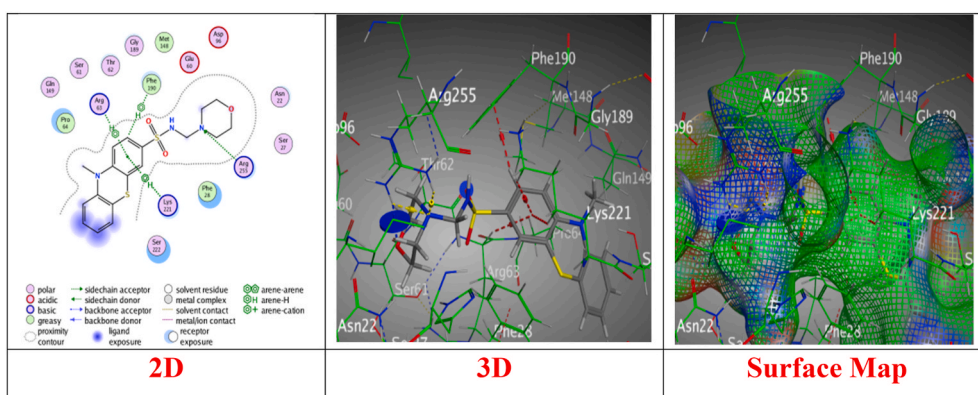


Fig. 4. Interaction images between derivative 5b with (PDB: 3tzf).

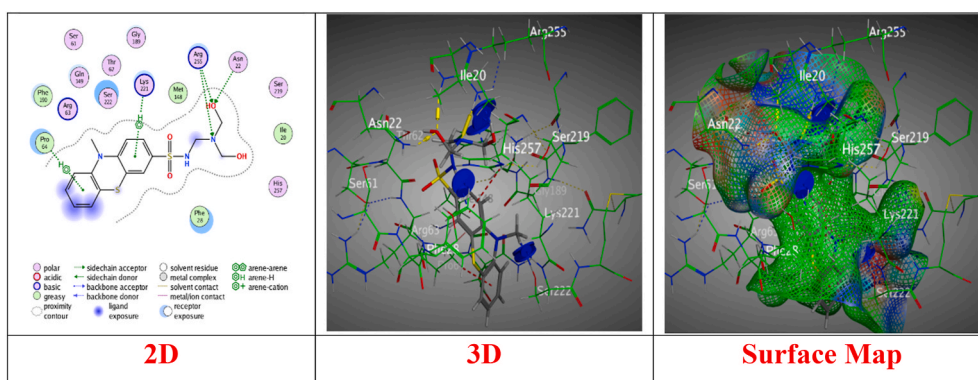


Fig. 5. Interaction images between derivative 5c with (PDB: 3tzf).

### 3. Experiment

#### 3.1. Synthesis of 10-methyl-10H-phenothiazine (1)

It was prepared in accordance with the reported work [48].

#### 3.2. Synthesis of 10-methyl-10H-phenothiazine-3-sulfonic acid (2)

It was prepared in accordance with the reported method [49].

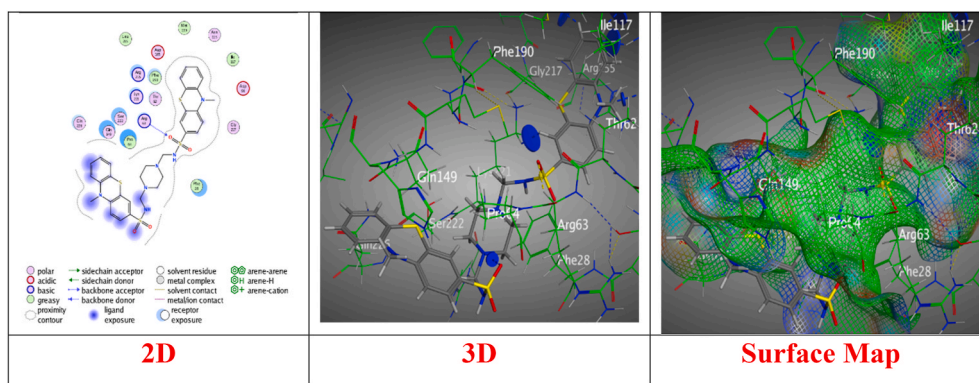


Fig. 6. Interaction images between derivative 6 with (PDB: 3tzf).

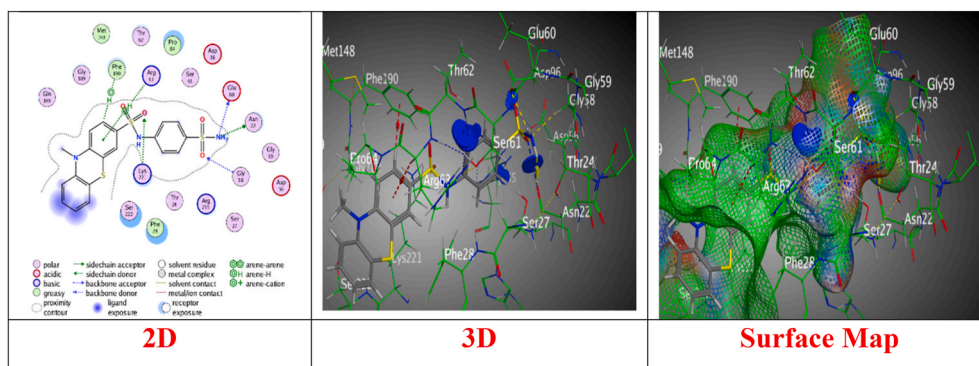


Fig. 7. Interaction images between derivative 13 with (PDB: 3tzf).

### 3.3. Synthesis of 10-methyl-10H-phenothiazine-3-sulfonyl chloride (3) and 10-methyl-10H-phenothiazine-3-sulfonamide (4)

The procedure of their preparation and analysis were displayed in the supplementary file.

### 3.4. Synthesis of mannich bases (5a-f)

#### 3.4.1. General procedure

In absolute ethanol (30 mL) containing formaldehyde 37% (0.1 g, 3 mmol), a mixture of compound 4 (0.87 g, 3 mmol) and the appropriate secondary amines (3 mmol) namely "piperidine, morpholine, diethanolamine, dimethyl amine, diethyl amine, and tetrahydrocarbazole" was heated for 30 min. Each mixture was heated at 70–75 °C for 7–8 h, depending upon the type of amine. The reaction mixture was left to stand at room temperature overnight. The obtained solid material was filtered off and crystallized from ethanol to give compounds 5a-f.

#### 3.5. 10-Methyl-N-(piperidin-1-ylmethyl)-10H-phenothiazine-3-sulfonamide (5a)

Yield, 74%; m.p. = 247–249 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3150 (NH), 1610 (C=N), 1342, 1171 ( $\text{SO}_2$ );  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  ppm: 1.60 (m, 6H, 3 $\text{CH}_2$ ), 2.25 (m, 4H, 2 $\text{CH}_2$ ), 3.28 (s, 3H,  $\text{CH}_3$ ), 4.25 (s, 2H,  $\text{CH}_2$ ), 6.99–7.16 (m, 4H, Ar-H), 7.45 (d, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.69 (d, 1H, Ar-H), 7.81 (s, 1H, NH). MS ( $m/z$ , %): 389 ( $\text{M}^+$ , 56). Anal. Calcd for  $\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_2\text{S}_2$  (389.53): C, 58.59; H, 5.95; N, 10.79%. Found: C, 58.52; H, 5.87; N, 10.72%.

#### 3.6. 10-Methyl-N-(morpholinomethyl)-10H-phenothiazine-3-sulfonamide (5b)

Yield, 68%; m.p. = 189–191 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3155 (NH), 1615 (C=N), 1339, 1169 ( $\text{SO}_2$ );  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  ppm: 36.5, 53.4 (2C), 64.9, 66.8 (2C), 115.0, 118.2, 120.0, 121.8, 123.7, 126.7, 128.2 (3C), 129.6, 144.4, 148.2. MS ( $m/z$ , %): 391 ( $\text{M}^+$ , 52). Anal. Calcd for  $\text{C}_{18}\text{H}_{21}\text{N}_3\text{O}_3\text{S}_2$  (391.50): C, 55.22; H, 5.41; N, 10.73%. Found: C, 55.15; H, 5.35; N, 10.66%.



### 3.7. *N*-((bis(2-hydroxyethyl)amino)methyl)-10-methyl-10H-phenothiazine-3-sulfonamide (5c)

Yield, 71%; m.p. = 177–179 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3450 (OH), 3145 (NH), 1609 (C=N), 1345, 1175 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.30 (t, 4H, 2CH<sub>2</sub>), 3.31 (s, 3H, CH<sub>3</sub>), 3.69 (t, 4H, 2CH<sub>2</sub>), 4.22 (s, 2H, 2OH), 4.40 (s, 2H, CH<sub>2</sub>), 7.09–7.25 (m, 4H, Ar-H), 7.55 (d, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.79 (d, 1H, Ar-H), 7.92 (s, 1H, NH). MS (*m/z*, %): 409 (M<sup>+</sup>, 42). Anal. Calcd for C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> (409.52): C, 52.79; H, 5.66; N, 10.26%. Found: C, 52.71; H, 5.55; N, 10.19%.

### 3.8. *N*-((dimethylamino)methyl)-10-methyl-10H-phenothiazine-3-sulfonamide (5d)

Yield, 68%; m.p. = 157–159 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3144 (NH), 1600 (C=N), 1342, 1171 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.20 (s, 6H, 2CH<sub>3</sub>), 3.19 (s, 3H, CH<sub>3</sub>), 4.47 (s, 2H, CH<sub>2</sub>), 6.95–7.12 (m, 4H, Ar-H), 7.41 (d, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.64 (d, 1H, Ar-H), 7.80 (s, 1H, NH). MS (*m/z*, %): 349 (M<sup>+</sup>, 61). Anal. Calcd for C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub> (349.47): C, 54.99; H, 5.48; N, 12.02%. Found: C, 54.93; H, 5.40; N, 11.93%.

### 3.9. *N*-((diethylamino)methyl)-10-methyl-10H-phenothiazine-3-sulfonamide (5e)

Yield, 77%; m.p. = 171–173 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3150 (NH), 1610 (C=N), 1342, 1171 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.10 (t, 6H, 2CH<sub>3</sub>), 2.84 (q, 4H, 2CH<sub>2</sub>), 3.21 (s, 3H, CH<sub>3</sub>), 4.37 (s, 2H, CH<sub>2</sub>), 7.00–7.20 (m, 4H, Ar-H), 7.49 (d, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.70 (d, 1H, Ar-H), 7.83 (s, 1H, NH). MS (*m/z*, %): 377 (M<sup>+</sup>, 66). Anal. Calcd for C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub> (377.52): C, 57.27; H, 6.14; N, 11.13%. Found: C, 57.19; H, 6.05; N, 11.08%.

### 3.10. *N*-((1,2,3,4,4a,9a-hexahydro-9H-carbazol-9-yl)methyl)-10-methyl-10H-phenothiazine-3-sulfonamide (5f)

Yield, 78%; m.p. = 215–217 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3140 (NH), 1605 (C=N), 1335, 1166 (SO<sub>2</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 25.9 (2C), 29.0, 32.0, 36.0, 44.5, 60.1, 64.0, 109.9, 114.4, 117.0 (2C), 120.1, 121.9 (2C), 123.8, 125.7 (2C), 128.7 (3C), 130.5, 138.0, 144.0, 146.4, 150.2. MS (*m/z*, %): 477 (M<sup>+</sup>, 45). Anal. Calcd for C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>S<sub>2</sub> (477.64): C, 65.38; H, 5.70; N, 8.80%. Found: C, 65.31; H, 5.61; N, 8.73%.

### 3.11. Synthesis of *N,N'*-(piperazine-1,4-diylbis(methylene))bis(10-methyl-10H-phenothiazine-3-sulfonamide) (6)

In 30 mL of ethanol containing 37% formalin (0.18 g, 6 mmol), compound 4 (1.74 g, 6 mmol) and piperazine (0.25 g, 3 mmol) were reacted and heated for 45 min. After 10 h of stirring at standard temperature (25 °C), such a mixture was allowed to stand overnight. Compound 6 was obtained by filtering the isolated product and purifying it chromatographically with an ethyl acetate-diethyl ether (2:1) eluent.

Yield, 81%; m.p. = 251–253 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3160 (NH), 1611 (C=N), 1342, 1171 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.27 (s, 8H, 4CH<sub>2</sub>), 3.10 (s, 6H, 2CH<sub>3</sub>), 4.05 (s, 4H, 2CH<sub>2</sub>), 7.17–7.40 (m, 8H, Ar-H), 7.58 (d, 2H, Ar-H), 7.70 (s, 2H, Ar-H), 7.82 (d, 2H, Ar-H), 7.95 (s, 2H, 2NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 35.9 (2C), 52.4 (4C), 63.9 (2C), 116.1 (2C), 119.8 (2C), 121.5 (2C), 122.9 (2C), 123.9 (2C), 128.0 (2C), 129.9 (6C), 131.0 (2C), 145.9 (2C), 149.8 (2C). MS (*m/z*, %): 694 (M<sup>+</sup>, 40). Anal. Calcd for C<sub>32</sub>H<sub>34</sub>N<sub>6</sub>O<sub>4</sub>S<sub>4</sub> (694.90): C, 55.31; H, 4.93; N, 12.09%. Found: C, 55.25; H, 4.85; N, 12.00%.

### 3.12. Synthesis of *N,N'*-(propane-1,3-diylbis(piperidine-4,1-diyl))bis(methylene))bis(10-methyl-10H-phenothiazine-3-sulfonamide) (7)

In ethanol (30 mL) containing 37% formalin (0.18 g, 6 mmol), compound 4 (1.74 g, 6 mmol) and 4,4'-trimethylenedipiperidine (0.63 g, 3 mmol) were heated for 30 min. After 10 h of stirring at 25 °C, such a mixture was allowed to stand overnight. Compound 7 was obtained by filtration and the isolated solid was purified chromatographically with an eluent of ethyl acetate – diethyl ether (2:1).

Yield, 73%; m.p. = 266–268 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3155 (NH), 1616 (C=N), 1343, 1174 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.42–1.62 (m, 16H, 7CH<sub>2</sub> + 2CH), 2.22–2.38 (m, 8H, 4CH<sub>2</sub>), 3.25 (s, 6H, 2CH<sub>3</sub>), 4.11 (s, 4H, 2CH<sub>2</sub>), 7.23–7.49 (m, 8H, Ar-H), 7.66 (d, 2H, Ar-H), 7.78 (s, 2H, Ar-H), 7.89 (d, 2H, Ar-H), 8.00 (s, 2H, 2NH). MS (*m/z*, %): 819 (M<sup>+</sup>, 38). Anal. Calcd for C<sub>41</sub>H<sub>50</sub>N<sub>6</sub>O<sub>4</sub>S<sub>4</sub> (819.13): C, 60.12; H, 6.15; N, 10.26%. Found: C, 60.02; H, 6.09; N, 10.17%.

### 3.13. Synthesis of *N,N'*-(1,5-bis(dimethylamino)pentane-1,5-diyl)bis(10-methyl-10H-phenothiazine-3-sulfonamide) (8)

Compound 4 (1.74 g, 6 mmol), glutaraldehyde (0.27 g, 3 mmol), and dimethyl amine (0.27 g, 6 mmol) were dissolved in 30 mL of ethanol and heated in a water bath for 2 h. After 10 h of stirring at 25 °C, such a mixture was allowed to stand overnight. Compound 8 was obtained by filtration and the isolated product was purified chromatographically with an *n*-hexane-ethyl acetate (6:1) eluent.

Yield, 71%; m.p. = 218–220 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3120 (NH), 1600 (C=N), 1340, 1174 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.30 (m, 2H, CH–CH<sub>2</sub>–CH<sub>2</sub>–CH<sub>2</sub>–CH), 1.55 (q, 4H, CH–CH<sub>2</sub>–CH<sub>2</sub>–CH<sub>2</sub>–CH), 2.15 (s, 12H, 4CH<sub>3</sub>), 3.15 (s, 6H, 2CH<sub>3</sub>), 3.80 (t, 2H, CH–CH<sub>2</sub>–CH<sub>2</sub>–CH<sub>2</sub>–CH), 6.78–7.00 (m, 8H, Ar-H), 7.19 (d, 2H, Ar-H), 7.30 (s, 2H, Ar-H), 7.40 (d, 2H, Ar-H), 7.51 (s, 2H, 2NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 14.5, 31.0 (2C), 35.5 (2C), 42.0 (4C), 82.5 (2C), 115.6 (2C), 118.8 (2C), 120.1 (2C), 122.0 (2C), 124.0 (2C), 127.0 (2C), 128.7 (6C), 129.9 (2C), 144.9 (2C), 148.6 (2C). MS (*m/z*, %): 739 (M<sup>+</sup>, 46). Anal. Calcd for C<sub>35</sub>H<sub>42</sub>N<sub>6</sub>O<sub>4</sub>S<sub>4</sub> (739.00): C, 56.89; H, 5.73; N, 11.37%. Found: C, 56.82; H, 5.66; N, 11.31%.

### 3.14. Synthesis of *N,N'*-((1,4-phenylenebis(azanediyl))bis(methylene))bis(10-methyl-10H-phenothiazine-3-sulfonamide) (9)

After being refluxed for 1 h in 30 mL of ethanol, compound **4** (1.74 g, 6 mmol), formaldehyde 37% (0.2 g, 6 mmol), and *p*-phenylenediamine (0.35 g, 3 mmol) were combined. After 20 h of stirring at 25 °C, such a mixture was allowed to stand overnight. Compound **9** was obtained by filtration and the isolated product was purified chromatographically with an eluent of *n*-hexane - diethyl ether (6:1).

Yield, 76%; m.p. = 273–275 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3152 (NH), 1612 (C=N), 1342, 1171 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 3.20 (s, 6H, 2CH<sub>3</sub>), 5.05 (s, 4H, 2CH<sub>2</sub>), 5.91 (s, 2H, 2NH), 6.60 (s, 4H, Ar-H), 7.18–7.41 (m, 8H, Ar-H), 7.58 (d, 2H, Ar-H), 7.70 (s, 2H, Ar-H), 7.81 (d, 2H, Ar-H), 7.93 (s, 2H, 2NH). MS (*m/z*, %): 716 (M<sup>+</sup>, 50). Anal. Calcd for C<sub>34</sub>H<sub>32</sub>N<sub>6</sub>O<sub>4</sub>S<sub>4</sub> (716.91): C, 56.96; H, 4.50; N, 11.72%. Found: C, 56.90; H, 4.42; N, 11.66%.

### 3.15. Synthesis of *N*-((1,2,3,4,4a,9a-hexahydro-9H-carbazol-9-yl)methyl)-10-methyl-*N*-(piperidin-1-ylmethyl)-10H-phenothiazine-3-sulfonamide (10)

A 30-min water bath heating process was used to combine compound **5a** (0.39 g, 1 mmol), formaldehyde 37% (0.03 g, 1 mmol), and 1,2,3,4-tetrahydrocarbazole (0.17 g, 1 mmol) in glacial acetic acid (15 mL). After being diluted with water, such a mixture was left to stand at 25 °C. Compound **10** was obtained by crystallizing the isolated product with ethanol to purify it.

Yield, 74%; m.p. = 191–193 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 1610 (C=N), 1339, 1171 (SO<sub>2</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 23.0, 25.0 (2C), 25.1 (2C), 29.4, 31.5, 36.3, 44.8, 54.3 (2C), 60.9, 69.0 (2C), 110.7, 115.8, 118.0 (2C), 120.6, 123.1 (3C), 125.0, 126.5, 129.8 (3C), 131.5, 139.0, 145.1, 148.0, 151.5. MS (*m/z*, %): 574 (M<sup>+</sup>, 54). Anal. Calcd for C<sub>32</sub>H<sub>38</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub> (574.80): C, 66.87; H, 6.66; N, 9.75%. Found: C, 62.80; H, 6.58; N, 9.69%.

### 3.16. Synthesis of *N*-((2,7-dioxo-2,3,4,5,6,7-hexahydro-1H-benzo[*b*]azonin-1-yl)methyl)-10-methyl-*N*-(piperidin-1-ylmethyl)-10H-phenothiazine-3-sulfonamide (11)

A solution of sodium periodate (0.44 g, 2 mmol) in water (5 mL) was mixed with **10** (0.57 g, 1 mmol) in a mixture of methanol and acetone (60 mL, 1:1). Compound **11** crystallized from methanol after being stirred at 25 °C overnight. The solvent was then extracted under decreased pressure, and the result was repeatedly washed with water (3 x 10 mL).

Yield, 64%; m.p. = 216–218 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 1608 (C=N), 1342, 1174 (SO<sub>2</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 22.0 (2C), 25.7 (2C), 27.0, 36.8, 37.8, 42.1, 55.1 (2C), 64.0, 68.0, 116.0 (2C), 118.8, 123.8 (2C), 125.4 (2C), 127.0 (4C), 130.2 (2C), 132.0, 135.9, 139.7, 145.8, 148.2, 171.1, 194.0. MS (*m/z*, %): 604 (M<sup>+</sup>, 60). Anal. Calcd for C<sub>32</sub>H<sub>36</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub> (604.78): C, 63.55; H, 6.00; N, 9.26%. Found: C, 63.49; H, 5.91; N, 9.18%.

### 3.17. Synthesis of 10-methyl-*N*-((9-oxo-1,2,3,9-tetrahydro-4H-cyclopenta[*b*]quinolin-4-yl)methyl)-*N*-(piperidin-1-ylmethyl)-10H-phenothiazine-3-sulfonamide (12)

Compound **11** (0.60 g, 1 mmol) was dissolved in a cold 2 N sodium hydroxide solution (15 mL). After a few seconds of heat, the yellow solution became colorless. Compound **12** was produced by neutralizing ethyl acetate, drying it, and crystallizing the white powder.

Yield, 71%; m.p. = 200–202 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 1616 (C=N), 1340, 1176 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.40 (m, 6H, 3CH<sub>2</sub>), 1.95 (m, 2H, CH<sub>2</sub>), 2.25 (t, 2H, CH<sub>2</sub>), 2.31 (t, 4H, 2CH<sub>2</sub>), 2.75 (t, 2H, CH<sub>2</sub>), 3.14 (s, 3H, CH<sub>3</sub>), 4.45 (s, 2H, CH<sub>2</sub>), 4.99 (s, 2H, CH<sub>2</sub>), 7.15–7.80 (m, 11H, Ar-H). MS (*m/z*, %): 586 (M<sup>+</sup>, 48). Anal. Calcd for C<sub>32</sub>H<sub>34</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub> (586.77): C, 65.50; H, 5.84; N, 9.55%. Found: C, 65.45; H, 5.79; N, 9.45%.

### 3.18. Synthesis of 10-methyl-*N*-(4-sulfamoylphenyl)-10H-phenothiazine-3-sulfonamide (13)

Compound **3** (0.93 g, 3 mmol) was portion-wise added at 0 °C to a solution of sulphanilamide (0.25 g, 3 mmol) in THF (30 mL) and stirred for 40 min at the same temperature. Lower pressure was used to remove the excess solvent. Compound **13** was obtained by dissolving the crude solid material in 30 mL of ethyl acetate, followed by 100 mL of water, 30 mL of diluted HCl, 50 mL of water, and 50 mL of saturated sodium chloride solution. The solid material was then dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure.

Yield, 80%; m.p. = 240–242 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3410 (NH<sub>2</sub>), 3120 (NH), 1610 (C=N), 1342, 1171 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.97 (s, 3H, CH<sub>3</sub>), 6.84 (s, 2H, NH<sub>2</sub>), 7.25–8.08 (m, 11H, Ar-H), 10.10 (s, 1H, NH). MS (*m/z*, %): 447 (M<sup>+</sup>, 55). Anal. Calcd for C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S<sub>3</sub> (447.54): C, 50.99; H, 3.83; N, 9.39%. Found: C, 50.91; H, 3.75; N, 9.33%.

### 3.19. Synthesis of mannich bases (14a-f)

They were prepared according to the previously mentioned procedure for the synthesis of compounds **5a-f**.

### 3.20. 10-Methyl-N-(4-(N-(piperidin-1-ylmethyl)sulfamoyl)phenyl)-10H-phenothiazine-3-sulfonamide (14a)

Yield, 68%; m.p. = 210–212 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3130 (NH), 1620 (C=N), 1350, 1168 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.55 (t, 6H, 3CH<sub>2</sub>), 2.25 (t, 4H, 2CH<sub>2</sub>), 3.21 (s, 3H, CH<sub>3</sub>), 4.20 (s, 2H, CH<sub>2</sub>), 7.11 (s, 1H, NH), 7.28–7.70 (m, 11H, Ar-H), 10.15 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 24.0, 25.5 (2C), 37.0, 54.5 (2C), 64.6, 115.7, 118.9 (3C), 120.2, 122.1, 123.9, 127.1, 128.8 (3C), 130.0 (3C), 134.9, 141.0, 145.0, 148.7. MS (*m/z*, %): 544 (M<sup>+</sup>, 50). Anal. Calcd for C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub>S<sub>3</sub> (544.70): C, 55.13; H, 5.18; N, 10.29%. Found: C, 55.05; H, 5.09; N, 10.21%.

### 3.21. 10-Methyl-N-(4-(N-(morpholinomethyl)sulfamoyl)phenyl)-10H-phenothiazine-3-sulfonamide (14b)

Yield, 77%; m.p. = 231–233 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3125 (NH), 1617 (C=N), 1340, 1176 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.33 (t, 4H, 2CH<sub>2</sub>), 3.26 (s, 3H, CH<sub>3</sub>), 3.65 (t, 4H, 2CH<sub>2</sub>), 4.15 (s, 2H, CH<sub>2</sub>), 7.05 (s, 1H, NH), 7.11–7.65 (m, 11H, Ar-H), 10.22 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 37.2, 53.0 (2C), 64.2, 66.6 (2C), 116.5, 119.9 (3C), 121.5, 123.3, 125.1, 128.1, 130.0 (3C), 131.1 (3C), 136.0, 142.0, 146.1, 150.0. MS (*m/z*, %): 546 (M<sup>+</sup>, 42). Anal. Calcd for C<sub>24</sub>H<sub>26</sub>N<sub>4</sub>O<sub>5</sub>S<sub>3</sub> (546.68): C, 52.73; H, 4.79; N, 10.25%. Found: C, 52.65; H, 4.70; N, 10.16%.

### 3.22. N-(4-(N-((bis(2-hydroxyethyl)amino)methyl)sulfamoyl)phenyl)-10-methyl-10H-phenothiazine-3-sulfonamide (14c)

Yield, 73%; m.p. = 218–220 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3430 (OH), 3130 (NH), 1605 (C=N), 1350, 1166 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.80 (t, 4H, 2CH<sub>2</sub>), 3.28 (s, 3H, CH<sub>3</sub>), 3.59 (t, 4H, 2CH<sub>2</sub>), 4.25 (s, 2H, 2OH), 4.44 (s, 2H, CH<sub>2</sub>), 7.21 (s, 1H, NH), 7.40–7.84 (m, 11H, Ar-H), 10.40 (s, 1H, NH). MS (*m/z*, %): 564 (M<sup>+</sup>, 53). Anal. Calcd for C<sub>24</sub>H<sub>28</sub>N<sub>4</sub>O<sub>6</sub>S<sub>3</sub> (564.69): C, 51.05; H, 5.00; N, 9.92%. Found: C, 50.99; H, 4.93; N, 9.85%.

### 3.23. N-(4-(N-((dimethylamino)methyl)sulfamoyl)phenyl)-10-methyl-10H-phenothiazine-3-sulfonamide (14d)

Yield, 77%; m.p. = 197–199 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3110 (NH), 1613 (C=N), 1338, 1175 (SO<sub>2</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 36.3, 44.9 (2C), 69.0, 117.0, 120.1 (3C), 121.9, 123.8, 125.5, 128.6, 130.4 (3C), 131.8 (3C), 136.1, 142.2, 146.6, 150.1. MS (*m/z*, %): 504 (M<sup>+</sup>, 66). Anal. Calcd for C<sub>22</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub>S<sub>3</sub> (504.64): C, 52.36; H, 4.79; N, 11.10%. Found: C, 52.29; H, 4.71; N, 11.00%.

### 3.24. N-(4-(N-((diethylamino)methyl)sulfamoyl)phenyl)-10-methyl-10H-phenothiazine-3-sulfonamide (14e)

Yield, 71%; m.p. = 216–218 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3115 (NH), 1614 (C=N), 1344, 1177 (SO<sub>2</sub>); MS (*m/z*, %): 532 (M<sup>+</sup>, 64). Anal. Calcd for C<sub>24</sub>H<sub>28</sub>N<sub>4</sub>O<sub>4</sub>S<sub>3</sub> (532.69): C, 54.11; H, 5.30; N, 10.52%. Found: C, 54.05; H, 5.24; N, 10.45%.

### 3.25. N-(4-(N-((1,2,3,4,4a,9a-hexahydro-9H-carbazol-9-yl)methyl)sulfamoyl)phenyl)-10-methyl-10H-phenothiazine-3-sulfonamide (14f)

Yield, 73%; m.p. = 187–189 °C; IR (KBr):  $\nu_{\max}$ ,  $\text{cm}^{-1}$ : 3111 (NH), 1611 (C=N), 1343, 1173 (SO<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.00–1.90 (m, 10H, 4CH<sub>2</sub> + 2CH), 3.29 (s, 3H, CH<sub>3</sub>), 5.35 (s, 2H, CH<sub>2</sub>), 7.00 (s, 1H, NH), 7.17–7.59 (m, 15H, Ar-H), 10.30 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 25.8 (2C), 29.5, 31.9, 35.9, 44.9, 60.5, 65.0, 110.0, 114.8, 117.5 (4C), 120.3, 122.0 (2C), 124.0, 125.9 (2C), 129.0 (3C), 130.6 (3C), 134.9, 138.2, 141.0, 144.1, 150.7 (2C). MS (*m/z*, %): 632 (M<sup>+</sup>, 48). Anal. Calcd for C<sub>32</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>S<sub>3</sub> (632.81): C, 60.74; H, 5.10; N, 8.85%. Found: C, 60.67; H, 5.01; N, 8.78%.

#### 3.25.1. Molecular docking

the synthesized derivatives with good antibacterial activities were created using the software version (MOE.2019.01). The ligands were subjected to computational docking with the DHPS (dihydropteroate synthase) protein structure (PBD: 3TZF) to establish the most preferred mode of interaction [47].

## 4. Conclusion

In conclusion, it was synthesized four series of phenothiazine derivatives linked to Mannich bases by mixing two active pharmacophores, phenothiazine, and alkyl amines. All the newly synthesized compounds were evaluated for *in vitro* growth inhibition activity against *P. aeruginosa*, *E. coli*, and *S. aureus*. Eight compounds showed good antibacterial activity with a MIC value of 12.5 µg/mL. The bis Mannich bases 6–9 showed moderate to weak activity against the tested strains with a MIC value of 12.5–100 µg/mL indicating that larger compounds may face challenges in entering bacterial cells, but their antimicrobial activity can still be influenced by other factors. In general, compounds 14a-f exhibited significant activity due to the presence of sulfonamoyl phenyl group which may enhance the antibacterial activity. These results were further supported by molecular docking studies, which showed the presence of many derivatives with high binding affinities and distinct interaction patterns with the target protein.

## Ethical approval

Not applicable.

## Consent to participate

All authors participated directly in the current research work.

## Consent to publish

The authors agree to publish the article under the Creative Commons Attribution License.

## Availability of data and materials

All relevant data are within the manuscript and available from the corresponding author upon request.

## CRediT authorship contribution statement

**Ahmed M. Abdula:** Resources, Methodology, Data curation, Conceptualization. **Ahmad Fawzi Qarah:** Validation, Software, Resources, Methodology. **Kahdr Alatawi:** Methodology, Investigation, Formal analysis, Data curation. **Jihan Qurban:** Writing – original draft, Software, Methodology, Investigation. **Matokah M. Abualnaja:** Writing – original draft, Validation, Software, Methodology. **Hanadi A. Katuah:** Writing – original draft, Resources, Methodology, Formal analysis. **Nashwa M. El-Metwaly:** Writing – review & editing, Supervision, Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e28573>.

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