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Synthesis, Antimicrobial and Molecular Modeling Studies of Some Benzophenone-based Thiazole and 4-Thiazolidinone Derivatives

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Abstract

New series of thiazolyl hydrazones were designed and synthesized via the reaction of benzophenone thiosemicarbazone **2** with chloroacetic acid, (un)substituted phenacylbromide and ethyl-2-chloroacetoacetate to yield compounds **3**, **5a-d** & **6** respectively. Furthermore, reaction of the thiazolidin-4-one **3** with aromatic aldehydes afforded compounds **4a-g**. Characterization data, along with *in vitro* antimicrobial activity for all compounds are herein reported. All the synthesized compounds were screened against *Methicillin- Resistant Staphylococcus aureus (MRSA), E. coli, K. pneumonia, P. aeruginosa, A baumannii, C. albicans and C. neoformans* var.*grubii*. Compounds **2** and **4e** showed the highest bacterial growth inhibition with 28.6% and 28.7% against *MRSA*, respectively. Moreover, the trisubstituted thiazole derivative **6 was** the most active compound against Gram-negative bacteria *A. baumannii* with 59% growth inhibition. Furthermore, compounds **4e & 6** showed 22.5% and 17.3% decrease in peptidoglycan density, respectively. Molecular docking into bacterial MurB enzyme active site was used to determine their binding modein which they showed good interactions with Gln229, Arg225 and Ser82 amino acid residues.

Keywords: Thiazoles, 4- thiazolidinone, antimicrobial activity, molecular modelling.

1. Introduction

One of the critical new aspect of chemistry is designing and development of novel heterocyclic compounds with diverse biological activities. Thiazoles are five membered heterocyclic derivatives that have a various spectrum of biological activities such as antitumor[1], anti-inflammatory [2], enzyme inhibitors [3], antioxidant [4],….etc, thus they play a significant role in medicinal and pharmaceutical chemistry. Moreover, thiazole containing compounds are also used as multitarget agents in Alzheimer's disease [5] as well as potent antimicrobial agents [6].

Examples of thiazoles based derivatives that have been studied for their antimicrobial activities (**I**, **Fig. 1**).

Additionally, the antimicrobial activity of 1,3-thiazolidin-4-one scaffold has gain attention in the research projects not only relied on its structure pharmacophore, but also depends on the position and nature of its substituents [7-9]. For instance, the

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availability of electron withdrawing group substitutions on the aromatic moieties of biphenyl-4-thiazolidinone derivatives showed a remarkable antibacterial activity [10].

Methicillin- Resistant Staphylococcus aureus (MRSA) is a utilizing pathogen that can cause a vast spectrum of infections, ranging from severe to potentially fatal or invasive diseases. Recently, literature survey reveled that the progression of infection due to *MRSA* seems to be elevated in neonates, immune compromised, and/or hospitalized individuals with a mortality rate reaches 50%. Currently, a wide range of FDA approved drugs are obtainable ranging from ciprofloxacin to oritavancin to treat *MRSA* infections. Unfortunately, acquiring resistance to the antibiotics by *MRSA* has recently raised the number of untreatable infections [11]. Given the alarming situation of drug resistant *MRSA*, the search for new drugs against *MRSA*, especially strains resistant to multiple antibiotics, is a higher priority medical need. Additionally, the *A. baumannii* resistance to many drugs have been increased and developed a major public health problem and isolates exhibiting multidrug resistance are emerging in clinical settings. At the present time, the resistance of *A. baumannii* to ceftazidime is as high as >85%, while that for ciprofloxacin (CIP) reached 90% [12].

Indeed, Vicini *et al*. designed and synthesized a new series of 2-thiazolylimino- 5-arylidene-4-thiazolidinone derivatives (**II**, **Fig. 1**) and their precursor 2-thiazolylimino-4-thiazolidinone for antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts and moulds [13]. The authors suggested that either the substituted or the unsubstituted 5-arylidene moiety plays a crucial role in enhancing the antimicrobial activity of this class of compounds. On the other hand, Zhou *et al*. reported ten cytoselective compounds from many thiazolidinone analogues (**III**, **Fig. 1**) by applying iterative library approaches [14].

Structure activity relationship studies revealed that the nitrogen atom on the 4-thiazolidinone ring couldn't be substituted; several substituents R´´´are tolerated at various positions.

Based on the above results, herein we report the synthesis and antimicrobial activity of the (5-Substituted benzylidene-2-((diphenylmethylene)hydrazono)thiazolidin-4-one **4a-g** and their precursor 2-((diphenylmethylene)hydrazono)thiazolidin-4-one **3** (Scheme 1) against Gram positive and Gram negative bacteria and fungi.

Also, the thiazolyl hydrazones **5a-d & 6** were synthesized and screened for *in vitro* antibacterial and antifungal activities.

In the same context, peptidoglycan is considered as one of the essential component of the bacterial cell wall, in which its biosynthesis is catalyzed by a series of Mur enzymes. This encouraged us to explore the binding mode of the targeted compounds to the bacterial MurB enzyme by performing the molecular modeling studies through docking the synthesized compounds into the crystal structure of *S. aureus* MurB (PDB 1HSK) [15].

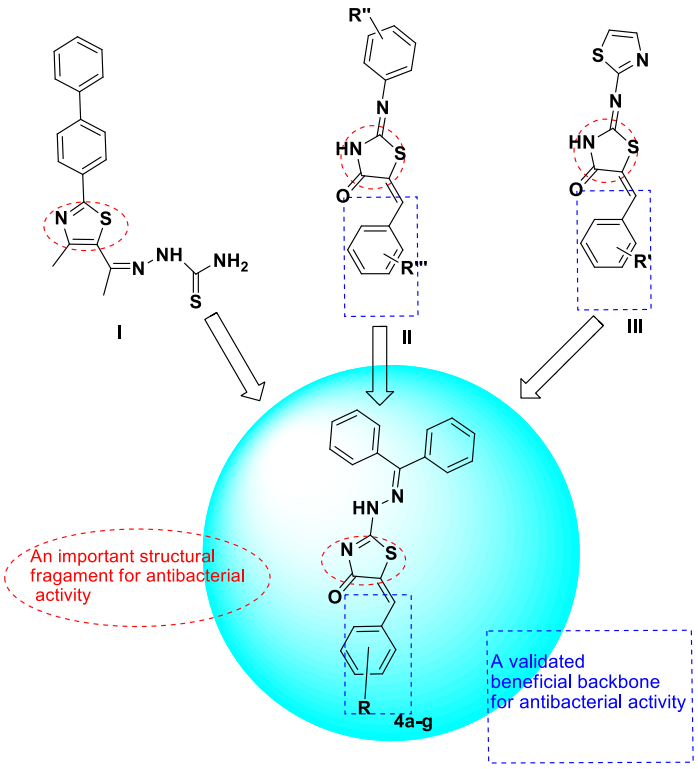


Fig. 1: Design of the targeted structures.

2. Experimental

2.1. Chemistry

Starting materials and reagents were purchased from Sigma – Aldrich or Acros Organics. Gallen Kamp apparatus used to record melting points and were uncorrected. Shimadzu IR 435 spectrophotometer was used to make record for FT-IR spectra. ^{1}HNMR spectra were recorded in δ scale given in ppm on a Varian 400 MHz spectrophotometer or a Varian 300 MHz spectrophotometer. Coupling patterns are described as coming after: s, singlet; d, doublet; t, triplet; m, multiplet. *J* describes a coupling constant. The coupling constants were rounded off to one decimal place. MS spectra mass were recorded on Hewlett Packard 5988 spectrometer (70 eV). Elemental analyzes were performed at the Microanalytical Center, Al-Azhar University.

2.1.1. Synthesis of 2-(diphenylmethylene)hydrazinecarbothioamide (2)

To a stirred solution of thiosemicarbazide (1 mmol) in an ethanol water mixture, an ethanolic solution of benzophenone (1 mmol) was added slowly, few drops of conc. HCl and refluxed for 10–20 mins. After cooling the reaction mixture to ambient temperature, the mixture was filtered to provide a solid crude product, which was crystallized from ethanol to furnish pure compound **2** buff crystals.

Buff crystals, yield 64 %, m.p. 168-170°C. **^{1}HNMR (400 MHz, DMSO-d_6)**: δ 8.51 (3H,s, -NH_2, D_2O exchangeable), δ 7.71-7.70 (2H, d, *J*= 8 Hz, Ar-H), δ 7.69-7.67 (3H, m, Ar-H), δ 7.57-7.55 (3H, m, Ar-H), δ 7.40-7.39 (2H, d, *J*= 8 Hz, Ar-H), δ 7.29 (1H,s, -NH D_2O exchangeable); FT-IR (ύ max, cm-^{1}): 3526 (NH_2), 3367 (NH); Anal. Calcd for $C_{14}H_{13}N_3S$: C, 65.85; H, 5.13; N, 16.46; Found: C, 65.69; H, 5.28; N, 16.70.

2.1.2. Synthesis of 2-((diphenylmethylene)hydrazono)thiazolidin-4-one (3)

A solution of 1.70 mmol of thiosemicarbazones **2**, 1.90 mmol of 2-chloroacetic acid, and 6.9 mmol of sodium acetate anhydrous in 20 mL of ethanol was stirred till reflux till the completion of the reaction (16 h). After, the solution was cooled down to 0 °C, and the precipitate was collected with filter under vacuum and washed with hot methanol and water.

Buff crystals, yield 73%, m.p. 175-177°C. **^{1}H-NMR (400 MHz, DMSO-d_6)**: δ 11.80 (1H,s, -NH D_2O exchangeable), δ 7.75-7.73 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.69-7.67 (3H, m, Ar-H), δ 7.57-7.55 (3H, m, Ar-H), δ 7.37-7.36 (2H, d, *J*= 8.5 Hz, Ar-H), δ 3.89 (2H, s, CH_2); FT-IR (ύ max, cm-^{1}): 3325 (NH), 1716 (C=O); Anal. Calcd for $C_{16}H_{13}N_3OS$: C, 65.06; H, 4.44; N, 14.23; Found: C, 65.30; H, 4.32; N, 14.51.

2.1.3. Synthesis of (5-Substituted benzylidene-2-((diphenylmethylene)hydrazono)thiazolidin-4-one (4a-g)

General procedure:

A solution of 0.34 mmol of 4-thiazolidinones **3**, 0.34 mmol of corresponding aromatic aldehyde, and catalytic few drops of piperidine in 10 mL of ethanol was stirred and reflux till the completion of the reaction (3-6 h). The solution was allowed to cool to 0 °C, and the precipitate was collected with filter under vacuum and washed with hot ethanol and water.

2.1.3.1. (E)-5-benzylidene-2-((diphenylmethylene)hydrazono)thiazolidin-4-one (4a)

Pale yellow solid, yield 51%, m.p. 209-211°C. **^{1}H-NMR (400 MHz, DMSO-d_6)**: δ 7.75 (1H, s, -NH D_2O exchangeable), δ 7.74 (1H,d, CH-benzylidene), δ 7.69-7.67 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.59-7.57 (3H, m, Ar-H), δ 7.48-7.46 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.44-7.42 (3H, m, Ar-H), δ 7.41-7.39 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.37-7.35 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.33-7.30 (1H, d, *J*= 8.5 Hz, Ar-H); FT-IR (ύ max, cm-^{1}): 3394 (NH), 1651 (C=O); Anal. Calcd for $C_{23}H_{17}N_3OS$: C, 72.04; H, 4.47; N, 10.96; Found: C, 72.39; H, 4.43; N, 11.28.

2.1.3.2. (E)-2-((diphenylmethylene)hydrazono)-5-(2-hydroxybenzylidene)thiazolidin-4-one (4b)

Yellow crystals, yield 88%, m.p. 194-196°C. **^{1}H-NMR (400 MHz, DMSO-d_6)**: δ 10.82 (1H,s, -OH D_2O exchangeable),δ 8.26 (1H,s, -NH D_2O exchangeable), δ 8.23 (1H,d, CH-benzylidene), δ 8.09-8.07 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.61-7.59 (3H, m, Ar-H), δ 7.61 (1H, d, *J*= 8.5 Hz, Ar-H), δ 7.57-7.55 (3H, m, Ar-H), δ 7.49-7.48 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.46-7.45 (1H, d, *J*= 8.5 Hz, Ar-H),δ 7.42-7.41 (1H, d, *J*= 8.5 Hz, Ar-H), δ 7.40-7.39 (1H, d, *J*= 8.5 Hz, Ar-H); FT-IR (ύ max, cm-^{1}): 3421 (OH), 3286 (NH), 1651 (C=O); Anal. Calcd for $C_{23}H_{17}N_3O_2S$: C, 69.15; H, 4.29; N, 10.52; Found: C, 69.37; H, 4.51; N, 10.83.

2.1.3.3. (E)-2-((diphenylmethylene)hydrazono)-5-(4-methoxybenzylidene)thiazolidin-4-one (4c)

Pale yellow crystals, yield 60%, m.p. 198-200°C. **^{1}H-NMR (400 MHz, DMSO-d_6)**: δ 7.75 (1H,s, -NH D_2O exchangeable), δ 7.74 (1H,d, CH-benzylidene), δ 7.73-7.71 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.69-7.67 (3H, m, Ar-H), δ 7.59-7.57 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.48-7.47 (3H, m, Ar-H), δ 7.37-7.35 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.30-7.28 (2H, d, *J*= 8.5 Hz, Ar-H),δ 3.33(3H, s, OCH_3); FT-IR (ύ max, cm-^{1}): 3421 (NH), 1651 (C=O); Anal. Calcd for $C_{24}H_{19}N_3O_2S$: C, 69.71; H, 4.63; N, 10.16; Found: C, 69.45; H, 4.81; N, 10.42.

2.1.3.4. (E)-2-((diphenylmethylene)hydrazono)-5-(3-methoxybenzylidene)thiazolidin-4-one (4d)

Pale yellow crystals, yield 48%, m.p. 205-207°C. **^{1}H-NMR (400 MHz, DMSO-d_6)**: δ 8.44 (1H,s, -NH D_2O exchangeable), δ 8.41 (1H,d, CH-benzylidene), δ 8.18-8.16 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.75-7.73 (3H, m, Ar-H), δ 7.69-7.67 (1H, d, *J*= 8.5 Hz, Ar-H), δ 7.59-7.57 (3H, m, Ar-H), δ 7.48-7.46 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.41-7.39 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.30-7.28 (1H, d, *J*= 8.5 Hz, Ar-H), δ 3.34 (3H, s, OCH_3); FT-IR (ύ max, cm-^{1}): 3421 (NH), 1654 (C=O); Anal. Calcd for $C_{24}H_{19}N_3O_2S$: C, 69.71; H, 4.63; N, 10.16; Found: C, 69.89; H, 4.79; N, 10.34.

2.1.3.5. (E)-2-((diphenylmethylene)hydrazono)-5-(4-nitrobenzylidene)thiazolidin-4-one (4e)

Brown crystals, yield 49%, m.p. 175-177°C. **^{1}H-NMR (400 MHz, DMSO-d_6)**: δ 8.31-8.22 (2H, d, *J*= 8.5 Hz, Ar-H), δ 8.03-8.10 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.75 (1H,s, -NH D_2O exchangeable), δ 7.73 (1H,d, CH-benzylidene), δ 7.69-7.67 (2H, d, *J*= 8.5

Hz, Ar-H), δ 7.59-7.57 (3H, m, Ar-H), δ 7.50-7.48 (3H, m, Ar-H), δ 7.46-7.44 (2H, d, *J*= 8.5 Hz, Ar-H); **^{13}C-NMR (100 MHz, DMSO)***:* δ 138.22, 129.68, 129.59, 129.41, 129.28, 129.92, 127.12, 125.90, 56.50, 40.62, 40.42, 40.21, 40.00, 39.79, 39.58, 39.37, 21.24, 19.02; FT-IR (ύ max, cm-^{1}): 3367 (NH), 1705 (C=O); Anal. Calcd for $C_{23}H_{16}N_4O_3S$: C, 64.47; H, 3.76; N, 13.08; Found: C, 64.59; H, 3.89; N, 13.31.

2.1.3.6. (E)-5-(4-bromobenzylidene)-2-((diphenylmethylene)hydrazono)thiazolidin-4-one (4f)

Yellow crystals, yield 46%, m.p. 168-170°C. **^{1}H-NMR (400 MHz, DMSO-d$_6$)**: δ 7.75 (1H,s, -NH D_2O exchangeable), δ 7.74 (1H,d, CH-benzylidene), δ 7.73-7.71 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.69-7.67 (3H, m, Ar-H), δ 7.59-7.57 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.50-7.48 (3H, m, Ar-H), δ 7.46-7.44 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.30-7.28 (2H, d, *J*= 8.5 Hz, Ar-H); FT-IR (ύ max, cm-^{1}): 3367 (NH), 1651 (C=O); Anal. Calcd for $C_{23}H_{16}BrN_3OS$: C, 59.75; H, 3.49; N, 9.09; Found: C, 59.91; H, 3.71; N, 9.26.

2.1.3.7. (E)-5-(4-(dimethylamino)benzylidene)-2-((diphenylmethylene)hydrazono)thiazolidin-4-one (4g)

White crystals, yield 74%, m.p. 210-212°C. **^{1}H-NMR (400 MHz, DMSO-d$_6$)**: δ 9.67 (1H,s, -NH D_2O exchangeable), δ 7.75 (1H,d, CH-benzylidene), δ 7.73-7.71 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.70-7.69 (3H, m, Ar-H), δ 7.68-7.67 (3H, m, Ar-H), δ 7.59-7.57 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.55-7.53 (2H, d, *J*= 8.5 Hz, Ar-H), δ 6.80-6.78 (2H, d, *J*= 8.5 Hz, Ar-H); FT-IR (ύ max, cm-^{1}): 3444 (NH), 1654 (C=O); Anal. Calcd for $C_{25}H_{22}N_4OS$: C, 70.40; H, 5.20; N, 13.14; Found: C, 70.67; H, 5.04; N, 13.01.

2.1.4. Synthesis of 2-(2-(diphenylmethylene)hydrazinyl)-4-substituted phenylthiazole (5a-d)

General procedure:

A mixture of thiosemicarbazone **2** (1 mmol) and (un)substituted phenacylbromide (1 mmol) in ethanol was refluxed for 3–6 hours and the reaction was monitored by TLC . The resulting product started to separate out during the reaction. The solid product was then filtered, washed with water and purified by crystallization in a N,N-dimethylformamide (DMF), affording pure 1,3-thiazole derivatives at yields of 55–77%.

2.1.4.1. 2-(2-(diphenylmethylene)hydrazinyl)-4-phenylthiazole (5a)

Pale yellow crystals, yield 55%, m.p. 172-174°C. **^{1}H-NMR (400 MHz, DMSO-d$_6$)**: δ 10.61 (1H,s, -NH D_2O exchangeable), δ 7.83-7.81 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.60-7.57 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.54-7.52 (3H, m, Ar-H), δ 7.42-7.40 (3H, m, Ar-H), δ 7.39-7.38 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.36-7.34 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.26-7.24 (1H, d, *J*= 8.5 Hz, thiazole); FT-IR (ύ max, cm-^{1}): 3404 (NH), 1651 (C=O); Anal. Calcd for $C_{22}H_{17}N_3S$: C, 74.34; H, 4.82; N, 11.82; Found: C, 74.59; H, 4.97; N, 11.98.

2.1.4.2. 2-(2-(diphenylmethylene)hydrazinyl)-4-p-tolylthiazole (5b)

Yellow solid, yield 62%, m.p. 185-187°C. **^{1}H-NMR (400 MHz, DMSO-d$_6$)**: δ 10.56 (1H,s, -NH D_2O exchangeable), δ 7.71-7.70 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.60-7.58 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.54-7.52 (3H, m, Ar-H), δ 7.49-7.47 (3H, m, Ar-H), δ 7.42-7.40 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.39-7.38 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.19-7.17 (1H, d, *J*= 8.5 Hz, thiazole), δ 2.30 (3H, s, CH_3); FT-IR (ύ max, cm-^{1}): 3419 (NH), 1652 (C=O); Anal. Calcd for $C_{23}H_{19}N_3S$: C, 74.77; H, 5.18; N, 11.37; Found: C, 74.43; H, 5.26; N, 11.19.

2.1.4.3. 4-(4-chlorophenyl)-2-(2-(diphenylmethylene)hydrazinyl)thiazole (5c)

White solid, yield 71%, m.p. 190-192°C. **^{1}H-NMR (400 MHz, DMSO-d$_6$)**: δ 10.65 (1H,s, -NH D_2O exchangeable), δ 7.85-7.83 (2H, d, *J*= 8.5 Hz, Ar-H), 7.60-7.59 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.57-7.54 (3H, m, Ar-H), δ 7.48-7.46 (3H, m, Ar-H), δ 7.44-7.42 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.40-7.38 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.36-7.34 (1H, d, *J*= 8.5 Hz, thiazole); FT-IR (ύ max, cm-^{1}): 3421 (NH), 1651 (C=O); Anal. Calcd for $C_{22}H_{16}ClN_3S$: C, 67.77; H, 4.14; N, 10.78; Found: C, 68.04; H, 4.28; N, 10.23.

2.1.4.4. 2-(2-(diphenylmethylene)hydrazinyl)-4-(4-nitrophenyl)thiazole (5d)

White solid, yield 77%, m.p. 201-203°C. **^{1}H-NMR (400 MHz, DMSO-d$_6$)**: δ 10.65 (1H,s, -NH D_2O exchangeable), δ 7.75-7.73 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.71-7.69 (2H, d, *J*= 8.5 Hz, Ar-H), 7.68-7.67 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.59-7.57 (3H, m, Ar-H), δ 7.48-7.46 (3H, m, Ar-H), δ 7.37-7.35 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.30-7.28 (1H, d, *J*= 8.5 Hz, thiazole); FT-IR (ύ max, cm-^{1}): 3434 (NH), 1654 (C=O); Anal. Calcd for $C_{22}H_{16}N_4O_2S$: C, 65.73; H, 5.24; N, 11.50; Found: C, 65.44; H, 5.38; N, 11.67.

2.1.5. Ethyl 2-(2-(diphenylmethylene)hydrazinyl)-4-methylthiazole-5-carboxylate (6)

General procedure:

A mixture of thiosemicarbazone **2** (1 mmol) and 4-chloro-ethyl acetoacetate (1 mmol) in addition to CH_3COONa (0.02 mmol) in EtOH (20 mL) was refluxed at 80 °C for 4 h. TLC was used to confirm the completion of the reaction that was cooled at room temperature. The precipitated product was separated

and filtered off to be purified by recrystallization from EtOH to afford the desired product **6** in good yield.

Orange solid, yield 75%, mp 209-211°C. **^{1}H-NMR (400 MHz, DMSO-d$_6$)**: δ 11.74 (1H,s, -NH D_2O exchangeable), δ 7.52-7.50 (2H, d, *J*= 8.5 Hz, Ar-H), δ 7.49-7.46 (3H, m, Ar-H), δ 7.43-7.40 (3H, m, Ar-H), δ 7.29-7.27 (2H, d, *J*= 8.5 Hz, Ar-H), δ 4.23 (2H, q, CH_2), δ 2.38 (3H, s, CH_3), δ 1.29 (3H, t, CH_3); **^{13}C-NMR (100 MHz, DMSO)***:* δ 162.49, 129.77, 129.28, 129.05, 128.88, 127.63, 60.69, 40.63, 40.42, 40.21, 40.00, 39.79, 39.58, 39.37, 14.79; FT-IR (ύ max, cm-^{1}): 3438 (NH), 1715 (C=O); Anal. Calcd for $C_{20}H_{19}N_3O_2S$: C, 65.98; H, 4.03; N, 13.99; Found: C, 66.21; H, 4.02; N, 14.26.

2.2. Biological Evaluation

The antimicrobial screening of the tested compounds was performed by CO-ADD following the procedures of Blaskovich *et al*. regarding the statistical methods of calculation [16].

2.2.1. Antibacterial data collection

A Tecan M1000 Pro monochromator plate reader and measuring the absorbance at 600 nm (OD600) used to determine the inhibition of bacterial growth. The negative control (media only) and positive control (bacteria without inhibitors) on the same plate used as references to calculate the percentage of growth inhibition for each well.

2.2.2. Antifungal data collection

Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references.

2.2.3. Inhibition

Percentage growth inhibition of an individual sample is calculated based on Negative controls (media only) and Positive Controls (bacterial/fungal media without inhibitors). Please note negative inhibition values indicate that the growth rate (or OD600) is higher compared to the Negative Control (Bacteria/fungi only, set to 0% inhibition). The growth rates for all bacteria and fungi has a variation of -/+ 10%, which is within the reported normal distribution of bacterial/fungal growth. Any significant variation (or outliers/hits) is identified by the modified Z-Score, and actives are selected by a combination of inhibition value and Z-Score.

2.2.4. Z-Score

Z-Score analysis is done to investigate outliers or hits among the samples. The Z-Score is calculated based on the sample population using a modified Z-Score method which accounts for possible skewed sample population. The modified method uses median and MAD (median average derviation) instead of average and sd, and a scaling factor [Iglewicz, B. & Hoaglin, D. C. Volume 16: How to Detect and Handle Outliers. The ASQC Basic Reference in Quality Control: Statistical Techniques, 1993]: M(i) = 0.6745 * (x(i) - median(x))/MAD). M(i) values of > |2.5| (absolute) label outliers or hits.

2.2.5. Growing of Staphylococcus aureus ATCC 43300 under stress conditions

S. aureus ATCC 43300 was sub-cultured in 300 mL brain heart infusion broth starting with inoculum size 1%. Prior to incubation, it was distributed into three groups with each 100 mL; a control non-treated group, a treated group with sub-MIC concentration of compound 4 (15 µg/mL) and a treated group with sub-MIC concentration of compound 6 (15 µg/mL). All groups were allowed to grow for 14 hrs. A viable count technique was applied to count the number of bacteria and adjusted to $4x10^6$ CFU/mL in all cases.

2.2.6. Peptidoglycan isolation and dye release assay

Both treated and control non-treated groups were used in this experiment and repeated in triplicates. Bacteria were harvested by centrifugation 1 mL of adjusted bacterial inoculum at 4000Xg at 4 degree C for 30 minutes. After removing the supernata, the cell pellets were re-suspended in 1 mL ice cold water. A boiling SDS (8%) was prepared and an equal volume of boiling SDS was added drop wisely with vigorous stirring in boiling water bath to the re-suspended cell pellets. Cells were allowed to be boiled for 30 minutes (to solubilize membranes and degrade DNA). A centrifugation step was applied at 18.000 Xg for 60 minutes at 4 degree C to precipitate the peptidoglycan.

All isolated peptidoglycan originated from the equal adjusted number of bacteria were stained with 500 uL of 1% aqueous solution of crystal violet and incubated for 3 minutes at room temperature. Many centrifugation and washing steps with phosphate buffer saline (PBS) were applied in order to remove the unbound dye until obtaining colorless PBS supernata.

The stained peptidoglycans were allowed to dry and 1 mL of 70% ethanol was used to extract the bounded dye. A centrifugation at 18,000 Xg was applied and a 100 uL of extracted dye in ethanol was transferred into

96 wells microtiter plate and the absorbance at 570 nm was recorded.

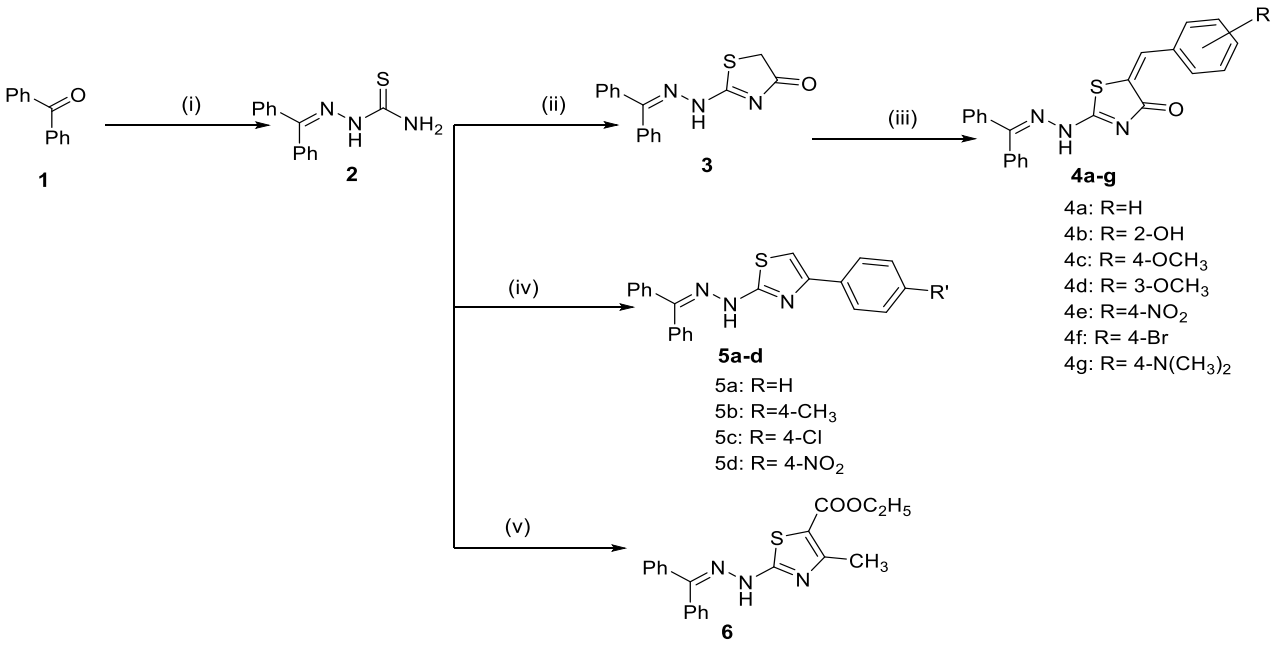
2.3. Molecular Modelling Studies

The 3D structures of the compounds were constructed by using the MOE of (Chemical Computing Group software, Canada). The structure of the compounds was established by Chemdraw Ultra 11.0. The crystal structure of bacterial *S. aureus* MurB (PDB Entry Code 1HSK) (Benson et al. 2001) was identified from the Brookhaven Protein Database (PDB http://www.rcsb.org/pdb). During the molecular docking process, the enzyme crystal structure was checked for missing atoms and bonds. Also, the ligand and water molecules were deleted in the crystal structures, except for the FAD co-factor for the protein PDB Code 1HSK. The parameters of the docking was adjusted as such all of the hydrogens were added, and a refinement protocol carried out for the enzyme in which the constraints were minimized until the RMSD gradient was 0.01 kcal/mol Å. Minimization of the energy was accomplished using the molecular mechanics force field "CHARMm". To guarantee the reliability of this docking protocol, the co-crystallised ligand, was redocked into the binding site of bacterial MurB enzyme. This procedure was performed three times and the best pose of the ligand exhibited RMSD values of 1.09 Å was determined. Additionally, the lowest energy aligned conformation(s) were recognized.

3. Results and Discussion

3.1. Chemistry

The synthesis of the target derivatives employ a straightforward strategy that adopts the advantage of the well-known condensation of benzophenone and thiosemicarbazide to yield 2-(diphenylmethylene)hydrazinecarbothioamide **(2).** Thus, cyclization of the key intermediate thiosemicarbazone **(2)** with chloroacetic acid yield the thiazolidine derivative **(3)**. Furthermore, 5-Substituted benzylidene-2-((diphenylmethylene)hydrazono)thiazolidin-4-one **(4a-g)** were achieved via reacting compound **(3)** with the appropriate aromatic aldehydes . Moreover, the thiazolyl hydrazones **(5a-d)** were obtained by refluxing compound **(2)** with (un)substituted phenacyl bromide. Finally, ethyl 2-(2-(diphenylmethylene)hydrazinyl)-4-methylthiazole-5-carboxylate **(6)** was synthesized via refluxing the thiosemicarbazone **(2)** with 2-chloroacetoacetic acid ethyl ester to obtain compound **(6)** in a good yield, **(Scheme 1)**



Scheme 1. Reagents and reaction conditions: (i) $H_2NNHCSNH_2$, C_2H_5OH, heating; (ii) $ClCH_2COOH$; (iii) Aromatic aldehydes, C_2H_5OH, heat (iv) (Un) substituted phenacylbromide, C_2H_5OH, heat; (v) $CH_3COCH(Cl)COOC_2H_5$, heat.

3.2. Antimicrobial Activity

The antimicrobial screening of all compounds was performed at the CO-ADD (The Community for Antimicrobial Drug Discovery) at the University of Queensland, Australia. Since antimicrobials are agents that either kill microorganisms or inhibit their growth [17-19], a systematic study to evaluate the antimicrobial

Table 1: Antimicrobial activity of compounds (3a–m) with the concentration set at 32 µg/mL in DMSO

Cpd #	Conc mmol/L	Antibacterial activity					Antifungal activity	
		Gram- positive bacteria		**Gram-negative Bacteria**				
		S. aureus	***Ecoli***	***K. pneumoniae***	***P. aeruginosa***	***A. baumannii***	***C. albicans***	***C. neoformans***
2	0.125	**28.61**	5.4	21.60	9.52	26.88	0.45	-63.36
3	0.108	25.33	-5.09	2.67	-13.06	36.77	2.82	-65.34
4a	0.083	18.53	0.27	24.87	8.3	42.34	-0.20	-61.88
4b	0.080	16.31	-0.19	**27.07**	6.53	45.66	8.86	-181.20
4c	0.077	24.83	0.97	14.92	11.69	40.98	4.82	-60.39
4d	0.077	25.08	1.6	20.32	12.4	46.47	1.91	-75.29
4e	0.074	**28.76**	2.97	23.79	16.95	**52.22**	0.13	-58.41
4f	0.069	23.11	-0.79	12.85	12.03	32.36	1.65	-61.88
4g	0.075	23.53	-1.71	18.49	8.41	43.49	-0.45	-71.29
5a	0.090	17.28	-8.78	19.09	12.73	30.02	5.17	-86.63
5b	0.086	14.52	-6.08	**27.29**	12.66	47.76	6.7	-104
5c	0.840	26.44	5.53	16.01	15.23	43.31	1.56	-76.73
5d	0.079	18.30	-3.23	17.3	2.27	24.90	-2.18	-72.77
6	0.087	22.72	3.90	16.2	-1.76	**59.04**	3.99	-80.19
Ciprofloxacin*	0.096	80>	80>	80>	80>	80>	-	-
Fluconazole*	0.104	-	-	-	-	-	80>	80>

**Ciprofloxacin (MIC (µg/ml) values: S. aureus (MRSA)=0.25; E. coli=0.007; K. pneumonia=0.5; A. Baumannii=1; P. Aeruginosa=0.25. Fluconazole (MIC (µg/ml) values: C. albicans=0.125; C. Neoformans=8.*

The used microorganisms are: *Escherichia coli (E. coli)* ATCC 25922, *Klebsiella pneumoniae (K. pneumoniae) MDR* ATCC 700603, *Acinetobacter baumannii (A. Baumannii)* ATCC 19606, *Pseudomonas aeruginosa (P. Aeruginosa)* ATCC 27853 and *Staphylococcus aureus (S. aureus) MRSA* ATCC 43300, and *two fungi: Candida albicans (C. albicans)* ATCC 90028 and *Cryptococcus neoformans (C. Neoformans)* ATCC 208821

effects of the synthesized organic compounds was designed [20-21]. This study measured the percentage of inhibition of microbial growth for each compound. Specifically, the *in vitro* experiments to elucidate the antimicrobial activity of compounds **(2, 3, 4a-g, 5a-d & 6)** were performed utilizing whole cell growth inhibition assays [22-23]. The inhibition of growth was measured against the following five bacteria: *Escherichia coli (E. coli)* ATCC 25922, *Klebsiella pneumoniae (K. pneumoniae) MDR* ATCC 700603, *Acinetobacter baumannii (A. Baumannii)* ATCC 19606, *Pseudomonas aeruginosa (P. Aeruginosa)* ATCC 27853 and *Staphylococcus aureus (S. aureus) MRSA* ATCC 43300, and *two fungi: Candida albicans (C. albicans)* ATCC 90028 and *Cryptococcus neoformans (C. Neoformans)* ATCC 208821 [24]. The standard concentration employed for screening was 32 µg/mL in DMSO [25-26]. **Table 1** record the *in vitro* antimicrobial activities of our synthesized products.

The growth inhibition, in percentile, for the Gram-positive bacteria *MRSA* with the target compounds is summarized in **Fig. 2**. Notably, compounds **2** and **4e** showed the highest growth inhibition with 28.6% and 28.7%, respectively whereas the compound **5b** showed the least growth inhibition. The enhanced activity of the compounds **2** and **4e** is due to the fact that easy solubility in DMSO. Similarly, **Fig. 3** includes the growth inhibition for the Gram-negative bacteria *A. baumannii* where the top three compounds were **6, 4e & 5e** with percentiles ranging from 59.0 to 47.7%. On the other hand, the growth inhibition for the fungi *C. albicans* with the target compounds is reported in **Fig. 4**. From the values obtained, we can conclude that the compounds are not effective as fungi

inhibitors, with only adducts **4b** and **5b** exhibiting minor antifungal agent growth inhibition of ~8%.

From the results presented in **Table 1**, medium to good antibacterial activity was observed for all the compounds **2, 3, 4a-g, 5a-d & 6** against Gram-positive bacteria *MRSA* and the two *MDR* Gram-negative bacteria (*E. coli*, and *K. pneumonia*) and *A. baumannii*. The increased activities are due to easy penetration of compounds into lipid membranes of organisms *MRSA*, *MDR* and *A. baumannii*. [24]. The tested compounds have shown weak to medium antibacterial activity against Gram-negative bacteria *P. aeruginosa*. On the other hand, almost no antimicrobial activity was observed for the target compounds against Gram-negative bacteria *E.coli* and the two fungi: *C. albicans* and *C. Neoformans*. This inactivity stems from the higher lipid content in the cell membrane of *C. albicans*, and *C. Neoformans*, which prevents easy diffusion of compounds into the cell [27-29]. Overall, the thiazole derivative **6** was found to be the most promising bioactive compound against Gram-negative bacteria *A. baumannii* with percentage of inhibition 59.0 while compound **4e** showed 28.7% of growth inhibition against Gram-positive bacteria *MRSA*

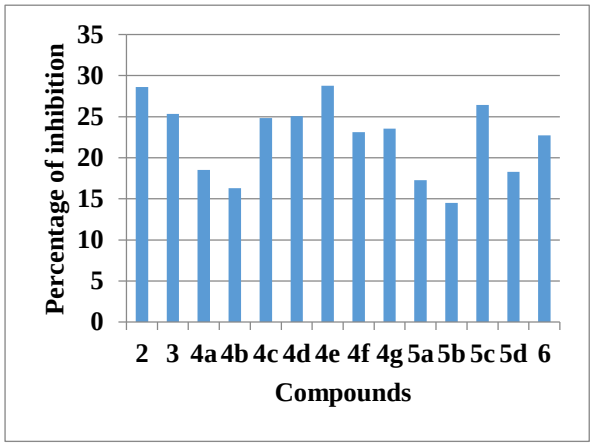


Fig. 2: Percentage of inhibition of *MRSA* growth by the tested compounds.

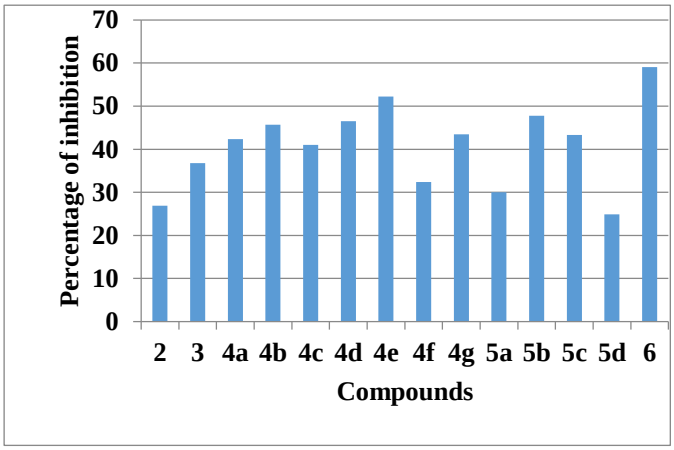


Fig. 3: Percentage of inhibition of *A. baumannii* growth by the tested compounds.

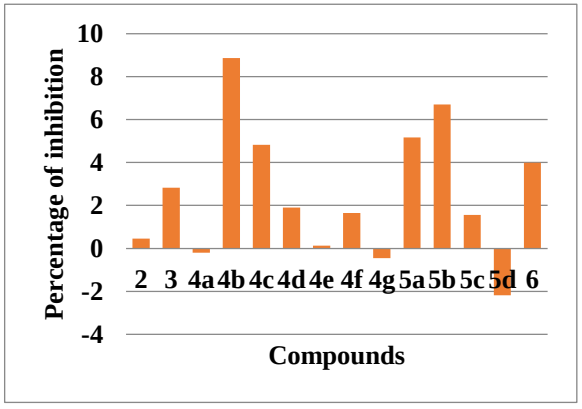


Fig. 4: Percentage of inhibition of *C. albicans* growth by the tested compounds.

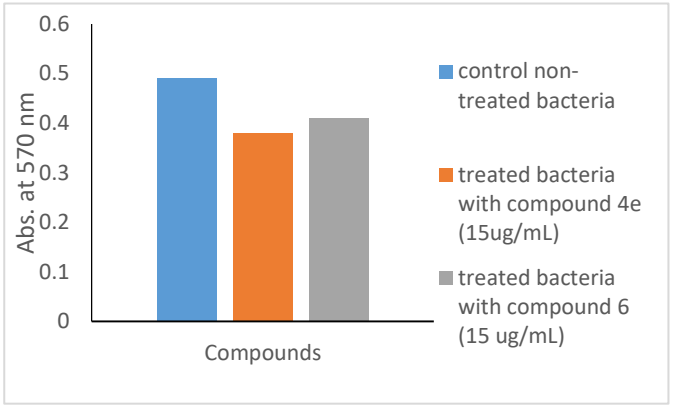


Fig. 5: Decrease in peptidoglycan density for compounds 4e and 6.

Peptidoglycan isolation and dye release assay

Peptidoglycan biosynthesis is considered as one of the most known and validated targets for antimicrobial therapy as well as Mur enzymes which are excellent candidates for drug discovery. From this point of view, in an attempt to confirm the mechanism of action of the highest two antibacterial activity observed for the tested compounds, peptidoglycan

isolation and dye release assay was carried out. It was found that compound **4e** at 15 ug/mL; there was a 22.5% decrease in peptidoglycan density compared to control group. And for derivative **6**; a 17.3% decrease in peptidoglycan density was determined (**Fig. 5**).

3.3. Molecular Docking Study

The diphosphate moiety was replaced by the x-ray crystallographic data of UDP-sugar substrate with MurB as guide replacement. It was noticed that the role of FAD cofactor is in intermediating hydride transfer from the NADPH to the UDP-sugar substrate. It was also realized that both substrates of Bacterial MurB fit in the same binding site of the bacterial enzyme [30]. The number of poses selected per ligand was adjusted to 10, and no constraints were used to perform molecular docking. The binding affinity score of the poses in addition to its orientation into the active site is selected in a manner similar to the co-crystallized ligands orientation; and keeping the binding interactions, especially hydrogen bond formation and hydrophobic groups. The estimated binding affinity of the co-crystallized ligand was -8.965 Kcal/mol with of complex hydrogen network with a number of amino acids including, Arg224, Gln229, Tyr155, Arg225, Gly139, Arg242, Arg188, Ser238, and Gly153 giving an evidence about the importance of hydrogen bond formation for effective enzyme binding. Modeling studies suggest that Compound **6** that showed 59.09% growth inhibition against *A. baumannii* binds to the MurB active site with a binding affinity of -6.262 Kcal/mol via the hydrogen bonding interaction of the carbonyl group of ester group with Gln229 and Asn83 with the NH group of the thiazole ring (**Table 2, Fig. 6**). Compound **4e** showed a hydrogen bond interaction network with amino acids Gly79 and Gly81 via the NO_2 group of the phenyl ring with a binding affinity of -7.811 Kcal/mol (**Table 2**); in addition to two cationic arene interactions of the 2 aromatic rings with the Arg225 and Ser82 amino acids (**Fig. 7**). This is consistent with the 52.22 % growth inhibition of the latter derivative against *A. baumannii* and 28.86 % growth inhibition against *MRSA*. As for compound **5b** with a binding affinity of -8.243 Kcal/mol through the cationic arene interaction with Arg225 and Ser82 via aromatic ring and the aromatic ring substituted from thiazole ring, respectively showed 27.29 % growth inhibition against *MDR* (**Table 2, Fig. 8**). Compound **4b** gives the same cationic arene interactions with Ser82, Phe274, in addition to 2 hydrogen bond interactions with Pro141 via sulphur atom of thiazolidine ring and the nitrogen atom and this with a binding affinity of -8.243 Kcal/mol (**Table 2, Fig. 9**). The docking study indicated that the important interactions of the tested compounds have resemblance to the binding mode with the FAD flavin ring system co-crystallized with enzyme with Gln229, Arg225 and Ser82 amino acids. Therefore, the proximity of the tested compounds to FAD and its hydrogen bond to the main chain of these amino acids appear to provide a structural platform. Analysis of the docking results also suggests that their good antimicrobial activity could be attributed to their high binding affinity, although still less than the reference ligand. Also, it can be referred to their

Table 2: The binding energy score ranked results of target compounds – MurB complex binding conformations.

Compound Name	Binding energy score	Average number of poses per run
2	-5.268	9
3	-5.841	10
4a	-5.947	10
4b	-7.073	8
4c	-7.666	10
4d	-5.656	9
4e	-7.811	9
4f	-7.851	9
4g	-6.432	9
5a	-7.814	9
5b	-8.243	7
5c	-8.088	7
5d	-8.563	9
6	-6.262	10

* The shown score is the mean of three consecutive runs.

* The docking method was validated by successful pose-retrieval docking experiment of the ligand (score: -8.965).

binding with the same amino acids as the co-crystallized ligand, especially the hydrogen bonding interactions and cationic arene interaction. These findings are considered encouraging, providing a possible mechanism of action for the tested compounds and indicating the possibility of developing novel antibacterial agents targeting the biosynthesis of peptidoglycan.

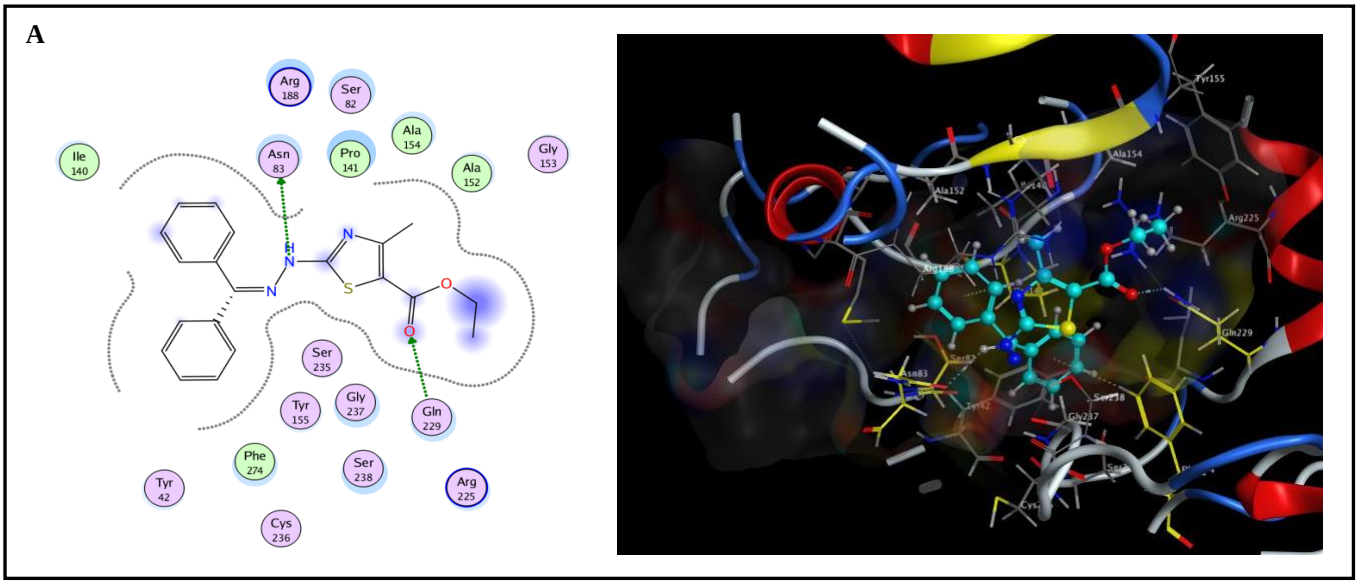


Fig. 6: Computer modeling of compound 6 binding to Mur-B (1HSK). Compound 6 was colored in blue.

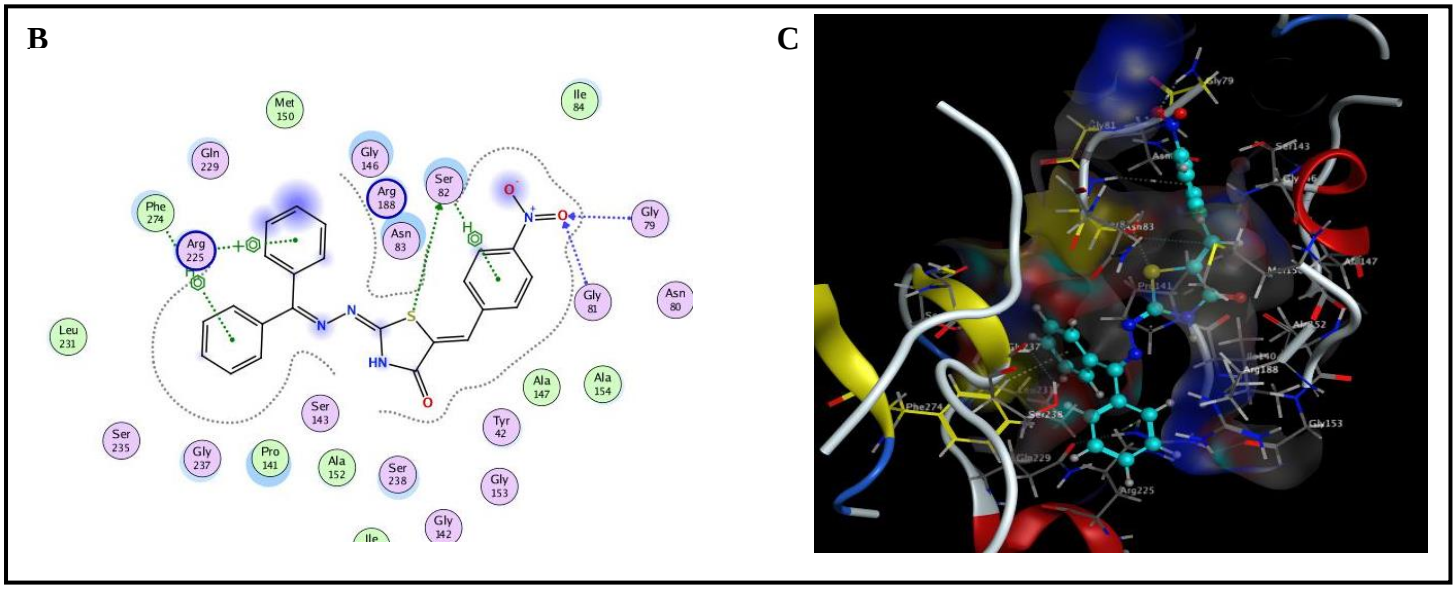


Fig. 7: (B) The 2D caption of compound 4e binding to the active site of Mur-B. (C) Binding pattern of compound 4e colored by element, ball and stick into Mur-B showing hydrogen bond interactions and cationic arene interactions(dotted lines).

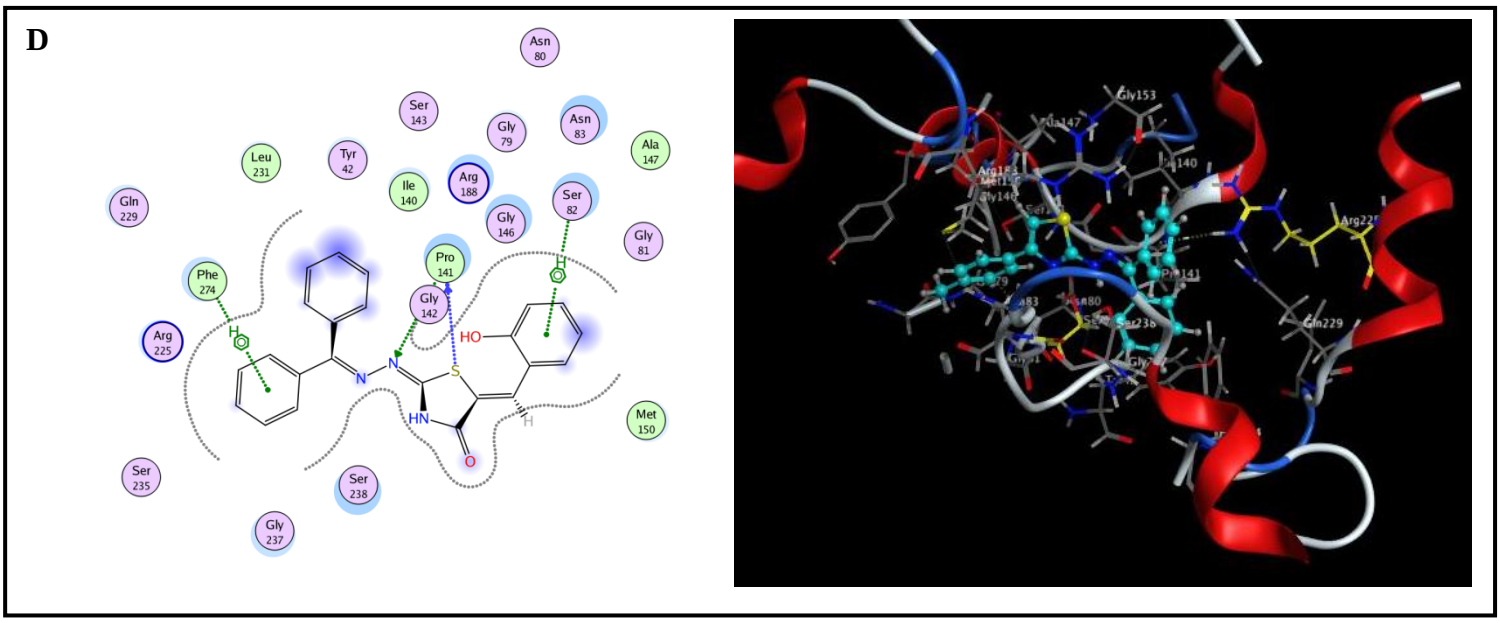


Fig. 8: Computer modeling of compound 5b binding to to Mur-B (1HSK). Compound 5b was colored in blue.

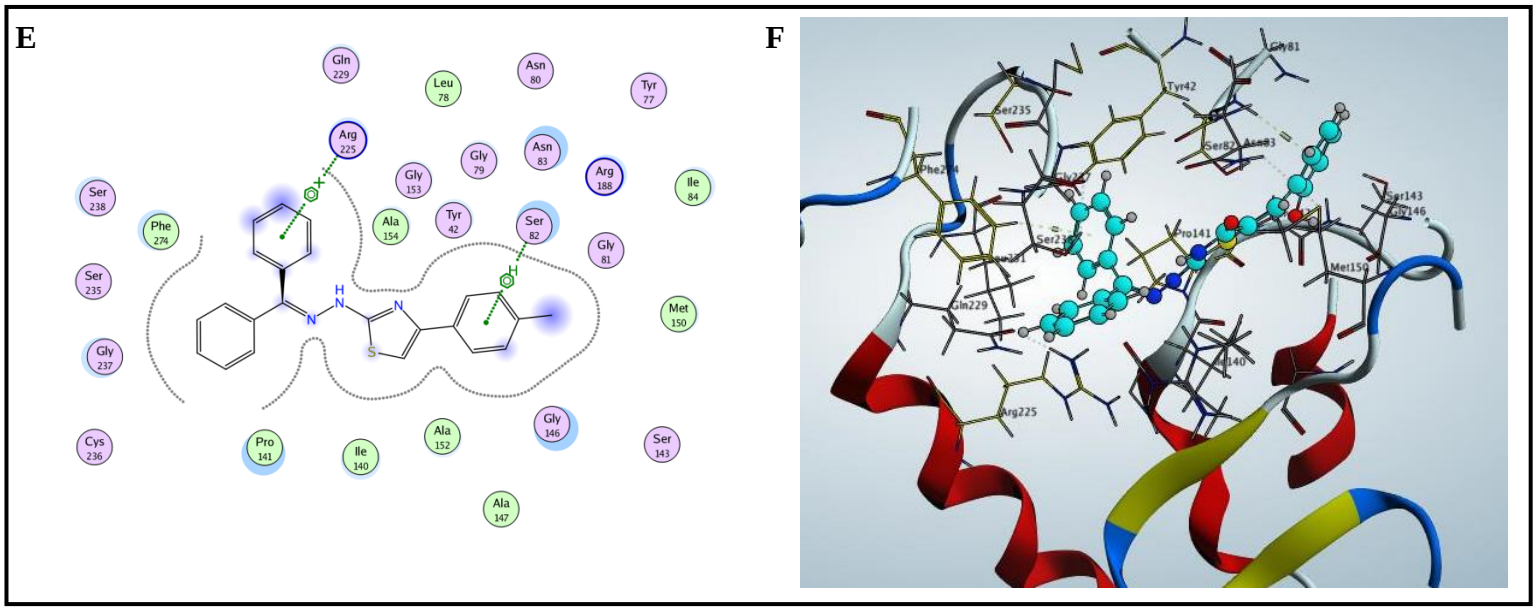


Fig. 9: (E) 3D caption of compound 4b colored by element, ball and stick into Mur-B showing hydrogen bond and cationic arene interactions (dotted lines), Compound 4b colored in blue. (F) Binding pattern of compound 4b colored by element, ball and stick into Mur-B.

4. Conclusions

In conclusion, we have documented the synthesis of several thiazole compounds from the key intermediate thiosemicarbazones **2**. The synthesis is simple, facile and high yielding to afford the corresponding products.. This work extends the examination of biological activity of the thiazole derivatives. Lastly, all the compounds were screened against an unusually wide range of microorganism, including one Gram-positive and four Gram-negative bacteria, plus two fungi. Thus compound 6 showed good antibacterial activity against *MRSA* in addition to activity towards extensively resistant *A. baumannii*, on the contrary compound 5d which showed low activity against both *MRSA* and *A. baumannii*.

5. Conflicts of interest

The authors declare that they have no conflict of interest.

6. Formatting of funding sources

Not applicable.

7. Acknowledgments

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