SYNTHESIS OF SOME NEW AZOLE DERIVATIVES AS ANTIBACTERIAL AGENTS

Saeed S. Atwa[1], Mohamed Hagras[1], Abdelrahman S. Mayhoub[1,2], Mohamed M. Elsebaei[1]*.

[1]Department of Pharmaceutical Organic Chemistry, College of Pharmacy, Al-Azhar University, Cairo, 11884, Egypt

[2]Nanoscience Program, University of Science and Technology, Zewail City of Science and Technology, Giza, Egypt

***Corresponding author: m.elsebaei@azhar.edu.eg**

ABSTRACT

Antibiotic resistance is a growing global health threat and requires extensive research to combat this urgent problem. Phenylthiazoles, known for their diverse biological activities including anthelmintic, insecticidal, and antimicrobial properties, have recently gained particular attention as potential anti-MRSA lead compounds. This class of compounds is an established pharmacophore for the development of new antibacterial agents, particularly against multidrug-resistant bacteria such as MRSA, a notorious pathogen resistant to most first-line antibiotics. In-depth structure-activity relationship (SAR) studies of phenylthiazoles revealed two key features critical to their antibacterial activity: a nitrogen-containing head and a lipophilic tail. In this study, we aimed to reduce the lipophilicity of phenylthiazoles and improve their overall physicochemical and pharmacokinetic profiles by synthesizing a new series bearing primary amines at the phenyl-4 position. Notably, compounds **5m** exhibited bactericidal activity against MRSA, exhibiting a minimum inhibitory concentration (MIC) of only 8 µg/mL against the prevalent MRSA strain USA300.

Keywords: Antibiotic resistance, Phenylthiazole, anti-MRSA.

1. Introduction

The number of fatal infections caused by antibiotic-resistant bacteria has steadily increased in recent years and represents a major global health challenge(Ventola 2015, Aslam, Wang et al. 2018, Biondo 2023). Staphylococcus aureus, a notorious bacterial pathogen, is responsible for a wide range of infections, from minor skin lesions to life-threatening invasive diseases such as soft tissue infections, pneumonia, endocarditis, and osteomyelitis. The emergence of methicillin-resistant S. aureus (MRSA) in the 1960s, triggered by resistance to lactam antibiotics, particularly methicillin, triggered numerous hospital epidemics(Shanson, Kensit et al. 1976, Levy and Marshall 2004, Rivera and Boucher 2011, Thati, Shivannavar et al. 2011, Elsebaei, Mohammad et al. 2018). Physicians today face enormous difficulties in treating MRSA infections due to the emergence of strains resistant to various classes of antibiotics, including tetracyclines, aminoglycosides, macrolides, lincosamides and fluoroquinolones(Jones, Karlowsky et al. 2003, Jones, Draghi et al. 2004, Pantosti, Sanchini et al. 2007). The situation has been further exacerbated by the recent emergence of drug-resistant S. aureus strains with intermediate susceptibility or resistance to vancomycin (VISA or VRSA)(Howden, Davies et al. 2010). Until recently, vancomycin served as the primary treatment option for MRSA infections(Tang, Hu et al. 2015).

As antimicrobial resistance reaches alarming levels, personal responsibility for combating it is becoming increasingly important. Our laboratory set out to find effective antibacterial agents, starting with the promising phenylthiazole scaffold. Phenylthiazoles were originally identified as a thiazole core with a guanidine head and an alkyl chain tail(Elsebaei, Mohammad et al. 2018, Elsebaei, Abutaleb et al. 2019, Elsebaei, Mohammad et al. 2019, Hosny, Abutaleb et al. 2020) have undergone extensive research and optimization to improve their effectiveness and metabolic profile. While they showed anti-biofilm activity, the poor permeability of the early phenylthiazoles likely contributed to their narrow spectrum(Eid, Elsebaei et al. 2017, Helal, Sayed et al. 2019, Mancy, Abutaleb et al. 2019, Elsebaie, El-Din et al. 2022, Sayed, Abutaleb et al. 2023). Consequently, changes to the tail led to the third generation, which showed intracellular effectiveness but required further optimization of the metabolic profile(El-Din, Elsebaie et al. 2023, Omara, Hagras et al. 2023, Shahin, Mohamed et al. 2023, Elbakry, Harras et al. 2024). This study delves deeper into scaffold modifications to elucidate their influence on activity against various microbial organisms and expands our understanding of the structure-activity relationship within this promising class of antibacterial agents. Therefore, this study aims to achieve two goals: first, to modify the scaffold by inserting a polar nitrogen atom into the lipophilic part to understand its activity against various microbial organisms, and second, to improve our knowledge of the structure-activity relationship to expand within this new class of antibacterial agents. The underlying concept for the current scaffold modification is to replace the benzylic carbon responsible for the short half-life with a nitrogen atom, which, according to previous studies, possesses broad-spectrum antibacterial activity and improves its physicochemical properties.

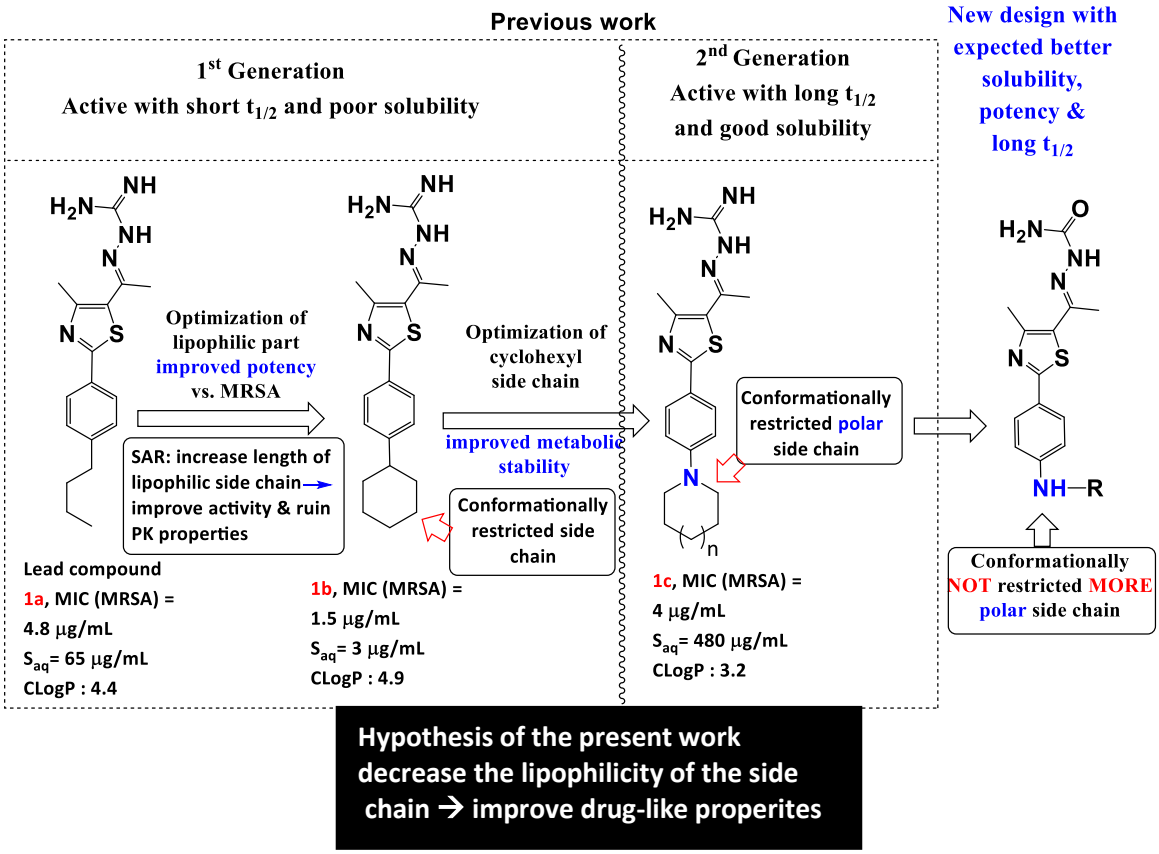


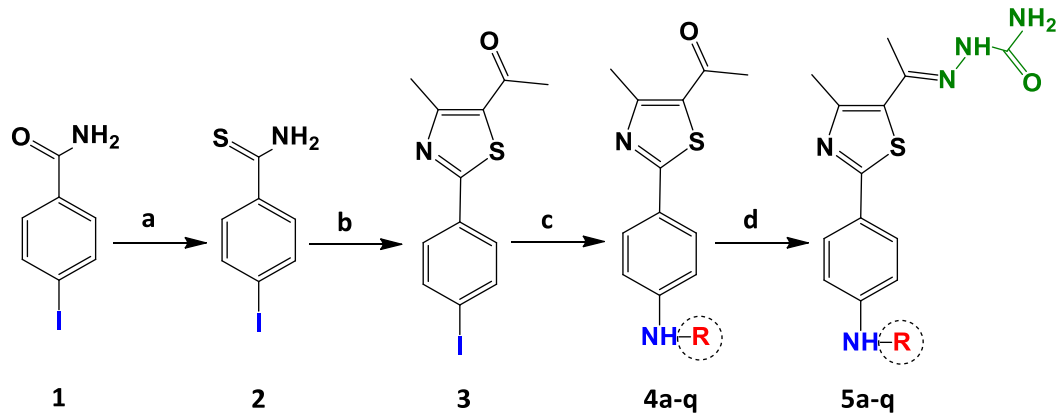
Figure 1. Progress of phenylthiazole antibiotics development and the general idea of the present work.

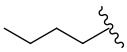
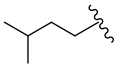
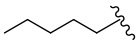
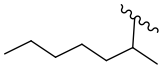
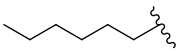
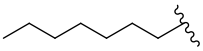
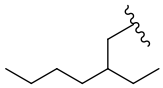
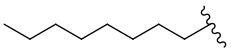
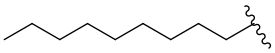
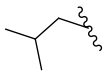
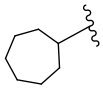
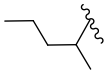
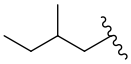
An initial investigation of the SAR of the phenylthiazole compounds prompted us to cyclize the terminal n-butyl moiety of the lead drug **1a** to enhance its antibacterial activity(Mohammad, Mayhoub et al. 2014). Unfortunately, while this modification indeed enhanced potency, it compromised the physicochemical properties and pharmacokinetic profile of the resulting analogues (**Figure 1**). However, this increase in potency was accompanied by a significant increase in lipophilicity, as evidenced by the increased clogP value. This led to a drastic decrease in water solubility, which dropping from 65 µg/mL for **1a** to mere 3 µg/mL for **1b**. Consequently, formulation of **1b** for oral or parenteral administration became a major challenge due to its poor solubility. The introduction of nitrogen atom to **1b** afford compound **1c,** which enhances its solubility and physicochemical properties by 160-fold higher than **1b**. **Scheme (1).**

2. Result and discussion

2.1. Chemistry

Treatment of para -iodobenzothioamide (**2**) with α-chloroacetylacetone yielded the key starting compound **3**, as previously reported(Mohammad, Mayhoub et al. 2014). The corresponding 1ry-amine derivatives **4a-q** were obtained using the Buchwald carbon-nitrogen cross-coupling conditions, using the Pd-catalyzed protocol, in which Pd(II) and X-Phos ligand in a polar aprotic solvent provided the best yields of compounds **4a-q** (**Scheme 1**). Condensation of **4a-q** with semicarbazide hydrochloride gave the final products **5-21** (**Scheme 1**).



	R		R
4a,5a		4k,5k	
4b,5b		4l,5l	
4c,5c			
4d,5d		4m,5m	
4e,5e		4n,5n	
4f,5f		4o,5o	
4g,5g		4p,5p	
4h,5h		4q,5q	
4i,5i			
4j,5j			

Reagents and conditions: **(a) Dry THF, Lawesson's reagent (b) Absolute EtOH, 3-chloro-2,4-pentandione, heat to reflux, 6 h, (c) $Pd(OAc)_2$ (5% mol), X-phos (10% mol), KOtBu (2.5 equiv.), appropriate 1ry amine, dioxane, heat at 200°C for 3 h; (d) Semicarbazide HCl, EtOH, conc. HCl,**

2.2. Antimicrobial Evaluation

The newly synthesized phenylthiazole derivatives **5a-q** were initially tested against methicillin-resistant *Staphylococcus aureus* (MRSA) USA300, which is a significant source of MRSA skin and soft tissue infections (SSTIs) worldwide. The phenylthiazole derivatives were also tested against an *Acinetobacter baumannii* AB5075 strain to examine the activity of the compounds against gram-negative bacteria. In this study, vancomycin was used as a positive control in the antibacterial evaluation. The initial screening against MRSA USA300 revealed an abundance of structure-activity relationships (SAR) as MIC values ranged from 8 to over 32 µg/mL (Table 1). The 2- ethylhexyl amine analog (compound **5m**) represented the best side chain as it provided the most potent analog from this series (MIC value of 8 µg/mL) **Table 1**, meaning that the target protein is at this point cannot adjust specific position for side chains with more than 8 carbon atoms. The SAR appears clear because it relates to the size of the side chain on the nitrogen atom of the primary amine. Briefly, the shrinkage of the nitrogen-containing side chain gradually impaired the anti-MRSA activity of the compounds, as the MIC value increased to four times 32 µg/mL for the 2-ethylhexylamine-containing derivative **5m** and for the sec-butylamine-containing one derivative was completely abolished analogue **5g**. Similarly, extension of the nitrogen-containing side chain yielded the nonylamine-containing derivatives 10 without inhibition for MRSA **Table 1**. This observation confirms our previous hypothesis that the active site of the target receptor cannot accommodate side chains larger than octylamine. The MIC for the control antibiotic vancomycin against MRSA USA300 was 1 µg/ml.

Table 1; Antimicrobial activities of compounds 5-21.

Cp.ID	**MIC (*µ*g/mL)**	
	S. aureus **USA300**	A. baumannii **AB5075**
5a	32	No inhibition
5b	16	32
5c	16	No inhibition
5d	16	No inhibition
5e	32	No inhibition
5f	No inhibition	No inhibition
5g	No inhibition	No inhibition
5h	32	64
5i	32	No inhibition
5j	32	64
5k	16	32
5l	16	No inhibition
5m	8	No inhibition
5n	16	32
5o	16	32
5p	16	No inhibition
5q	32	64
Vancomycin	1	NT

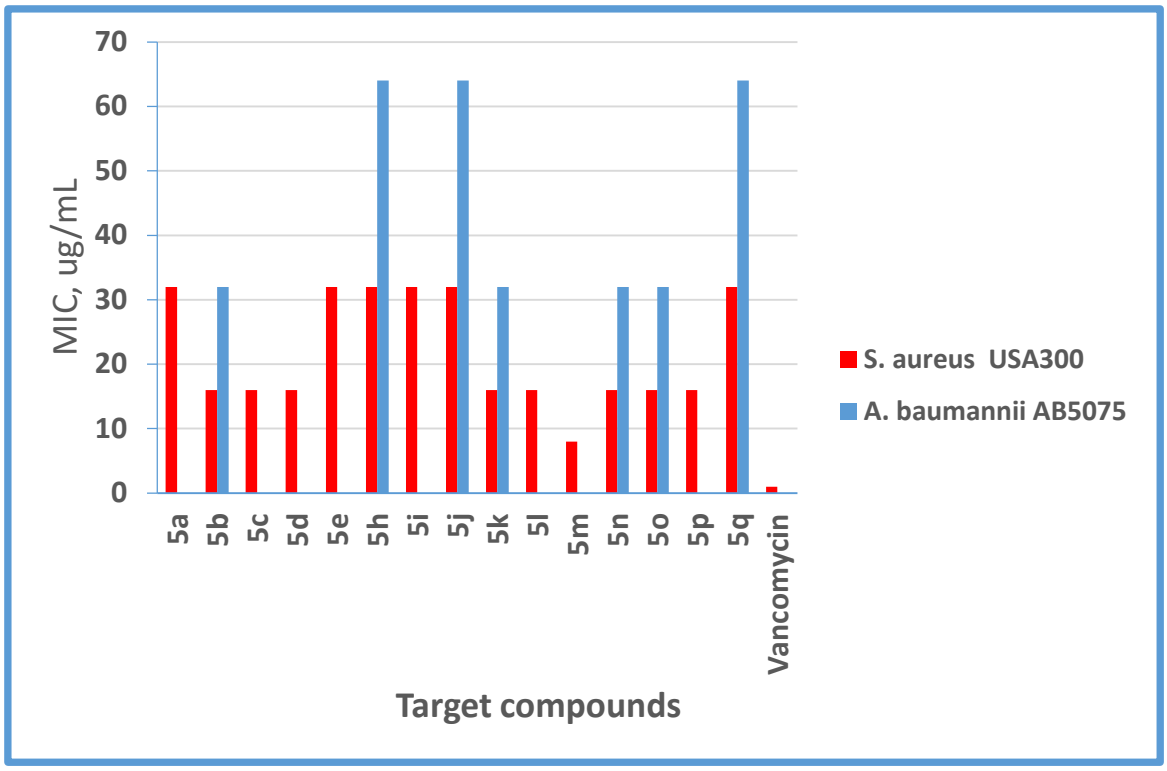


Figure 2. MIC values of tested compounds

2.3. ***Computational ADMET* study**

The ability to predict pharmacokinetics is an important step in drug research development. It helps drug manufacturers get the optimal edge themselves, or even encourages others to continue the fight. In the last two decades(Amin, El-Saadi et al. 2021), there have been online applications and tools for predicting absorption, distribution, metabolism and excretion (ADME). In this particular study, those properties for the best three candidates in our study were calculated and the results are summed up in the **tables 1-3**. To study the ADME, probability and physiochemical properties of these three candidates, three different programs were used: PreAdME, SwissAdme and Molsoft.

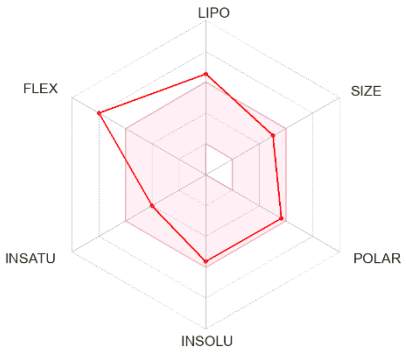


Figure 3. Egg radar for **5m** analog

To evaluate the contribution of molecular rigidity to oral bioavailability, the number of rotatable bonds was chosen as the most appropriate parameter. In general, the number of rotatable bonds corresponds to the molecular weight, i.e. if this number increases as the molecular weight increases, and vice versa. The molecular weight of our candidates is less than 500, the logP (calculated octanol/water partition coefficient) is less than 5, indicating their initial good oral bioavailability. A further division was made by dividing the number of rotatable bonds: this number measures the molecule flexibility and indicates the oral bioavailability. It can be calculated from the number of individual non-ring bonds that are not bonded to either a terminal or a hydrogen atom. It is divided into three groups: number of rotatable bonds 7 or less, 8-10 and more than 10. Our research has shown that the number of rotatable bonds for the three compounds was over 10, which means that it has predominantly low bioavailability. The number of H-bond donors and acceptors is less than 5 for all candidates. Therefore, all three compounds plausibly have good absorption(Veber, Johnson et al. 2002).

In this study, we also measured the topological polar surface area (tPSA) which allows us to predict the permeability of polar atoms through membranes. The tPSA values for the three compounds are all similar, at 120.64. This suggests that the compounds are all similarly polar(Veber, Johnson et al. 2002). Since a molecules that have a PSA value above 140 Å usually also have poor permeability (Lipinski, Lombardo et al. 2012), all three investigated compounds showed a PSA value low 140 Å indicating their moderated permeability. Swiss ADME was the software of choice to predict the physicochemical parameters. The data retrieved are listed in **Table 1.**

Table 1: Physiochemical properties prediction of selected analogs.

Code	tPSA[a]	H-bond Donor	H-bond Acceptor	NORTB[b]	MW[c]	Log P
5d	120.64	4	4	11	387.5	1.95
5l	120.64	4	4	11	401.5	1.4
5m	120.64	4	4	12	415.6	0.8

aTPSA, topological polar surface area; bNORTB, number of rotatable bonds; cMW, molecular weight

NORTB is a measure of the flexibility of a molecule. The NORTB values for the three compounds are 11, 11, and 12, respectively. This suggests that the compounds differ slightly in their flexibility. Compound **5m** has the most rotatable bonds, which suggests that it may be the most flexible of the three compounds. Log P is a measure of the lipophilicity of a molecule. Lipophilic molecules are soluble in nonpolar solvents and insoluble in polar solvents. The log P values for the three compounds are 1.95, 1.4, and 0.8, respectively. This suggests that the compounds differ in their lipophilicity, with compound **5m** being the least lipophilic.

Lipiniski rule(Lipinski, Lombardo et al. 2012): states that the oral drug must have only one violation of the following criteria: not more than 5 HBD and not more than 10 HBA, molecular weight less than 500 Daltons and logP is less than the value of 5. At using SwissADME and Molsoft, our compounds met Lipinski's rules for drug similarity; For this reason, we believe they have good absorption. Drug solubility is a critical factor in determining the rate of dissolution that enables oral bioavailability; Therefore, the solubility of the drug is tested, which improves when the solubility values are less than 10 mg/L. Since our compounds have values of 2-4 mg/L, they are poorly soluble in water(Ustaoğlu, Taş et al. 2021).

Table 2: Predicting drug-likeness of selected analogs.

Code	Solubility (mg/L)	Drug Likeness model score	Lipinski's rule violation	Bioavailability score
5d	2.28	0.93	0	0.55
5l	2.41	1.14	0	0.55
5m	3.62	0.9	0	0.55

Caco-2 Permeability: This measures how easily a compound can pass through the intestinal wall and enter the bloodstream. Compound **5d** has the highest bCaco-2 permeability, followed by compound **5m** and then compound **5l**. HIA (%): This measures the percentage of a compound that is absorbed from the intestine. Compound **8** has the highest CHIA, followed by compound **5m** and then compound **5l**. MDCK permeability: This measures how easily a compound can pass through the kidney membrane and enter the bloodstream. Compounds **5d** and **5m** have similar MDCK permeability, while compound **16** has the lowest MDCK permeability. PPB (%): This measures the percentage of a compound bound to plasma proteins. Compound **5m** has the highest PPB, followed by compound **5d** and then compound **5l**. CYP2D6 Metabolism: This measures whether a compound is metabolized by the enzyme CYP2D6. Compound **5d** is not metabolized by CYP2D6, while compound **5l** is a substrate for CYP2D6 and compound **5m** is not metabolized by CYP2D6. In order to determine those properties, PreADMET[133] is used as a tool to investigate as deeply as we can from those pharmacokinetics. Compound **8** has the highest permeability and absorption, followed by compound **5m** and then compound **5l**. Compound **5m** has the highest plasma protein binding, followed by compound **5d** and then compound **5l**. Compound **5l** is metabolized by CYP2D6 while compound **5d** is not the case and compound **5m** is not metabolized by CYP2D6. These differences in physicochemical properties may impact the biological activity and pharmacokinetics of the compounds. For example, compound **5d** may have a longer half-life in the body than compound **16** due to its lower plasma protein binding. The pre-ADMET program was used to generate the data in **Table 3.**

Table 3: Pharmacokinetics prediction through pre-ADME

	Pharmacokinetics					
Code	**[a]BBB**	**[b]Caco-2 (× 10^6cm/s)**	**[c]HIA(%)**	**[d]MDCK (nm/s)**	**[e]PPB(%)**	**[f]CYP2D6**
5d	2.45	20.2	92.26	67.95	99.33	Non
5l	0.78	17.45	92.32	10.62	99.48	Substrate
5m	1.15	19.25	92.51	13.00	100	Non
Vancomycin	0.03	20.64	1.12	0.04	42.20	Non

aBBB:blood brain barrier penetration; bCACO-2: permeability through cells derived from human colon adenocarcinoma; cHIA: percentage human intestinal absorption; dMDCK: permeability through Madin-Darby canine kidney cells; ePPB: plasma protein binding; f CYP2D6:cytochromeP4502D6**.**

3. Experimental of chemistry section

3.1.General.

All biologically tested compounds are with purity of 98% or more.^{1}H NMR spectra were run at 400 MHz and ^{13}CNMR spectra were determined at 100 MHz in deuterated dimethyl sulfoxide (DMSO-*d6*) on a Varian Mercury VX-400 NMR spectrometer. Chemical shifts are given in parts per million (ppm) on the delta (□) scale. Chemical shifts were calibrated relative to those of the solvents. Column chromatography was performed on 230-400 mesh silica. The progress of reactions was monitored with Merck silica gel IB2-F plates (0.25 mm thickness). Melting points were determined using capillary tubes with a Stuart SMP30 apparatus and are uncorrected. All yields reported refer to isolated yields. Compound (**3**) was prepared as reported (Mohammad, Mayhoub et al. 2014).

3.1.1. 1-(2-(4-(*1ry*-amine derivatives-1-yl)phenyl-4-methylthiazol-5-yl)ethan-1-one (4a-q).

***General procedure*:**

to dioxane (10 mL) in a 75-mL sealed tube compound **3** (200 mg, 1 mmol), palladium acetate (20 mg, 5 mol%), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (**X**-phos) (60 mg, 10 mol%) and potassium *tert*-butoxide (120 mg, 2.5 equiv.). After the reaction mixture was purged with dry nitrogen gas for 15 min at 100 ^{o}C, appropriate *primary* amines (3 equiv.) were added. The sealed tube was then heated and stirred at 200 ^{o}C for 3 hrs and monitored by thin-layer chromatography (TLC). After completion of the reaction, the reaction mixture was poured on water then extracted with ethyl acetate (3 × 15 mL) then dried over $MgSO_4$, the organic materials were then concentrated under reduced pressure. The crude materials were purified *via* silica gel flash column chromatography using hexane-ethyl acetate (8:2) as yellowish viscous oil.

3.1.1.1.1-(2-(4-(butylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4a)

Orange oil (91 mg, 54.2 %);^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 3.45 (br, 1H), 3.08-3.03 (m, 2H), 2.45 (s, 3H), 2.28 (s, 3H), 1.58-1.51 (m, 2H), 1.43-1.34 (m, 2H), 0.94-0.90 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 192.83, 159.72, 151.08, 147.85, 143.83, 132.51, 127.59, 121.16, 112.07, 42.66, 31.19, 20.27, 18.66, 16.67, 14.26; MS (*m/z*); 288 (M+, 100.00%).

3.1.1.2.1-(4-methyl-2-(4-(pentylamino)phenyl)thiazol-5-yl)ethanone (4b)

Yellow oil (69 mg, 39.2 %);^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 3.48 (br, 1H), 3.07-3.02 (m, 2H), 2.50 (s, 3H), 2.31 (s, 3H), 1.74-1.52 (m, 2H) , 1.39-1.34 (m, 2H), 1.34-1.30 (m, 2H), 0.91 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (DMSO-*d6*) δ: 198.38, 159.80, 151.06, 147.82, 143.72, 132.53, 127.59, 121.16, 112.06, 42.95, 29.32, 28.75, 24.43, 22.44, 18.66, 16.67, 14.43; MS (*m/z*); 302 (M+, 100.00%).

3.1.1.3.1-(2-(4-(hexylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4c)

Yellow oil (87 mg, 40.1%); ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 3.45 (br, 1H), 3.07-3.02 (m, 2H), 2.31 (s, 3H), 2.28 (s, 3H), 1.57-1.51 (m, 2H) , 1.39-1.33 (m, 2H), 1.31-1.27 (m, 2H), 0.90 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 203.02, 159.79, 151.06, 147.73, 143.71, 132.61, 127.58, 121.18, 112.06, 42.99, 31.59, 29.04, 26.80, 22.60 , 18.66 , 16.62 , 14.41; MS (*m/z*); 316 (M+, 100.00%).

3.1.1.4.1-(2-(4-(heptylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4d)

Brown oil (89 mg, 46.3%); ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 3.45 (br, 1H), 3.07-3.02 (m, 2H), 2.50 (s, 3H), 2.28 (s, 3H), 1.59-1.52 (m, 2H) , 1.34-1.28 (m, 2H), 0.89 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 196.48, 159.81, 151.05, 147.73, 143.70, 132.61, 127.58, 121.16, 112.06, 42.98, 31.77, 29.07, 29.02, 27.09 , 22.54 , 18.65 , 16.62 , 14.43 ; MS (*m/z*); 330 (M+, 100.00%).

3.1.1.5. 1-(4-methyl-2-(4-(octylamino)phenyl)thiazol-5-yl)ethanone (4e).

Brown oil (72 mg, 35.9 %); ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 3.50 (br, 1H), 3.07-3.02 (m, 2H), 2.34 (s, 3H), 2.29 (s, 3H), 1.89 (s,), 1.57-1.53 (m, 2H), 1.38-1.31 (m, 2H), 1.29-1.26 (m, 2H) , 0.88-0.85 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 196.94, 159.20, 151.16, 148.60, 144.54, 131.78, 127.65, 121.02, 112.06, 42.97, 31.74, 29.32, 29.19, 29.06, 27.13 , 22.57, 18.67, 16.96, 14.42; MS (*m/z*); 344 (M+, 100.00%).

3.1.1.6. 1-(4-methyl-2-(4-(nonylamino)phenyl)thiazol-5-yl)ethanone (4f)

Brown oil (98 mg, 46.9%); ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 12.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 3.50 (br, 1H), 3.05 (d, *J* = 12.0 Hz,), 2.45 (s, 3H), 2.28 (s, 3H), 1.55-1.53 (m, 2H), 1.26 (m, 2H), 0.86 (t, J = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 198.94, 159.81, 151.05, 147.09, 143.67, 132.64, 127.58, 121.17, 112.06, 42.97, 31.76, 29.48, 29.35, 29.14, 29.05, 27.10, 22.56, 18.65, 16.60, 14.42 ; MS (*m/z*); 358 (M+, 100.00%).

3.1.1.7. 1-(2-(4-(sec-butylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4g)

Yellow oil (89 mg, 52.9 %); ^{1}H NMR (DMSO-*d6*) δ: 7.95 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 4.35 (br, 1H), 3.53 (t, *J* = 8.0 Hz, 3H), 2.65 (s, 3H), 2.45 (s, 3H), 1.67-1.63 (m, 2H), 1.48 (m, 1H), 1.18 (d, *J* = 8.0 Hz, 3H), 0.92 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 198.94, 159.38, 151.21, 150.44, 147.74, 143.69, 132.57, 127.66, 112.06, 57.80, 29.09, 20.08, 11.30 ; MS (*m/z*); 288 (M+, 100.00%).

3.1.1.8. 1-(2-(4-(isobutylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4h)

Yellow oil (33 mg, 19.7 %); ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 3.45 (br, 1H), 2.90-2.86 (t, *J* = 8.0 Hz , 2H), 2.45 (s, 3H), 2.28 (s, 3H), 1.89-1.82 (m, 1H), 0.95-0.93 (d, *J* = 8.0 Hz, 6H); ^{13}C NMR (DMSO-*d6*) δ: 189.70, 159.70, 151.38, 147.84, 143.86, 132.48, 127.59, 121.06, 112.06, 50.84, 27.85, 20.85, 18.65, 16.64; MS (*m/z*); 288 (M+, 100.00%).

3.1.1.9. 1-(4-methyl-2-(4-(pentan-2-ylamino)phenyl)thiazol-5-yl)ethanone (4i)

Yellow oil (75 mg, 42.6 %); ^{1}H NMR (DMSO-*d6*) δ: 7.60 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 3.50 (br, 1H), 2.50 (s, 3H), 2.28 (s, 3H), 1.51 (d, *J* = 12.0 Hz, 2H), 1.41-1.37 (m, 2H), 1.12 (d, *J* = 8.0 Hz, 3H), 0.91 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 198.94, 159.38, 151.21, 150.44, 147.74, 143.69, 132.57, 127.66, 112.06, 57.80, 39.70, 29.09, 26.80, 20.08, 14.30; MS (*m/z*); 302 (M+, 100.00%).

3.1.1.10. 1-(4-methyl-2-(4-((2-methylbutyl)amino)phenyl)thiazol-5-yl)ethanone (4j)

Brown oil (78 mg, 44.3 %); ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 8.0 Hz, 2H), 3.45 (br, 1H), 3.01-3.00 (d, *J* = 4.0 Hz, 2H), 2.45 (s, 3H), 2.38 (s, 3H), 1.20-1.15 (m, 2H), 1.13-1.11 (d, *J* = 8.0 Hz, 3H), 0.93-0.88 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 198.94, 159.38, 151.21, 150.44, 147.74, 143.69, 132.57, 127.66, 57.80, 35.30, 28.24, 26.80, 18.10 , 16.86 , 11.30; MS (*m/z*); 302 (M+, 100.00%).

3.1.1.11. 1-(2-(4-(isopentylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4k)

Dark-brown oil (88 mg, 50%);^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.61 (d, *J* = 8.0 Hz, 2H), 3.48 (br, 1H), 3.09-3.04 (m, 2H), 2.50 (s, 3H), 2.38 (s, 3H), 2.28-1.65 (m, 1H), 1.49-1.43 (m, 2H), 0.93 (d, *J* = 8.0 Hz, 6H); ^{13}C NMR (DMSO-*d6*) δ: 195.35, 159.83, 151.05, 147.76, 143.67, 132.60, 127.59, 121.20, 112.08, 41.17, 38.01, 25.81, 22.93, 19.69, 18.67, 16.65; MS (*m/z*); 302 (M+, 100.00%).

3.1.1.12. 1-(2-(4-(heptan-2-ylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4l)

Dark-brown oil (77 mg, 40 %); ^{1}H NMR (DMSO-*d6*) δ: 7.64 (d, *J* = 12.0 Hz, 2H), 6.57 (d, *J* = 4.0 Hz, 2H), 3.45 (br, 1H), 2.45 (s, 3H), 2.31 (s, 3H), 1.90-1.64 (m, 1H), 1.62-1.56 (m, 2H), 1.53-1.47 (m, 2H), 1.46-145 (m, 2H); ^{13}C NMR (DMSO-*d6*) δ: 194.25,

159.80, 150.44, 147.69, 143.68, 132.57, 127.64, 120.88, 112.32, 47.63, 36.63, 31.82, 25.78, 22.59 , 20.82 , 18.66, 16.60, 14.40; MS (*m/z*); 330 (M+, 100.00%).

3.1.1.13. 1-(2-(4-((2-ethylhexyl)amino)phenyl)-4-methylthiazol-5-yl)ethanone (4m)

Yellow oil (75 mg, 37.4 %); ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 8.0 Hz, 2H), 3.45 (br, 1H), 2.97-2.94(t, *J* = 8.0 Hz, 2H), 2.45 (s, 3H), 2.28 (s, 3H), 1.57-1.39 (m, 1H), 1.39-1.34 (m, 2H), 1.32-1.30 (m, 2H), 1.30-1.26 (m, 2H), 0.90-0.88 (t, , *J* = 4.0 Hz, 3H), 0.87-0.85 (t, *J* = 4.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 200.39, 159.79, 151.25, 147.71, 143.71, 132.60, 127.57, 121.06, 112.01, 46.31, 31.00, 28.83, 24.21, 23.06 , 18.85, 16.60, 14.45, 11.14; MS (*m/z*); 344 (M+, 100.00%).

3.1.1.14. 1-(2-(4-(cyclobutylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4n)

Light-yellow oil (116 mg, 69.8%); ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 12.0 Hz, 2H), 6.55 (d, *J* = 8.0 Hz, 2H), 3.91-3.85 (m, 1H), 3.45 (br, 1H), 2.50 (s, 3H), 2. 38 (s, 3H), 1.89-1.81 (m, 4H), 1.75-1.62 (m, 2H); ^{13}C NMR (DMSO-*d6*) δ: 192.25, 159.88, 149.45, 147.73, 143.63, 132.77, 127.61, 121.51, 112.42, 48.06, 30.67, 18.64, 16.61, 15.34; MS (*m/z*); 286 (M+, 100.00%).

3.1.1.15. 1-(2-(4-(cyclopentylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4o)

Light-yellow oil (118 mg, 67.5 %); ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 4.0 Hz, 2H), 6.61 (d, *J* = 4.0 Hz, 2H), 3.76-3.72 (m, 1H), 3.45 (br, 1H), 2.30 (s, 3H), 2. 28 (s, 3H), 1.98-1.90 (m, 4H), 1.73-1.68 (m, 4H), 1.66-1.41 (m,1H); ^{13}C NMR (DMSO-*d6*) δ: 198.88, 150.58, 147.68, 143.57, 132.64, 127.53, 121.07, 112.52, 53.85, 32.93, 24.14, 18.66, 16.61; MS (*m/z*); 300 (M+, 100.00%).

3.1.1.16. 1-(2-(4-(cyclohexylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4p)

Yellow oil (118 mg, 64.4 %); ^{1}H NMR (DMSO-*d6*) δ: 7.58 (d, *J* = 8.0 Hz, 2H), 6.61 (d, *J* = 8.0 Hz, 2H), 3.45 (br, 1H), 2.32 (s, 3H), 2.28 (s, 3H), 1.95-1.89 (m, 1H), 1.75-1.70 (m, 4H), 1.63-1.58 (m, 4H), 1.40-1.30 (m, 4H), 1.30-1.12 (m, 2H); ^{13}C NMR (DMSO-*d6*) δ: 198.28, 159.72, 150.06, 147.86, 143.86, 132.42, 127.65, 120.93, 112.38, 50.78, 32.95, 25.97, 24.96, 18.66, 16.68; MS (*m/z*); 314 (M+, 100.00%).

3.1.1.17. 1-(2-(4-(cycloheptylamino)phenyl)-4-methylthiazol-5-yl)ethanone (4q)

Orange oil (76 mg, 39.7 %); ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.57 (d, *J* = 8.0 Hz, 2H), 3.45 (br, 1H), 2.50 (s, 3H), 2. 38 (s, 3H), 1.90-1.78 (m, 1H), 1.66-1.58 (m, 4H), 1.55-1.48 (m, 4H), 1.47-1.45 (m, 4H); ^{13}C NMR (DMSO-*d6*) δ: 196.83, 159.72, 147.96, 143.93, 132.36, 127.65, 127.62, 120.89, 112.55, 52.77, 34.32, 28.48, 24.27, 23.31, 18.65, 16.71; MS (*m/z*); 328 (M+, 100.00%).

3.1.2. 2-(1-(2-(4-(Substituted *primary* amine-1-yl)phenyl)-4-methylthiazol-5-yl)ethylidene) hydrazinecarboxamide (5a-q).

General procedure: Acetylphenylthiazole derivatives **4a-p** (0.31 mmol) were dissolved in absolute ethanol (15 mL), concentrated hydrochloric acid (1 mL), semicarbazide hydrochloride (175 mg, 1.5 mmol, 5 equiv.), were added. The reaction mixture was heated at reflux for 2 hrs. The solvent was concentrated under reduced pressure, then poured in crushed ice and neutralized with sodium carbonate to pH 7-8, and the formed precipitated was collected by filtration, washed with copious amount of water. Crystallization from absolute ethanol afforded the desired products as solids.

3.1.2.1.2-(1-(2-(4-(butylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbo

xamide (5a). Orange solid (93 mg, 85.6%); mp = 165-167 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 5.73 (brs, 2H), 5.64 (brs, 2H), 3.45 (br, 1H), 3.08-3.03 (m, 2H), 2.45 (s, 3H), 2.28 (s, 3H), 1.58-1.51 (m, 2H), 1.43-1.34 (m, 2H), 0.94-0.90 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.27, 159.72, 151.08, 147.85, 143.83, 132.51, 127.59, 121.16, 112.07, 42.66, 31.19, 20.27, 18.66, 16.67, 14.26; HRMS (EI) *m/z* 344.1783 M^+, calc. for $C_{17}H_{24}N_6S$ 344.4777 M^+; Anal. Calc. for: $C_{17}H_{24}N_6S$ (344): C, 59.27; H, 7.02; N, 24.40; S, 9.31%; HPLC RT: 23.133; Area 99.33%.

3.1.2.2.2-(1-(2-(4-(pentylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5b). Yellow solid (60 mg, 73.4%); mp =168-169 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 5.78 (brs, 2H), 5.68 (brs, 2H), 3.48 (br, 1H), 3.07-3.02 (m, 2H), 2.50 (s, 3H), 2.31 (s, 3H), 1.74-1.52 (m, 2H) , 1.39-1.34 (m, 2H), 1.34-1.30 (m, 2H), 0.91 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (DMSO-*d6*) δ: 164.18, 159.80, 151.06, 147.82, 143.72, 132.53, 127.59, 121.16, 112.06, 42.95, 29.32, 28.75, 24.43, 22.44 , 18.66 , 16.67 , 14.43; HRMS (EI) *m/z* 358.1940 M^+, calc. for $C_{18}H_{26}N_6S$ 358.5042 M^+; Anal. Calc. for: $C_{18}H_{26}N_6S$ (358): C, 60.30; H, 7.31; N, 23.44; S, 8.94 %; HPLC RT: 23.806; Area 99.01%.

3.1.2.3.2-(1-(2-(4-(hexylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5c). Yellow solid (84 mg, 82 %); mp =170-172 °C. Yellow oil (87 mg, 40.1%); ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 5.70 (brs, 2H), 5.57 (brs, 2H), 3.45 (br, 1H), 3.07-3.02 (m, 2H), 2.31 (s, 3H), 2.28 (s, 3H), 1.57-1.51 (m, 2H) , 1.39-1.33 (m, 2H), 1.31-1.27 (m, 2H), 0.90 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.20, 159.79, 151.06, 147.73, 143.71, 132.61, 127.58, 121.18, 112.06, 42.99, 31.59, 29.04, 26.80, 22.60 , 18.66 , 16.62 , 14.41; HRMS (EI) *m/z* 372.2096 M^+, calc. for $C_{19}H_{28}N_6S$ 372.2130 M^+; Anal. Calc. for: $C_{19}H_{28}N_6S$ (372): C, 61.26; H, 7.58; N, 22.65; S, 8.61 %; HPLC RT: 24.714; Area 98.97%.

3.1.2.4.2-(1-(2-(4-(heptylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5d). Orange solid (72 mg, 69.2%); mp = 173-174 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 5.71 (brs, 2H), 5.57 (brs, 2H), 3.45 (br, 1H), 3.07-3.02 (m, 2H), 2.50 (s, 3H), 2.28 (s, 3H), 1.59-1.52 (m, 2H) , 1.34-1.28 (m, 2H), 0.89 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.22, 159.82, 151.05, 147.73, 143.70, 132.61, 127.58, 121.16, 112.06, 42.98, 31.77, 29.07, 29.02, 27.09 , 22.54 , 18.65 , 16.62 , 14.43; HRMS (EI) *m/z* 386.2253 M^{+}, calc. for $C_{20}H_{30}N_6S$ 386.5574 M^{+}; Anal. Calc. for: $C_{20}H_{30}N_6S$ (386): C, 62.14; H, 7.82; N, 21.74; S, 8.30 %; HPLC RT: 25.798; Area 98.23%.

3.1.2.5.2-(1-(2-(4-(octylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5e). Canary-yellow solid (75 mg, 89.6%); mp =175-177 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 5.96 (brs, 4H), 3.50 (br, 1H), 3.07-3.02 (m, 2H), 2.34 (s, 3H), 2.29 (s, 3H), 1.89 (s,), 1.57-1.53 (m, 2H), 1.38-1.31 (m, 2H), 1.29-1.26 (m, 2H) , 0.88-0.85 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.22, 159.20, 151.16, 148.60, 144.54, 131.78, 127.65, 121.02, 112.06, 42.97, 31.74, 29.32, 29.19, 29.06, 27.13 , 22.57, 18.67, 16.96, 14.42; HRMS (EI) *m/z* 400.2409 M^{+}, calc. for $C_{21}H_{32}N_6S$ 400.5840 M^{+}; Anal. Calc. for: $C_{21}H_{32}N_6S$ (400): C, 62.96; H, 8.05; N, 20.98; S, 8.00%; HPLC RT: 26.413; Area 98.97%.

3.1.2.6.2-(1-(2-(4-(nonylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5f). Brown solid (85 mg, 75%); mp =178-180 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 12.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 6.15 (s,), 5.71 (brs, 2H), 5.56 (brs, 2H), 3.50 (br, 1H), 3.05 (d, *J* = 12.0 Hz,), 2.45 (s, 3H), 2.28 (s, 3H), 1.55-1.53 (m, 2H), 1.26 (m, 2H), 0.86 (t, J = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.18, 159.82, 151.05, 147.69, 143.54, 131.67, 132.64, 127.58, 121.17, 112.06, 42.97, 31.76, 29.48, 29.35, 29.14, 29.05, 27.10 , 22.56, 18.65, 16.60, 14.42; HRMS (EI) *m/z* 414.2566 M^{+}, calc. for $C_{22}H_{34}N_6S$ 414.6106 M^{+}; Anal. Calc. for: $C_{22}H_{34}N_6S$ (414): C, 63.73; H, 8.27; N, 20.27; S, 7.73 %; HPLC RT: 27.080; Area 98.13%.

3.1.2.7.2-(1-(2-(4-(sec-butylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5g). Yellow solid (85 mg, 79.9 %); mp = 160-161 °C. ^{1}H NMR (DMSO-*d6*) δ: 11.69 ( ), 9.45 (brs, 2H), 9.10 (brs, 2H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.37 (s,), 7.09 (d, *J* = 8.4 Hz, 2H), 4.35 (br, 1H), 3.53 (t, *J* = 8.0 Hz, 3H), 2.65 (s, 3H), 2.45 (s, 3H), 1.67-1.63 (m, 2H), 1.48 (m, 1H), 1.18 (d, *J* = 8.0 Hz, 3H), 0.92 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.21, 159.38, 151.21, 150.44, 147.74, 143.69, 132.57, 127.66, 112.06, 57.80, 29.09, 20.08, 11.30; HRMS (EI) *m/z* 344.1783 M^{+}, calc. for $C_{17}H_{24}N_6S$ 344.4777 M^{+}; Anal. Calc. for: $C_{17}H_{24}N_6S$ (370): C, 59.27; H, 7.02; N, 24.40; S, 9.31 %; HPLC RT: 23.909; Area 99.74%.

3.1.2.8.2-(1-(2-(4-(isobutylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5h). Canary-yellow solid (17 mg, 43.1%); mp =158-159 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 5.72 (brs, 2H), 5.63 (brs, 2H), 3.45 (br, 1H), 2.90-2.86 (t, *J* = 8.0 Hz, 2H), 2.45 (s, 3H), 2.28 (s, 3H), 1.89-1.82 (m, 1H), 0.95-0.93 (d, *J* = 8.0 Hz, 6H); ^{13}C NMR (DMSO-*d6*) δ: 164.32, 159.70, 151.38, 147.84, 143.86, 132.48, 127.59, 121.06, 112.06, 50.84, 27.85, 20.85, 18.65, 16.64; HRMS (EI) *m/z* 344.1783 M^+, calc. for $C_{17}H_{14}N_6S$ 344.4777 M^+; Anal. Calc. for: $C_{17}H_{14}N_6S$ (344): C, 61.59; H, 7.07; N, 22.68%; Found: C, 59.27; H, 7.02; N, 24.40; S, 9.31%; HPLC: RT: 22.875; Area 98.61%.

3.1.2.9.2-(1-(2-(4-(pentan-2-ylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine

carboxamide (5i). Yellow solid (70 mg, 78.7%); mp =163-165 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.60 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 8.0 Hz, 2H), 5.71 (brs, 2H), 5.58 (brs, 2H), 3.50 (br, 1H), 2.50 (s, 3H), 2.28 (s, 3H), 1.51 (d, *J* = 12.0 Hz, 2H), 1.41-1.37 (m, 2H), 1.12 (d, *J* = 8.0 Hz, 3H), 0.91 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.22, 159.38, 151.21, 150.44, 147.74, 143.69, 132.57, 127.66, 112.06, 57.80, 39.70, 29.09, 26.80, 20.08, 14.30; HRMS (EI) *m/z* 358.1940 M^+, calc. for $C_{18}H_{26}N_6S$ 358.5042 M^+; Anal. Calc. for: $C_{18}H_{26}N_6S$ (358): C, 60.30; H, 7.31; N, 23.44; S, 8.94 %; HPLC RT: 23.857; Area 99.37%.

3.1.2.10. 2-(1-(2-(4-(2-methylbutylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine carboxamide (5j). Orange solid (75 mg, 81.1%); mp =162-164 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 8.0 Hz, 2H), 5.77 (brs, 2H), 5.63 (brs, 2H), 3.45 (br, 1H), 3.01-3.00 (d, *J* = 4.0 Hz, 2H), 2.45 (s, 3H), 2.38 (s, 3H), 1.20-1.15 (m, 2H), 1.13-1.11 (d, *J* = 8.0 Hz, 3H), 0.93-0.88 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.24, 159.83, 151.21, 150.44, 147.74, 143.69, 132.57, 127.66, 127.58, 57.80, 35.30, 28.24, 26.80, 18.10 , 16.86 , 11.30; HRMS (EI) *m/z* 358.1940 M^+, calc. for $C_{18}H_{26}N_6S$ 358.5042 M^+; Anal. Calc. for: $C_{18}H_{26}N_6S$ (358): C, 60.30; H, 7.31; N, 23.44; S, 8.94 %; HPLC RT: 23.857; Area 99.37%.

3.1.2.11. 2-(1-(2-(4-(isopentylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine carboxamide (5k). Yellow solid (85 mg, 81.5%); mp =163-164 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 8.0 Hz, 2H), 6.61 (d, *J* = 8.0 Hz, 2H), 5.73 (brs, 2H), 5.59 (brs, 2H), 3.48 (br, 1H), 3.09-3.04 (m, 2H), 2.50 (s, 3H), 2.38 (s, 3H), 2.28-1.65 (m, 1H), 1.49-1.43 (m, 2H), 0.93 (d, *J* = 8.0 Hz, 6H); ^{13}C NMR (DMSO-*d6*) δ: 164.18, 159.83, 151.05, 147.76, 143.67, 132.60, 127.59, 121.20, 112.08, 41.17, 38.01, 25.81, 22.93, 19.69, 18.67 , 16.65; HRMS (EI) *m/z* 358.1940 M^+, calc. for $C_{18}H_{26}N_6S$ 358.5042 M^+; Anal. Calc. for: $C_{18}H_{26}N_6S$ (358): C, 60.30; H, 7.31; N, 23.44; S, 8.94 %; HPLC RT: 23.909; Area 99.74%.

3.1.2.12. 2-(1-(2-(4-(heptan-2-ylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine carboxamide (5l).

Brown solid (85 mg, 93.4%); mp =171-172 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.64 (d, *J* = 12.0 Hz, 2H), 6.57 (d, *J* = 4.0 Hz, 2H), 5.73 (brs, 2H), 5.62 (brs, 2H), 3.45 (br, 1H), 2.45 (s, 3H), 2.31 (s, 3H), 1.90-1.64 (m, 1H), 1.62-1.56 (m, 2H), 1.53-1.47 (m, 2H), 1.46-145 (m, 2H); ^{13}C NMR (DMSO-*d6*) δ: 164.21, 159.80, 150.44, 147.69, 143.68, 132.57, 127.64, 120.88, 112.32, 47.63, 36.63, 31.82, 25.78, 22.59 , 20.82 , 18.66, 16.60, 14.40; HRMS (EI) *m/z* 386.2253 M^+, calc. for $C_{20}H_{30}N_6S$ 386.5574 M^+; Anal. Calc. for: $C_{20}H_{30}N_6S$ (386): C, 62.14; H, 7.82; N, 21.74; S, 8.30%; HPLC RT: 25.191; Area 99.21%.

3.1.2.13. 2-(1-(2-(4-(2-ethylhexylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine carboxamide (5m).

Yellow-white solid (63 mg, 72.2%); mp =172-174 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 8.0 Hz, 2H), 6.62 (d, *J* = 8.0 Hz, 2H), 5.73 (brs, 2H), 5.62 (brs, 2H), 3.45 (br, 1H), 2.97-2.94(t, *J* = 8.0 Hz, 2H), 2.45 (s, 3H), 2.28 (s, 3H), 1.57-1.39 (m, 1H), 1.39-1.34 (m, 2H), 1.32-1.30 (m, 2H), 1.30-1.26 (m, 2H), 0.90-0.88 (t, , *J* = 4.0 Hz, 3H), 0.87-0.85 (t, *J* = 4.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ: 164.22, 159.79, 151.25, 147.71, 143.71, 132.60, 127.57, 121.06, 112.01, 46.31, 31.00, 28.83, 24.21, 23.06 , 18.85, 16.60, 14.45, 11.14; HRMS (EI) *m/z* 400.2409 M^+, calc. for $C_{21}H_{32}N_6S$ 400.58400 M^+; Anal. Calc. for: $C_{21}H_{32}N_6S$ (400): C, 62.96; H, 8.05; N, 20.98; S, 8.00%; HPLC RT: 26.055; Area 99.39%.

3.1.2.14. 2-(1-(2-(4-(cyclobutylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine carboxamide (5n).

Yellow-white solid (120 mg, 86%); mp =156-157 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.62 (d, *J* = 12.0 Hz, 2H), 6.55 (d, *J* = 8.0 Hz, 2H), 5.73 (brs, 2H), 5.58 (brs, 2H), 3.91-3.85 (m, 1H), 3.45 (br, 1H), 2.50 (s, 3H), 2. 38 (s, 3H), 1.89-1.81 (m, 4H), 1.75-1.62 (m, 2H); ^{13}C NMR (DMSO-*d6*) δ: 164.18, 159.88, 149.75, 147.73, 143.63, 132.77, 127.61, 121.51, 112.42, 48.06, 30.67, 18.64, 16.61, 15.34; HRMS (EI) *m/z* 342.1627 M^+, calc. for $C_{17}H_{22}N_6S$ 342.4618 M^+; Anal. Calc. for: $C_{17}H_{22}N_6S$ (342): C, 59.62; H, 6.48; N, 24.54; S, 9.36 %; HPLC RT: 22.256; Area 99.21%.

3.1.2.15. 2-(1-(2-(4-(cyclopentylamino)phenyl)-4-methylthiazol-5-

yl)ethylidene)hydrazine carboxamide (5o). Brown solid (108 mg, 77.1%); mp = 158-159 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.61 (d, *J* = 4.0 Hz, 2H), 6.61 (d, *J* = 4.0 Hz, 2H), 5.77 (brs, 2H), 5.62 (brs, 2H), 3.76-3.72 (m, 1H), 3.45 (br, 1H), 2.30 (s, 3H), 2. 28 (s, 3H), 1.98-1.90 (m, 4H), 1.73-1.68 (m, 4H), 1.66-1.41 (m, 4H); ^{13}C NMR (DMSO-*d6*) δ: 164.24, 150.58, 147.68, 143.57, 132.64, 127.53, 121.07, 112.52, 53.85, 32.93, 24.14, 18.66, 16.61; HRMS (EI) *m/z* 356.1783 M^+, calc. for $C_{18}H_{24}N_6S$ 356.4884 M^+; Anal. Calc. for: $C_{18}H_{24}N_6S$ (356): C, 60.65; H, 6.79; N, 23.57; S, 8.99%; HPLC RT: 23.199; Area 99.33%.

3.1.2.16. 2-(1-(2-(4-(cyclohexylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine carboxamide (5p).

Yellow solid (128 mg, 92.1%); mp = 160-161 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.58 (d, *J* = 8.0 Hz, 2H), 6.61 (d, *J* = 8.0 Hz, 2H), 5.71 (brs, 4H), 3.45 (br, 1H), 2.32 (s, 3H), 2.28 (s, 3H), 1.95-1.89 (m, 1H), 1.75-1.70 (m, 4H), 1.63-1.58 (m, 4H), 1.40-1.30 (m, 4H), 1.30-1.12 (m, 2H); ^{13}C NMR (DMSO-*d6*) δ: 164.23, 159.72, 150.06, 147.86, 143.86, 132.42, 127.65, 120.93, 112.38, 50.78, 32.95, 25.97, 24.96, 18.66, 16.68; HRMS (EI) *m/z* 370.1940 M^+, calc. for $C_{19}H_{26}N_6S$ 370.5149 M^+; Anal. Calc. for: $C_{19}H_{26}N_6S$ (370): C, 61.59; H, 7.07; N, 22.68; S, 8.99%; HPLC RT: 23.774; Area 98.16%.

3.1.2.17. 2-(1-(2-(4-(cycloheptylamino)phenyl)-4-methylthiazol-5-yl)ethylidene)hydrazine carboxamide (5q).

Orange solid (85 mg, 95.5%); mp =162-163 °C. ^{1}H NMR (DMSO-*d6*) δ: 7.64 (d, *J* = 12.0 Hz, 2H), 6.57 (d, *J* = 8.0 Hz, 2H), 5.73 (brs, 2H), 5.62 (brs, 2H), 3.45 (br, 1H), 2.50 (s, 3H), 2. 38 (s, 3H), 1.90-1.78 (m, 1H), 1.66-1.58 (m, 4H), 1.55-1.48 (m, 4H), 1.47-1.45 (m, 4H); ^{13}C NMR (DMSO-*d6*) δ: 164.26, 159.72, 149.91, 147.96, 143.93, 132.36, 127.65, 127.62, 120.89, 112.55, 52.77, 34.32, 28.48, 24.27, 23.31, 18.65, 16.71; HRMS (EI) *m/z* 384.2096 M^+, calc. for $C_{20}H_{28}N_6S$ 384.5415 M^+; Anal. Calc. for: $C_{20}H_{28}N_6S$ (384): C, 62.47; H, 7.34; N, 21.85; S, 8.34 %; HPLC RT: 24.953; Area 98.07%.

4. Experimental of biological section

4.1. Stocks preparation:

Vancomycin stock solution:

The contents of one vancomycin vial (1000 mg, Vancomycine, Mylan USA) were dissolved in 10 ml water for injection, a final concentration of 100 mg/mL solution was aliquoted in small volumes and stored at -20 °C.

A 5120 ug/ml stock was prepared then concentrations from 64 to 0.125 μg/ml were prepared according to the CLSI standards.

4.2. Inoculum preparation:

Streak *S. aureus* USA 300 (Diep, *et al.*, 2006) and *A. baumannii* AB5075 (Jacobs, *et al.*, 2014) on BHA and incubate ON at 37ºC. Make ON cultures in 3 ml media from each organism. Incubate ON at 37 ºC with shaking at 180 rpm. Adjust the OD_{600} of the cultures at 0.5 Mcfarland standard (0.08-0.1) (1×10^8 CFU/mL) for all cultures. Dilute the adjusted culture a further 1:20 (100 uL in 2000 uL) using MH broth. (5×10^6 CFU/mL)

4.3.Plate preparation

Add 75 uL double strength MH broth to each well. Add 75 uL of each tested compound dilution to the broth in the wells, using the same tip starting from the low to the high concentration. Add 15 uL of the adjusted cultures to each well using the same tip starting from the low to the high concentration (5×10^5 CFU/mL). Positive control: Media+ DMSO "the highest used conc"+ organism. Negative control "sterility check": Media only. All compounds were prepared from a stock concentration of 512 ug/mL, starting from a concentration of 64 ug/mL and up to 0.125 ug/mL. Compounds that showed ppt in the 512 ug/mL concentration were directly prepared from the original stock 5000 ug/mL, starting from a concentration of 64 ug/mL and up to 0.5 ug/mL. DMSO was used in the same concentrations used in the compounds. All MIC was done twice in duplicates. TTC was used to distinguish compounds with low solubility or color from those with positive bacterial growth. Antimicrobial activity was detected by adding 10 µL of 0.5% TTC (2,3,5-triphenyl tetrazolium chloride) aqueous solution. The viable bacterial cells reduced the yellow TTC to pink/red 1,3,5-triphenylformazan (TPF) (Radaelli, *et al.*, 2016). MIC was defined as the lowest concentration of the tested compounds that inhibited visible growth, as indicated visually or by the TCC staining (yellow= no growth, red= microbial growth).

4.4. Colony counts validation of inoculum suspensions

Laboratories are encouraged to perform colony counts on inoculum suspensions at least quarterly to ensure that the final inoculum concentration routinely obtained closely approximates 5×10^5 CFU/mL. Do this by removing a 0.01-mL aliquot from the growth control well or tube immediately after inoculation and diluting it in 10 mL of saline (1:1000 dilution). After mixing, remove a 0.1-mL aliquot and spread it over the surface of a suitable agar medium. After incubation, the presence of approximately 50 colonies indicates an inoculum density of 5×10^5 CFU/mL. The MIC was done in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI documents M100-S24, and M07-A9) (Wayne, 2014).

5. Conclusion

From the above-mentioned results in table (3) it was found that (for the on MRSA-US300 activity) the highest activity obtained with compounds **5m** with MIC value about **8 µg/mL** followed by compound **5b, 5c, 5d, 5k, 5l, 5n, 5o** and **5p** with MIC value about 16 µg/mL. The other compounds show very low activity. On the other hand, most synthesized compounds gave very weak activity against *Acinetobacter baumannii* **AB**5075 except compounds **5b, 5k, 5n and 5o** which has moderate activity with MIC 32 µg/mL followed by compound **5h, 5j** and **5q** with MIC value about 64 µg/mL. This means that the presence of terminal hydrogen bond acceptor group is essential for activity against resistant gram- positive bacteria.

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تخليق بعض مشتقات الآزول الجديدة كعوامل مضادة للبكتيريا

[١]سعيد صبري عطوة، [١]محمد هجرس،[١,٢]عبد الرحمن صلاح ميهوب، [١]محمد مصطفى السباعي.

[١]قسم الكيمياء العضوية الصيدلية- كلية الصيدلة (بنين)- جامعة الازهر- القاهرة- مصر

[٢] برنامج علوم النانو جامعة العلوم والتكنولوجيا – مدينة زويل للعلوم والتكنولوجيا – الجيزة - مصر

البريد الالكتروني للباحث الرئيسي : m.elsebaei@azhar.edu.eg

- تشكّل مقاومة المضادات الحيوية تهديداً عالمياً للصحة والتنمية، وهي من أكبر الأزمات التي يهتم بها كل العاملين والمهتمين بمجال الصحة العامة.
- أصبحت قدرتنا على علاج حالات العدوى الشائعة مهددة بسبب ظهور وانتشار مسببات الأمراض المقاومة للأدوية والتي اكتسبت طرق مقاومة جديدة، مما أدى إلى ظهور مقاومة لمضادات الميكروبات. ومما يثير القلق بشكل خاص الانتشار العالمي السريع للبكتيريا المقاومة للأدوية المتعددة أو الكاملة (المعروفة أيضا باسم "الجراثيم المستعصية")، والتي تسبب حالات عدوى لا يمكن علاجها بالأدوية المضادة للميكروبات المتاحة حاليا، مثل المضادات الحيوية .
- لذلك اصبح من الضرورة اكتشاف وتصنيع مركبات جديدة قادرة على حفظ التوازن بين حركية الدواء ومعالجة الأمراض المختلفة الناتجة عن المقاومة البكتيرية.
- إيجاد التوازن الأمثل بين المتطلبات الهيكلية للعمل الدوائي والخصائص الدوائية للنشاط البيولوجي يمثل تحديا كبيرا في تطوير الأدوية. يمكن أن يؤدي ترابط الأجزاء المحبة للدهون في البنية الأساسية للمركب إلى تعزيز النشاط البيولوجي ولكن يكون له تأثير ضار على الخصائص الشبيهة بالدواء. في هذا البحث، تم تقليل الجزء المحب للدهون من مركبات فينيل ثيازول التي تم تحديدها مسبقًا كعوامل مضادة للبكتيريا عن طريق إدخال أمين أولي في الجزء الجانبي المحب للدهون. وفي هذا الصدد فإن معظم المشتقات المحضرة عززت بشكل ملحوظ خصائص الذوبان في الماء للمركبات الجديدة مقارنة بالمركب الأصلي الأول (**٣**). ولذلك، تم تحديد المركب (**5m**) ليكون التناظرية الأكثر فعالية ضد المكورات العنقودية الذهبية سيئة السمعة المقاومة للميثيسيلين(MRSA) ، مع قيمة تركيز مثبط أدنى (MIC) منخفضة تصل إلى (8 ميكروغرام / مل).
- وهكذا، نجحت التعديلات التي أدخلت على الفينيل ثيازول المذكورة في تحسين الحركة الدوائية لهذه السلسلة الجديدة مع الحفاظ على النشاط البيولوجي للمركبات ضد. MRSA

الكلمات المفتاحية: مقاومة المضادات الحيوية , الفينيل ثيازول , مضاد المكورات العنقودية الذهبية المقاومة للميثيسيلين