SYNTHESIS OF VIRTUALLY SCREENED POTENT NOVEL PRIMAQUINE DERIVATIVES AND EVALUATE THE BIOLOGICAL ACTIVITY AGAINST ANTIBACTERIAL RESISTANCE MICROORGANISMS

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Article History: **Received**: 25.06.2022 **Revised**: 23.07.2022 **Accepted**: 21.08.2022

Abstract: The emergence of multidrug resistance among pathogens has become a global challenge for bacterial infection treatment. The methicillin-resistant *Staphylococcus aureus*, multi-drug resistant tuberculosis and antibacterial resistant *E. coli* is the leading cause of mortality among infectious diseases worldwide. Finding a novel chemical to combat antibiotic resistance microorganisms is critical right now in the world. In this study we synthesized five novel primaquine derivatives and evaluated their efficacy against methicillin-resistant *Staphylococcus aureus*, multi-drug resistant tuberculosis and antibacterial resistant *E. coli.* All the molecules show excellent antibacterial activity against antimicrobial resistance microorganisms compared to standard drugs.

Keywords: Primaquine, Synthesis, Antimicrobial resistance, MRSA, *E. coli*, MDR-TB

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DOI: 10.31838/ecb/2022.11.06.014

INTRODUCTION

Antibiotics' efficacy, which has revolutionized medicine and saved millions of lives, is being jeopardized by the fast rise of resistant bacteria around the world [1-3]. Bacterial illnesses have resurfaced many decades after the initial patients were treated with antibiotics. The overuse and misuse of antibiotics, as well as a lack of new drug research by the pharmaceutical sector due to limited economic incentives and difficult regulatory requirements, have all been blamed for the antibiotic resistance crisis [4-6]. The Centers for Disease Control and Prevention (CDC) has identified a number of bacteria as posing urgent, serious, and worrying dangers, many of which have already wreaked havoc on the US health-care system, patients, and their families [7-10]. Coordinated efforts to enact new regulations, restart research initiatives, and explore crisis-management strategies are critical.

Antimicrobial resistance (AMR) is a severe global danger to human, animal, and environmental health that is gaining traction. This is related to multidrug-resistant (MDR) bacteria's development, dissemination, and persistence [11-13]. MDR bacteria can be found in the animal, human, and environmental niches, and these pathogens are all related in this trio. Excessive use of antibiotics in animals (food, pets, aquatic), antibiotics sold over the counter, increased international travel, poor sanitation/hygiene, and release of nonmetabolized antibiotics or their residues into the environment through manure/feces are all possible causes of "the global resistome" or AMR [14-18]. These variables lead to the emergence of MDR bacterial illnesses in the community due to genetic selection pressure. The global use of antimicrobials in cattle has recently shown hotspots of antibiotic usage across continents, which will have economic and public health implications in the coming years. Antibiotics are commonly used in food animals such as cattle, fowl, and pigs, and it is predicted that by 2030, their use would have increased by 67 percent in the world's most populous countries. [19].

To overcome of these AMR problems, we have planned to discovery of novel drugs against antimicrobial resistance strains. In our previous study, we have already reported ten PQ-13, PQ-24, PQ-36, PQ-38, PQ-53 as potential molecules against *E. coli*, *M. tuberculosis*, *S. aureus*, Ciprofloxacin resistant *E. coli* (CPR *E. coli*), Methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *tuberculosis* (MDR TB) by *in silico* study. In this study we synthesized these novel primaquine derivatives and evaluate the antibacterial activity of major antimicrobial resistance strains.

METHODOLOGY

Instrumentation

The melting points were calculated in an open capillary tube and are unadjusted. On a PerkinElmer spectrometer, IR spectra were recorded in KBr. On a Gemini 300 MHz instrument, 1H NMR spectra were recorded in DMSOd6 as the solvent and TMS as the internal standard. A Shimadzu LC/MS spectrometer was used to record the mass spectra.

Synthesis of Schiff base intermediate molecules

Equimolar (0.01M) amounts of primaquine and various aldehydes were dissolved in methanol (15 mL), then acetic acid (0.5 mL) was added and the mixture was refluxed for two hours. After the reaction was completed (as measured by TLC), the reaction mixture was cooled and put into water, where the solid separated. To obtain equivalent Schiff bases

intermediates compounds, the solid was filtered, washed with water, and crystallised from ethanol.

General procedure of substituted 4-thiazolidinone molecules (1 a-g)

In dioxane, a solution of Schiff's base intermediates (0.01 mol) and thioglycolic acid (0.01 mol) was prepared (20 ml). After adding a pinch of anhydrous zinc chloride, the mixture was refluxed for 8 hours. The solid was separated, filtered, rinsed in sodium bicarbonate solution, and then recrystallized from ethanol.

General procedure of substituted 2-azetidinones molecules (2 a-c)

The Schiff's base intermediates (0.01 mol) and triethylamine (0.01 mol) was dissolved in dioxane (40 ml) and kept in an ice bath. To this, cold solution of Chloro acetyl chloride (0.01 mol) was added slowly at 0^0, stirred for 10-12 h and left-over night. Filtration was used to remove the precipitated triethylammonium chloride, and distillation was used to extract the dioxane. The residue was put into cold water, and the solid that resulted was dried and crystallised using ethanol.

Antibacterial activity of the MRSA, MD-TB, CPR *E. coli*

Bacteria Strains Preparation

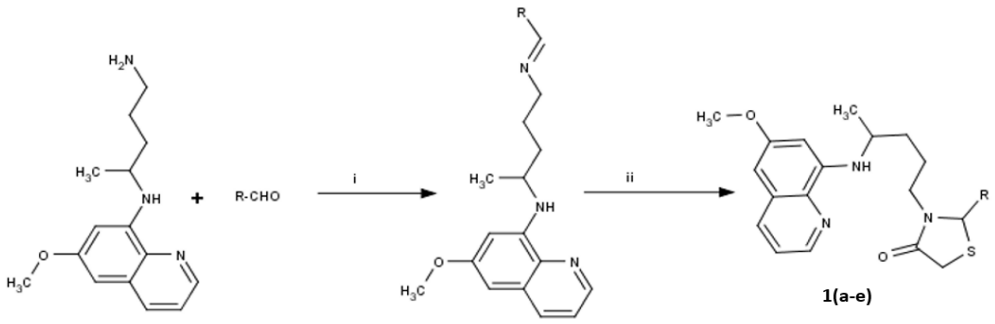
The wild-type *E. coli*, *Staphylococcus aureus* (SA), *Mycobacterium tuberculosis* (MTB) Ciprofloxacin resistant *E. coli* (CPR *E. coli*), Methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *tuberculosis* (MDR TB) were obtained from Department of Microbiology, Bharathidasan University, Trichy. All the bacteria strains were cultured in Mueller Hinton broth (MHB) (Merck, Germany) at 37°C for 24 h with 200 rpm agitation.

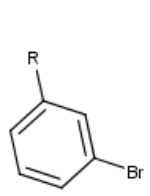
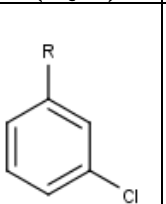
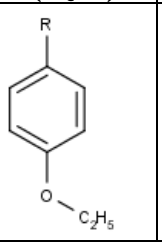
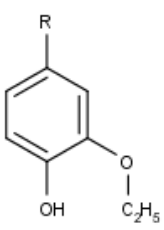
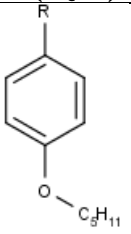
MIC determination

The antibacterial effectiveness of synthesized compounds was investigated using the standard broth dilution method (CLSI M07-A8), which measured visible microorganism growth in agar broth. Serial two-fold dilutions of synthesized compounds in different concentrations with adjusted bacterial concentration (108 CFU/ml, 0.5 McFarland's standard) were used to determine MIC. The control contained only inoculated broth and incubated for 24 h at 37 °C. in this analysis Primaquine were used as a standard drugs and compare the MIC of synthesized compounds. To confirm the MIC value, the optical turbidity of the tubes was measured before and after incubation.

RESULT AND DISCUSSION

In our previous work, we have screened and identified ten novel primaquine derivatives (PQ-13, PQ-24, PQ-36, PQ-38, PQ-53) from designed 100 novel primaquine derivatives with the aid of molecular docking and ADMET analysis. These five molecules shows good binding interaction with MRSA, MD-TB, CPR *E. coli* proteins as and good drug-likeness properties. Therefore, in this study we have synthesized by cyclo-condensation reaction of imines 1(a-e) (Scheme 1).



	1a (PQ-13)	1b (PQ-24)	1c (PQ-36)	1d (PQ-38)	1e (PQ-53)
R=					

Scheme 1.

Reaction Condition: (i) : Eq. mol of Primaquine and different aldehydes, EtOH, RT, 3hr, (ii) : Schiff's base and thioglycolic acid, 1,4 Dioxane, RT 8 hr.

Characterization study of synthesized compounds

PQ-13 : (2-(3-bromophenyl)-3-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-1,3-thiazolidin-4-one)

IR (KBr, cm^{-1}) υ_{max} : 3377 (-NH), 2922 (O-CH_3) 1606 (C=O), 613 (C-Br): ^{1}H NMR: δ 1.17 (3H,d, *J* = 6.6 Hz), 1.67 (4H, t), 3.60-3.79 (7H, m), 4.3 (1H, t, *J* = 7.0Hz), 5.9 (1H, s), 6.90-6.91 (7H,m), 7.91 (1H, dt, *J* = 8.6 Hz), 8.72 (1H, dd, *J* = 4.7 Hz). ^{13}C NMR: δ 18.2 (1C, s), 22.1 (1C, s), 33.11 (1C, s), 34.3 (1C, s), 47.46 (1C, s), 50.1 (1C, s), 56.20 (1C, s), 65.2 (1C, s), 101.4 (1C, s), 106.5 (1C, s), 118.8 (1C, s), 122.8 (1C, s), 128.35 (1C, s), 128.3 (1C, s), 131.2 (3C), 135.2 (1C, s), 136.1 (1C, s), 136.1 (1C, s), 139.5 (1C, s), 149.7 (1C, s), 155.7 (1C, s), 169.8 (1C, s). LC-MS analysis for $C_{24}H_{26}BrN_3O_2S$ calculated (EI, m/z (%): 500.46, found: 501.23 [M+1]. Melting point is 69-70ºC.

PQ-24 (2-(4-chlorophenyl)-3-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-1,3-thiazolidin-4-one)

IR (KBr, cm^{-1}) υ_{max} : 3415 (-NH), 2981 (O-CH_3) 1797 (C=O), 875 (C-Cl), ^{1}H NMR: δ ^{1}H NMR: δ 1.18 (3H,d, *J* = 6.4 Hz), 1.60 (4H, t), 3.66-3.78 (7H, m), 4.3 (1H), 6.01 (1H, s), 6.91-6.92 (7H,m), 8.15 (1H, dt, *J* = 8.6 Hz), 8.81 (1H,

dd, *J* = 4.7 Hz). ^{13}C NMR: δ 18.3 (1C, s), 22.7(1C, s), 33.1 (1C, s), 34.5 (1C, s), 47.3 (1C, s), 50.1 (1C, s), 56.2 (1C, s), 65.4 (1C, s), 101.9 (1C, s), 106.2 (1C, s), 122.8 (1C, s), 128.3 (2C, s), 131.2 (2C), 133.5 (1C, s), 136.1 (1C, s), 136.1 (1C, s), 139.4 (1C, s), 140.3 (1C, s), 149.2 (1C, s), 155.1 (1C, s), 169.1 (1C, s). LC-MS analysis for $C_{24}H_{26}ClN_3O_2S$ calculated (EI, m/z (%): 455. 14 Found: 456.87 [M+1]. Metling point is 72-73 ºC.

PQ-36 (2-(4-ethoxyphenyl)-3-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-1,3-thiazolidin-4-one)

IR (KBr, cm^{-1}) υ_{max} : 3415 (-NH), 2981 (O-CH_3), 2669 (-SH) 1797 (C=O),1181 (C-O-C_2HH_5), ^{1}H NMR: δ ^{1}H NMR: δ 1.16-1.18 (10H,m), 1.60 (4H, t), 3.66-3.69 (7H, m), 4.1-4.2 (3H, m), 6.06 (1H, s), 6.91-6.92 (7H,m), 8.15 (1H, dt, *J* = 8.6 Hz), 8.79 (1H, dd, *J* = 4.7 Hz). ^{13}C NMR: δ 14.2 (1C, s), 18.8 (1C, s), 22.5 (1C, s), 33.4 (1C, s), 34.0 (1C, s), 47.6 (1C, s), 50.1 (1C, s), 56.0 (1C, s), 64.3 (1C, s), 65.2 (1C, s), 101.4 (1C, s), 106.4 (1C, s), 114.3 (2C, s), 122.4 (1C, s), 127.8 (2C, s), 129.6 (1C, s), 135.7 (1C, s), 136.3 (1C, s), 139.5 (1C, s), 140.2 (1C, s), 149.8 (1C, s), 155.8 (1C, s), 158.5 (1C, s), 169.8 (1C, s). LC-MS analysis for $C_{26}H_{31}N_3O_3S$ calculated (EI, m/z (%): 465.20 Found : 466.61 [M+1]. Metling point is 70-71ºC.

PQ-38 (2-(3-ethoxy-4-hydroxyphenyl)-3-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-1,3-thiazolidin-4-one)

IR (KBr, cm^{-1}) υ_{max} : 3356 (-OH), 2918 (O-CH_3), 2851 (-SH) 1797 (C=O), 1171 (C-O-C_2HH_5): ^{1}H NMR: δ ^{1}H NMR: δ 1.17-1.18 (10H,m), 1.60 (4H, t), 3.66-3.69 (7H, m), 4.1-4.2 (3H, m), 6.06 (1H, s), 6.91-6.92 (7H,m), 8.15 (1H, dt, *J* = 8.6 Hz), 8.79 (1H, dd, *J* = 4.7 Hz), 8.72 (1H, dd, *J* = 4.7 Hz).^{13}C NMR: δ 14.2 (1C, s), 18.40 (1C, s), 22.7 (1C, s), 33.5 (1C, s), 34.6 (1C, s), 47.3 (1C, s), 50.7 (1C, s), 56.32 (1C, s), 64.47 (1C, s), 65.2 (1C, s), 101.9 (1C, s), 101.5 (1C, s), 110.5 (1C, s), 115.3 (1C, s), 122.8 (1C, s), 128.3 (1C, s), 128.39 (1C, s), 131.2 (2C, s), 136.2 (1C, s), 136.2 (1C, s), 137.15 (1C, s), 139.29 (1C, s) 146.2 (1C, s), 149.2 (1C, s), 155.2(1C, s), 169.11 (1C, s). LC-MS analysis for $C_{26}H_{31}N_3O_4S$ calculated (EI, m/z (%): 481.20 and Found: 482.61 [M+1]. Melting point is 87-88 ºC.

PQ-53 (3-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-2-[4-(pentyloxy)phenyl]-1,3-thiazolidin-4-one)

IR (KBr, cm^{-1}) υ_{max} : 3472 (-NH), 2075 (O-CH_3), 1641 (C=O): ^{1}H NMR: δ 0.3 (3H, t, *J* = 7.0 Hz), 1.12-1.6 (7H, 1.17 m), 3.62-3.78 (2H, m), 3.51 (1H, d, *J* = 16.0 Hz), 3.66-3.86 (7H, m), 4.20 (3H, tq, *J* = 7.0, 6.6 Hz), 5.99 (1H, s), 6.9-7.95 (7H, m), 7.92 (1H, dt, *J* = 8.1 Hz), 8.73 (1H, dd, *J* = 4.8 Hz). ^{13}C NMR: δ 14.0 (1C, s), 18.2 (1C, s), 22.3 (1C, s), 22.3 (1C, s), 28.9 (1C, s), 29.0 (1C, s), 33.11 (1C, s), 34.3 (1C, s), 47.5 (1C, s), 50.1 (1C, s), 56.1 (1C, s), 65.1 (1C, s), 69.3 (1C, s), 101.4 (1C, s), 106.3 (1C, s), 118.3 (2C, s), 122.3 (1C, s), 128.38 (2C, s), 128.4 (1C, s), 131.2-131.36 (3C, s), 135.2-136.1 (3C, s), 139.5 (1C, s), 146.2 (1C, s), 155.6 (1C, s), 169.4 (1C, s). LC-MS analysis for $C_{29}H_{37}N_3O_3S$ calculated (EI, m/z (%): 507.25 and Found: 508.69 [M+1]. Melting point is 81-82 ºC

Antibacterial activity of the MRSA, MD-TB, CPR *E. coli*

Antibacterial activity was investigated by finding the MICs of the virtually screened potent five primaquine derivatives against *E. coli*, *M. tuberculosis*, *S. aureus*, CPR *E. coli*, MDR-TB, MRSA strains and comparing with Primaquine, Ciprofloxacin, Methicillin, amikacin as standard drugs. The MIC values of the synthesized compounds and standard drugs are given in Table 1. The MIC values were within the range are 80-135 µg/ml for normal strains and 125-155 µg/ml for resistant strains. The synthesized all the primaquine derivatives shows excellent antibacterial activity against *E. coli*, *M. tuberculosis*, *S. aureus*, CPR *E. coli*, MDR-TB, MRSA strains compared to standard drugs. The antibacterial activity of all tested compounds demonstrated acceptable antibacterial effects. Particularly MIC of CPR *E. coli*, MDR-TB, MRSA strains could not predictable due to the drug resistant actions of these strains. Moreover, in the normal strains, the PQ-13, PQ-24, PQ-36, PQ-38, PQ-53 synthesized compounds show more active than standard drugs.

Table 1. Minimum inhibitory concentrations (MICs) (µg/ml) of synthesized compound on antibiotic resistant-bacterial strains.

Mol. No	*E. coli*	CPR-*E. coli*	MTB	MDR-TB	SA	MRSA
PQ-13	80	125	110	150	95	135
PQ-24	90	135	125	145	100	145
PQ-36	70	130	115	155	90	140
PQ-38	85	130	125	160	110	130
PQ-53	85	120	120	135	95	135
Primaquine	120	500	300	350	130	550

CONCLUSION

The present study revealed that the newly synthesized PQ-13, PQ-24, PQ-36, PQ-38, PQ-53 compounds having good anti-bacterial activity against *E. coli*, *M. tuberculosis*, *S. aureus*, CPR *E. coli*, MDR-TB, MRSA strains. In future these compounds will be a promising new drug molecules for antibacterial resistant microorganisms.

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