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Antibacterial Activity of Phosphangold(I) Thiolate and Enhancing Activity in Combination with Available Antibiotics Against *Staphylococcus aureus*

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Abstract: With the widespread increase of bacterial resistance to available antibiotics and the lack of resources for the discovery of new classes of antibiotics, the multidrug-resistant S. aureus has become a global concern. The methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported as a serious threat in health settings. There is now a strong demand for new antibacterial agents as only a few antibiotics can combat MRSA infections. This article aims to in vitro investigate the antibacterial properties of new (phosphanegold(I) thiolate) compounds against *S. aureus* strains. The disc diffusion method was applied as a preliminary screening method of the gold compounds against different Gram-positive and Gram-negative bacteria. In contrast, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-killing assay methods were carried out to investigate the antibacterial activity of selected compounds against different Methicillin-resistant *Staphylococcus aureus* (MRSA) species. Moreover, combinations of available antibiotics and selected (phosphanegold(I) thiolate) compound were investigated using the checkerboard method. Two out of five compounds belong to phosphangold(I) thiolate named 3F2 and 3F3 were showing antibacterial activity against Gram-positive bacteria, predominantly *Staphylococcus aureus* strains with MIC values within the range from 4 µg/mL- 8 µg/mL and 30 µg/mL – 60 µg/mL, respectively. Only 3F2 was exhibited good and promising antibacterial activity against different *S.aureus* strains including MRSA strains. Additionally, the 3F2 was exhibited synergistic activity and positive drug interaction when combine with ciprofloxacin at sub-inhibitory concentration. These findings suggested that the new investigated phosphanegold(I) thiolate, mainly 3F2 compound, may have great potential to act as an antibacterial agent with action against Gram-positive bacteria that mainly prevent or control MRSA infection's growth. Thus, further investigations are needed to fully understand the mechanism of action of tested (phosphanegold(I) thiolate against *S.aureus*.

Keywords: Antibacterial activity; Drug combinations; MRSA; Gold; phosphanegold(I) thiolate.

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Introduction

The emergence of antimicrobial resistance phenotypes in bacteria worldwide, in particular those that are resistant to antibiotics, and the appearance of new infectious diseases have made it vital. In fact, antibacterial resistance is responsible for the increased mortality observed in infectious diseases due to the lack of effective antibiotics for prevention and treatment (1–3). Recently, the World Health Organization (WHO) suggested prioritizing the fight against

ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species (4).

The methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the most difficult infections to treat due to its ability to develop resistance against available antibiotics, despite the introduction of new derivatives. Many of these antibiotics and their derivatives are linked to dose limitations, development of resistance, and expensive costs (5). Over the last two decades, there has been a noticeable increase in the number of cases of vancomycin-resistant *Staphylococcus aureus* (VRSA) around the world, and the resistance mechanisms of the aforementioned strain are quite different, thus, making it difficult to treat them with the same antibiotics (6,7). Vancomycin is still the drug of choice for the treatment of MRSA infections, despite the fact that resistance to this antibiotic has been increasing noticeably (8–10) . Therefore, serious steps must be taken to discover novel, safe and effective antimicrobial agents.

Metallodrugs have been used for a long time prior to the discovery of organic antibiotics. Since then, there has been a general decline in the use of metal-based drugs, particularly antibacterial compounds (1,11,12). However, the incorporation of metal ions into unique designed ligands aims to enhance their biological properties, antimicrobial activity, overcoming drug resistance and toxicity (1,13). Moreover, the use of metal-based antibacterial agents is one of the strategies adopted by many researchers to combat the challenges of bacterial resistance due to their diverse mode of action to organic antibiotics beside that the combination of metals with organic antibiotics may enable an extra mode of action compared to the single antibiotic (1,13,14). The attractive biological properties of gold-based compounds as an antibacterial agent have been reported in many studies (13,15,16). As the compounds belong to NHC-gold showed interesting antibacterial activity but these studies were lacking deep information on its mechanism of action despite multitargets that have been identified which suggested to have multi antibacterial mode of action of NHC-gold compounds(1). However, NHC-gold compounds have promising anticancer activity greater than antibacterial activity, this is may attributed to the multitarget anticancer agent of NCH-gold compounds (14). Moreover, Au(I) and Au(III) compounds have been used for their anticancer activity, but aslo they may be exhhibte promosing antibacterial agents (1). The interset in gold-phosphine compounds has been increased in particular gold(I)-phosphie compound due to their antibacterial activity. Howeever, less number of gold-phosphine compounds have been investigated for their anntibacterial effect compared to anticancer activity (17).

The first FDA approved gold (I) phosphine drug, auranofin was clinically used as a treatment for the rheumatic disease. Later on, auranofin were screened and evaluated for its potential antibacterial activity against multi-drug resistant bacteria which was more potent on Gram-positive bacteria than on Gram-negative (1,2,18). Later, many Au (I) and Au (III)- phosphine compounds were tested against a broad spectrum of bacterial pathogens. However, the majority of the studies were carried out on gold(I) compounds, perhaps because of their greater stability compared to gold(III) compounds (18).moreover, majority of

Au(I) phosphine tested in vitro were shown stronger antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria (19). Recently, efforts have been concentrated on development of antibacterial activity of Au(III)- phosphine type in particular through the stabilization of gold ion with different ligands (2). The interest in development of gold-based drugs as has been increased recently, trying to fill the gap of not enough new antibacterial drugs and to minimize the development of bacterial resistance toward available antibiotics. Herein this research article, we report on the extension of our cowork team efforts on development of new phosphanegold(I) thiolate compounds named 3F series by screening and evaluating its antibacterial activity against *Staphylococcus aureus* strains. However, the synthesis, structure and the chemical characteristics of the phosphanegold(I) thiolate compounds (3F series) are explained by our co-worker team (20–24).

Materials and Methods

Gold compound Compounds

A total of six new Phosphanegold (I)Thiolate compounds belong to 3F series, contain same ligand, which is thiolate group, were obtained from Distinguish Professor Dr. Tiekink Edward chemistry group from Faculty of Sciences and Technology, Sunway University (20–24).

Table (1): Chemical Formula, Molecular Weight and preferable solvent for the investigated Phosphanegold(I) Thiolate.

Compound Code	Chemical Formula	Molecular Weight(gmol-1)	Solvent
3FL (Ligand)	MeOC(=S)NHC6H4F-3	185.22	DMSO
3F2	Cy3PAu(SC(OMe)=NC6H4F-3)	661.61	DMSO
3F3	DPPFeAu2((SC(OMe)=NC6H4F-3))2	1316.73	DMSO
3F4	DPPEtAu2((SC(OMe)=NC6H4F-3))2	1160.77	DMSO
3F5	DPPButAu2((SC(OMe)=NC6H4F-3))2	1188.82	DMSO
3F6	DPPHexAu2((SC(OMe)=NC6H4F-3))2	1216.88	DMSO

Preparation of bacteria

In total of sixty-four bacterial samples used in this study, sixteen human important pathogens were purchased from American Type Culture Collection (ATCC). Nine Gram negative bacterial species and seven Gram positive bacterial species were used for preliminary screening. Beside that fourteen Methicillin-resistant *Staphylococcus aureus* as genetically different reference strains were gifted from Professor.Dr. Hiramasato from Juntendo University-Japan and one strain of Vancomycin intermediate *Staphylococcus aureus* (ViSA) was obtained from UMBI institute, University Kebangsaan Malaysia. All pathogens were revived from -80° C using agar plate media Tryptic soya Agar (TSA) (Merck), Nutrient Agar (NA) (Merck) or Brain Heart Infusion agar (BHI) (Merck) for 24 hours at optimum condition for each pathogen as working stocks. Then all the pathogens were cultured using corresponding media and grown overnight at 37°C before the day of the experiment.

Preliminary Screening

The disk diffusion method was carried out to screen the antibacterial activity of the gold compounds F series (3F2, 3F3, 3F4, 3F5, 3F6 and 3FL) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The pathogens were cultured using Muller-Hinton Broth (MHB) (Merck) at 37°C for overnight in shaker incubator 200 rpm. The overnight cultures were diluted, and inoculum suspension of each bacterial strain was adjusted to 0.5 McFarland standard turbidity (corresponding to approximately 108 CFU/ml) using fresh media. Then suspension of each bacterial strain was applied evenly onto Mueller-Hinton agar (MHA) (Merck) plates (30mm in thickness) using a sterile cotton swab. The 3F series gold compounds were dissolved in 100% Dimethylsulphoxide (DMSO) (Merck) to reach a test concentration of 2 mg/mL. Sterile 6-mm filter paper discs were sited on the surface of MHA plate, then 10 µl of each dissolved gold compound was directly added to the discs to give final concentration of 20 µg/disc for each gold compound. The final concentration of each gold compound used to be enough to show if there is any antibacterial activity. Every plate contained one standard antibiotic paper disc, serving as the positive control, one disc worked as negative control (5 µl MHB) and one disc worked as solvent control (5 µl DMSO). The plates then were incubated at 37 °C for 24 h. Antibacterial activity after incubation was assessed by measuring the size of inhibition zone against bacterial strain. The experiment was carried out in triplicate for statistical analysis

Determination of MIC and MBC

A broth micro-dilution method was performed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of gold compound 3F2 and 3F3 according to the CLSI guidelines. This experiment was performed using Methicillin-resistant *Staphylococcus aureus* MRSA strains. In brief, the tested compounds were serially diluted two-fold in DMSO to reach the final range of concentration 0.0125 µg/ml to 512 µg/ml and then placed into each well of a 96-well micro-plate. The inoculum suspension with density of 105 CFU/ml exponentially growing bacterial cells was added into each well. The 96-well microplates were incubated at 37 °C for 24 h. The experiment was performed in triplicate. Standard antibiotics (Ampicillin, Chloramphenicol and Vancomycin) were used as positive control, medium + DMSO as solvent control and broth inoculum bacterial cells as negative control. The growth of bacterial cells was detected by adding 50 µl of a 0.2 mg/ml p-iodonitrotetrazolium violet (INT) (Sigma-Alrdich) as indicator agent into each of the micro-plate wells and incubated in dark at 37 °C for 30 min under aerobic agitation. The MIC was determined at the lowest concentration that has no changes in color to pink. To determine the MBC values of each gold compounds after MIC determination, an aliquot of 100µl from each well which showed no visible growth will be spread onto MHA plate and further incubated at 37 °C for 24 hours. The MBC defined as the minimum concentration of the tested compound that produced a 99.9 % reduction in bacterial viable count on the MHA plate. The experiment was carried out in triplicate for statistical analysis.

Time kill Assays

To determine the kinetics of the gold compound 3F2 in vitro and the time required to kill the bacterial cells. Time killing assay was performed using macro-dilution method and drop plating in accordance to CLSI protocols. Briefly, an inoculum of MRSA strains represent different *SCCmec* , with 105 CFU/ml of log phase growing bacterial cells in MHB were exposed to the gold com-

pound 3F2 and Chloramphenicol as positive control, at final concentration of MIC and 1/2MIC for 3F2 and MIC value of Chloramphenicol. The culture tubes were incubated for 24 hours at 37°C inside shaker incubator 200 rpm. A growth culture control as vehicle cells only without exposing to treatment was used in each trial. A total of 100µl of culture was removed in time interval (0, 4, 8, 12, 16 and 24 hours) and serially ten-fold diluted in 0.85% normal saline, then 25µl of each dilution was plated on MHA plates. The plates were incubated in 37°C for 24 hours. The viable cells were determined by counting the colony forming unit. The experiment was repeated three times. To analyse the time killing curve data, the average of log10 colony forming unit (CFU/ml) were plotted against time (hours) and the changes in viable cells number was determined. In general, ≥ 3 log10 (CFU/ml) reduction relative to the initial inoculation is consider the antibacterial agent has bactericidal activity against the microbe, whereas < 3 log10 reduction relative to the initial inoculation is consider the antibacterial agent has bacteriostatic activity. The experiment was carried out in triplicate for statistical analysis

Synergistic Effects of Phosphanegold (I)Thiolate on MRSA

The antibacterial effects of 3F2 gold compound in combination with ciprofloxacin, chloramphenicol, Ampicillin, and vancomycin (Sigma-Aldrich, USA) were evaluated using checkerboard method as described by Schwalbe with some modification (64,65). The results of this method were calculated mathematically using the below equation and expressed in form of fractional inhibitory concentration (FIC) index equal to the total of the FIC of each antibacterial agent then the FICI(s) were used to plot the isobologram. Combinations were incubated for 20 hours at 37º C, MIC of 3F2 and reference antibiotics that inhibit the growth of MRSA were determined. And FIC indices were calculated from the FIC of 3F2 and FIC of the reference antibiotics. The FIC index range as follow: FICI < 0.5, synergy; 0.5 ≤ FICI <1 partial synergy; FICI =1 additive; FICI > 1,antagonism (64–67). The experiment was carried out three times for statistical analysis.

$$\sum FICI = \frac{MIC\ drug\ A\ in\ combniation}{MIC\ drug\ A\ alone} + \frac{MIC\ drug\ B\ in\ combniation}{MIC\ drug\ B\ alone}$$

Statistical analysis

Each experiment was carried out in triplicate for statistical analysis. All data are expressed as the mean ± standard deviation (SD).

Results

Preliminary Screening

Phosphanegold(I) thiolate compounds belong to 3F series were preliminary evaluated for their antibacterial activity against important Gram-positive and Gram-negative pathogens using the Kirby-Bauer disc diffusion method, which was performed on 20 µg/disc of each gold compound. Qualitative results were obtained after 24 hours of incubation. The antibacterial properties of the gold compounds were tested on fifteen Gram-positive and Gram-negative pathogens that pose a threat to humans and animals (Table 1). The screening and evaluation results showed that the Gram-positive bacteria had a greater sensitivity to the gold compounds compared to the Gram-negative bacteria. Interestingly, only MRSA *(*ATCC 43300) showed an obvious sensitivity to all the gold compounds, including the ligands alone. Both the 3F2 and 3F3 gold compounds were the most potent against MRSA,

with an inhibition zone size of 12 mm, while for the ligands, the inhibition zone size was 8 mm (Figure 3.2). Meanwhile, the other gold compounds were shown to have an inhibition zone size of 7 mm to 8 mm against MRSA. Moreover, MSSA was also sensitive to 3F2, 3F3 and 3FL, with inhibition zone sizes of 10 mm, 12 mm and 8 mm, respectively (Figure 3.3). However, the other Gram-positive bacteria, namely *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC10876) and *Enterococcus faecium* (ATCC19434), were highly resistant to all the compounds, including the ligands, except in the case of *Enterococcus faecium* (ATCC19434), which showed a mild inhibition zone of about 7 mm and 6 mm against the 3F3 and 3FL gold compounds, respectively. The zone of inhibition of *Staphylococcus saprophyticus* could not be determined. However, most of the Gram-negative pathogens were resistant to the gold compounds, with a few exceptions, perhaps due to the nature of their outer cell membrane structure (Table 1). It is worth noting that only the *S.aureus* strains were sensitive towards both the ligands and the compounds.

Table (2): Preliminary screening of gold compound compounds against important pathogens. The concentration of each gold compound is 20µg/disc. The diameter of inhibition zones in millimeters (mm) and the standard deviation (SD) were measured around the disc after 24 h incubation. The rustles represent the average of three replicates.

Bacterial Pathogens Bacteria name (ATCC.No)	Gold compound					
	3F2	3F3	3F4	3F5	3F6	3FL
Gram positive pathogen						
Bacillus subtilis (6633)	-	-	-	-	-	-
Bacillus cereus (10876)	-	-	-	-	-	-
Enterococcus faecium (19434)	-	7 (±0.8)	-	-	-	6 (±1.5)
Staphylococcus aureus (25923)	10 (±0.5)	12 (±0.5)	-	-	-	8 (±0.3)
MRSA (43300)	12 (±0.0)	12 (±0.0)	8 (±0.5)	7 (±0.5)	8 (±0.7)	8 (±1.5)
Staphylococcus saprophyticus	ND	ND	ND	ND	ND	ND
Gram negative pathogens						
Acinetobacter baumannii (19606)	-	-	-	-	-	-
Escherichia Coli (25922)	-	-	-	-	-	-
Enterobacter aerogenes (14028)	-	7 (±1.0)	-	-	-	5 (±0.5)
Salmonella typhimurium (14028)	6 (±0.5)	-	-	-	-	-
Shigella sonnei (9290)	-	-	8 (±0.6)	-	-	-
Klebsiella pneumonia (700603)	-	-	-	-	-	-
Stenotrophomonas maltophilia (13637)	-	-	-	-	-	-
Pseudomonas aeruginosa (27853)	-	-	-	-	-	-
Proteus vulgaris (13315)	-	9 (±0.5)	-	-	-	-

Anti-Staphylococcus aureus activity of Phosphanegold(I) thiolate compound

Based on the preliminary screening of the gold compound compounds, 3F3 and 3F2 were shown to possess a high level of antibacterial activity against the *Staphylococcus aureus* species. Thus, further investigations were carried out with regard to the anti-*Staphylococcus aureus* activity of the 3F3 and 3F2 gold compounds. Seventeen reference strains (Table 2) and twenty-eight clinical strains belonging to *Staphylococcus aureus* (S1) were used to test and confirm the antibacterial activity of 3F3 and 3F2 compounds. Fifteen reference strains were Methicillin-resistance *Staphylococcus aureus* (MRSA) carrying different *SCCmec*, one strain was Methicillin sensitive *Staphylococcus aureus* (MSSA), and one reference strain was

Vancomycin intermediate resistance (ViSA). At the same time, the clinical strains were all identified as Methicillin resistant *Staphylococcus aureus* (MRSA).

The 3F3 and 3F2 gold compounds showed an average zone of inhibition ranging between 8 mm to 12 mm with a standard deviation (SD) ranging between 0.5 – 1 towards all the *S.aureus* reference strains, which indicated good and promising antibacterial activity, despite the smaller zone of inhibition compared with the other reference antibiotics used as a positive control in this study. However, the *S.aureus* has developed a resistance against most of the antibiotics that are currently in use, including vancomycin. All the *S.aureus* reference strains showed resistance against ampicillin and chloramphenicol, with the zone of inhibition being ≤ 22 mm and ≤ 18 mm, respectively, except for the Mu50 strain, which showed a zone of inhibition of 29 mm against ampicillin and 17 mm against chloramphenicol, closely matching the most reecent CLSI breakpoint report. The ViSA strain revealed a moderate sensitivity towards 3F2 and 3F3, with the zone of inhibition being 9.5 mm and 8 mm, respectively. Meanwhile, vancomycin was more potent towards ViSA, with a zone of inhibition 16 mm. It should be mentioned that the gold compounds used in this experiment had a concentration of 20µg/disc compared to commercial vancomycin, which is 30µg/disc, and this may have impacted the results (Table 2).

Table (3): Antibacterial activity measured by the zone of inhibition of gold compounds 3F3, 3F2, and standard antibiotics. The diameter of inhibition zones in millimeters mm and the standard deviation (SD) were measured around the disc after 24 h incubation. The rustles represent the average of three replicates. MRSA strains are different in terms of *SCCmec*.

MRSA strains	3F2 20ug/disc	3F3 20ug/disc	C 30ug/disc	AMP 10ug/disc	VAN 30ug/disc
JCSC9884	10.0(±0.5)	9.5 (± 0.9)	16.0 (±1.5)	-	15.0 (±0.0)
JCSC9885	8.5 (± 1.0)	7.5(± 0.5)	15.0 (±1.0)	12.0 (±1.0)	17.0 (±1.0)
JCSC9889	10.0 (± 0.5)	9.0 (± 0.5)	-	9.0 (±1.0)	16.0 (±0.5)
JCSC9890	9.0 (± 0.8)	9.0 (± 0.9)	13.0 (±1.0)	10.0 (±0.0)	15.0 (±0.0)
JCSC9892	9.0 (± 0.9)	9.5 (± 0.5)	14.0 (±1.0)	10.0 (±0.0)	16.0 (±.00)
JCSC9893	10.0(± 1)	8.5(± 0.6)	15.0 (±0.5)	10.0 (±1.0)	15.0 (±0.5)
JCSC9894	9.5(± 0.5)	9.5(± 0.6)	15.0 (±1.0)	-	16.0 (±0.5)
JCSC10004	10.0(± 0.5)	10.0(± 0.8)	16.0 (±2.0)	11.0 (±0.0)	16.0 (±0.0)
JCSC6673	9.0 (±0.8)	8.5 (± 1.0)	14.0 (±1.5)	8.0 (±1.0)	16.0 (±1.0)
JCSC6674	10.0(± 0.5)	8.5 (± 0.5)	12.0 (±1.0)	11.0 (±0.5)	17.0 (± 0.5)
JCSC9897	9.5 (±0.5)	8.0 (± 0.9)	15.0 (±1.0)	10.0 (±0.0)	15.0 (±0.0)
JCSC9900	9.5 (±0.5)	9.0 (± 0.5)	16.0 (±2.0)	12.0 (±1.0)	15.0 (±0.0)
JCSC9901	9.9 (± 0.9)	9.5 (± 0.5)	14.0 (±2.0)	10.5 (±1.0)	17.0 (±1.0)
JCSC9902	9.0 (± 0)	8.0 (± 0.9)	13.0 (±2.0)	10.0 (±0.0)	15.0 (±0.0)
ATCC 43300	12.0 (± 0.5)	12.0 (±0.5)	14.0 (±1.0)	11.0 (±1.0)	16.0 (±1.0)
MSSA ATCC 25923	10.0 (± 0.5)	9.0 (±0.9)	15.0 (±1.0)	22.0 (±1.0)	17.0 (±1.0)
ViSA Mu50	9.5 (±0.5)	8.0 (±0.0)	17.0 (±1.0)	29.0 (±1.0)	16.0 (±1.0)

3F2, 3F2; gold compounds, **Amp**; Ampicillin, **C**; Chloramphenicol, **VAN**; Vancomycin; **MSSA** Methicillin Sensitive *Staphylococcus aureus;* **MRSA**; Methicillin Resistance *Staphylococcus aureus;* **ViSA** Vancomycin intermediate-resistant *Staphylococcus aureus.*

The 3F2 and 3F3 gold compounds showed zone of inhibition against all the MRSA clinical samples ranged between 8 mm to 11 mm, with the exception of

MRSA samples (A6) and (A92), which exhibited resistance against both 3F2 and 3F3 (S1). However, the same MRSA clinical strains were resistant to penicillin, as the average zone of inhibition was between 8 mm to 21 mm, whlie greater than 29 mm is considered as sensitive based on the most recent CLSI breakpoint report (S1). All the *S.aureus* reference and clinical strains were resistant against chloramphenicol, with a zone of inhibition of ≤ 20 mm, which is considered as resistant based on the most recent CLSI breakpoint report. The experiment was repeated three times and the average size of the zone of inhibition with the standard deviation was used to represent the results.

Determination of MIC and MBC

The antibacterial activity of the 3F2 and 3F3 gold compounds were quantitatively measured against the MRSA reference strains only by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is defined as the lowest concentration of a compound that is required to inhibit bacterial growth, while the MBC is defined as the lowest concentration of a compound that is required to kill 99% of bacteria. The micro-dilution method was adapted for this test. The MIC values of the 3F2 gold compound against all the MRSA reference strains were in the range of 4 µg/ml to 8 µg/ml (Table 3) However, according to the distribution of the MIC values among the MRSA strains, approximately 47% of the strains exhibited sensitivity towards the 3F2 compound at 4 µg/ml, 35% of them were sensitive at 6 µg/ml, and about 12% and 6% were sensitive at 7 µg/ml and 8 µg/ml, respectively (S2 In the case of the 3F3 gold compound, the MIC values against the pathogens were between 30 µg/ml to 60 µg/ml (Table 3.6). About 70% of the MRSA strains were sensitive towards the 3F3 compound at 30 µg/ml, whereas 24% and 6% were sensitive at 40 µg/ml and 60 µg/ml, respectively (S2) In contrast, the antibiotics used in this experiment as the positive control were less potent than the gold compounds, especially the 3F2 compound. However, the MIC values for ampicillin ranged between 16 µg/ml to 124 µg/ml, which was very high compared to the breakpoint values based on the most recent CLSI breakpoint report. Moreover, chloramphenicol showed better antibacterial activity against all the MRSA strains compared to ampicillin, with MIC values ranging between 8 µg/ml to 64 µg/ml, which was in agreement with the CLSI report. It is worth noting that the 3F2 gold compound exhibited a lower minimum inhibitory concentration against all the MRSA strains, even in the case of the Mu50 strain, which is vancomycin intermediate resistant, with a vancomycin MIC value of ≥ 5µg/ml, while the 3F2 compound had an MIC value of only 4 µg/m (Table 3). The broth dilution test was used to determine the MBC/MIC ratio. The MBC/MIC ratio was analysed to determine the mode of antibacterial activity of the phosphanegold(I) thiolate compounds. If the MBC value is no more than four-fold the value of the MIC, then the antibacterial agent is considered to be bactericidal (MBC/MIC ≤ 4). The results in Table 3 show that the 3F2 and 3F3 gold compounds exhibited bactericidal activity against all the MRSA reference strains compared to the standard antibiotics, which showed a different mode of antibacterial activity ranging between bacteriostatic and bactericidal.

Table (4): Minimum inhibitory concentration (Minimum Bactericidal Concentration) and MIC/MBC ratio of 3F2, 3F3 and standard antibiotics against *Staphylococcus aureus* reference strains.

MRSA Strains		ANTIBACTERIAL ACTIVITY									
		3F3		3F2		Amp		C		Van	
	SCCmec type	MIC (MBC)	MBC/MIC	MIC (MBC)	MBC/MIC	MIC (MBC)	MBC /MIC	MIC (MBC)	MBC/ MIC	MIC (MBC)	MBC/ MIC
JCSC9884	I (1B)	60 (120)	2◊	4(14)	4◊	112(128)	1◊	8(24)	4◊	-	-
JCSC9885	IIa (2A)	30(60)	2◊	4(14)	4◊	32(128)	4◊	8(16)	2◊	-	-
JCSC9889	III (3A)	30(60)	2◊	6(6)	1◊	64(128)	2◊	64(64)	2◊	-	-
JCSC9890	IVa(2B)	40(60)	2◊	6(18)	3◊	16(128)	8●	8(32)	4◊	-	-
JCSC9892	IVa	40(40)	1◊	6(8)	1◊	43(75)	2◊	12(24)	2◊	-	-
JCSC9893	IVb	30(60)	2◊	4(8)	2◊	16(96)	6●	8(24)	3◊	-	-
JCSC9894	IVc	30(50)	2◊	6(11)	2◊	128(128)	1◊	8(24)	3◊	-	-
JCSC 10004	IVd	40(40)	1◊	6(12)	2◊	106(106)	1◊	12(24)	2◊	-	-
JCSC6673	IVg	30(30)	1◊	8(24)	3◊	64(256)	4◊	8(32)	4◊	-	-
JCSC6674	IVh	30(30)	1◊	4(8)	2◊	16(32)	2◊	16(32)	2◊	-	-
JCSC9897	V	30(30)	1◊	6(18)	3◊	64(128)	2◊	8(64)	8●	-	-
JCSC9900	VI	30(40)	2◊	7(8)	1◊	16(64)	6●	8(16)	2◊	-	-
JCSC9901	VII (5C1)	40(40)	1◊	7(16)	4◊	32(128)	4◊	8(32)	4◊	-	-
JCSC9902	VIII (4A)	30(30)	1◊	4(8)	2◊	16(128)	8●	8(16)	2◊	-	-
ATCC 43300	II	30(80)	2◊	4(16)	4◊	64(128)	8●	8(16)	2◊	-	-
*ATCC 25923	-	30(30)	1◊	4(16)	4◊	12(128)	16●	8(16)	2◊	-	-
†ATCC Mu50	II	30(60)	2◊	4(16)	4◊	16(128)	8●	8(32)	4◊	5(>5)	>1◊

(3F2, 3F2); Gold compounds; (-) Not tested; (*) MSSA Methicillin Sensitive *Staphylococcus aureus*; (†) ViSA Vancomycin intermediate resistant *Staphylococcus aureus*; (◊) Bactericidal; (●) Bacteriostatic; (AMP) Ampicillin; (C) Chloramphenicol; (VAN) Vancomycin. .

Time killing assay

This test is widely used for the determination of novel antibacterial agents in vitro to provide information about the kinetics of the antibacterial agents. The 3F2 gold compound was selected for this test as it showed a higher antibacterial activity against different *S.aureus* strains compared to the 3F3. Moreover, the 3F2 gold compound exhibited lower MIC values compared to the 3F3 gold compound. The kinetic interaction between the 3F2 gold compound and the selected *S.aureus* strains was tested at the MIC and ½ MIC concentrations of each strain. The kinetic profile of the 3F2 gold compound (Figure 1, A-H) showing a reduction of viable bacterial cells of ≥ 3 log10 compared to the initial inoculum after 4 hours of exposure. Meanwhile, the time-kill profile of the 3F2 gold compound at ½ MIC concentration for each strain exhibited varying bactericidal and bacteriostatic activities against the different *S.aureus* strains. The 3F2 gold compound displayed bactericidal activity against three strains (MRSA JCSC9900, MRSA JCSC9901 and MRSA JCSC9890) in the first 12 hours after exposure. After that, the killing rate slowed down, and the bacterial cells started to grow again to ≤ 3 log10 of the initial inoculum after 24 hours, except for the MRSA JCSC9890 strain, which grew less than the initial inoculum (Figure 1, A, B and D). A shorter time of bactericidal activity was shown by the 3F2 gold compound against four strains (MRSA JCSC9889, MRSA JCSC9885, MRSA JCSC9884 and MRSA ATCC43300), but the killing rate slowed after approximately 8 hours of exposure, and the growth of bacterial cells was even higher than 3 log10 of the initial inoculum (Figure 1, E-H). Interestingly, the 3F2 gold compound showed bacteriostatic activity against only one strain (MRSA JCSC 9902) at ½ MIC concentration and only for 8 hours. After that, the bacterial cells started to grow 3 log10 higher than the initial inoculum (Figure1, C).

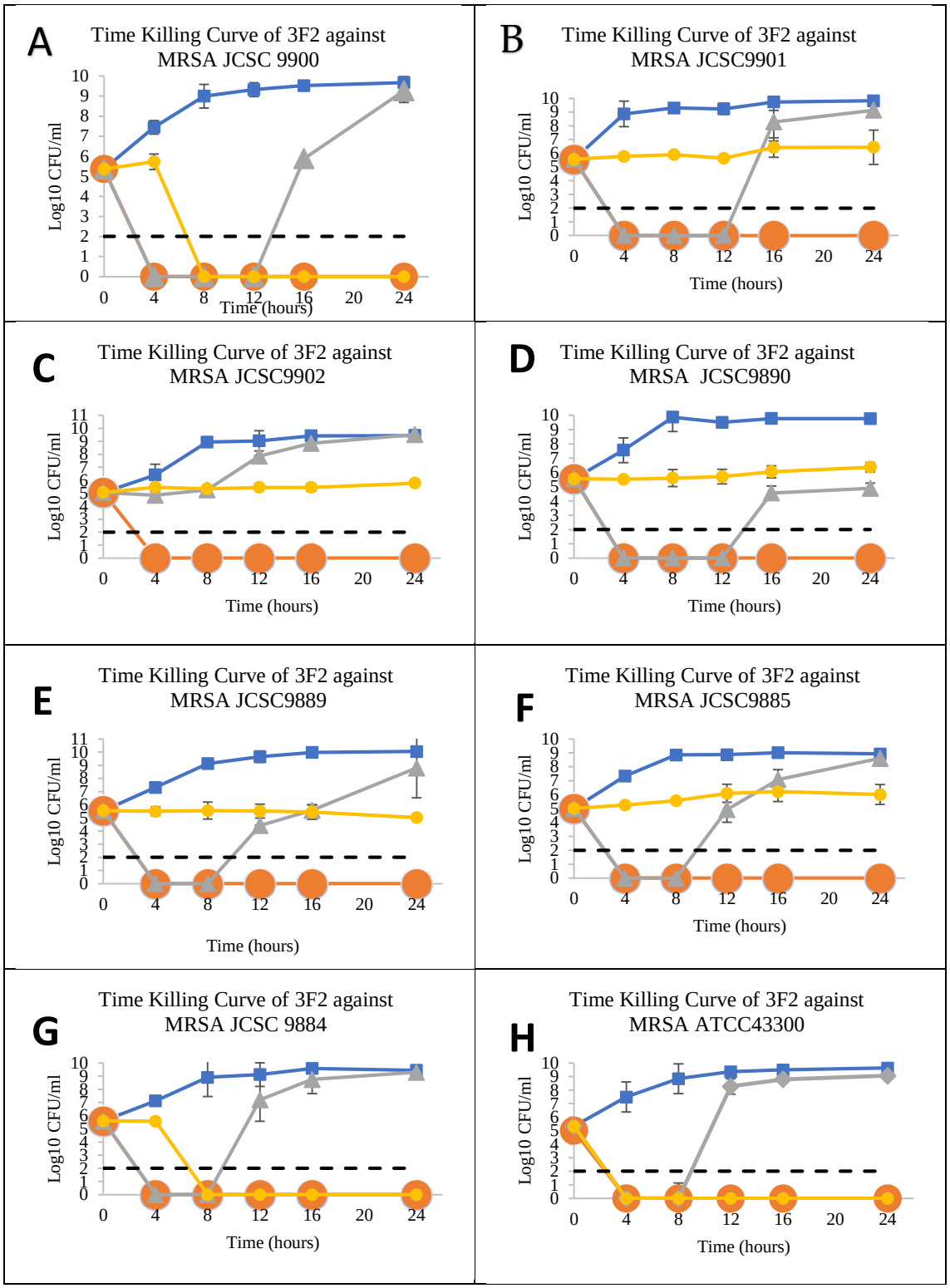

Figure (1): Time killing profile of 3F2 Gold compound against different MRSA strains. (Orang line) 3F2 MIC 4 µg/ml; (Gray line) 1/2 MIC 2 µg/ml; (Yellow line) Chloramphenicol MIC 8 µg/ml; (Dashed line) indicate the bactericidal level; (Blue line) vehicle cells without treatment (negative control).

Table (5): Combination of 3F2 and Ciprofloxacin and Fractional inhibitory concentration index.

Combination	3F2 (µg/ml)	CIP (µg/ml)	FIC 3F2	FIC CIP	FICI	Interpretation
Combination 1	2	0.2	0.5	0.5	1	Additive
Combination 2	1	0.2	0.25	0.5	0.75	Partially Synergistic
Combination 3	0.5	0.2	0.125	0.5	0.625	Partially Synergistic
Combination 4	0.25	0.2	0.0625	0.5	0.562	Partially Synergistic
Combination 5	0.125	0.2	0.0312	0.5	0.531	Partially Synergistic
Combination 6	2	0.1	0.5	0.25	0.75	Partially Synergistic

3F2: Gold compound; CIP: Ciprofloxacin; FIC: Fractional inhibitory concentration (Mean of the FIC of at three independent experiments); FICI: Fractional inhibitory concentration index.

F2 Combination with Antibiotics

A checkerboard assay was performed to investigate the antibacterial activity of the 3F2 gold compound in combination with the systemic antibiotics, namely, ampicillin, chloramphenicol, and ciprofloxacin, against MRSA(ATCC43300), and vancomycin against ViSA (ATCC Mu50). Each assay for each antibiotic was tested separately using a 96-well plate containing a range of concentrations of between 4X MIC to 1/32 X MIC for both the antibiotic and the 3F2 gold compound. Of the four antibiotics, ciprofloxacin and ampicillin interacted positively and showed a synergistic reaction with the 3F2 gold compound, while chloramphenicol and vancomycin were not antagonistic, but rather did not show any drug interaction.

The interaction between the 3F2 gold compound and ciprofloxacin was shown in various combinations with different sub-inhibitory concentrations. Six combinations showed inhibition of bacterial growth after 24 hours of incubation under fixed conditions (Table 4). The first combination, which represented a sub-inhibitory concentration of half the minimum inhibitory concentration (1/2 X MIC) for both agents with a fractional inhibitory concentration index (FICI) of 1.0, showed enhanced antibacterial activity or drug interaction on MRSA (ATCC 43300), which was noted when the data points were plotted on the isobologram (Figure 2). The concentration of 3F2 to ciprofloxacin was in the ratio of 10:1, meaning that 3F2 was ten times more concentrated than ciprofloxacin

Combination 2 (with sub-inhibitory concentrations of 3F2 (1 µg/ml), Cip (0.2 µg/ml)) and combination 6 (with sub-inhibitory concentrations of 3F2 (2 µg/ml), Cip (0.1 µg/ml)) exhibited the same drug interaction activity with a fractional inhibitory concentration index (FICI) of 0.75, which indicated partial synergistic activity or general synergism when the plotted FIC values of the combinations fell below the independent MIC line of the constructed isobologram (Figure 2). The ratio of the concentration of 3F2 to ciprofloxacin in combination 2 was 5:1, while in combination 6 the ratio was 20:1.

Enhanced drug interactions were observed in combinations 3, 4 and 5, with slightly different fractional inhibitory concentration indices (FICI) of 0.625, 0.562 and 0.531, respectively. The ratios of the concentration of the 3F2 gold compound to ciprofloxacin in the abovementioned combinations were 2.5:1, 1.25:1 and 1:1.6, respectively. The best synergism was noted in combination 5, where the FICI was 0.531. However,

all the data points that were plotted fell below the line of enhancement on the constructed isobologram (Figure 2). It should be noted that the little differences in the FICI and the ratio of the 3F2 gold compound to ciprofloxacin did not change the classification of the drug interaction from synergistic activity (Table 4). It is important to mention that combinations where the FICI > 1, which were classified as indifferent or antagonistic activity, will not be represented and discussed in this study since they did not give significant or promising results.

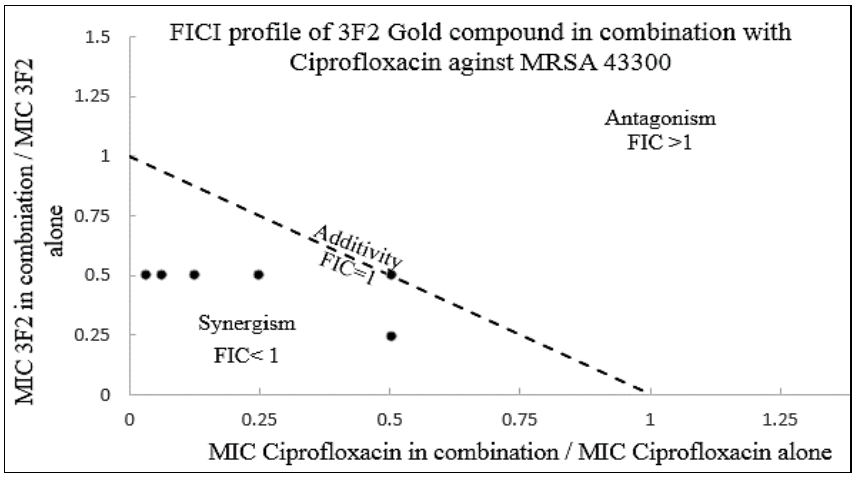

Figure (2): Isobologram illustrates the FICI profile of the 3F2 Gold compound in combination with Ciprofloxacin. The black dots represent the mean of FIC values for both the 3F2 gold compound and Ciprofloxacin.

The best synergy profile was noted when the 3F2 gold compound was combined with ciprofloxacin predominantly at a ratio of 1.24:1 and at sub-inhibitory concentrations of 0.125 µg/ml and 0.2 µg/ml, respectively. A time-kill assay was performed to assess the bactericidal rate of the combination and to confirm the 3F2 gold compound/ciprofloxacin interaction (Figure 3). it is clear that the tested combination (green line) synergistically showed bactericidal activity at the abovementioned ratio with a bacterial inhibition of higher than 3 log10 CFU/ml after 24 hours of exposure under fixed conditions. This bactericidal activity of the combination was similar to that of the 3F2 gold compound alone at its MIC value (yellow line). However, the 3F2 gold compound alone (purple line) at its ½ MIC value showed a temporary bactericidal activity since the bacterial cells regrew after 8 hours of exposure to reach the maximum bacterial growth of 3 log10 CFU/ml higher than that of the initial inoculum. Meanwhile, ciprofloxacin alone (grey line) at its ½ MIC value did not show any bactericidal activity, but instead exhibited bacteriostatic activity for almost 12 hours after exposure, after which, bacterial cells grew at a maximum rate of 3 log10 CFU/ml higher than the initial inoculum under the same fixed conditions (Figure 3).

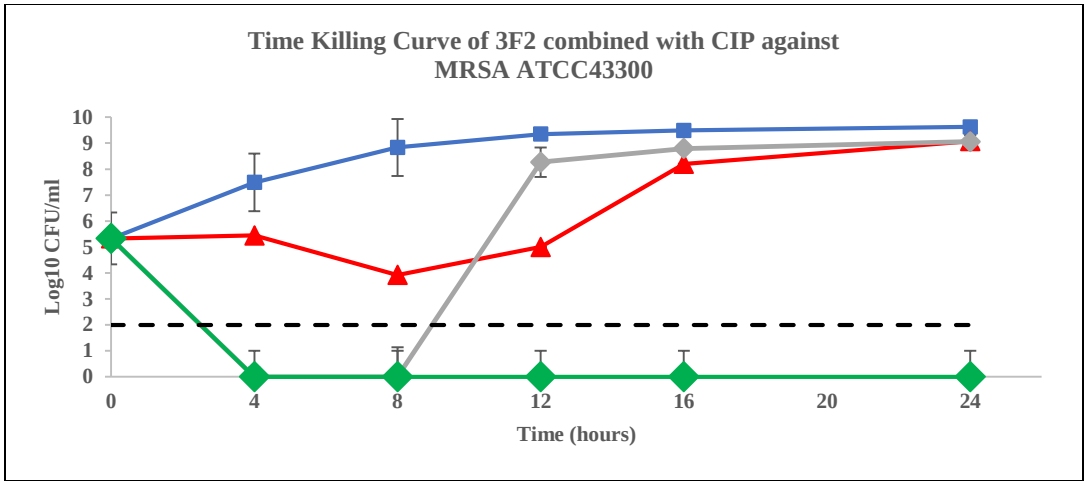

Figure (3): Time killing curve for synergistic combination activity between 3F2 Gold compound and Ciprofloxacin under fixed condition for 24 hours exposure. Combination (green line) with sub-MIC of both agents; 3F2 (2 µg/mL) ; CIP (0.2 µg/mL) showed enhanced activity in comparison with sub-MIC individually grey (3F2) and red (CIP) lines; (Blue line) vehicle cells without treatment (negative control). The dotted line represents the bactericidal threshold. The data is presented as a mean of three replicates.

Discussion

Lately, the interest in the synthetic metalo-drug that exhibit biological characteristics, such as antibacterial effects has increased and several studies have been carried out for better understanding the process and the course of action (25). Moreover, the ability of the metals to bind with ligands and free them in certain process make them significant for use in biological mechanisms(26). Therefore, our cooperative team has synthesized new Phosphanegold(I) thiolate compounds. Both the ligand and its gold compound compounds were examined and analysed for their scope of antibacterial effects against range of Gram-negative and Gram-positive important pathogens by using the Kirby-Bauer disc diffusion technique. All such pathogens are clinically significant and cause serious infection and illnesses to human and animals like burn, wound and skin infections, urinary tract disorders, pneumonia, as well as with nosocomial contagions and infections of seriously ill patients. The technique Kirby-Bauer disc diffusion illustrated that 3FL as a ligand has comparatively weak or no antibacterial activity in comparison to its compounds for both Gram-negative and Gram-positive pathogens. It is also suggested that the same ligand combined with gold atoms in compounds gave greater antibacterial activity under same experimental conditions. Our findings also mentioned that metal compounds combined with ligand could show higher activity than only the ligand (26–28). This may indicate that the chelating enables the attributes of compounds to cross the cell membrane's lipid bilayers to get to the target and this is possibly explained by Tweedy's theory of chelating (28). Chelation reduces the metal ion's polarity mostly because of the sharing of positive charge (electron) with donor groupings and the likelihood of electron delocalisation over the whole ring, as per the theory. Consequently, the chelates turn more lipophilic, enabling them to be more permeable through the bacterial

membrane's lipid layers (28). Compounds 3F4, 3F5 and 3F6 showed no antibacterial activity at all with respect to both the Gram-negative and Gram-positive bacteria, though those compounds showed trivial activity with respect to MRSA strain while only 3F4 showed activity against *Shigella sonnei* (ATCC 9290). Just two compounds among the phosphangold(I) thiolate (3F series) demonstrated a variety of antibacterial spectrum; 3F2 and 3F3 exhibited between mild and good antibacterial activity against *Enterococcus faecium* (ATCC19434), *Staphylococcus aureus* (ATCC25923), Methiciline-resistant *Staphylococcus aureus* (ATCC 43300), *Entrobacter aerogenes* (ATCC14028), *Salmonella typhimurium*(ATCC 14028) and *Proteus vulgaris* (ATCC13315). The outcome of preliminary examination indicated that compounds phosphanegold(I) thiolate were having a capability to penetrate the thick stratum of peptidoglycan in the Gram-positive bacteria to activate the antibacterial action and indicated that the ligand (3FL) combined to gold compound to create the compound together enhanced scope of antibacterial activity against both Gram-negative and Gram-positive bacteria. This is because of the fact that some antibacterial drugs behave differently against Gram-negative and Gram-positive bacteria since cell thickness is different for each; nonetheless, the cell wall of bacteria is the initial target for majority of the antibacterial drugs (29). Curiously, some of the properties of antibacterial agents can be described by their capability to diffuse through the membrane and cell wall and in some way to interact to bring about changes in the structure that ultimately lead to bacterial cell death.

Despite the fact that compounds 3F2 and 3F3 show potential antibacterial activity with respect to Gram-positive bacteria, specifically *Staphylococcus aureus*, it was insignificant on Gram-negative bacteria, possibly since Gram-negative bacteria's outer cell wall acts as a barrier to guard the cell from any attack like the influx of testing compounds to target areas (25,27,29–31)

To outline the outcomes obtained by the primary screening, compounds 3F2 and 3F3 were further examined for their antibacterial effects toward *S.aureus*. Curiously, 3F2 and 3F3 compounds displayed a strong and potential activity against various strains of *S.aureus* like carrier strains and showed the same activity against clinical strains of *S.aureus*. Both 3F2 and 3F3 compounds brought about similar outcomes against all strains of *S.aureus* in terms of inhibition zone. This might be due to the resemblance in the chemical structure and also because they have the same ligand. Nonetheless, 3F2 and 3F3 together with their thiolate ligand exhibited comparable outcomes in the previous studies (27,29,30,32) indicating that 3F2 and 3F3 phosphingold(I) thiolate shown promising antibacterial activity against *S.aureus* strains.

MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) examinations of 3F2, 3F3 compounds are carried out to determine bacteriostatic (to inhibit) or bactericidal (to kill) activity. The significance of this examination should not be regarded as an assumption that bactericidal drug operation is superior to bacteriostatic drug operation in majority of the cases, and investigators must realise that these drugs could be bactericidal (kill-

ing) for one bacterial strain while bacteriostatic for another strain or species (33). Nonetheless, other studies proposed that treating bacterial Gram positive infections with bactericidal treatment is more preferable than bacteriostatic drugs (34). Alternatively, concentration dependent drugs were more preferred to concentration independent (dependent on time) drugs (33). The distinction between MBC and MIC in vitro was well explained; a compound having MBC value ≤ 4 times compared to MIC value is regarded as a bactericidal agent while it is considered as a bacteriostatic agent if value of MBC is ≥ 4 times than that of MIC (35,36).

Based on the above-mentioned description and considering growing resistance to antibiotics specifically Gram-positive antibiotics, the advance of bactericidal agents has still been the priority in majority of the cases instead of the bacteriostatic drugs. Compound 3F2 and 3F3 were regarded as bactericidal for majority of the *S.aureus* varieties including the ViSA strain (Mu50). Both compounds were particularly shown to have an MBC/MIC ratio of value less than 4. The compounds had distinctively different behaviour against various *Staphylococcus aureus* strains. Despite the difference in values of MBC and MIC of 3F2 compound and 3F3 compound, both compounds exhibited same activity against same bacterial strain.

Previous studies indicated that antibacterial agents that are positively charged and which can form compound bonds and interact with cell wall interactively may have bactericidal effects (37,38). Curiously, this is more likely in case of Gram-positive bacteria because the peptidoglycan cell wall pore may fit enough of the compound to cross (39). Other study illustrated that pyrazine gold compound, the functionalized Au(I)-NHC group, gets attached to the amino acids in peptidoglycan layer of the cell wall, which is the reason for the damage to the cell wall (40). Nevertheless, the anti-staphylococcus aureus activity is not just related to the gold atom, but also to the nature of both the aminothiol and the phosphine to which the metal is attached. (41).

These results indicate that 3F2 and 3F3 show varying scope of bactericidal activity as MRSA strains may vary from each other via relaying different type *SCCmec* which may affect the antibacterial endurance against heavy metal compounds and play a significant role in *S.aureus* bacterial resistance. Even though the *SCCmec* was out of scope in this investigation, it was good to state this reason as other studies also reported the same (42–44). Nonetheless, bacteriostatic action of any compound was not recorded in any strain; only certain standard antibiotics displayed bacteriostatic activity.

Time killing analyses have been used, like the in vitro standard technique, to determine the antibacterial agents' pharmacodynamics information. This analysis gives qualitative report about the time required for the antibacterial drug to inhibit or kill the development of bacteria at fixed concentration (45). Besides MIC and MBC outcomes, 3F2 compound was the preferred phosphinegold (I) thiolate compound for time killing analysis; two concentrations of 3F2 compound were examined to determine the dynamic correlation of the antibacterial action of 3F2 compound and *S.aureus* strains; 1 time MIC (1 x MIC) and half time MIC (½ x MIC) were administered to a fixed inoculum as per

CLSI guideline. An antibacterial drug is considered bactericidal in case it can inhibit the initial inoculum of bacterial cells by 3log10 CFU/ml or higher.

In this investigation, a high rate of time killing was recorded in all the examined *S.aureus*. The kinetic outline of 3F2 compound at value of MIC displayed a comparatively rapid bactericidal action against all examined strains and decrease in viable cells ≥ 3log10 was recorded after 4 hours exposure to 3F2 compound in comparison to the initial inoculum. Alternatively, the half MIC concentration value showed ≥ 3log10 decrease of viable cell at initial 8 hours; then the bacterial cells redevelop aggressively after 20 hours exhibiting increment in viable cells count more compared to the initial inoculum. This phenomenon took place in all tested strains excluding MRSA JCSC9902, in case of this strain, the 3F2 compound at (½x MIC) concentration displayed bacteriostatic activity that is where the number of viable cells remain almost consistent at first 8 hours and then increase dramatically after that.

The data that time killing analysis gave is that compound 3F2 at 1x MIC concentration value was considered as a bactericidal drug against all *S.aureus* strains, while it is a concentration depended drug; nonetheless, at the 1/2 MIC concentration value, the 3F2 compound was considered as a bacteriostatic (time dependent) drug, where the effect of compound 3F2 disappeared after some time of exposure. The redevelopment occurrence was common in several previous investigations and can be explained by the term 'subpopulation'. Generally, there are 2 different bacteria subpopulations with varied susceptibility in each bacterial population wherein the selective increase in resistant subpopulation takes over the preferred killing of vulnerable subpopulation at a particular time of interaction (30,46,47). Apart from that, the unstable nature of the compound, which is dependent on the organisation of metal-ligand, also may lead to bacterial redevelopment after initial decrease in viable cells (27,48).

Drug combination therapy used to be a substitute to single drug therapy which can reduce the resistance and toxicity and displaying synergistic effects, since the combination therapies owing these advantages, has become a standard strategy to treat various diseases (49). Antimicrobial effectiveness usually change when mixing two or more drugs simultaneously and discovering antibiotic combinations with improved efficiency (synergistic effect) is an longstanding goal despite the antibiotic resistance which is remain the serious threatens for individual antibiotics (50,51). The 3F2 gold compound demonstrated additive activity as well as positive drug interaction when combined with antibiotic systematically employed for the treatment of MRSA infection via the checkerboard method. Moreover, the 3F2 gold compound showed additive as well as promising positive drug interaction in terms of inhibiting the in vitro growth pertaining to MRSA when used in combination with ciprofloxacin, which agrees with the studies reported earlier (31,52). Sreedharan and Singh (2019) evaluated the antibacterial activity pertaining to conjugated ciprofloxacin along with gold nanoflower (a new kind of developed nanoparticles). As per the results, the combination of gold nanoflower with ciprofloxacin demonstrated improved bactericidal activity to

fight against numerous Gram-positive including MRSA strains (53). The 3F2 gold compound demonstrated positive drug interaction as well as excellent synergy distribution when combined with ciprofloxacin, which can also be employed as a dual therapy for MRSA infection treatment. A previous study has reported employing gold-based drug-ciprofloxacin or other antibiotics combination for the treatment of *N. gonorrhoeaa* (54). Furthermore, as per a different study, gold-based drug auranofin as well as five other analogous gold compounds demonstrated synergistic activity with standard antibiotics that are commonly employed against *Helicobacter pylor* infections. Decreasing the cell membrane's integrity via standard antibiotic could also result in increasing the uptake as well as the accumulative concentration of gold-based drug, which encourage additional damage to *Helicobacter pylori*. (55). Combining 3F2 gold compound with ciprofloxacin demonstrated higher potent anti-Staphylococcus aureus, which could be similar to ciprofloxacin's mechanism of action. Ciprofloxacin belongs to the class of fluoroquinolones (FQs) and is broadly employed as antibiotic for the treatment of respiratory, urinary and enteric infections. This broad usage of ciprofloxacin can be attributed to its ability to passively diffuse through the cell membranes and bacterial cell wall. It applies a series of mechanism of actions like DNA gyrase inhibition, cellular membrane penetration and bacterial efflux pump inhibition (56–59). Subsequently, a possible mechanism of action pertaining to the combination of 3F2 gold compound and ciprofloxacin could be that both the agents would attack two different targets. For instance, ciprofloxacin enables 3F2 gold compound entry via disruption of the cell membrane integrity, which results in increased uptake of 3F2 gold compound as well as accumulation within the cell until the killing concentration is achieved

Chohan *et al*. (60) carried out a study in which they suggested a different possible killing pathway with regards to the metal compound when combined with ciprofloxacin. They identified various chemical coordination pertaining to the combined agents from the parent drugs, which improved the antibacterial activity versus ciprofloxacin alone to fight against various pathogens, including *Staphylococcus aureus*. The author suggested that this activity could be because of the changes occurring in the polarity as well as the chelating system pertaining to the metal ion within combination with ciprofloxacin. These changes enhance the metal compound’s lipophilicity nature, which it turn enable passive transportation via the bacterial cell membrane’s lipid layers, thus enhancing the activity pertaining to drugs combination (60). The aforementioned proposed killing pathway seems more suitable to our finding and suggest that 3F2 gold compound in combination with ciprofloxacin may result different chemical specification than the parent agents which promote the penetration of combined drugs through cell membrane thus increased the uptake of 3F2 gold compound to killing concentration level.

Nazari and her colleagues carried out another study and reported that the gold compounds slightly improved the antibacterial activity pertaining to various antibiotics, including ciprofloxacin as well as beta-lactam antibiotics class to fight against *S.aureus* strain. The same

study demonstrated that various antibiotics could exhibit different activities when gold compound is introduced (61). In this study, 3F2 gold compound exhibited different effects on the evaluated antibiotics. This could be due to the varying interaction of gold compound with different orientation or result in formation of a coordination compound with free donor groups like phosphorous or sulphur in combination with various antibiotics. This chemical interaction could also alter the morphology pertaining to both antibiotics as well as gold compound itself, thus enhancing antibacterial activity. This put forward mechanism pertaining to gold compound plus antibiotics has been reported many times (61,62). Furthermore, gold-based drugs have demonstrated enhancement of bactericidal activity pertaining to antibiotics, when both drugs are combined together for the treatment of MSSA and MRSA strains in vivo subcutaneous abscess mice model (63). With regards to combination or chemical conjugate of gold compound along with antibiotics, there can be considerable enhancement in pharmacokinetics and therapeutic index pertaining to the combination in contrast to individual drug.

Conclusions

Six phosphanegold(I) thiolate compounds were tested and displaying antibacterial activity against wide range of Gram-positive and Gram-negative bacteria. Two out of six compounds, 3F2 and 3F3 were showing obvious activity against Gram-positive bacteria *Staphylococcus aureus* strains. Only 3F2 exhibited promising antibacterial activity against different MRSA strains, as evidenced by its lower or comparable MIC values against standard reference antibiotics. The time-kill curve provided valuable information about the 3F2 gold compound, which is considered as a bactericidal agent against different MRSA strains, depending on its concentration and the time. The interesting antibacterial activity of phosphangold(i) thiolate proposed a fundamental role of phosphanegold(I) structure in exaggerating antibacterial activity of the compound and may lead to new possible finding that phosphangold(i) thiolate compound more active against Gram positive than Gram negative. The metal compounds such as gold compound are capable to combinatorial synthetic methods, phosphanegold(I)thiolate (3F2) was exhibited synergistic activity and positive drug interaction when combine with ciprofloxacin which can be used as dual therapy to treat MRSA infection. In general, metal compounds seem to offer a rich stage for the design of novel antibacterial agent. Thus phosphanegold(I)thiolate (3F2) compound may possessed potential use as a single agent and as a combinatorial agent with the available antibiotics to treat or inhibit the growth of *S.aureus*.

Supplementary Materials

Table S1: Antibacterial activity measured by zone of inhibition of 3F2 and 3F3 gold compounds against clinical MRSA samples**, Table S2**: MIC distribution of 2F2 and 3F3 against *Staphylococcus aureus* strains.

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Conflicts of Interest

The authors declare no conflict of interest.

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