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Original article

The synthesis of phenylalanine-derived C5-substituted rhodanines and their activity against selected methicillin-resistant *Staphylococcus aureus* (MRSA) strains

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ABSTRACT

A series of rhodanine compounds containing various substituents at the N3- and C5-positions were synthesized and their *in vitro* activity against a panel of clinically relevant MRSA strains was determined. The anti-MRSA activity of compounds **21** (MIC = 3.9 μg/mL, MBC = 7.8 μg/mL) and **22** (MIC = 1.95 μg/mL, MBC = 7.8 μg/mL) was significantly greater than that of the lead compounds, **1**–**3** and reference antibiotics penicillin G (MIC = 31.25 μg/mL) and ciprofloxacin (MIC = 7.8 μg/mL) and comparable to that of vancomycin (MIC = 0.97 μg/mL). Compounds **21** and **22** were found to be bactericidal at only 2–4-fold higher than their MIC concentrations. In addition, their MIC values remained unchanged in the presence or absence of 10% serum. Overall, the results suggest that compounds **21** and **22** may be of potential use in the treatment of MRSA infections.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become endemic in most hospitals and health care facilities in Western nations. In the United States, MRSA infections cause an estimated 19,000 deaths per annum [1]. The two major types, hospital (HA)- and community-acquired (CA) MRSA, are significantly different in their epidemiological and genetic characteristics [2–4]. HA-MRSA occurs in patients with specific risk factors [5–7], whereas CA-MRSA can occur in healthy individuals that do not have predisposing factors [8–10]. Typically, HA-MRSA infections occur in the urine, lungs, bloodstream and surgical sites whereas CA-MRSA primarily produces skin and skin structure infections [4,11]. The HA-MRSA strains contain the mobile class I, II and III staphylococcus chromosome cassettes mec (SCCmec) and resistance to the β-lactam antibiotics is due to the encoding of the penicillin binding protein (PBP) 2a by the mecA gene [12–14]. In contrast, the resistance to non-β-lactams is due to the presence of transposons and other genes. PBP2a has an elevated rate constant for dissociation and decreased acylation rate constant and these together significantly decrease the acylation of PBP2a, producing β-lactam resistance [15]. This, in combination with other genes, has allowed MRSA to become resistant to many different classes of antibiotics [16,17]. In contrast, CA-MRSA strains harbor SCCmec IV [18], a smaller and more mobile SCCmec than classes I–III and this may contribute to its widespread dissemination [19,20]. The CA-MRSA strains, like HA-MRSA, are broadly resistant to β-lactam and macrolide/azalide antimicrobials but responsive to certain non-β-lactam antibiotics [3,21]. However, resistance rates are increasing and there are other limitations in the use of these drugs. Thus, given the widespread dissemination and mortality caused by MRSA, the synthesis and development of new drugs is imperative.

Rhodanine-based molecules have been shown to be small molecule inhibitors of numerous targets such as hepatitis C virus (HCV) NS3 protease [22], aldose reductase [23,24], β-lactamase [25], UDP-N-acetylmuramate/L-alanine ligase [26], penicillin binding protein (PBP) [27], antidiabetic agents [28], cathepsin D [29] and histidine decarboxylase [30]. In addition, we have reported that rhodanine analogs are inhibitors of the HCV enzyme NS5B polymerase [31]. In a search for new antibacterial compounds from our in-house rhodanine library, we initially identified three rhodanine derivatives [**1**: MIC = 62.5 μg/mL; **2**: MIC = 250 μg/mL and **3**: MIC = 125 μg/mL] exhibiting modest activity against selected MRSA strains (Chart 1). Here, we report the subsequent synthesis, anti-MRSA activity and SAR optimization focusing on N3- and C5-positions of the rhodanine core of these lead compounds. A variety of strains of *S. aureus* were used for this study and were obtained from the American Type Tissue

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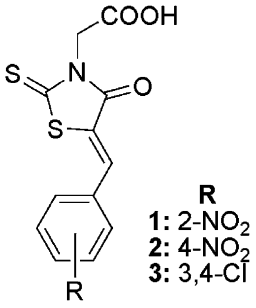


Chart 1. Structures of the anti-MRSA rhodanine hits.

Collection (ATTC). Strains were selected to represent the scope of *Staphylococcus* variants that might be present in a number of clinical, community and quality control situations. ATCC strain 34404 is commonly used as a quality control organism for susceptibility testing. Strains 700698, 700787, and BAA-39, represent nosocomial or hospital-acquired strains from distant global locations and body sites (i.e., pneumonia patient from Japan, blood culture from New York and nasal culture from Hungary, respectively). Strain BAA-1680 represents a community-acquired MRSA (CA-MRSA) strain from the skin of a patient from Michigan, U.S.A.

Although the SAR of rhodanine derivatives has been studied extensively, there is a paucity of information about the contribution of the amino acid side chain at N3-position and uniquely substituted arylidenes at C5-position to their antibacterial action [25,27,32–34]. We hypothesized that the phenylalanine or other hydrophobic amino acid substituents at N3-position would increase the likelihood of penetration through bacterial cell wall and therefore potentially improving their anti-MRSA activity.

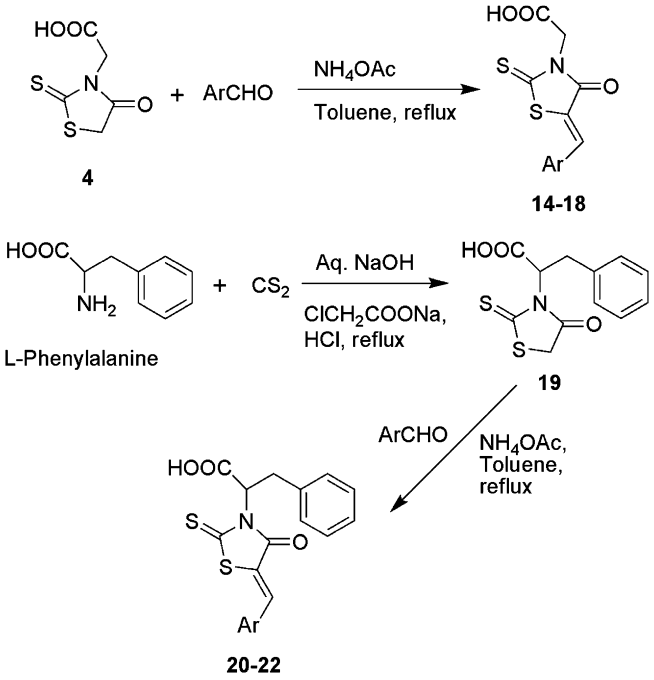
2. Results and discussion

2.1. Chemistry

A group of rhodanine analogs (**14**–**18** and **20**–**22**) was synthesized according to Scheme 1. Rhodanine-3-acetic acid (**4**) was subjected to Knoevenagel condensation with varied aldehydes to form the target compounds (**14**–**18**) as per the procedure reported by us previously [31]. The phenylalanine-derived rhodanine intermediate (**19**) needed for the preparation of target compounds **20**–**22** was synthesized by first cyclizing the L-phenylalanine with CS_2 and sodium α-chloroacetate to form **19**, using a previously reported procedure [35]. The resulting rhodanine intermediate **19** was subjected to Knoevenagel condensation with varied aldehydes as mentioned above to form the target compounds **20**–**22**. The Knoevenagel condensation reaction with aromatic aldehydes provided only the *Z* isomer, as determined by the chemical shift of the methine proton ranging from 7.7 to 8.5 as a singlet (except cinnamylidene analog **16** which showed a doublet) whereas the methine proton of the *Z* isomer of cyclohexylmethylidene analog (**18**) appeared at 6.9 instead of at the calculated chemical shift of 6.3 for the *E* isomer [28,36]. This downfield movement of methine proton in (*Z*) C5-benzylidene and alkylmethylidene analogs was reported to be due to the deshielding effect of the adjacent carbonyl group [28,36]. The synthesized compounds **14**–**18** and **20**–**22**, along with commercially available rhodanine analogs **5**–**13**, were subjected to evaluation against various strains of MRSA (Table 1).

2.2. Anti-MRSA activity

An *in vitro* evaluation of in-house rhodanine analogs [31] against MRSA strains resulted in the identification of the moderately potent



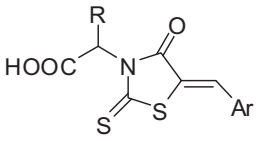
Scheme 1. Synthesis of compounds 14–18 and 20–22.

lead compounds **1**–**3**. In an effort to rapidly obtain SAR data, we decided to purchase structural analogs of compounds **1**–**3**. Compounds **5**–**13** were purchased from Sigma–Aldrich, whereas compounds **14**–**18** and **20**–**22** were synthesized and tested for their *in vitro* activity against a panel of MRSA strains together with reference antibiotics ciprofloxacin, vancomycin and penicillin G.

Following the initial hits with rhodanines **1**–**3**, commercially available rhodanine analogs (compounds **5**–**13**) were obtained for exploration of the SAR around the N3- and C5-position of the rhodanine scaffold, leading to the development of SAR data (Table 1) and identification of a relatively potent analog (compound **12**, MIC = 15.6 μg/mL) as described below. Compounds **5**–**8** contained the α-isopropylacetic acid substituent at N3-position of the rhodanine ring, as well as a series of substituted C5-benzylidenes. While 2-chlorobenzylidene (compound **5**), 4-fluorobenzylidene (compound **6**) and naphthyledene-1-yl (compound **7**) analogs each exhibiting MIC value of 62.5 μg/mL were as active as lead compound **1**, 2–4-fold improvement in inhibitory activity was observed in the presence of the 2,4-dichlorobenzylidene substituent (compound **8**, MIC = 15.6 μg/mL). Based on these findings, we obtained compounds **9**–**13** that had a 2,4-dichlorobenzylidene substituent at the C5-position and various substituents at the N3-position of the rhodanine ring. The bulky α-*n*-propylacetic acid (compound **9**, MIC = 15.6 μg/mL), α-methylthioethylacetic acid (compound **10**, MIC = 31.25 μg/mL), α-isobutylacetic acid (compound **11**, MIC = 15.6 μg/mL) and α-benzylacetic acid (compound **12**, MIC = 15.6 μg/mL) analogs exhibited either comparable or a 2-fold improvement in MIC values. In contrast, the less bulky α-methylacetic acid analog (compound **13**, MIC = 125 μg/mL) exhibited a significant decrease in activity.

Based on the SAR information gathered, we synthesized the next series of rhodanines in the hopes of identifying analogs with greater potency. We chose rhodanine-3-acetic acid 4 as the scaffold for further SAR exploration, focusing on variations at the C5-benzylidene substituent. The 4-methoxybenzylidene (compound **14**, MIC = 250 μg/mL), quinolinylidene-4-yl (compound **15**, MIC = 250 μg/mL) and styryl (compound **16**, MIC = 250 μg/mL) analogs exhibited a 4-fold decrease in activity versus the lead compound **1** clearly

Table 1
Anti-MRSA activity of compounds **1**–**3**, **5**–**18** and **20**–**22**.



Compd[a]	R	Ar	MIC μg/mL[b]				
			MRSA ATCC 34404	MRSA ATCC 700787	MRSA ATCC 700698	MRSA ATCC BAA-39	MRSA CA ATCC BAA-1680
1	-H	2-nitrophenyl	62.5	62.5	62.5	62.5	62.5
2	-H	4-nitrophenyl	250	250	250	250	250
3	-H	3,4-dichlorophenyl	125	125	125	125	125
5	-*i*-Pr	2-chlorophenyl	ND	62.5	62.5	62.5	ND
6	-*i*-Pr	4-fluorophenyl	ND	62.5	62.5	62.5	ND
7	-*i*-Pr	naphthalene-1-yl	62.5	62.5	62.5	62.5	62.5
8	-*i*-Pr	2,4-dichlorophenyl	15.6	15.6	15.6	15.6	15.6
9	-n-Pr	2,4-dichlorophenyl	15.6	15.6	15.6	15.6	15.6
10	-methylthioethyl	2,4-dichlorophenyl	31.25	31.25	31.25	31.25	31.25
11	-*i*-But	2,4-dichlorophenyl	15.6	15.6	15.6	15.6	15.6
12	-benzyl	2,4-dichlorophenyl	15.6	15.6	15.6	15.6	15.6
13	-Me	2,4-dichlorophenyl	ND	125	125	125	ND
14	-H	4-methoxyphenyl	200	250	250	250	250
15	-H	quinolin-4-yl	200	250	500	500	250
16	-H	styryl	250	250	250	250	250
17	-H	3-phenoxypheny	31.25	31.25	31.25	31.25	31.25
18	-H	cyclohexyl	31.25	31.25	31.25	31.25	31.25
20	-benzyl	2-nitrophenyl	125	125	62.5	62.5	62.5
21	-benzyl	3,4-dichlorophenyl	3.9	3.9	3.9	3.9	3.9
22	-benzyl	3-phenoxyphenyl	1.95	3.9	3.9	3.9	1.95
Cipro			7.8	62.5	7.8	7.8	7.8
Vanco			≤0.97	1.95	≤0.97	≤0.97	≤0.97
Pen-G			31.25	31.25	15.6	31.25	62.5

[a] Compounds **1**–**3** were synthesized previously [31], compounds **5**–**13** were acquired from Sigma–Aldrich whereas compounds **14**–**22** were synthesized in this report, ND: not determined.
[b] Results of average values obtained from two independent experiments in duplicates; Cipro = ciprofloxacin, Vanco = vancomycin, Pen-G = penicillin G.

demonstrating the important role of electron withdrawing groups on C5-benzylidene moiety in influencing the anti-MRSA activity. The replacement of the 2-nitrobenzylidene moiety in lead compound **1** with 3-phenoxybenzylidene (compound **17**, MIC = 31.25 μg/mL) and with cyclohexylidene (compound **18**, MIC = 31.25 μg/mL) moieties produced a 2-fold increase in activity. Subsequently, we used a molecular hybrid approach, where we chose compound **12** carrying α-benzyl group at N3 substituent, while varying the C5-position substituents present in compounds **1**, **3** and **17**. The molecular hybrid of compounds **12** and **1** resulted in a new analog (compound **20**, MIC = 125 μg/mL) with 8-fold decreased activity compared to **12** and only 2-fold less active compared to **1**. However, when this approach was utilized for compounds **12** and **3**, it resulted in a new analog (compound **21**, MIC = 3.9 μg/mL) with a 4- and 30-fold improvement in activity compared to the respective parent compounds. Finally, the application of the molecular hybrid approach to compounds **12** and **17**, resulted in the most potent analog of the entire series (compound **22**, MIC = 3.9 μg/mL against MRSA ATCC 700787, MRSA ATCC 700698 and MRSA ATCC BAA-39 and MIC = 1.95 μg/mL against MRSA ATCC 34404, MRSA CA ATCC BAA-1680).

Ligands binding to site II (also called the indole-benzodiazepine site) on human serum albumin frequently contain aromatic carboxylic acids with a negatively charged acidic group at one end of the molecule located away from a hydrophobic center (e.g., non-steroidal anti-inflammatory drugs [NSAIDs]) [37]. Since these structural features (hydrophobic group at C5-position and the acidic function at the N3-position) are present in our rhodanine analogs, we hypothesized that these compounds may have the propensity to bind avidly to serum proteins and may influence their anti-MRSA activity. The binding of the molecules to serum proteins can significantly reduce their free concentrations in plasma, thereby potentially decreasing their antimicrobial efficacy. The common indirect approach to evaluate the propensity of a molecule to bind serum proteins is the determination of its MIC in the presence of serum. In order to evaluate our serum protein binding hypothesis, we determined MIC values of the most potent compounds, **8**, **21** and **22** against all MRSA strains in the presence of 10% fetal bovine serum (FBS). The results indicated that the MIC values for these compounds were identical in the presence and absence of 10% FBS. This finding suggests that these compounds do not significantly bind to serum proteins and this would increase the amount of free drug and thus the anti-MRSA activity.

The MIC values obtained for ciprofloxacin, vancomycin and penicillin G against the various MRSA strains in this study are consistent with previous studies. For example, the MIC value for vancomycin is typically 0.5–2 μg/mL [21], similar to our results (MIC = ≥0.97 μg/mL). As expected, the MIC values for penicillin G were relatively high (15.6–62.5 μg/mL) and this is consistent with the fact that MRSA strains are resistant to penicillin G. The MIC value for ciprofloxacin (7.8 μg/mL for all strains, except ATCC 700787, which was 62.5 μg/mL) is similar to that of earlier report [38]. However, the *in vitro* susceptibility of MRSA strains is highly variable and furthermore, resistance to the fluoroquinolone antibiotics is increasing in CA- and HA-MRSA. Consequently, they are not

routinely recommended for use in the treatment of MRSA. The activity of compounds **21** and **22** against the CA-MRSA strain used in this study is important as it is a USA300 genotype. The USA300, a highly virulent strain, is characterized by its widespread dissemination and greater likelihood to produce necrotizing pneumonia, invasive skin and skin structure infections and sepsis [2,39]. In addition, compounds **21** and **22** were active against MRSA BAA-39, a strain that has *in vitro* resistance to clindamycin, erythromycin, gentamicin, tobramycin, imipenem, tetracycline, amoxicillin and five different cephalosporins. This suggests that these compounds may be effective against MRSA strains that are multi-drug resistance. It has been reported that several arylalkylidene rhodanines interact with PBPs [27]. In addition, all MRSA strains harbor the *mec* gene that codes for the PBP2a protein [12,18]. Therefore, it is possible that these inhibitors may be blocking functionality of PBP2a, although this remains to be proven experimentally.

3. Conclusions

We report the synthesis and anti-MRSA activity of a series of glycine and phenylalanine-derived rhodanine analogs. The SAR study clearly suggests the presence of the hydrophobic side chains of valine (compound **8**), leucine (compound **11**) and phenylalanine (compounds **12**, **21**–**22**) at the N3-position and the electron deficient benzylidene moiety at C5-position of the rhodanine scaffold improves the activity of these compounds against MRSA. This could be due to these substituents increasing their ability to penetrate the bacterial cell wall. The antibacterial activity of compounds **21** and **22** against a panel of MRSA strains was significantly greater than that of the reference antibiotics penicillin G and ciprofloxacin. Most importantly, compound **22** exhibited only a 2–4-fold higher MIC value than that of vancomycin. The anti-MRSA activity of the most potent compounds **8**, **21** and **22** remained unchanged in the absence or presence of 10% FBS. Currently, the mechanism of action of the compounds tested in this study is unknown. Compounds **21** and **22** did produce a bactericidal action on selected MRSA strains. Typically, antibiotics can produce a bactericidal effect by inhibiting cell wall synthesis or DNA synthesis. Thus, it is possible that the bactericidal compounds in this study could produce their anti-MRSA activity via these mechanisms, although this remains to be proven. We conclude from this study that the phenylalanine-derived compounds **21** and **22** are promising templates for the development of new drugs to treat MRSA infections.

4. Experimental

4.1. Chemistry-general

Melting points (mp) were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The reagents for organic synthesis were purchased from Aldrich Chemical Co. (Milwaukee, WI), TCI America (Portland, OR), Alfa Aesar (Ward Hill, MA), and Acros Organics (Antwerp, Belgium) and were used as received. All compounds were checked for homogeneity by TLC using silica gel as a stationary phase. NMR spectra were recorded on a Bruker 400 Avance DPX spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz) outfitted with a *z*-axis gradient probe. The chemical shifts for ^{1}H and ^{13}C are reported in parts per million (δ ppm) downfield from tetramethylsilane (TMS) as an internal standard. The ^{1}H NMR data are reported as follows: chemical shift, multiplicity (s) singlet, (d) doublet, (t) triplet, (m) multiplet. The C, H, and N analyses were performed by Atlantic Microlabs, Inc., (Norcross, GA) and the observed values were within ±0.4% of calculated values.

All strains of MRSA (BAA-39, BAA-1680, 34404, 700698 and 700787) were purchased from ATCC (Manassas, VA). Mueller Hinton II cation-adjusted broth was purchased from Teknova (Hollister, CA). Mueller Hinton agar was purchased from HiMedia (India). *N*,*N*-dimethyl formamide (DMF) was purchased from USB Corporation (Cleveland, OH), and fetal bovine serum from Atlanta Biologicals (Atlanta, GA). Antibiotics were purchased as follows: vancomycin hydrochloride and penicillin G (potassium salt) from Calbiochem (LaJolla, CA) and ciprofloxacin from USP (Rockville, MD). Plates used from microdilution techniques were purchased from Becton–Dickinson (FranklinLakes, NJ).

4.2. General procedure for the preparation of 5-benzylidene rhodanine-3-acetic acid derivatives (**14**–**18**)

Rhodanine-3-acetic acid **4** (0.3 g, 1.57 mmol) was added to a three neck reaction flask equipped with a reflux condenser containing 6 mL anhydrous toluene, followed by the addition of ammonium acetate (1.60–3.20 mmol) and 1.57 mmol of the corresponding aromatic aldehydes. The reaction mixture was refluxed for 1–2 h under inert conditions and the reaction was monitored by TLC. The reaction mixture was evaporated under vacuum, and the residue was dissolved in water. The aqueous solution was then acidified with concentrated HCl and the precipitated product was extracted into ethyl acetate, dried over sodium sulfate and evaporated under vacuum to obtain the purified target compounds **14**–**18** (compounds **20**–**22** were purified by column chromatography).

4.3. 2-(5-(4-Methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid (**14**)

Yellow solid. Yield = 0.39 g, (81%), mp 210–214 °C (lit. mp 190–193 °C) [40]; R_f = 0.37 (DCM:MeOH 90:10), ^{1}H NMR (DMSO-d_6) δ 3.84 (3H, s), 4.72 (2H, s), 7.13 (2H, d, J = 4.4 Hz), 7.66 (2H, d, J = 5.2 Hz), 7.86 (1H, s, CH=), 13.43 (1H, s); ^{13}C NMR (DMSO-d_6) δ 45.5, 56.1, 115.7, 118.9, 125.8, 133.6, 134.6, 162.2, 166.9, 167.8, 193.7. Anal. Calcd. for $C_{13}H_{11}NO_4S_2$: C, 50.47; H, 3.58; N, 4.53. Found: C, 50.61; H, 3.49; N, 4.44.

4.4. 2-(5-(4-Quinolinylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid quinolin-4-yl (**15**)

Yellow solid. Yield = 0.42 g, (84%), mp 97–102 °C; R_f = 0.35 (DCM:MeOH 90:10), ^{1}H NMR (DMSO-d_6) δ 4.44 (2H, s), 7.73 (1H, d, J = 4.64 Hz), 7.79 (1H, t, J = 7.66 Hz), 7.95 (1H, t, J = 7.64 Hz), 8.18 (1H, d, J = 8.20 Hz), 8.29 (1H, d, J = 8.12 Hz), 8.51 (1H, s, CH=), 9.10 (1H, d, J = 4.68 Hz), 13.34 (1H, s); ^{13}C NMR (DMSO-d_6) δ 47.8, 120.3, 124.4, 125.6, 127.1, 128.3, 130.7, 138.7, 148.5, 150.9, 166.3, 167.8, 174.4, 193.6. Anal. Calcd. for $C_{14}H_{11}NO_3S_2$. $^3/_4$ H_2O: C, 52.39; H, 3.37; N, 8.15. Found: C, 52.65; H, 3.57; N, 7.83.

4.5. 2-(5-(Cinnamylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid styryl (**16**)

Yellow solid. Yield = 0.37 g, (77%), mp 220–223 °C (lit. mp >217 °C) [41]; R_f = 0.34 (DCM:MeOH 94:6), ^{1}H NMR (DMSO-d_6) 4.45 (2H, s), 7.16 (1H, m), 7.45 (4H, m), 7.64 (1H, d), 7.74 (2H, d, CH= and ArCH=), 13.44 (1H, s); ^{13}C NMR (DMSO-d_6) δ 45.4, 123.6, 124.0, 128.7, 129.4, 130.7, 134.9, 135.9, 146.5, 166.2, 167.8, 193.4. Anal. Calcd. for $C_{14}H_{11}NO_3S_2$. 1/5H_2O: C, 54.53; H, 3.70; N, 4.54. Found: C, 54.53; H, 3.34; N, 4.63.

4.6. 2-(5-(3-Phenoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid (**17**)

Brown solid. Yield = 0.45 g, (87%), mp 178–180 °C [31]; R_f = 0.62 (DCM:MeOH 80:20), ^{1}H NMR (DMSO-d_6) δ 4.73 (2H, s), 7.10 (2H, d,

J = 8.52 Hz), 7.22 (2H, m), 7.28 (1H, s), 7.45 (3H, t, J = 7.68 Hz), 7.58 (1H, t, J = 7.98 Hz), 7.90 (1H, s, CH=), 13.46 (1H, s); ^{13}C NMR (DMSO-d_6) δ 48.4, 119.7, 120.0, 120.9, 124.2, 124.6, 125.6, 130.7, 131.6, 132.0, 135.4, 156.2, 158.0, 167.1, 167.3, 193.3. Anal. Calcd. for $C_{18}H_{13}NO_4S_2$. 1/2H_2O: C, 56.83; H, 3.71; N, 3.68. Found: C, 57.10; H, 3.75; N, 3.72.

4.7. 2-(5-(Cyclohexylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (**18**)

Brown solid. Yield = 0.39 g, (86%), mp 138–141 °C (lit. mp 209–211 °C) [36]; R_f = 0.89 (DCM:MeOH 80:20), ^{1}H NMR (DMSO-d_6) δ 1.28 (5H, m), 1.66 (6H, m), 4.66 (2H, s), 6.94 (1H, d, J = 9.52, CH=), 13.40 (1H, s); ^{13}C NMR (DMSO-d_6) δ 25.1, 25.5, 30.8, 31.7, 41.5, 45.3, 72.8, 124.3, 144.6, 165.5, 167.8, 194.2. Anal. Calcd. for $C_{12}H_{15}NO_3S_2$: C, 50.51; H, 5.30; N, 4.91. Found: C, 50.81; H, 5.44; N, 4.80.

4.8. Preparation of 2-(4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**19**)

In a round-bottomed flask equipped with a magnetic stirrer, phenylalanine (5 g, 30.3 mmol) was dissolved with sodium hydroxide (1.21 g, 30.3 mmol) in water (25 mL). Then, carbon disulfide (2.3 g, 30.3 mmol) was added to the reaction mixture, which was stirred vigorously overnight. An aqueous solution of sodium chloroacetate (3.51 g, 30.3 mmol) was added and stirring was continued at 23 °C for 3 h. Then the reaction mixture was acidified with dilute HCl until pH 1.0 and refluxed overnight. The reaction mixture was neutralized with saturated $NaHCO_3$ solution. The resultant solution was acidified again with dilute HCl. The cyclized product was extracted in ethyl acetate, dried over anhydrous sodium sulfate and evaporated under vacuum and the residue was purified by column chromatography to afford a brown liquid. Yield = 2.55 g, (30%); R_f = 0.7 (DCM:MeOH 95:05), ^{1}H NMR (DMSO-d_6) δ 3.41 (2H, m), 4.27 (2H, m), 5.65 (1H, s), 7.14 (1H, m), 7.17 (3H, t, J = 6.82), 7.23 (1H, m), 13.37 (1H, s).

4.9. 2-(5-(2-Nitrobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**20**)

Yellow solid. Yield = 0.087 g, (25%), mp 170–175 °C; R_f = 0.68 (DCM:MeOH 95:5), ^{1}H NMR (DMSO-d_6) δ 3.48 (2H, m), 5.87 (1H, s), 7.21 (5H, m), 7.69 (1H, d, J = 7.52 Hz), 7.75 (1H, t, J = 7.78 Hz), 7.87 (1H, t,J = 7.44 Hz), 8.04 (1H, s, CH=), 8.22 (1H, d, J = 8.08 Hz), 13.59 (1H, s); ^{13}C NMR (DMSO-d_6) δ 33.6, 58.9, 115.6, 117.9, 122.0, 125.6, 126.1, 127.2, 128.8, 129.0, 129.4, 130.0, 131.3, 132.1, 135.2, 148.4, 166.2, 169.1, 193.6. Anal. Calcd. for $C_{19}H_{14}N_2O_5S_2$. 2 CH_2Cl_2: C, 43.17; H, 3.10; N, 4.79. Found: C, 43.33; H, 3.09; N, 4.98.

4.10. 2-(5-(3,4-Dichlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**21**)

Yellow solid. Yield = 0.41 g, (89%), mp 138–140 °C; R_f = 0.54 (DCM:MeOH 95:5), ^{1}H NMR (DMSO-d_6) δ 3.51 (2H, d, J = 5.32 Hz), 5.86 (1H, s), 7.18 (5H, m), 7.54 (1H, d, J = 8.4 Hz), 7.79 (2H, m), 7.94 (1H, s, CH=), 13.42 (1H, s); ^{13}C NMR (DMSO-d_6) δ 33.6, 59.2, 116.1, 116.5, 123.4, 127.2, 128.8, 129.4, 130.0, 131.5, 132.1, 132.7, 133.4, 133.9, 134.0, 137.2, 166.7, 169.0, 192.8. Anal. Calcd. for $C_{19}H_{13}Cl_2NO_3S_2$.: C, 52.06; H, 2.99; N, 3.20. Found: C, 51.93; H, 3.09; N, 3.34.

4.11. 2-(5-(3-Phenoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (**22**)

Yellow solid. Yield = 0.22 g, (44%), mp 63–65 °C; R_f = 0.6 (DCM:MeOH 95:5), ^{1}H NMR (DMSO-d_6) δ 3.49 (2H, d, J = 4.6 Hz), 5.85 (1H, s), 7.08 (2H, d, J = 7.84 Hz), 7.18 (8H, m), 7.37 (1H, d, J = 7.68 Hz), 7.44 (2H, t, J = 7.2 Hz), 7.55 (1H, t, J = 7.88 Hz), 7.79 (1H, s, CH=), 13.52 (1H, s); ^{13}C NMR (DMSO-d_6) δ 29.0, 33.1, 58.2, 119.3, 120.0, 121.0, 121.6, 123.0, 124.2, 125.3, 126.8, 128.3, 129.0, 130.3, 131.3, 133.3, 134.5, 136.5, 140.8, 155.8, 157.6, 166.3, 168.7, 192.6. Anal. Calcd. for $C_{25}H_{19}NO_4S_2$. 1/5C_6H_{14}: C, 65.78; H, 4.51; N, 2.93. Found: C, 65.48; H, 4.89; N, 2.79.

4.12. Test for antibacterial activity

All purchased and synthesized compounds were tested for their *in vitro* antibacterial activity against all strains of MRSA by performing a microdilution minimal inhibitory concentration (MIC) technique. Initially, a 2 mg/mL concentration of a compound was made by dissolving it in *N*,*N*-dimethyl formamide (DMF) and then adding cation-adjusted Mueller Hinton broth. The quantity of DMF added never exceeded 0.5% of the total media volume. The MIC assays were carried out in 96 well sterile plates. Serial dilution of the compounds was made in Mueller Hinton broth (100 μL). Appropriate cell concentrations of each strain of MRSA were made in Mueller Hinton broth by adjusting the turbidity to a 0.5 McFarlane Standard and then adding 0.2 mL of the suspension to 40 mL of fresh Mueller Hinton Broth. One hundred microliters of cell suspension was added to each well. Control wells contained Mueller Hinton broth and cell suspension but no compound. Each compound was tested against all strains of MRSA minimally in triplicate. The MIC of each compound was recorded as the well showing no turbidity when compared to control wells.

MBCs were established by extending the MIC procedure to the evaluation of bactericidal activity. After 24 h, 5 μL was drawn from the wells and spotted onto suitable agar plates. The plates were incubated at 37 °C overnight. The MBC read 18 h later was defined as the lowest concentration of compound or standard reference antibiotics that resulted in 0.1% survival in the subculture. All the experiments were performed in triplicate.

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References

[1] R.M. Klevens, M.A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L.H. Harrison, R. Lynfield, G. Dumyati, J.M. Townes, A.S. Craig, E.R. Zell, G.E. Fosheim, L.K. McDougal, R.B. Carey, S.K. Fridkin, For the active bacterial core surveillance (ABCs) MRSA investigators, invasive methicillin-resistant staphylococcus aureus infections in the United States, JAMA 298 (2007) 1763–1771.
[2] R. Gordon, F. Lowy, Pathogenesis of methicillin-resistant staphylococcus aureus infection, Clin. Infect. Dis. 46 (2008) S350–S359.
[3] M. Patel, Community-associated methicillin-resistant staphylococcus aureus infections: epidemiology, recognition and management, Drugs 69 (2009) 693–716.
[4] T.T. Chavez, C.F. Decker, Health care-associated MRSA versus community-associated MRSA, Dis.-Mon. 54 (2008) 763–768.
[5] W. Brumfitt, J. Hamilton-Miller, Methicillin-resistant staphylococcus aureus, N. Engl. J. Med. 320 (1989) 1188–1196.
[6] F.D. Lowy, Staphylococcus aureus infections, N. Engl. J. Med. 339 (1998) 520–532.
[7] T.S. Naimi, K.H. LeDell, K. Como-Sabetti, S.M. Borchardt, D.J. Boxrud, J. Etienne, S.K. Johnson, F. Vandenesch, S. Fridkin, C. O'Boyle, R.N. Danila, R. Lynfield, Comparison of community- and health care-associated methicillin-resistant staphylococcus aureus infection, JAMA 290 (2003) 2976–2984.
[8] H. Boucher, G.A. Corey, Epidemiology of methicillin-resistant staphylococcus aureus, Clin. Infect. Dis. 46 (2008) S344–S349.

[9] B.C. Herold, L.C. Immergluck, M.C. Maranan, D.S. Lauderdale, R.E. Gaskin, S. Boyle-Vavra, C.D. Leitch, R.S. Daum, Community-acquired methicillin-resistant staphylococcus aureus in children with no identified predisposing risk, JAMA 279 (1998) 593–598.
[10] C.D. Salgado, B.M. Farr, D.P. Calfee, Community-acquired methicillin-resistant staphylococcus aureus: a meta-analysis of prevalence and risk factors, Clin. Infect. Dis. 36 (2003) 131–139.
[11] F.R. DeLeo, M. Otto, B.N. Kreiswirth, H.F. Chambers, Community-associated methicillin-resistant staphylococcus aureus, Lancet 375 (2010) 1557–1568.
[12] T. Ito, Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, K. Hiramatsu, Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant staphylococcus aureus, Antimicrob. Agents Chemother. 45 (2001) 1323–1336.
[13] Y. Katayama, T. Ito, K. Hiramatsu, A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in staphylococcus aureus, Antimicrob. Agents Chemother. 44 (2000) 1549–1555.
[14] R.H. Deurenberg, C. Vink, S. Kalenic, A.W. Friedrich, C.A. Bruggeman, E.E. Stobberingh, The molecular evolution of methicillin-resistant staphylococcus aureus, Clin. Microbiol. Infect. 13 (2007) 222–235.
[15] C. Fuda, M. Suvorov, S.B. Vakulenko, S. Mobashery, The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant staphylococcus aureus, J. Biol. Chem. 279 (2004) 40802–40806.
[16] H.F. Chambers, The changing epidemiology of staphylococcus aureus, Emerg. Infect. Dis. 7 (2001) 178–182.
[17] S. Deresinski, Methicillin-resistant staphylococcus aureus: an evolutionary, epidemiologic, and therapeutic odyssey, Clin. Infect. Dis. 40 (2005) 562–573.
[18] K. Okuma, K. Iwakawa, J.D. Turnidge, W.B. Grubb, J.M. Bell, F.G. O'Brien, G.W. Coombs, J.W. Pearman, F.C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito, K. Hiramatsu, Dissemination of new methicillin-resistant staphylococcus aureus clones in the community, J. Clin. Microbiol. 40 (2002) 4289–4294.
[19] R.S. Daum, T. Ito, K. Hiramatsu, F. Hussain, K. Mongkolrattanothai, M. Jamklang, S. Boyle-Vavra, A novel methicillin-resistance cassette in community-acquired methicillin-resistant "staphylococcus aureus" isolates of diverse genetic backgrounds, J. Infect. Dis. 186 (2002) 1344–1347.
[20] X.X. Ma, T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R.S. Daum, K. Hiramatsu, Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant staphylococcus aureus strains, Antimicrob. Agents Chemother. 46 (2002) 1147–1152.
[21] J.P. Powell, R.P. Wenzel, Antibiotic options for treating community-acquired MRSA, Expert Rev. Anti. Infect. Ther. 6 (2008) 299–307.
[22] W.T. Sing, C.L. Lee, S.L. Yeo, S.P. Lim, M.M. Sim, Arylalkylidene rhodanine with bulky and hydrophobic functional group as selective HCV NS3 protease inhibitor, Bioorg. Med. Chem. Lett. 11 (2001) 91–94.
[23] G. Bruno, L. Costantino, C. Curinga, R. Maccari, F. Monforte, F. Nicolò, R. Ottanà, M.G. Vigorita, Synthesis and aldose reductase inhibitory activity of 5-arylidene-2,4-thiazolidinediones, Bioorg. Med. Chem. 10 (2002) 1077–1084.
[24] H. Fujishima, K. Tsubota, Improvement of corneal fluorescein staining in post cataract surgery of diabetic patients by an oral aldose reductase inhibitor, ONO-2235, Br. J. Ophthalmol. 86 (2002) 860–863.
[25] E.B. Grant, D. Guiadeen, E.Z. Baum, B.D. Foleno, H. Jin, D.A. Montenegro, E.A. Nelson, K. Bush, D.J. Hlasta, The synthesis and SAR of rhodanines as novel class C beta-lactamase inhibitors, Bioorg. Med. Chem. Lett. 10 (2000) 2179–2182.
[26] M.M. Sim, S.B. Ng, A.D. Buss, S.C. Crasta, K.L. Goh, S.K. Lee, Benzylidene rhodanines as novel inhibitors of UDP-N-Acetylmuramate/-Alanine ligase, Bioorg. Med. Chem. Lett. 12 (2002) 697–699.
[27] A. Zervosen, W. Lu, Z. Chen, R.E. White, T.P. Demuth Jr., J. Frere, Interactions between penicillin-binding proteins (PBPs) and two novel classes of PBP inhibitors, arylalkylidene rhodanines and arylalkylidene iminothiazolidin-4-ones, Antimicrob. Agents Chemother. 48 (2004) 961–969.
[28] Y. Momose, K. Meguro, H. Ikeda, C. Hatanaka, S. Oi, T. Sohda, Studies on antidiabetic agents. X. Synthesis and biological activities of pioglitazone and related compounds, Chem. Pharm. Bull. (Tokyo) 39 (1991) 1440–1445.
[29] C.A. Whitesitt, R.L. Simon, J.K. Reel, S.K. Sigmund, M.L. Phillips, J. Kevin Shadle, L.J. Heinz, G.A. Koppel, D.C. Hunden, S.L. Lifer, D. Berry, J. Ray, S.P. Little, X. Liu, W.S. Marshall, J.A. Panetta, Synthesis and structure-activity relationships of benzophenones as inhibitors of cathepsin D, Bioorg. Med. Chem. Lett. 6 (1996) 2157–2162.
[30] C.A. Free, E. Majchrowicz, S.M. Hess, Mechanism of inhibition of histidine decarboxylase by rhodanines, Biochem. Pharmacol. 20 (1971) 1421–1428.
[31] T.T. Talele, P. Arora, S.S. Kulkarni, M.R. Patel, S. Singh, M. Chudayeu, N. Kaushik-Basu, Structure-based virtual screening, synthesis and SAR of novel inhibitors of hepatitis C virus NS5B polymerase, Bioorg. Med. Chem. 18 (2010) 4630–4638.
[32] E. Andre, L. Bastide, S. Michaux-Charachon, A. Gouby, P. Villain-Guillot, J. Latouche, A. Bouchet, M. Gualtieri, J. Leonetti, Novel synthetic molecules targeting the bacterial RNA polymerase assembly, J. Antimicrob. Chemother. 57 (2006) 245–251.
[33] M. Gualtieri, L. Bastide, P. Villain-Guillot, S. Michaux-Charachon, J. Latouche, J. Leonetti, In vitro activity of a new antibacterial rhodanine derivative against staphylococcus epidermidis biofilms, J. Antimicrob. Chemother. 58 (2006) 778–783.
[34] O. Bozdag-Dündar, Ö. Özgen, A. Mentese, N. Altanlar, O. Atll, E. Kendi, R. Ertan, Synthesis and antimicrobial activity of some new thiazolyl thiazolidine-2,4-dione derivatives, Bioorg. Med. Chem. 15 (2007) 6012–6017.
[35] (a) F. Zuber, E. Sorkin, Über die Herstellung einiger N-substituierter Rhodaninderivate, Helv. Chim. Acta 35 (1952) 1744–1747;
(b) C. Xing, L. Wang, X. Tang, Y.Y. Sham, Development of selective inhibitors for anti-apoptotic Bcl-2 proteins from BHI-1, Bioorg. Med. Chem. 15 (2007) 2167–2176;
(c) V.I. Yakubich, L.V. Gritsyuk, Synthesis of methionine-based rhodanines, Farm. Zh. 1 (1984) 40–43.
[36] Y. Ohishi, T. Mukai, M. Nagahara, M. Yajima, N. Kajikawa, K. Miyahara, T. Takano, Preparations of 5-alkylmethylidene-3-carboxymethylrhodanine derivatives and their aldose reductase inhibitory activity, Chem. Pharm. Bull. (Tokyo) 38 (1990) 1911–1919.
[37] U. Kragh-Hansen, V. Chuang Tuan Giam, M. Otagiri, Practical aspects of the ligand-binding and enzymatic properties of human serum albumin, Biol. Pharm. Bull. 25 (2002) 695–704.
[38] L. Hu, M.L. Kully, D.W. Boykin, N. Abood, Optimization of the central linker of dicationic bis-benzimidazole anti-MRSA and anti-VRE agents, Bioorg. Med. Chem. Lett. 19 (2009) 3374–3377.
[39] C.F. Decker, Pathogenesis of MRSA infections, Dis.-Mon. 54 (2008) 774–779.
[40] J. Zhou, Y. Song, F. Zhu, Y. Zhu, Facile synthesis of 5-benzylidene rhodamine derivatives under microwave irradiation, Synth. Commun. 36 (2006) 3297–3303.
[41] T. Tadao, M. Kawamura, A. Ajima, T. Mohri, M. Hayashi, H. Terashima, F. Hirata, T. Morimura, Rhodanine derivatives and an aldose reductase inhibitor containing the rhodanine derivatives as active ingredients. Patent # EP47109, Appln. # EP1981–303816, August 21, 1981.