Original Article

Antibacterial properties of new 5-substituted derivatives of rhodanine-3-propanoic acid

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ABSTRACT

Bacterial infections present a serious challenge to health care practitioners due to the emergence of resistance to numerous conventional antibacterial drugs. Therefore, new bacterial targets and new antimicrobials are unmet medical needs. Rhodanine derivatives have been shown to possess potent antimicrobial activity through a novel mechanism. However, their antibacterial potential has not been completely explored. Therefore, the present study series of rhodanine-3-propanoic acid derivatives possessing hydroxyl substituted benzylidene moiety at the C-5 position of the rhodanine core was synthesized through Knoevenagel condensation of rhodanine-3-propanoic acid with various hydroxyl substituted aromatic aldehydes. All the compounds were structurally characterized and evaluated *in vitro* for their antibacterial activity against two Gram-positive and two Gram-negative bacterial strains. Biological data showed that various synthesized derivatives exhibited potent antibacterial activity against various tested bacterial strains.

Keywords: Rhodanine, rhodanine-3-propanoic acid, synthesis, antimicrobial, antibacterial

INTRODUCTION

Diseases caused by microbial infection are a serious menace to the health of human beings and is a major cause of death in developed and developing countries.[1] Over the past several years, the emergence of organisms resistant to nearly all the classes of antimicrobial agents has become a serious health concern worldwide. One of the major driving forces for the development and spread of bacterial resistance is the high-antibiotic consumption and sometimes their random use.[2] Some important examples of microbial agents becoming resistant toward the first- and second-line antimicrobial therapies are vancomycin-resistant enterococci, multidrug-resistant *Staphylococcus aureus* (SA), and organism related to *Candida* spp. and *Aspergillus niger*.[3] To overcome resistance, in addition to the prudent use of available drugs, new groups of compounds which may be useful as antibacterial agents have been examined.

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The 2-thiazolidine-4-one derivatives traditionally named rhodanine have been known for over 100 years, and due to their fascinating properties, they are still examined.[4] The molecules based on the 2-thioxo-thiazolidine-4-one (rhodanine) core, as an imperious privileged scaffold, are known for their broad range of pharmacological profiles.[5-7] The structural diversity among 2-thioxo-1,3-thiazolidine-4-one derivatives is exclusively located in two positions, that is, the first is the N-3 position, and the second is the nature of arylidene moiety at the C-5 position. These introduction of the lipophilic or hydrophilic or aromatic group at one of the two key positions (or at both) offers a panel of possibilities for drug design and also for the physicochemical property studies.

Krátký *et al*. reported the synthesis and antimicrobial potential of various phenolic substituted rhodanine derivatives. Results showed that compound **2** was the most promising candidate against tested bacterial strains with MIC values in the range of 32–62.5 μg/mL.[8] Xu *et al*. described antimicrobial properties of rhodanine derivatives with a carboxyalkyl acid moiety at the N-3 position.[9] The research conducted by Miao *et al*. and Patel *et al*. indicated that antibacterial activity of the acid derivatives occurred when a major hydrophobic group was introduced to the arylidene substituent at the C-5 position.[10,11] The best results were achieved when the aryl moiety was additionally substituted with an electron-withdrawing group. Taking into account the data presented by above authors and inspired from our ongoing research related to the design and development of novel antimicrobial agents, we decided to synthesize some new derivatives of rhodanine bearing propanoic acid moiety at *N*-3 position and various hydroxyl substituted benzylidene moiety at C-5 position [Figure 1].

RESULTS AND DISCUSSION

Chemistry

Proposed compounds were synthesized using a synthetic procedure involving two steps. The synthetic scheme was shown in Scheme 1. The synthesis of rhodanine-3-propanoic acid was achieved using β-alanine, carbon disulfide, and sodium chloroacetate as the reactants. In the FT-IR spectrum, a broad absorption band appeared at 3513 cm^{-1} due to O-H stretching of the carboxyl group. Another prominent absorption bands due to two carbonyl groups (C=O) appeared at 1744 and 1698 cm^{-1}. In ^{1}H NMR spectra, two singlets appeared at δ 4.71 and 4.12 ppm due to methylene protons of -N-CH2 and protons of a rhodanine ring methylene group. The spectral data were in full agreement with the structure of rhodanine-3-propanoic acid. Rhodanine propanoic acid was condensed with various substituted aromatic aldehydes. Satisfactory results were obtained when rhodanine propanoic acid was condensed with various aromatic aldehydes using ethanol as solvent and piperidine as a base at 80–90°C. Using the optimized reaction conditions, various proposed compounds have been synthesized successfully. Various title compounds were characterized using physico-chemical data [Table 1] and various spectral data such as IR and ^{1}H NMR. The present synthesis led to the formation of thermodynamically more stable *Z*-isomer in all the cases, as confirmed by the more downfield shift value of methine proton and complies with the literature reported data.[9,12]

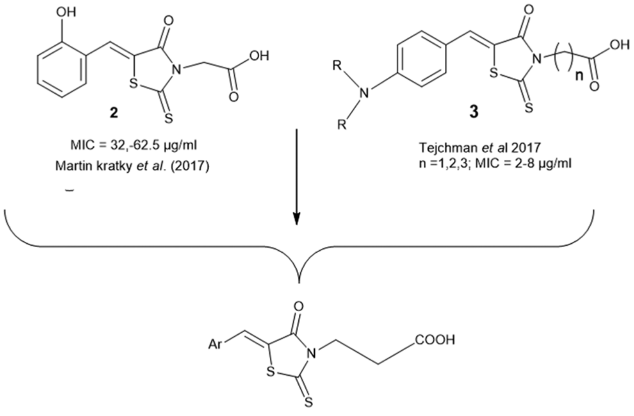
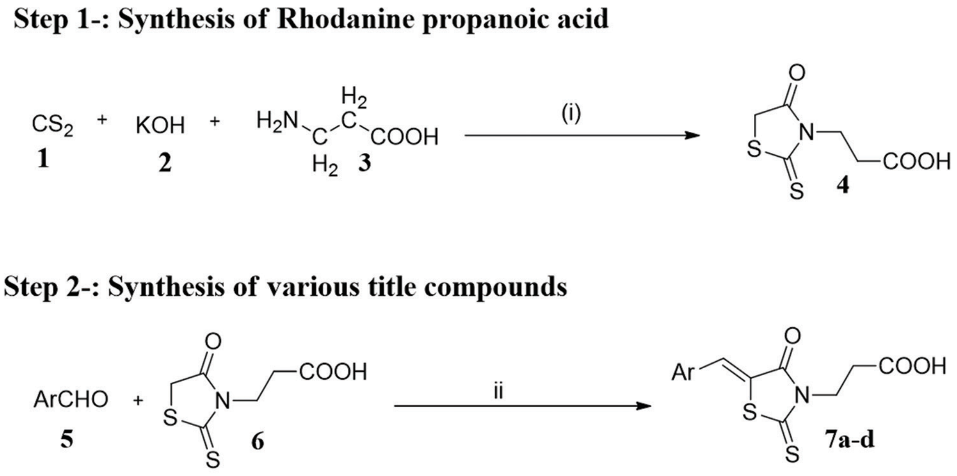


Figure 1: Design of proposed compounds

Antibacterial activity

Antibacterial activity (minimum inhibitory concentration [MIC]) was evaluated using broth dilution method against four bacterial strains, namely, SA and *Streptococcus pyogenes* (SP), representatives of Gram-positive bacteria, while Gram-negative strains involved in the study were *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA). Ciprofloxacin and ampicillin were used as reference drugs for the comparison of antibacterial activity. Results of antimicrobial evaluation data indicated that tested compounds (**7a-d**) were active against various tested microbial strains [Table 2]. Tested compounds displayed excellent to good activity as compare to the standard drugs ampicillin and ciprofloxacin. Some compounds exhibited superior activity against tested bacterial strains as compare to ampicillin; however, all were less potent than the standard drug ciprofloxacin.

Among various tested compounds, **7d** exhibited the highest potency against SA having MIC = 62.5 μg/mL, while other compounds such as **7a** and **7c** also displayed excellent activity against this strain, having MIC of 100 μg/mL and were 2-2.5-fold more potent than the standard drug ampicillin (MIC = 250 μg/mL). Compound **7d** was almost equipotent against SA when compared to ciprofloxacin (MIC= 50 μg/mL). Compounds **7b** was the least active compound against this strain with MIC of 200 μg/mL. Compounds **7c** was the most potent candidate (MIC= 100 μg/mL) against SP and exhibited equipotent activity as compare to standard drug ampicillin



Scheme 1: (i) H2SO4 stirr for 8 h (ii) EtOH, Piperidine, GAA, reflux, 3–4 h

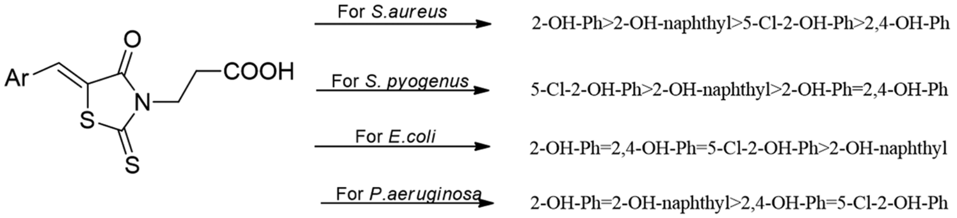
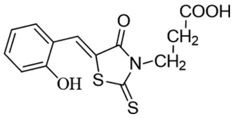
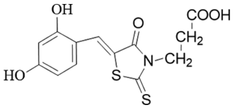
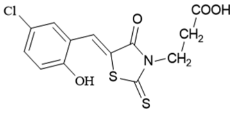
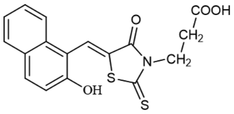


Figure 2: Structure-activity relationship for antibacterial activity

Table 1: The physicochemical data of various title compounds (7a-d)

No.	Structure	Chemical formula	Color	Yield	M. P.	R_f^a
7a		$C_{13}H_{11}NO_4S_2$	Yellow	62%	210–211°C	0.61
7b		$C_{13}H_{11}NO_5S_2$	Yellow	64%	246–247°C	0.67
7c		$C_{13}H_{10}ClNO_4S_2$	Yellow	63%	219–220°C	0.66
7d		$C_{17}H_{13}NO_4S_2$	Yellow	67%	246–248°C	0.64

[a]Hexane: Ethyl acetate (3:2)

Table 2: Antibacterial activity of various title compounds 7a-d

Code	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*
7a	100	250	200	100
7b	200	250	100	125
7c	100	100	100	125
7d	62.5	200	125	100
Ampicillin	250	100	100	--
Ciprofloxacin	50	50	25	25

(MIC = 100 µg/mL). Other compounds were found to be moderately active against this strain (MIC = 200–250 µg/mL) as compared to the standard drugs. Compound **7b** and **7c** presented the highest potency (MIC = 100 µg/mL) against Gram-negative bacteria EC followed by **7d** having MIC of 125 µg/mL. Against PA, derivatives **7a** and **7d** presented significant potency having MIC of 100 µg/mL and were 4-fold less potent as compared to the standard drug ciprofloxacin (MIC = 50 µg/mL), whereas other two compounds (**7b** and **7c**) were 5-fold less (MIC = 125 µg/mL) potent as compared to ciprofloxacin. The structure-activity correlation for various tested strains is shown in Figure 2.

From the antimicrobial activity data, it can be concluded that the nature of substituent present at the C-5 position of the rhodanine core has a crucial impact on the activity exhibited by these compounds against tested bacterial strains. Substitution with hydroxyl substituted arylidene moiety at the C-5 position of rhodanine core provided compounds with potent antibacterial microbial activity against a tested panel of bacterial strains. The introduction of halogen atom on the arylidene moiety improves the antibacterial activity against various tested bacterial strains except PA. Further, the replacement of hydroxyphenyl moiety (**7a**) with hydroxynaphthyl (**7d**) improves the spectrum of antibacterial potency against SA. Our observations were in accordance with research findings reported in the literature.[8,12]

CONCLUSION

The present work involved design, synthesis, and characterization and *in vitro* evaluation studies of new rhodanine derivatives as potent antibacterial agents. Four new derivatives were synthesized, structurally characterized, and evaluated *in vitro* for antibacterial activity. Results revealed that the synthesized compounds exhibited potent to good antibacterial activity. In particular, compounds **7c** and **7d** presented the most promising antibacterial profile against various tested bacterial strains with low micromolar values (MIC 62.5–200 µg/mL) and were active against all the tested strains of bacteria. The antimicrobial activity was modulated by the nature of substituent attached to the 5th position of the rhodanine core. The present study identified new rhodanine propanoic acid derivatives as potential antibacterial agents, and further studies with the identified lead molecules would be helpful in the design and development of candidates with more potent antimicrobial profile.

Experimental

All the intermediates and title compounds were synthesized using solution-phase chemistry. The progress of reactions was monitored by TLC. From the TLC, we ensured to declare the completion of the reaction. The TLC plates were visualized by viewing in UV and iodine chamber. The reaction products were purified by different work-up processes to remove unreacted starting material and impurities. Recrystallization or repeated recrystallization was done using suitable solvents to get a pure sample of title compounds. In few cases, intermediate or title compounds were purified by column chromatography. Melting points and R$_f$ values of all the compounds and intermediates were determined. The structure and purity of the anticipated compounds were characterized by physical constants and FT-IR spectral studies initially followed by ^{1}H-NMR spectroscopic data. The synthetic scheme consists of two steps.

General procedure for the synthesis of rhodanine-3-propanoic acid [2-(4-oxo-2- thioxothiazolidin-3-yr) propanoic acid, 4] (Step-1)

In a 50 mL round-bottomed flask provided with a magnetic stirrer and condenser, β-alanine (1 g, 11.22 mmol) was solubilized in a solution of 22% potassium hydroxide at room temperature. To this, a homogeneous solution was added carbon disulfide (0.7 mL, 11.22 mmol) drop-wise during 10 min at 25˚C. After addition, peach to orange color appeared, and the contents were stirred over a period of 3h. Now, a solution of chloroacetic acid (1.06 gm, 11.22 mmol) in water was added in small portions to the reaction mixture. The yellow color appeared, and the solution was vigorously stirred at 25 ˚C for 3 h. The resulting solution was acidified to pH 4 with concentrated hydrochloric acid, and the suspension was stirred over a period of 8 h. After this, the contents were cooled and the resulting solid was collected by filtration, washed with water, and dried under high vacuum. The crude product was purified by recrystallization from ethanol to afford crystals of pure product.[13]

*General procedure for the synthesis of arylidene derivatives of rhodanine propionic acid (**7a-d**, Step-2)*

A mixture of rhodanine propionic acid (0.1 mmol) and substituted aromatic aldehyde (0.1mmol) was suspended in absolute alcohol (10 mL). To this, catalytic amount of piperidine and glacial acetic acid was added. The mixture was then allowed to reflux with stirring at 80°C for 4–7 h. The completion of the reaction was monitored with the help of TLC. On completion, the precipitated solid was filtered, washed with absolute ethanol, than with aqueous ethanol solution (2 × 20 mL) and finally with water. After this, the solid was dried. Moreover, the crude product was re-crystallized with aqueous ethanol to afford pure compound in good to excellent yield.

(Z)-3-(5-(2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (***7a***)

IR (KBr) ν_{max} (cm^{-1}): 3218 (O-H), 3050 (aromatic C-H stretching), 2907(aliphatic C-H stretching), 1705 and 1681 (C=O), 1659 (C=N), 1590 (C=C), 1071 (C-N), 669 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): 10.25 (s, 1H, OH), 7.66–7.31 (m, 5H, Ar-H and -CH=), 4.23–4.18 (t, *J* = 7.8 Hz, 2H, N-CH$_2$), 2.61–2.56 (t, *J* = 7.8 Hz, 2H, -CH$_2$-COOH).

(Z)-3-(5-(2,4-dihydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (7b)

IR (KBr) ν_{max} (cm^{-1}): 3318 (O-H), 3050 (aromatic C-H stretching), 2907 (aliphatic C-H stretching), 1705 and 1681 (C=O), 1659 (C=N), 1590 (C=C), 1071 (C-N), 669 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): 10.68 (s, 1H, OH), 8.26–8.19 (bs, 1H, OH), 7.83 (s, 1H, -CH=), 7.55–7.52 (d, *J* = 7.8 Hz, 1H, Ar-H), 6.79–6.71 (m, 1H, Ar-H), 4.27–4.22 (t, *J* = 7.6 Hz, 2H, N-CH$_2$), 2.38–2.27 (m, 2H, -CH$_2$-COOH).

(Z)-3-(5-(5-chloro-2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)propanoic acid (7c)

IR (KBr) ν_{max} (cm^{-1}): 3434 (O-H), 3055 (aromatic C-H stretching), 2924 (aliphatic C-H stretching), 1717 and 1679 (C=O), 1590 (C=C), 1171 (C-N), 794 (C-Cl). 669 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.54 (bs, 1H, COOH), 7.90 (s, 1H, -CH=), 7.47–7.37 (m, 2H, Ar-H), 7.01–6.98 (d, *J* = 8.6 Hz, 1H, Ar-H), 4.28–4.23 (t, 2H, *J* = 7.5 Hz, N-CH$_2$-CH$_2$-), 2.68–2.63 (t, *J* = 7.5 Hz, 2H, N-CH$_2$-CH$_2$-).

(Z)-3-(5-((2-hydroxynaphthalen-1-yl)methylene)-4-oxo-2-thioxothiazoli-din-3-yl)propanoic acid (***7d***)

IR (KBr) ν_{max} (cm^{-1}): 3450 (O-H), 3050 (aromatic C-H stretching), 2857 (aliphatic C-H stretching), 1700 and 1696 (C=O), 1625 (C=N), 1548 (C=C), 1153 (C-N), 669 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25–9.21 (m, 1H, Ar-H), 8.56–8.53 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.16–8.13 (d, *J* = 9.0 Hz, 1H, Ar-H), 8.01–7.98 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.71–7.53 (m, 3H, Ar-H and -CH=), 4.27–4.22 (t, 2H, *J* = 7.2 Hz, N-CH$_2$-CH$_2$-), 2.67–2.62 (t, *J* = 7.2 Hz, 2H, N-CH$_2$-CH$_2$-).

Procedure for the determination of MIC values

The MICs of synthesized compounds were carried out by broth microdilution method as described by Rattan.[14] DMSO was used as diluents to get the desired concentration of drugs to test on standard bacterial strains. Antibacterial activity was screened against two Gram-positive SA, SP, and two Gram-negative (EC, PA) bacteria using ciprofloxacin as a standard antibacterial agent.

Mueller-Hinton broth and Sabouraud's broth were used as a nutrient medium to grow for bacteria and fungus, respectively. Inoculum size for test strain was adjusted to 106 CFU (colony forming unit) per milliliter by comparing the turbidity. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of a plate of medium suitable for the growth of test organism and put for incubation at 37°C for bacteria. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting the growth of the organism was recorded as the MIC. Each test compound was diluted obtaining 1000 μg/mL concentration, as a stock solution. In primary screening 500, 250, and 125 μg/mL, concentrations of the test compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all organisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, and 6.25 μg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC (Rattan *et al*., 2000).

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