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Original Article

DESIGN, SYNTHESIS AND ANTIMICROBIAL STUDIES OF 5-BENZYLIDENE SUBSTITUTED RHODANINE CONTAINING HETEROCYCLES

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ABSTRACT

Objective: The principal objective of the study was to synthesize and evaluate the biological activities of a novel class of 5-benzylidene substituted rhodanine derivatives as antimicrobial agents.

Methods: All the synthesized compounds (D1-D10) were screened for their antimicrobial activities using microdilution methods as per the reported procedure. All compounds were evaluated as potential antimicrobial agents against gram-positive bacteria: *Bacillus cereus, Staphylococcus aureus, gram negative bacteria: Escherichia coli Pseudomonas aeruginosa and Klebsiella pneumoniae* Fungal cultures used in the study were *Aspergillus niger, Candida albicans, Candida parapsilosis, Candida tropicalis and Candida glabrata*.

Results: Compound D6 showed good antifungal activity in the MIC range 16µg/ml against *Candida tropicalis* and Compound D10 showed good antifungal activity in the MIC range 16µg/ml against *Candida glabrata*. Compounds D2 and D5 showed good antibacterial activity at 32µg/ml. all the other compounds showed moderate antibacterial activity.

Conclusion: Based on the above results, it can be concluded that the compounds may lead to the development of more potent antimicrobial drug candidates in the near future.

Keywords: Anti-fungal, Rhodanine, Microdilution, Anti-bacterial

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INTRODUCTION

Design, synthesis and development of pharmaceutically active molecules have always been a primary objective of medicinal chemistry. Treatment of infectious diseases is a challenging task for researchers and due to the increasing number of multidrug-resistant microbial pathogens, the discovery of new molecules to combat drug resistance is always a necessity [1-6]. The existing antimicrobial drugs for the treatment of infectious diseases are insufficient to protect us for the long term because of an increasing number of resistant strains. Hence there is an emergent need for the development of newer antimicrobial agents with a new mode of action. Rhodanines are accepted as advantaged heterocycles in medicinal chemistry as one of the 4-thiazolidinones subtypes [7-11]. Thioxo-thiazolidinone (Rhodanine) and its derivatives [12, 13] are reported to have a broad spectrum of biological activities such as antibacterial [14-19], antifungal [20-22], anti-diabetic [23, 24], anti-tubercular [25-27] anticancer [28, 29] anti-HIV [30-38] and antimalarial [39]. Rhodanine derivatives have been investigated for Alzheimer's disease also [40, 41]. Rhodanine (2-thioxothiazolidin-4-one) can be used for chemical modifications such as N-3 and/or "active methylene" C-5 substitution can be found that are capable of generating potential for new bioactive compounds [42, 43]. We, therefore, took up designing new chemical entity of 3-[(dialkylamino) alkyl]-2-thioxothiazolidinone (fig. 1) by substituting various benzylidene derivatives at 5th position and the evaluation of their *in vitro* antimicrobial activity. In this study, we report *in vitro* activity of some very potent 3-(dialkylamino) alkyl substituted rhodanine derivatives.

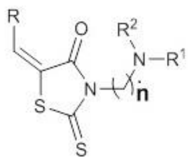


Fig. 1: General structure of designed molecular framework

MATERIALS AND METHODS

Synthesis

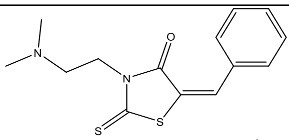
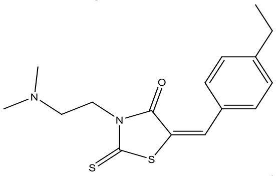
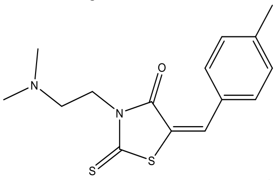
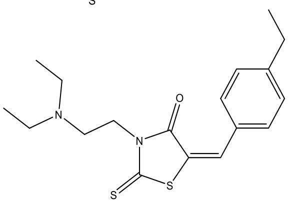
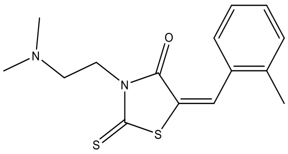
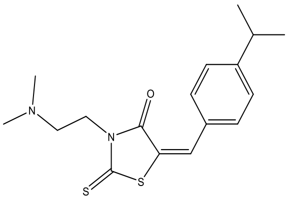
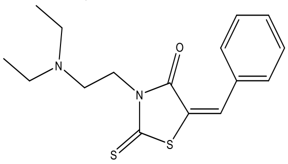
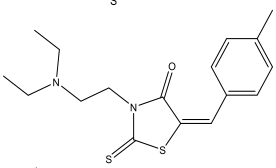
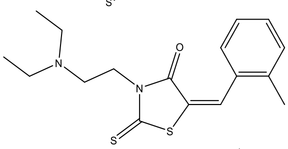
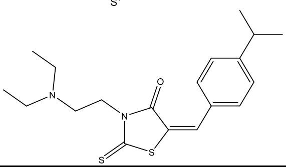
All the chemicals (reagent grade) were obtained from commercial sources and were used as supplied without further purification. Melting points were determined in open capillary tubes on an electrically heated block and are uncorrected. The reaction progress and purity of the synthesized compounds were monitored by analytical thin-layer chromatography (TLC) on pre-coated silica gel plates (Merck India Ltd). IR spectra (ν_{max} in cm^{-1}) of the compounds were recorded on Perkin Elmer's FT-IR RX1 PC spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE III spectrometers (operating at 500 MHz for ^1H, and 125 MHz respectively for ^{13}C) in deuterated solvents with TMS as internal reference (chemical shifts δ in ppm). Electron Spray Ionization Mass spectra (ESI-HRMS) were recorded on Thermo Scientific Exactive plus Orbitrap spectrometer. All spectral analysis data were under the assigned structures. All the compounds were characterised by TLC, IR, ^1H and ^{13}C NMR, and HRMS. All the chemicals and solvents were procured from Sigma-Aldrich/Merck India Ltd. 5-benzylidene substituted Rhodanine containing heterocycles were synthesized as per the reported procedure.

Different derivatives were synthesized (table 1) by the reaction of N–substituted Rhodanine (1), substituted benzaldehyde (2) and ammonium acetate in a minimum amount of acetic acid [44]. Stirred and refluxed the mixture at 80-85 °C. TLC was checked. After completion of the reaction, the reaction mixture was washed with ethyl acetate and dried in a rota vapour to get (3) in good to excellent yield

5-Benzylidene-3-(2-(dimethylamino) ethyl)-2-thioxothiazolidin -4-one (D1)

The title compound was synthesized from 3-(2-(dimethylamino) ethyl)-2-thioxothiazolidin-4-one (0.5g, 0.0024 mol), benzaldehyde (0.24 ml, 0.0024 mol) and ammonium acetate (0.37 g. 0.0048 mol) in a minimum amount of acetic acid. Stirred and refluxed the reaction mixture at 80-85 °C for 1.5 h. The reaction was monitored with TLC. After completion of the reaction, the reaction mixture was washed with ethyl acetate and the residue was dried under reduced pressure. Then the residue was dissolved in chloroform and washed with water. The chloroform layer was evaporated and then again, it was dissolved in a minimum amount of chloroform (5 ml). It was kept in a freezer overnight. Brown coloured solid was obtained, which was separated by filtering it with the help of sintered crucible.

Table 1: Structures of newly designed compounds (D1-D10)

Code	Structure	Name
D1		5-Benzylidene-3-(2-(dimethyl amino)ethyl)-2-thioxothiazolidin-4-one
D2		3-(2-(dimethyl amino) ethyl)-5-(4-ethylbenzylidene)-2-thioxothiazolidin-4-one
D3		3-(2-(dimethyl amino) ethyl)-5-(4-methylbenzylidene)-2-thioxothiazolidin-4-one
D4		3-(2-(diethylamino)ethyl)-5-(4-ethylbenzylidene)-2-thioxothiazolidin-4-one
D5		3-(2-(dimethylamino)ethyl)-5-(2-methylbenzylidene)-2-thioxothiazolidin-4-one
D6		3-(2-(dimethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one
D7		5-benzylidene-3-(2-(diethylamino)ethyl) -2-thioxothiazolidin-4-one
D8		3-(2-(diethylamino)ethyl)-5-(4-methylbenzylidene)-2-thioxothiazolidin-4-one
D9		3-(2-(diethylamino)ethyl)-5-(2-methylbenzylidene)-2-thioxothiazolidin-4-one
D10		3-(2-(diethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one

3-(2-(dimethylamino) ethyl)-5-(4-ethylbenzylidene)-2-thioxo-thiazolidin-4-one (D2)

The title compound was synthesized as per the procedure for compound D1. 3-(2-(dimethylamino) ethyl)-2-thioxothiazolidin-4-one (0.5g, 0.0024 mol), 4-ethylbenzaldehyde (0.328 ml, 0.0024 mol), and ammonium acetate (0.369g, 0.0048 mol) in minimum amount of acetic acid was taken as reaction mixture.

3-(2-(dimethylamino)ethyl)-5-(4-methylbenzylidene)-2-thioxothiazolidin-4-one (D3)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-(dimethylamino)ethyl)-2-thioxo-thiazolidin-4-one (0.5g, 0.0024 mol), 4-methylbenzaldehyde (0.254 ml, 0.00215 mol) and ammonium acetate (0.37g, 0.0048 mol) in minimum amount of acetic acid.

3-(2-(diethylamino)ethyl)-5-(4-ethyl benzylidene)-2-thioxo-thiazolidin-4-one (D4)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-diethylamino) ethyl-2-thioxothiazolidine-4-one (0.5g, 0.0021 mol), 4-ethylbenzaldehyde (0.28 ml, 0.0021 mol) and ammonium acetate (0.324g, 0.0042 mol) in minimum amount of acetic acid.

3-(2-(dimethylamino)ethyl)-5-(2-methylbenzylidene-2-thioxothiazolidin-4-one (D5)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-(diethylamino)ethyl)-2-thioxo-thiazolidin-4-one (0.5g, 0.0024 mol), 2-methylbenzaldehyde (0.277 ml, 0.0024 mol) and ammonium acetate (0.37g, 0.0048 mol) in minimum amount of acetic acid.

3-(2-(dimethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one (D6)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-(dimethylamino)ethyl)-2-thioxothiazolidin-4-one (0.5g, 0.0024 mol), isopropyl benzaldehyde (0.356g, 0.0024 ml), and ammonium acetate (0.37g, 0.0048 mol) in minimum amount of acetic acid.

5-benzyliden-3-(2-diethylamino)ethyl)-2-thioxothiazolidin-4-one (D7)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-(diethylamino)ethyl)-2-thioxothiazolidin-4-one (0.5g, 0.0021 mol), benzaldehyde (0.21 ml, 0.0021 mol), and ammonium acetate (0.3237g, 0.0042 mol) in minimum amount of acetic acid.

3-(2-(diethylamino)ethyl)-5-(4-methylbenzylidene)-2-thioxo-thiazolidin-4-one (D8)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-(diethylamino)ethyl)-2-thioxo-thiazolidin-4-one (0.5g, 0.0021 mol), 4-methylbenzaldehyde (0.252g, 0.0021 mol), and ammonium acetate (0.32g, 0.0042 mol) in minimum amount of acetic acid.

3-(2-(diethylaminoethyl)-5-(2-methylbenzylidene)-2-thioxothiazolidin-4-one (D9)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-(diethylamino)ethyl)-2-thioxothiazolidin-4-one (0.5g, 0.0021 mol), 2-methylbenzaldehyde (0.252g, 0.0021 mol) and ammonium acetate (0.32g, 0.0042 mol) in the minimum amount of acetic acid.

3-(2-(diethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one (D10)

The title compound was synthesized as per the procedure for compound D1 by taking 3-(2-(diethylamino) ethyl)-2-thioxothiazolidin-4-one (0.5g, 0.0021 mol), 4-isopropyl-benzaldehyde (0.32 ml, 0.0021 mol) and ammonium acetate (0.32g, 0.0042 mol) in the minimum amount of acetic acid.

Biological assays

Antifungal assay

Test fungal pathogens

Fungal cultures used in the study were *Aspergillus niger, Candida albicans, Candida parapsilosis,, Candida tropicalis and Candida glabrata.* All the strains used were MTCC and were procured from Microbial Type Culture Collection and Gene Bank, CSIR-IMTECH, Chandigarh, India.

Sub-culturing of test organisms

All reference fungal cultures were subcultured on *potato dextrose agar*. The fungal slant was incubated for 48h at 30 °C. Mcfarland density (0.5 on the Mcfarland scale) of fungal culture was adjusted in normal saline to achieve the final concentration of 1 x 10^5cfu/ml of each test organism individually. In the case of *Aspergillus niger*, conidial suspensions were harvested after isolates were subcultured on PDA at 30 °C to 7 d and were suspended in normal saline. *Aspergillus niger* inocula were then prepared spectrophotometrically and further diluted in normal saline to obtain a final inoculum concentration of 1 x 10^5cfu/ml. This had been used as adjusted inoculum for all the further studies.

Determination of MIC

MIC was done by broth microdilution method conferring to the reference of Clinical and Laboratory Standards Institute 2012, USA, using 96 well ELIZA plates [45]. To determine MIC, the compounds and the standard drug Ketoconazole (reference antimycotic drug) were dissolved in DMSO to give a stock concentration of 1 mg/ml. From these serial dilutions of the test, compounds were prepared in appropriate concentrations ranging from 0.5 to 1000µg/ml. Each well was inoculated with 50 µl of fungal suspension to give a final concentration of 1 x 10^5cfu/ml. The microtitre plates were incubated at 37 °C for 48 h. The fungal growth was measured by taking absorbance at 600 nm using a microtitre plate reader. 0.5% DMSO and sterile RPMI medium were used as blank control, which does not inhibit the growth of fungus. MIC was defined as the lowest concentration of drug showing no growth. The experiment was performed in triplicate.

Minimum fungicidal concentration (MFC)

MFC was determined as the lowest concentration of compound that that kills 99.9% of the fungal cells after 24 h incubation at 37 °C. MFC values were determined by removing 100 µl of fungal suspension from culture demonstrating no visible growth in MIC experiment and inoculating in fresh nutrient agar plates. Plates were incubated at 37 °C for a total period of 24 h. The MFC is determined with the wells whose concentrations are greater than MIC. Each experiment was repeated at least 3 times

Antibacterial assay

Test bacterial pathogens

The bacterial pathogens used in the study were gram-positive bacteria *Bacillus cereus, Staphylococcus aureus*, gram-negative bacteria, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. All the strains used were MTCC and procured from Microbial Type Culture Collection and Gene Bank, CSIR-IMTECH, Chandigarh, India.

Sub-culturing of test organism

All bacterial reference cultures were subcultured on nutrient agar. The bacterial slants were incubated overnight at 37 °C. Mcfarland density (0.5 on the McFarland scale) of bacterial culture was adjusted in normal saline to achieve the final concentration of 1 x 10^5cfu/ml of each test organism individually.

Determination of MIC

MIC was done by broth microdilution method recommended by the National Committee for Clinical Laboratory Standards Institute 2012 USA using 96 well ELIZA plates [46]. To determine the MIC, the compounds and standard drug ampicillin were dissolved in

0.5% DMSO to give a stock concentration of 1000 µg/ml. Colony suspensions equivalent to 0.5 McFarland standard were prepared and inoculated to yield an inoculum of 1 x 10^5cfu/ml. The microtitre plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of drug showing no growth. MIC was attained from there independent tests that were performed in triplicate.

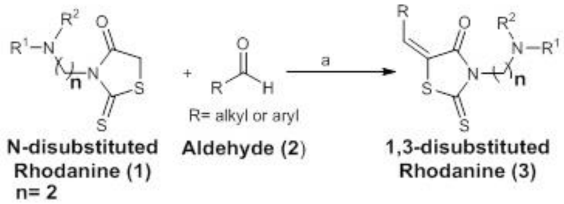
Minimal bactericidal concentration (MBC)

MBC was determined as the lowest concentration of compounds that kills 99.9% of the bacterial cells after 24h incubation at 37 °C. MBC values were determined by removing 100 µl of bacterial suspension from culture demonstrating no visible growth in MIC experiment and inoculating in fresh nutrient agar plates. Plates were incubated at 37 °C for a total period of 24 hr. The MBC is determined with the wells whose concentrations are greater than MIC. Each experiment was repeated at least 3 times.

RESULTS AND DISCUSSION

Chemistry

The designed molecular framework i.e. 5-benzylidene 3-(dialkylamino) alkyl-2-thioxothiazolidin-4-one has been synthesised as per scheme 1.



Scheme 1: Reagents and condition: a) Ammonium acetate, Acetic acid, 80-85 °C

Table 2: Synthesised derivatives

Compound code	NR^1R^2	n	R
D1	dimethyl amino	2	Phenyl
D2	dimethyl amino	2	4-ethyl,-phenyl
D3	dimethyl amino	2	4-methyl phenyl
D4	diethyl amino	2	4-ethyl phenyl
D5	dimethyl amino	2	2-methyl phenyl
D6	dimethyl amino	2	4-isopropyl phenyl
D7	diethyl amino	2	Phenyl
D8	diethyl amino	2	4-methyl phenyl
D9	diethyl amino	2	2-methyl phenyl
D10	diethyl amino	2	4-isopropyl phenyl

Spectral characterization of synthesised compounds

5-Benzylidene-3-(2-(dimethylamino) ethyl)-2-thioxo-thiazolidin-4-one (D1)

Brown solid (Yield 66%), mp 105 °C; IR (KBr) ν (cm^{-1}): 2932, 2851, 1701, 1236, 1175, 1120; ^1H NMR (500 MHz, DMSO): *δ*2.36 (6H, s), 2.69-2.72 (2H, t), 4.23-4.26 (2H, t), 7.35-7.40 (4H, m), 7.63 (1H, s), 7.80-7.84 (1H, m); ^{13}C (125 MHz, DMSO): *δ*193.55 (C=S), 167.87 (C=O), 139.30, 133.36, 133.22, 130.74, 130.64, 129.61, 129.34, 123.04, 114.07, 55.33, 45.34, 41.97, 29.70; HRMS (m/z) calculated for $C_{14}H_{16}N_2OS_2$: 292.0704, found: 293.0777 (MH^+).

3-(2-(dimethylamino) ethyl)-5-(4-ethylbenzylidene)-2-thioxo-thiazolidin-4-one (D2)

Dark brown solid (Yield 65%), mp 96 °C; IR (KBr) ν (cm^{-1}): 2928, 2859, 1563, 1328, 1282, 1235, 1231; ^1H NMR (500 MHz, DMSO): *δ*1.19 (3H, bs), 2.30 (6H, s), 2.61-2.67 (4H, m), 4.20-4.23 (2H, t), 7.19-7.23 (2H, m), 7.27-7.33 (2H, m), 7.62 (1H, s); ^{13}C (125 MHz, DMSO): *δ*192.02 (C=S), 167.92 (C=O), 133.43, 130.87, 129.98, 128.93, 128.55,121.80, 55.16, 45.12, 41.76, 28.92, 15.12; HRMS (m/z) calculated for $C_{16}H_{20}N_2OS_2$: 320.1017, found: 321.1086 (MH^+).

3-(2-(dimethylamino)ethyl)-5-(4-methylbenzylidene)-2-thioxothiazolidin-4-one (D3)

Yellow coloured solid (Yield 70%), mp 102 °C; IR (KBr) ν (cm^{-1}): 2917, 2855, 2786, 1701, 1590, 1334, 1180; ^1H NMR (500 MHz, DMSO), δ2.25 (6H, s), 2.33 (3H, s), 2.56-2.59 (2H, t), 4.17-4.20 (2H, t), 7.20-7.21 (2H, d), 7.31-7.32 (2H, d), 7.63 (1H, s); ^{13}C (125MHz, DMSO):δ 193.57 (C=S), 167.91 (C=O), 141.63, 133.35, 130.74, 130.67, 130.13, 121.81, 55.53, 45.61, 42.27, 21.64; HRMS (m/z) calculated for $C_{15}H_{18}N_2OS_2$: 306.0861 found: 307. 0.0930 (MH^+).

3-(2-(diethylamino)ethyl)-5-(4-ethyl benzylidene)-2-thioxo-thiazolidin-4-one (D4)

Yellow coloured solid (Yield 68%), mp 108 °C; IR (KBr) ν (cm^{-1}): 2928, 2855, 1699, 1594, 1283; ^1H NMR (500 mHz, DMSO), δ0.98-1.01 (6H, t), 1.18-1.19 (3H, t), 2.55-2.74 (10H=2H x 5, m), 7.23-7.24 (2H, d), 7.34-7.35 (2H, d), 7.63 (1H, s); ^{13}C (125 MHz, DMSO); δ 192.04 (C=S), 167.87 (C=O), 147.84, 133.27, 130.89, 128.94, 121.87, 48.36, 47.37, 42.01, 28.92, 15.26, 15.14, 14.12, 11.91; HRMS (m/z) calculated for $C_{18}H_{24}N_2OS_2$: 348.1330, found: 349.1415 (MH^+).

3-(2-(dimethylamino)ethyl)-5-(2-methylbenzylidene-2-thioxothiazolidin-4-one (D5)

Dark brown coloured solid (Yield 68%); mp 115 °C; IR (KBr) ν (cm^{-1}): 2627, 1725, 1431, 1356, 1310, 1291; ^1H NMR (500 MHz, DMSO): δ2.27 (6H, s), 2.39 (3H, s), 2.58-2.61 (4H, m), 7.19-7.33 (4H, m), 7.88 (1H, s), ^{13}C (125MHz, DMSO): δ194.10 (C=S), 167.61 (C=O), 139.47, 132.43, 131.17, 131.04, 130.71, 128.07, 126.70, 124.23, 55.50, 45.55, 42.18, 29.70, 19.99; HRMS (m/z) calculated for $C_{15}H_{18}N_2OS_2$: 306.0861, found: 307.0938 (MH^+).

3-(2-(dimethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one (D6)

Light brown coloured solid (Yield 70%); mp 110 °C; IR (KBr) ν (cm^{-1}): 2966, 2924, 2790, 1703, 1592, 1326;^1H NMR (500 MHz, DMSO); δ 1.21-1.22 (6H, d), 2.26 (6H, s), 2.58-2.61 (2H, t) 2.87-2.92 (2H, m), 4.84-4.94 (1H, m), 7.26-7.27 (2H, d), 7.35-7.36 (2H, d), 7.64 (1H, s); ^{13}C (125 MHz, DMSO): δ 192.04 (C=S), 167.95 (C=O), 152.41, 133.39, 131.01, 130.93, 130.02, 127.54, 127.15, 114.08, 55.47, 45.53, 42.19, 34.48, 34.22, 23.64; HRMS (m/z) calculated for $C_{17}H_{22}N_2OS_2$: 334.1174, found: 335.1249 (MH^+)

5-benzyliden-3-(2-diethylamino)ethyl)-2-thioxothiazolidin-4-one (D7)

Dark brown coloured solid (Yield 68%); mp 125 °C; IR (KBr) ν (cm^{-1}): 2928, 2855, 1699, 1592, 1283, 1176; ^1H NMR (500 MHz, DMSO), δ1.01-1.04 (6H, t), 2.62-2.66 (4H, m), 2.77-2.80 (4H, m) 7.19-7.24 (1H, m), 7.40-7.41 (4H, m), 7.65 (1H, s); ^{13}C (125 mHz, DMSO); δ 193.45 (C=S), 167.76 (C=O), 139.29, 133.13, 129.34, 127.91, 123.06, 48.02, 47.16, 41.62, 11.52; HRMS (m/z) calculated for $C_{14}H_{20}N_2OS_2$: 320.1017, found: 321.1083 (MH^+).

3-(2-(diethylamino)ethyl)-5-(4-methylbenzylidene)-2-thioxo-thiazolidin-4-one (D8)

Yellow coloured solid (Yield 68%); mp 112 °C; IR (KBr) ν (cm^{-1}): 2928, 2855, 1699, 1592, 1282; ^1H NMR (500MHz, DMSO); δ0.96-0.99 (6H, t), 2.33-2.71 (11H= 3H+2H+2H+4H, m), 7.19-7.22 (2H, t), 7.31-

7.33 (2H, d); 7.62 (1H, s); ^{13}C (125MHz, DMSO): δ 193.54 (C=S), 167.88 (C=O), 141.61, 133.20, 130.76, 130.13, 114.08, 48.55, 47.47, 42.24, 21.64, 12.11; HRMS (m/z) calculated for $C_{17}H_{22}N_2OS_2$: 334.1174, found: 335.1259 (MH$^+$).

3-(2-(diethylaminoethyl)-5-(2-methylbenzylidene)-2-thioxothiazolidin-4-one (D9)

Yellow coloured solid (Yield 70%); mp 112 °C; IR (KBr) ν (cm^{-1}): 2928, 2851, 1699, 1594, 1283; ^{1}H NMR (500MHz, DMSO); δ 0.97-1.00 (6H, t), 2.39 (3H, s), 2.52-2.71 (8H= 4H+4H, m), 7.19-7.25 (3H, m), 7.25-7.34 (1H, m), 7.84 (1H, s); ^{13}C (125MHz, DMSO): δ 194.05 (C=S), 167.57 (C=O), 139.46, 132.44, 128.07, 126.71, 114.08, 48.56, 47.53, 42.26, 20.00, 12.15; HRMS (m/z) calculated for $C_{17}H_{22}N_2OS_2$: 334.1174, found: 335.1249 (MH$^+$).

3-(2-(diethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one (D10)

Light brown coloured solid (Yield 65%); mp 118 °C; IR (KBr) ν (cm^{-1}): 2932, 2920, 2851, 1699, 1593, 1283, 1175; ^{1}H NMR (500 MHz, DMSO), δ 0.98 (6H, s), 1.18-1.21 (6H, t), 2.54-2.88 (8H= 4H+2H+2H, m), 4.83-4.94 (1H, m), 7.26-7.37 (4H, m), 7.63 (1H, s);^{13}C (125MHz, DMSO): δ 193.57 (C=S), 167.89 (C=O), 152.39, 141.14, 133.20, 130.93, 127.54, 121.91, 48.51, 47.45, 42.21, 34.22, 23.64, 12.08; HRMS (m/z) calculated for $C_{19}H_{26}N_2OS_2$: 362.1487, found: 363.1571 (MH$^+$).

Antifungal activity

All the compounds were screened for their *in vitro* antifungal activity against various species of *Candida* and *Aspergillus niger.* Ketoconazole was taken as the reference standard. Compounds with MIC>250µg/ml were considered as inactive. MIC between 250-125 µg/ml was indicative of low activity and MIC between 64 to 32µg/ml exhibited moderate activity. MIC less than 16µg/ml can be considered as the activities which are used in the clinical situation. The MIC values of the compounds (D1-D10) against fungal pathogens are given in table 3. D10 was observed to show MIC value at 16µg/ml against *Candida glabrata*. Similarly, D6 also showed MIC value at 16µg/ml against *Candida tropicalis* and are considered as lead compounds for further development. The MFC values of the compounds against fungal pathogens are given in table 4. Compound D10 was observed to show MFC value at 32µg/ml against *Candida glabrata* and compound D6 showed MFC value at 32µg/ml against *Candida tropicalis* The structure-activity relationship studies revealed that the most potent compounds D6 (3-(2-(dimethylamino)ethyl)-5-(4-isopropylbenzylidene)-2-thioxothiazolidin-4-one)and D10(3-(2-(diethylamino) ethyl)-5-(4-isopropyl benzylidene)-2-thioxothiazolidin-4-one) which are having 4–isopropyl benzylidene group at 5thposition of rhodanine showed MIC value 16 µg/ml against *candida albicans* and *Candida glabrata* respectively.

Table 3: Minimum Inhibitory concentration of derivatives of D1-D10 against fungal pathogens

Minimum Inhibitory concentration (µg/ml)*											
Fungal pathogens	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**ketoconazole**
Aspergillusniger	32	125	125	32	32	64	32	125	64	125	1
Candida albicans	32	32	125	32	32	32	32	64	32	32	0.5
Candida glabrata	64	64	125	64	32	64	32	64	64	16	0.5
Candida parapsilosis	32	64	125	64	32	34	32	125	64	64	0.5
Candida tropicalis	64	64	125	32	32	16	32	32	32	64	1

*Values are average of three readings

Table 4: Minimum fungicidal concentration of derivatives of D1-D10 against fungal pathogens

Minimum fungicidal concentration (µg/ml)*										
Fungal pathogens	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
Aspergillus niger	64	250	250	64	64	125	64	250	125	250
Candida albicans	64	64	250	64	64	64	64	125	64	64
Candida glabrata	125	125	250	125	64	125	64	125	125	32
Candida parapsilosis	64	125	250	125	64	125	64	250	125	125
Candida tropicalis	125	125	250	64	64	32	64	64	64	125

*Values are average of three readings

Antibacterial activity

The MIC values of the compounds (D1-D10) and the positive control Ampicillin against the gram-positive bacterial strains (*Bacillus cereus and Staphylococcus aureus*) and gram-negative strains *(Escherichia coli, Klebsiella pneumoniae, and Pseudomonas. aeruginosa*) are given. The synthesized compounds (D1-D10, table 5) were also been evaluated for their antibacterial activities against different species. Ampicillin was taken as the reference standard. Compounds with MIC>250µg/ml were considered as inactive and MIC between 250and 125µg/ml were indicative of low activity. MIC between 64 to 32µg/ml showed moderate activity. MIC less than 16µg/ml was supposed to be the activity which can be used in the clinical situation. In table 4, D2 elicited a moderate antibacterial activity at MIC value of 32µg/ml in gram-negative strains, i. e, against *Escherichia coli and Klebsiella pneumoniae.* Compound D5 showed activity against *Klebsiella pneumoniae* at MIC value of 32µg/ml. But other compounds were observed to be rather resistant towards gram-positive strains. In comparison with Ampicillin which gave a MIC value of 1µg/ml in *Escherichia coli, Klebsiella pneumonia*, the results obtained with D2 and D5 were not very significant. The MBC values of the compounds (D1-D10) against both gram-positive and gram-negative strains are given in table 6. The MBC values of all the compounds were ≥ 64 µg/ml against all bacterial strains.

Table 5: Minimum inhibitory concentration (µg/ml) of derivatives of D1-D10 against bacterial strains

Minimum inhibitory concentration (µg/ml)*											
Pathogens	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**Ampicillin**
Bacillus cereus	64	125	250	125	64	125	64	125	125	125	2
Staphylococcus aureus	64	62.5	250	125	64	125	64	125	125	125	0.5
Escherichia coli	64	32	125	125	64	64	64	125	125	125	1
Klebsiella pneumoniae	64	32	250	125	32	64	64	125	125	125	1
Pseudomonas aeruginosa	64	250	250	250	250	250	250	250	250	250	1

*Values are average of three readings

Table 6: Minimum bactericidal concentration (μg/ml) of derivatives of D1-D10 against bacterial strains

Minimum bactericidal concentration (μg/ml)*										
Pathogens	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
Bacillus cereus	125	250	500	250	125	250	125	250	250	250
Staphylococcus aureus	125	125	500	250	125	250	125	250	250	250
Escherichia coli	125	64	250	250	125	125	125	250	250	250
Klebsiella pneumoniae	125	64	500	250	64	125	125	250	250	250
Pseudomonas aeruginosa	125	500	500	500	500	500	500	500	500	500

*Values are average of three readings

Structural modifications of rhodanine derivatives results in compounds with a broad spectrum of pharmacological activities [47]. Substitution of various benzylidene derivatives at 5th position increases hydrophobicity and led to an increase in antimicrobial activity [48]. It has been reported that, the presence of the hydrophobic phenylalanine side chain at the N3-position and the electron-deficient benzylidene moiety at the C5-position of the rhodanine scaffold is responsible for the antibacterial activity of these compounds against methicillin-resistant Staphylococcus aureus [49]. In our study 4-ethylbenzylidene and 2-methylbenzylidene group at 5thposition of rhodanine showed good antibacterial activity at 32μg/ml. A series of benzylidene–rhodanines were reported to be acting as antifungal agents which are most active against Candida genus and Candida neoformans [50]. In our study, 4–isopropyl benzylidene group at 5thposition of rhodanine showed MIC value 16 μg/ml against *candida albicans* and *Candida glabrata,* respectively.

CONCLUSION

In the search for a novel antimicrobial agent different 5-benzylidene substituted rhodanine derivatives were synthesized from various N-substituted rhodanine derivatives. All the ten synthesized compounds were screened for antimicrobial activity using the microdilution method recommended by the National Committee for Clinical Laboratory Standards, USA (CLSI 2006) to assess their antimicrobial activity. All the compounds exhibited good antifungal activity and compound D6(3-(2-(dimethylamino)ethyl)-5-(4-isopropyl benzylidene)-2-thioxothiazolidin-4-one) and D10(3-(2-(diethylamino) ethyl)-5-(4-isopropyl benzylidene)-2 thioxothiazolidin-4-one) which are having 4–isopropyl benzylidene group at 5thposition of rhodanine showed MIC value 16 μg/ml against *candida tropicalis* and *Candida glabrata* respectively. Compounds D2 and D5 Showed good antibacterial activity at 32 μg/ml. All the other compounds showed moderate antibacterial activity. Based on these results, it can be suggested these compounds may lead to the development of more potent antimicrobial agents in the future.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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