



SYNTHESIS OF 2-((5-BENZYLIDENE-4-OXO-4,5-DIHYDROTHIAZOL-2-YL)-SUBSTITUTED AMINO ACIDS AS ANTICANCER AND ANTIMICROBIAL AGENTS

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A series of Rhodanine derivatives were synthesized by Knoevenagel condensation. All the synthesized compounds were tested for their in vitro anticancer activity against MCF-7 and BT-474 human breast cancer cell lines. All the synthesized compounds were characterized and screened for their antimicrobial activity against the bacterial and fungal strain. Majority of the compounds showed good to moderate anticancer and antimicrobial activity. Among these compounds, one showed promising activity against gram-positive bacteria *B. subtilis* and *S. aureus* when compared with ampicillin. Some of the most potent compounds possessed selective antimicrobial activity.

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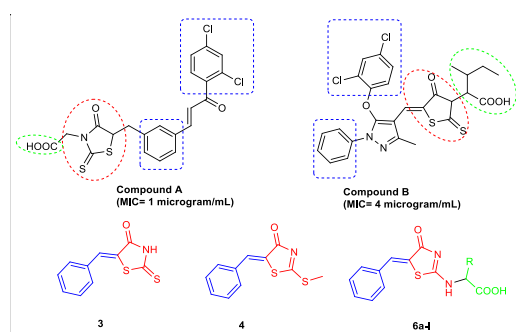


Figure 1. Previously reported antibacterial agents and synthesized compounds.

INTRODUCTION

The dealing of microbial infections remains a very challenging salutary problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Even though the many antibiotics, drugs and chemotherapeutic available, the emergence of old and newer antibiotic-resistant bacterial strains in the last two decades created an urgent need for the discovery and development of new antimicrobials with a broader spectrum of activity and lower toxicity.¹⁻³

The 2-thioxothiazolidin-4-one (Rhodanine) based molecules exhibit biological activities, such as antidiabetic,⁴ anticancer,⁵ antitubercular,^{6,7} anti-HIV,⁸⁻¹⁰ antiparasitic,¹¹ anticonvulsant,¹² and antiproliferative.^{13,14} Thiazole also has anti-inflammatory,¹⁵ anticancer,¹⁶ anti-fungal¹⁷ and antimicrobial¹⁸ activity. The Rhodanine derivatives have been known for over five decades, and there are various reports available on Rhodanine derivatives as antimicrobial agents.¹⁹⁻²⁴ These reports suggested that Rhodanine nuclei were essential to the observed levels of antimicrobial activity.²⁵⁻²⁸

We make a plan to synthesized Rhodanine derivatives as an antimicrobial agent by preparing hybrid molecules having similar features of reported potent antimicrobial agents (Figure 1).

Because of the facts mentioned above, Rhodanine derivatives were synthesized, characterized by different spectral analysis techniques and screened for their antimicrobial activity. All the synthesized compounds were also screened for their anticancer activity against cell line MCF-7. The results suggest that the compounds could be exploited as an antimicrobial agent. In continuation of our work,²⁹⁻⁴² on the synthesis of heterocyclic and bioactive compounds, we have synthesized some Rhodanine analogs.

MATERIALS AND METHODS

Rhodanine, benzaldehyde and various solvents were commercially available. The major chemicals were purchased from Sigma Aldrich and Avra labs. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded on an FT-IR (Bruker). Melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. The ¹³C NMR spectra were recorded on a 400 MHz Varian NMR spectrometer. The ¹H NMR spectra were recorded on a 400 MHz Varian NMR spectrometer. The chemical shifts are reported as δ (ppm) units (tetramethylsilane). The following

abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

Procedure for the synthesis of (Z)-5-benzylidene-2-thioxothiazolidin-4-one (3)

A mixture of benzaldehyde **1** (1 mmol), 2-thioxothiazolidin-4-one **2** (1 mmol), anhydrous sodium acetate (1 mmol) and glacial acetic acid (1 ml) was refluxed for 5 h. The progress of the reaction was monitored by TLC (20% ethyl acetate: *n*-hexane). After completion of the reaction, the reaction mixture was poured into the ice-cold water. The precipitate was filtered off and washed with water (3×10 ml), dried, and purified by recrystallization in ethanol as a solvent to give 82 % yield.

Orange solid, Yield: 82%. mp 204–206 °C; ES-MS *m/z*: 221.05. IR $\nu_{\max}/\text{cm}^{-1}$: 1670 (C=O), 1600 (C=C), 1585 (C=N), 1230 (C=S), 1192 (C–N). ¹H NMR: δ (ppm) = 7.40–7.55 (m, 5H, Ar–CH), 7.70 (s, 1H, =CH), 13.90 (s, 1H, NH). ¹³C NMR: δ (ppm) = 125.5, 128.5, 130.7, 130.9, 131.4, 133.5, 169.4, 194.7.

Procedure for the synthesis of (Z)-5-benzylidene-2-(methylthio)thiazol-4(5H)-one (4)

In a 50 ml round bottom flask, the compound (Z)-5-benzylidene-2-thioxothiazolidin-4-one (**3**) (1 mmol), triethylamine (1.2 mmol), iodomethane (1.2 mmol), and dichloromethane (10 ml) were stirred at room temperature for 2 h. The progress of the reaction was monitored by TLC (10% methanol: chloroform). After completion of the reaction, the reaction mixture was concentrated in-vacuo. The residue was washed with water (3×15 mL) to afford the crude product and it was recrystallized from ethanol. Orange solid, Yield: 85%. M.p 145–147 °C; ES-MS *m/z*: 235.00. IR $\nu_{\max}/\text{cm}^{-1}$: 3026 (CH–Ar), 1694 (C=O), 1590 (C=C), 1462 (C=N), 1151 (C–S), 979 (C–N). ¹H NMR: δ (ppm) = 2.85 (s, 3H, S–CH₃), 7.40–7.72 (m, 5H, Ar–CH), 7.90 (s, 1H, =CH). ¹³C NMR: δ (ppm) = 14.4, 126.5, 128.6, 131.4, 132.9, 133.5, 135.7, 152.3, 162.7, 169.2.

General procedure for the synthesis of (Z)-2-((5-benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino) acid (6a-1)

In a 50 ml round bottom flask, the compound (**4**) (1 mmol), amino acids (**5a-1**) (1.2 mmol), potassium carbonate (1 mmol) and ethanol (5 ml) were mixed and stirred for 20–60 min at room temperature. The progress of the reaction was monitored by TLC (10% methanol:chloroform). After completion of the reaction, the reaction mixture was concentrated in-vacuo. The residue was washed with water (3×15 mL) to afford the crude product. The (Z)-2-((5-benzylidene-4-oxo-4,5-dihydrothiazol-2-yl) substituted amino acids (**6a-1**) were recrystallized from ethanol and isolated as yellowish solids.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (6a).

Yellow solid, Yield: 92%, M.p 221–223 °C; ES-MS *m/z*: 277.00. IR $\nu_{\max}/\text{cm}^{-1}$: 3384 (OH), 3210 (NH), 3026 (CH–Ar), 1737 (HO–C=O), 1599 (C=O), 1553 (C=C), 1599 (C=N), 1006 (C–S), 1091 (C–N). ¹H NMR: δ (ppm) = 1.40–1.50 (d, 3H, C–CH₃), 4.60–4.70 (q, 1H, CH), 7.40–7.60 (m, 5H, Ar–CH), 7.70 (s, 1H, =CH), 9.15 (s, 1H, NH), 10.20 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 16.7, 53.4, 127.5, 128.7, 132.7, 135.7, 152.3, 158.6, 167.7, 179.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-methylbutanoic acid (6b).

Yellow solid, Yield: 90%, M.p 178–180 °C; ES-MS *m/z*: 304.50. IR $\nu_{\max}/\text{cm}^{-1}$: 3744 (OH), 3011 (NH), 1737 (HO–C=O), 1689 (C=O), 1553 (C=C), 1509 (C=N), 1232 (C–S), 1010 (C–N). ¹H NMR: δ (ppm) = 0.90–0.92 (d, 6H, CH–(CH₃)₂), 1.52–1.54 (m, 1H, CH), 4.44–4.46 (d, 1H, CH), 7.26–7.68 (m, 5H, Ar–CH), 7.76 (s, 1H, =CH), 9.18 (s, 1H, NH), 10.26 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 18.7, 30.4, 61.4, 127.9, 128.6, 128.9, 132.3, 135.9, 152.9, 158.1, 167.3, 178.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-methylpentanoic acid (6c).

Yellow solid, Yield: 94%, M.p 110–112 °C; ES-MS *m/z*: 319. IR $\nu_{\max}/\text{cm}^{-1}$: 3365 (OH), 3211 (NH), 3007 (CH–Ar), 1732 (HO–C=O), 1693 (C=O), 1556 (C=C), 1583 (C=N), 1014 (C–S), 1096 (C–N). ¹H NMR: δ (ppm) = 0.99–1.01 (t, 3H, CH₂–CH₃), 1.19–1.22 (d, 3H, CH₃), 1.52–1.60 (m, 2H, CH₂), 1.81–1.92 (m, 1H, CH), 4.43–4.45 (d, 1H, CH), 7.21–7.62 (m, 5H, Ar–CH), 7.78 (s, 1H, =CH), 9.12 (s, 1H, NH), 11.01 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 18.7, 30.4, 61.4, 127.9, 128.6, 128.9, 132.3, 135.9, 152.9, 158.1, 167.3, 178.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-phenylpropanoic acid (6d).

Yellow solid, Yield: 92%, M.p 187–189 °C; ES-MS *m/z*: 353. IR $\nu_{\max}/\text{cm}^{-1}$: 3395 (OH), 3213 (NH), 2990 (CH–Ar), 1737 (HO–C=O), 1691 (C=O), 1551 (C=C), 1581 (C=N), 1017 (C–S), 1098 (C–N). ¹H NMR: δ (ppm) = 2.51–2.53 (d, 2H, CH₂), 4.43–4.78 (q, 1H, CH), 7.21–7.72 (m, 10H, Ar–CH), 7.79 (s, 1H, =CH), 9.14 (s, 1H, NH), 11.02 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 36.4, 58.4, 125.9, 127.7, 128.6, 128.9, 135.3, 136.9, 152.2, 158.5, 167.1, 175.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-4-(methylthio)butanoic acid (6e).

Yellow solid, Yield: 90%, M. p 122–124 °C; ES-MS *m/z*: 337. IR $\nu_{\max}/\text{cm}^{-1}$: 3398 (OH), 3200 (NH), 2980 (CH–Ar), 1733 (HO–C=O), 1695 (C=O), 1541 (C=C), 1586 (C=N), 1011 (C–S), 1092 (C–N).

¹H NMR: δ (ppm)= 2.01–2.20 (q, 2H, CH₂), 2.20 (s, 3H, CH₃), 2.61–2.63 (t, 2H, CH₂), 4.43–4.78 (q, 1H, CH), 7.31–7.60 (m, 5H, Ar–CH), 7.78 (s, 1H, =CH), 9.16 (s, 1H, NH), 11.22 (s, 1H, COOH). ¹³C NMR: δ (ppm)=15.4, 29.8, 30.4, 56.4, 127.7, 128.6, 128.9, 132.3, 136.9, 152.2, 158.5, 167.5, 174.9.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-4-methylpentanoic acid (6f)

Yellow solid, Yield: 96%, M.p 209–211 °C; ES-MS m/z: 319. IR ν_{\max} /cm⁻¹: 3397 (OH), 3212 (NH), 3013 (CH–Ar), 1734 (HO–C=O), 1691 (C=O), 1555 (C=C), 1583 (C=N), 1013 (C-S), 1091 (C–N). ¹H NMR: δ (ppm)= 0.92–0.94 (d, 6H, CH–(CH₃)₂), 1.41–1.43 (m, 1H, CH), 1.71–1.73(t, 2H, CH₂), 4.44–4.46 (q, 1H, CH), 7.29–7.69 (m, 5H, Ar–CH), 7.76 (s, 1H, =CH), 9.14 (s, 1H, NH), 10.84 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 22.7, 24.4, 40.4, 55.2, 127.8, 128.9, 129.2, 132.1, 135.4, 152.2, 159.1, 167.1, 174.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-hydroxypropanoic acid (6g)

Yellow solid, Yield: 95%, M.p 195–197 °C; ES-MS m/z: 293. IR ν_{\max} /cm⁻¹: 3450 (OH), 3214 (NH), 3007 (CH–Ar), 1738 (HO–C=O), 1688 (C=O), 1553 (C=C), 1511 (C=N), 1019 (C-S), 1097 (C–N). ¹H NMR: δ (ppm)= 3.60 (s, 1H, CH), 4.01–4.03 (q, 1H, CH), 4.23–4.25 (d, 2H, CH₂), 7.31–7.60 (m, 5H, Ar–CH), 7.78 (s, 1H, =CH), 9.12 (s, 1H, NH), 10.86 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 59.2, 62.3, 127.1, 128.5, 129.2, 132.9, 135.1, 151.9, 158.1, 167.9, 173.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-mercaptopropanoic acid (6h)

Yellow solid, Yield: 92%, M.p 189–191 °C; ES-MS m/z: 309. IR ν_{\max} /cm⁻¹: 3455 (OH), 3201 (NH), 3017 (CH–Ar), 2500 (SH), 1739 (HO–C=O), 1698 (C=O), 1559 (C=C), 1501 (C=N), 1011 (C-S), 1099 (C–N). ¹H NMR: δ (ppm)= 1.50 (s, 1H, CH), 3.11–3.29 (d, 2H, CH₂), 4.13–4.38 (t, 1H, CH), 7.22–7.59 (m, 5H, Ar–CH), 7.68 (s, 1H, =CH), 9.18 (s, 1H, NH), 11.84 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 26.9, 60.3, 127.5, 128.8, 129.1, 132.5, 135.6, 152.9, 158.3, 167.2, 178.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)-amino)succinic acid (6i)

Yellow solid, Yield: 90%, M.p 169–171 °C; ES-MS m/z: 321. IR ν_{\max} /cm⁻¹: 3465 (OH), 3213 (NH), 3020 (CH–Ar), 1735 (HO–C=O), 1689 (C=O), 1549 (C=C), 1503 (C=N), 1030 (C-S), 1089 (C–N). ¹H NMR: δ (ppm)= 2.61–2.63 (d, 2H, CH₂), 3.71–3.73 (t, 1H, CH), 7.33–7.69 (m, 5H, Ar–CH), 7.78 (s, 1H, =CH), 9.30 (s, 1H, NH), 11.74 (s, 2H, COOH). ¹³C NMR: δ (ppm) = 26.9, 60.3, 127.5, 128.8, 129.1, 132.5, 135.6, 152.9, 158.3, 167.2, 178.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-(1H-imidazol-4-yl) propanoic acid (6j)

Yellow solid, Yield: 94%, M. p 162–164 °C; ES-MS m/z: 343. IR ν_{\max} /cm⁻¹: 3435 (OH), 3215 (NH), 3021 (CH–Ar),

1736 (HO–C=O), 1681 (C=O), 1552 (C=C), 1508 (C=N), 1032 (C-S), 1091 (C–N). ¹H NMR: δ (ppm)= 2.91–2.93 (d, 2H, CH₂), 3.72–3.74 (t, 1H, CH), 7.30–7.60 (m, 5H, Ar–CH), 7.64 (s, 1H, =CH), 7.78 (s, 1H, =CH), 8.74 (s, 1H, =CH), 9.30 (s, 1H, NH), 11.74 (s, 1H, COOH), 13.00 (s, 1H, NH). ¹³C NMR: δ (ppm) = 28.9, 58.3, 117.9, 124.7, 127.9, 128.6, 129.2, 132.1, 135.2, 136.7, 152.3, 158.2, 167.5, 176.2.

(Z)-2-((5-benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)-3-(4-hydroxyphenyl) propanoic acid (6k).

Yellow solid, Yield: 93%, mp 192–194 °C; ES-MS m/z: 369. IR ν_{\max} /cm⁻¹: 3465 (O=C–OH), 3395 (OH), 3218 (NH), 2994 (CH–Ar), 1739 (HO–C=O), 1699 (C=O), 1553 (C=C), 1591 (C=N), 1011 (C-S), 1089 (C–N). ¹H NMR: δ (ppm)= 2.81–2.99 (d, 2H, CH₂), 4.43–4.45(t, 1H, CH), 5.31 (s, 1H, OH), 7.30–7.60 (m, 5H, Ar–CH), 7.31–7.72 (m, 4H, Ar–CH), 7.72 (s, 1H, =CH), 9.32 (s, 1H, NH), 11.16 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 36.4, 58.6, 115.9, 127.7, 128.6, 128.9, 129.2, 130.2, 135.3, 136.9, 152.2, 155.7, 158.5, 167.7, 174.2.

(Z)-2-((5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)-amino)-3-hydroxybutanoic acid (6l).

Yellow solid, Yield: 94%, mp 209–211 °C; ES-MS m/z: 307. IR ν_{\max} /cm⁻¹: 3464 (OH), 3202 (NH), 3013 (CH–Ar), 1736 (HO–C=O), 1681 (C=O), 1555 (C=C), 1597 (C=N), 1046 (C-S), 1112 (C–N). ¹H NMR: δ (ppm)= 1.15–1.17 (d, 3H, CH₃), 3.52–3.54 (d, 1H, CH), 3.64 (s, 1H, OH), 3.93–4.18 (m, 1H, CH), 7.26–7.68 (m, 5H, Ar–CH), 7.74 (s, 1H, =CH), 9.68 (s, 1H, NH), 11.24 (s, 1H, COOH). ¹³C NMR: δ (ppm) = 19.7, 64.4, 66.6, 127.8, 128.7, 128.9, 132.4, 135.1, 152.9, 158.2, 167.8, 175.2.

Antimicrobial activity

The antibacterial activity was evaluated against two Gram-positive bacteria namely, *Bacillus subtilis* (NCIM-2063) and *Staphylococcus aureus* (NCIM-2901), and one Gram-negative bacterium *Escherichia coli* (NCIM-2256). The antibacterial activity of compounds was monitored by observing their Minimum Inhibitory Concentration (MIC, $\mu\text{g mL}^{-1}$) as previously mentioned by the broth dilution method using Ciprofloxacin and Ampicillin as standard drugs. The antifungal activity was evaluated against three fungal strains; *Candida albicans* (NCIM-3471), *Aspergillus flavus* (NCIM-539) and *Aspergillus niger* (NCIM-1196) using Fluconazole and Miconazole as standard drugs. Minimum inhibitory concentration (MIC, $\mu\text{g mL}^{-1}$) values for antifungal were determined using standard agar dilution method.⁴³ Methanol was used as solvent control for both antibacterial and antifungal testing. The MIC values of the tested compounds are presented in Table 3.

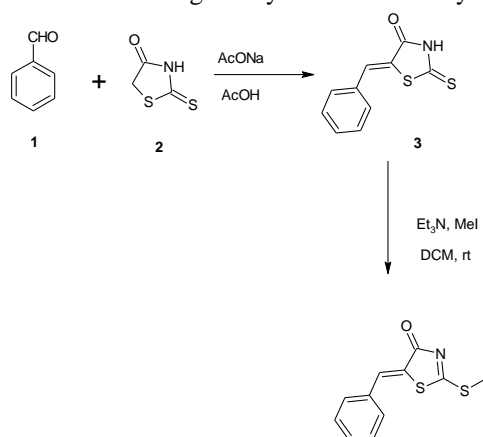
Anticancer activity

All the synthesized compounds were also tested for their anticancer activity on mammalian cell lines MCF-7 and BT-474 human breast cancer cell line. This test is performed as previously mentioned MTT colorimetric assay.⁴⁴ The anticancer activity of the compounds was determined by calculating their IC₅₀ values, the concentration of compound

required to inhibit 50% of cell growth compared to untreated control cells. The IC₅₀ values were presented in micromol per milliliter (μM). The Adriamycin was used as a positive control for the comparison of the anticancer activity of synthesized compounds.

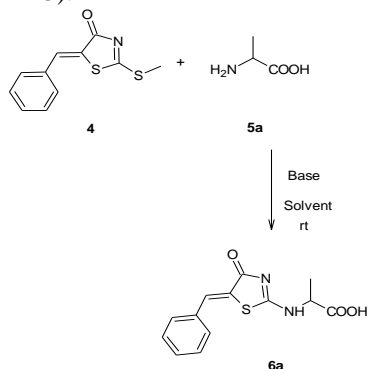
RESULTS AND DISCUSSION

The synthetic protocols employed for the synthesis of Rhodanine derivatives **3**, **4**, and **6a-1** is presented in Scheme 1. The compound (**3**) was prepared via a Knoevenagel condensation between benzaldehyde (**1**) and Rhodanine (**2**). The compound (**4**) (Scheme 1) was obtained via reaction of the compound (**3**) with iodomethane in dichloromethane using triethylamine as a catalyst.



Scheme 1. Synthesis of (Z)-5-benzylidene-2-(methylthio)thiazol-4(5H)-one (**4**)

We synthesized and screening of model reaction (Z)-2-((5-benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (**6a**) (Scheme 2, Table 1). The reaction in which the compound **4** (1 mmol) and compound **5a** (1.2 mmol), catalyzed by various bases and various solvents were selected as a model reaction to optimize the reaction conditions. In terms of the effect of solvents and bases on the condensation reaction, potassium carbonate was found to be the better base and ethanol was found to be the best solvent for the reaction (Table 1, entry 11); other solvents, including methanol, acetic acid, N,N-dimethylformamide (DMF) and toluene were less efficient (Table 1, entries 2–5, 7–10 and 12–15).



Scheme 2 Screening of model reaction (Z)-2-((5-benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)propanoic acid (**6a**)^{aa}Reaction condition (**6a**). Compound (**4**) (1 mmol), Compound (**5a**) (1.2 mmol), base (1 mmol), solvent 1 mL, stirring at rt for 20–80 min.

Table 1. Screening of catalyst, solvents, reaction time, and yield for the synthesis **6a**.

No.	Base	Solvent	Time, min	Yield, ^b %
1	Et ₃ N	Ethanol	50	82
2	Et ₃ N	Methanol	60	65
3	Et ₃ N	Acetic acid	65	60
4	Et ₃ N	DMF	70	60
5	Et ₃ N	Toluene	80	50
6	NaOAc	Ethanol	50	80
7	NaOAc	Methanol	55	50
8	NaOAc	Acetic acid	65	55
9	NaOAc	DMF	70	45
10	NaOAc	Toluene	90	55
11	K ₂ CO ₃	Ethanol	20	98
12	K ₂ CO ₃	Methanol	50	70
13	K ₂ CO ₃	Acetic acid	55	65
14	K ₂ CO ₃	DMF	70	60
15	K ₂ CO ₃	Toluene	80	60

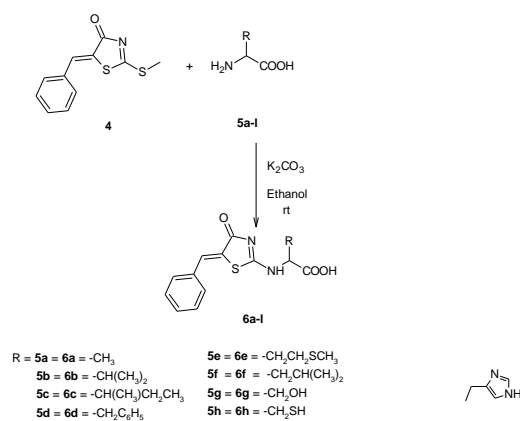
^aAll the reaction was carried out in equimolar amounts of each compound in 1 mL of solvent. ^bIsolated yield.

Nevertheless, all of these yields were best. Ethanol gave the corresponding product in 80–98% yield, which was the best among these solvents (Table 1, entries 1, 6 and 11). To increase the efficiency of the condensation reaction, the effects of different bases were investigated (Table 1, entries 1–15). Potassium carbonate exhibited the best performance with used solvents and gave a better yield, (Table 1, entries 11–15). Sodium acetate and triethylamine gave lower yields with other solvents but gave a better yield in combination with ethanol as a solvent (Table 1, entries 1 and 6). All the reactions were carried out in equimolar amounts of each compound in 1 mL of solvent. Among these reactions, same amounts of the solvent, namely 1 ml of ethanol turned out to be the best choice with yields of 82, 80 and 98% (Table 1, entries 1, 6 and 11). We would like to mention here that ethanol as a solvent with K₂CO₃ as base was the best choice with a yield of 98% and less time required for the completion of the reaction (Table 1, entry 11). Thus we decided to carry out the further reactions in ethanol with potassium carbonate. As a result, the reaction time was shortened; thermal decomposition was also minimized, at room temperature stirring, resulting in higher isolated yields.

Thus we decided the further series, substituted acid derivatives **6a-1** (Scheme 3, Table 2) were synthesized reacting from 5-benzylidene-2-(methylthio)thiazol-4(5H)-one (**4**) with various amino acids (**5a-1**) in ethanol by using K₂CO₃ as a catalyst. In this reaction, there was displacement of a methyl sulfinyl group by amino acids from the C2 position of the thiazolone ring.

The physical data of the synthesized compounds are presented in Table 2. All the reactions proceeded well in 20–60 min to give products in very good yields (82–98%).

The purity of the synthesized compounds was checked by TLC on silica gel precoated F254 Merck plates and melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. The structure of the synthesized compounds was confirmed by IR, ¹H NMR, ¹³C NMR and mass spectral analysis.



Scheme 3 Synthesis of Z)-2-((5-benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)amino)substituted acid (**6a-l**).

Antimicrobial activity

From the antibacterial activity data (Table 3), the synthesized compounds of present series showed moderate to good antibacterial activity. Amongst the synthesized series, the compounds **6g** (MIC= 15 $\mu\text{g mL}^{-1}$ and 12.5 $\mu\text{g mL}^{-1}$), **6h** (MIC= 10 $\mu\text{g mL}^{-1}$ and 15 $\mu\text{g mL}^{-1}$) and **6j** (MIC= 6 $\mu\text{g mL}^{-1}$ and 5.5 $\mu\text{g mL}^{-1}$) were found to be most active molecules and they are found to be specific towards the Gram-positive bacteria, *S. aureus* and *B. subtilis*. The compound **6j** (MIC= 6.0 and 5.5 $\mu\text{g mL}^{-1}$ against *B. subtilis* and *S. aureus*, respectively) was more active than both standards; Ciprofloxacin (MIC= 6.25 $\mu\text{g mL}^{-1}$) and Ampicillin (MIC= 12.5 $\mu\text{g mL}^{-1}$). The compounds **6g** (MIC= 15 and 12.5 $\mu\text{g mL}^{-1}$ against *B. subtilis* and *S. aureus*, respectively) and **6h** (MIC= 10 and 15 $\mu\text{g mL}^{-1}$ against *B. subtilis* and *S. aureus*, respectively) showed lower activity than Ciprofloxacin and comparative level of activity as Ampicillin. On the other hand, compound **6f** (MIC= 15 $\mu\text{g mL}^{-1}$) was found to be narrow spectrum molecule showing activity only against the bacterium *S. aureus*. Out of these, the remaining compounds of the series **3**, **4**, **6a**, **6i**, **6k**, and **6l** had very high MIC

values and therefore they are inactive as antibacterial agents. It is interesting to find out that bacterium *E. coli* is resistant to all compounds, suggesting that molecules of the series may be inactive against Gram-negative bacteria.

The results of in vitro antifungal activities showed that synthesized compounds have moderate activity. Most of the synthesized compounds were inactive against fungal strains. Compound **6d** (MIC= 20 $\mu\text{g mL}^{-1}$) had shown significant activity against *C. albicans* when compared with Miconazole (MIC= 10 $\mu\text{g mL}^{-1}$). The compounds **4** (MIC= 30 $\mu\text{g mL}^{-1}$) and **6e** (MIC= 30 $\mu\text{g mL}^{-1}$) showed moderate activity against *A. niger* when compared with Miconazole (MIC= 6.25 $\mu\text{g mL}^{-1}$). The compounds **6b** and **6c** (MIC= 25 and 20 $\mu\text{g mL}^{-1}$ against *A. flavus* and *A. niger*, respectively) were most active from the synthesized series. None of the synthesized compounds showed comparable activity with that of Fluconazole (MIC= 3.25 $\mu\text{g mL}^{-1}$).

Anticancer activity

The newly synthesized 14 compounds were screened for their in vitro growth inhibitory activities against two human breast cancer cell lines MCF-7 and BT-474, 100 μM (micromol mL^{-1}) by MTT assays method, (Table 4). The results are shown as percentage anticancer activity after 24 h.

The compounds found active in preliminary screening were further studied for their cytotoxic effect on human breast cancer cell lines MCF-7 and BT-474 cell lines and the results are expressed as IC_{50} . Among these 14 newly synthesized thiazole derivatives screened for their cytotoxic effect on MCF-7 and BT-474 cells, five compounds showed percentage cell death greater against cell lines used. Among the most active three compounds, two compounds exhibited cell death greater than 50% against both cell lines. The synthesized compounds **6e**, **6f**, **6g**, **6h**, **6k** and **6l** showed maximum percentage cytotoxicity 100 μM . The anticancer studies of compound (**6g**) and (**6l**), against MCF-7 and BT-474 cell lines exhibited IC_{50} values are 1.4, 0.7, 1.2, and 1.3 μM , respectively.

Table 2. Physical data for synthesized Rhodanine derivatives **3**, **4** and **6a-l**.

No.	R	Formula (Mol. wt.)	Time, min	Yield, %	M.P., °C
3	-	C ₁₀ H ₇ NOS ₂ (221)	300	82	204–206
4	-	C ₁₁ H ₉ NOS ₂ (235)	120	85	145–147
6a	methyl	C ₁₃ H ₁₂ N ₂ O ₃ S (276)	30	92	221–223
6b	isopropyl	C ₁₅ H ₁₆ N ₂ O ₃ S (304)	30	90	178–180
6c	sec-butyl	C ₁₆ H ₁₈ N ₂ O ₃ S (318)	35	94	110–112
6d	benzyl	C ₁₉ H ₁₆ N ₂ O ₃ S (352)	30	92	187–189
6e	2-(methylthio)ethyl	C ₁₅ H ₁₆ N ₂ O ₃ S ₂ (336)	40	90	122–124
6f	isobutyl	C ₁₆ H ₁₈ N ₂ O ₃ S (318)	50	96	209–211
6g	hydroxymethyl	C ₁₃ H ₁₂ N ₂ O ₄ S (292)	55	95	195–197
6h	mercaptomethyl	C ₁₃ H ₁₂ N ₂ O ₃ S ₂ (308)	50	92	189–191
6i	carboxymethyl	C ₁₄ H ₁₂ N ₂ O ₅ S (320)	60	90	169–171
6j	imidazolymethyl	C ₁₆ H ₁₄ N ₄ O ₃ S (342)	32	94	162–164
6k	4-hydroxybenzyl	C ₁₉ H ₁₆ N ₂ O ₄ S (368)	40	93	192–194
6l	1-hydroxyethyl	C ₁₄ H ₁₄ N ₂ O ₄ S (306)	30	94	209–211

The solvent of recrystallization was ethanol; Eluents used in TLC were chloroform: methanol (1:9) for all compounds.

Table 3. In vitro antimicrobial evaluation of synthesized Rhodanine derivatives **3**, **4**, and **6a-l**.

Entry	Antibacterial activity (MIC values in $\mu\text{g mL}^{-1}$)			Antifungal activity (MIC values in $\mu\text{g mL}^{-1}$)		
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. flavus</i>	<i>A. niger</i>
3	80	35	100	95	100	100
4	100	100	100	100	95	30
6a	70	60	75	100	80	90
6b	25	15	90	85	25	20
6c	15	20	70	90	25	20
6d	35	30	75	20	90	35
6e	70	100	80	95	35	30
6f	75	15	100	95	70	90
6g	15	12.5	60	95	75	65
6h	10	15	70	65	90	50
6i	100	80	70	95	85	100
6j	6.0	5.5	90	70	85	60
6k	55	65	90	100	80	100
6l	85	100	90	80	95	80
Ciprofloxacin	6.25	6.25	4.0	-	-	-
Ampicillin	12.5	12.5	12.5	-	-	-
Fluconazole	-	-	-	3.25	3.25	3.25
Miconazole	-	-	-	10	6.25	6.25

The data represents the mean values of three replicates; Standard errors were all within 10% of the mean; - denotes not tested.

While the compounds **6e**, **6g**, **6h**, **6k** and **6l** against BT-474 cell lines exhibited IC_{50} : 6.2, 0.7, 6.8, 12.1, and 1.3 $\mu\text{M mL}^{-1}$, respectively. The IC_{50} of reference drug Adriamycin against MCF-7 and BT-474 cells was found to be 0.9 and 0.5 $\mu\text{M mL}^{-1}$, respectively. The anticancer activity of all newly synthesized thiazole derivatives mainly depends on the type of substitution on thiazole moiety. The substitution pattern of amino acids showed variation in anticancer activity.

Table 4. *In vitro* anticancer activity of the studied compounds against the MCF-7 and BT-474 cells, after 24 h

Sr. No.	Compounds	IC_{50} , ^a μM^b	
		MCF-7 ^c	BT-474 ^d
1	3	68.7	61.3
2	4	65.8	62.1
3	6a	64.9	42.6
4	6b	59.4	63.7
5	6c	49.7	66.6
6	6d	80.5	42.6
7	6e	88.7	6.2
8	6f	7.1	55.8
9	6g	1.4	0.7
10	6h	7.2	6.8
11	6i	41.3	74.1
12	6j	68.7	64.5
13	6k	3.1	12.1
14	6l	1.2	1.3
	Adriamycin	0.9	0.5

^a GI_{50} (Growth inhibition of 50): Concentration of drug that decreases the growth of the cells by 50 compared to a non-treated control cell. ^bValues are the average of three readings ^cMCF-7: Human breast cancer cell line ^dBT-474: Human breast cancer cell line ^eAdriamycin: Positive control compound

The compounds with a substituted hydroxyl group attached to thiazole ring which contains amino acids showed the highest percentage of cell death. While the compounds having electron releasing alkyl chain, methyl-1H-imidazole ring group on thiazole rings resulted in the loss of activity.

CONCLUSIONS

We have reported at room temperature, less reaction time with good to excellent yields. All the synthesized compounds were also tested for their *in vitro* anticancer activity against MCF-7 and BT-474 human breast cancer cell lines. Among them most of the compounds show good to excellent anticancer activity, especially **6e**, **6f**, **6g**, **6h**, **6k** and **6l** are the most active compounds against tested cell line. The compound **6g** activity value is very close to standard drug, which can be regarded as the promising drug candidate for development of anticancer drugs. The investigation has revealed that a number of Rhodanine derivatives have promising antimicrobial properties.

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