Synthesis of thiophene derivatives and their anti-microbial, antioxidant, anticorrosion and anticancer activity

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Abstract

Background: A new series of thiophene analogues was synthesized and checked for their in vitro antibacterial, antifungal, antioxidant, anticorrosion and anticancer activities.

Results: A series of ethyl-2-(substituted benzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate derivatives were synthesized by using Gewald synthesis and their structures were confirmed by FTIR, MS and 1H-NMR. The synthesized compounds were further evaluated for their in vitro biological potentials i.e. antimicrobial activity against selected microbial species using tube dilution method, antiproliferative activity against human lung cancer cell line (A-549) by sulforhodamine B assay, antioxidant activity by using DPPH method and anticorrosion activity by gravimetric method.

Conclusion: Antimicrobial screening results showed that compound \( S_1 \) was the most potent antibacterial agent against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella typhi having MIC value 0.81 µM/ml and compound \( S_4 \) also displayed excellent antifungal activity against both Candida albicans and Aspergillus niger (MIC=0.91 µM/ml) when compared with cefadroxil (antibacterial) and fluconazole (antifungal) as standard drug. The antioxidant screening results indicated that compound \( S_4 \) and \( S_6 \) exhibited excellent antioxidant activity with IC\(_{50}\) values 48.45 and 45.33 respectively when compared with the ascorbic acid as standard drug. Anticorrosion screening results indicated that compound \( S_7 \) showed more anticorrosion efficiency (97.90%) with low corrosion rate. Results of anticancer screening indicated that compound \( S_8 \) showed effective cytotoxic activity against human lung cancer cell line (A-549) at dose of \( 10^{-4} \) M when compared with adriamycin as standard.

Keywords: Antimicrobial, Antioxidant, Anticorrosion, Anticancer

Background

Heterocyclic compound are extensively distributed in nature and have versatile synthetic applicability and biological activity which implement the new approaches for the medicinal chemist to plan and organize towards the discovery of novel drugs [1]. Thiophene and its derivatives showed extensive significance in pharmaceutical field because of its varied biological and clinical applications [2].

Serious life-threatening infections have been increased because of the increase in the resistance of microorganism agents which is mainly caused by multi-drug resistant of Gram-positive and Gram-negative pathogenic bacteria [3]. So, there is an pressing need to designed an effective, potent and novel antimicrobial agents with better pharmacodynamic and pharmacokinetic properties [4]. Moreover, cancer also remains one of the primary causes of death in the world therefore effective treatments with novel medicines having improved tumor selectivity, efficacy, and safety remains desirable [5]. Ideal anticancer drugs eliminate the cancer cells without harming normal tissues [6]. Unfortunately, no currently available anticancer agents with desired therapeutic index that earned
this criterion and clinical use of drugs which involves a weighing of benefits beside toxicity [7].

Recently, thiophene and its derivatives attracted researchers in expanding their potential in the field of antioxidant [8]. The main purpose of antioxidant is to neutralize free radicals in order to prevent various oxidative diseases like autoimmune, cardiovascular, and neurovascular diseases [9]. The setback of drug resistance and generation of free radicals has promoted the researchers to study and explore for new compounds to combat the problems of infection and the associated risks from generation of free radicals. Corrosion inhibition mechanism is a crucial problem which causes destruction of material (usually metal) by chemical and/or electrochemical reaction with its environment. Organic inhibitors generally containing heteroatom's such as sulphur is found to have higher basicity and electron donating ability which act by adsorption on the metal surface and slabs the active surface sites which leads in the reduction of corrosion rate [10, 11].

Thiophene derivatives show remarkable applications in different disciplines. In medicine, thiophene derivatives show antimicrobial [12], analgesic and anti-inflammatory [13], antihypertensive [14], and antitumor activity [15] while they are also used as inhibitors of corrosion of metals [16] or in the fabrication of light-emitting diodes in material science [17]. In light of afore-mentioned facts and in continuance of our research on the improvement of thiophene derivatives, we hereby report the synthesis and biological screening (i.e. antimicrobial, antioxidant, anticorrosion and anticancer) of thiophene derivatives with a variety of acceptor and donor groups.

Results and discussion

Chemistry

A series of ethyl-2-(substituted benzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate derivatives were synthesized (Scheme 1, S1–S18) by using Gewald synthesis. Initially, ethylcyanoacetate and cyclohexanone was reacted with sulphur at room temperature with continuous stirring in presence of diethylamine which resulted in the formation of intermediate-1. Later on intermediate-1 treated with substituted aromatic benzaldehyde and dioxane in presence of triethylamine yielded the final compounds (S1–S18). The structures of the entire newly synthesized compound were checked by IR data and 1H NMR which was in full favour with molecular structures assigned. Its physical characterizations were indicated in Table 1.

Antimicrobial activity

In vitro antimicrobial activity of novel synthesized compounds were screened against the microorganisms such as Gram positive (Staphylococcus aureus, Bacillus subtilis), Gram negative (Escherichia coli, Salmonella typhi) and fungal strains (Aspergillus niger and Candida albicans) using tube dilution method. The results after examined were expressed as minimum inhibitory concentration (MIC i.e. lowest conc. required for the test substances to complete growth inhibition). The MIC values of standard and synthesized compounds were presented in Table 2. Antimicrobial evaluation results indicated that the entire synthesized compounds were found to have significant antimicrobial activity and different compound were found to be active against different microorganisms.

Antimicrobial screening results showed that compound S1 was the most potent antibacterial agent against B. subtilis, S. aureus, E. coli and S. typhi having MIC value 0.81 µM/mL. Further having significant antibacterial activity, the synthesized compound S1 also displayed excellent antifungal activity against both A. niger and C. albicans and (MIC=0.91 µM/mL). On the whole, antimicrobial activity results (Table 2) indicated that compounds S1 and S4 were found to be most potent antimicrobial agents.

Antioxidant activity

Newly synthesized derivatives were investigated for their in vitro antioxidant activity by DPPH assay at absorbance 517. The percentage (%) inhibition was calculated from the Eq. 1 and IC50 value of newly synthesized compounds were obtained from the graph drawn between concentration with % inhibition of test compound given in Figs. 1, 2 and 3. From the results obtained it was known that all the newly derived compounds were found to have good to moderate antioxidant activity. Among them, compound S4 and S6 exhibited excellent antioxidant activity with IC50 values 48.45 and 45.33 respectively when compared with the ascorbic acid as standard drug. The results were shown in Table 3.

Anticorrosion activity

Corrosion inhibition of most of the active synthesized thiophene derivatives was evaluated by weight loss of mild steel in their presence and absence in 1.0 M HCl at 35 °C. The anticorrosion activity results of selected compound are indicated in Table 4.

The graph of inhibition efficiency Vs concentration at 35 °C for 12 h presented in Fig. 4. The result of inhibition efficiency after 12 h at 35 °C indicated that compounds exhibited good corrosion inhibition potential. Compound S1 showed more anticorrosion efficiency (97.90%) and low corrosion rate (0.98). The anticorrosion screening results indicated that the corrosion rate decreases with increasing concentration of inhibitors.
Anticancer activity
The synthesized derivatives were screened for cytotoxicity by sulforhodamine-B (SRB) assay method on A-549 cell line and the results were compared with adriamycin as a standard drug. Among the screened compounds, compound $S_8$ showed significant anticancer activity at dose $10^{-4}$ M against human lung cancer cell line (A-549) when compared with adriamycin as standard. Antitumor activity of the synthesized compound against human lung cancer cell line (A-549) was found to be improved because of the presence of electron withdrawing group (p-Br, Compound $S_8$) on benzylidene portion. Anticancer activity of ethyl-2-(substituted benzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate derivatives were presented in Tables 5 and 6. The results obtained from graph drawn between tested compound and standard drug indicated in Fig. 5.

Structure activity relationship (SAR) studies
The subsequent structure activity relationship of newly synthesized ethyl-2-(substituted benzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate derivatives can be derived on the basis of the result
obtained from antimicrobial, antioxidant, anticorrosion and anticancer testing as follows:

1. Antibacterial activity of the synthesized compound was enhanced due to the presence of electron withdrawing group (–Cl, Compound $S_1$) on benzylidene portion against $S. aureus$, $B. subtilis$, $E. coli$ and $S. typhi$. Because of the absence of hyper conjugation of methyl group favours the increase in activity of lead molecule. The structural variations such as chloro group at o and p position to the aromatic linkage favours the activity in positive manner.

2. Antifungal activity of the synthesized compound was enhanced because of the presence of electron releasing group (o-OCH$_3$, Compound $S_4$) in benzylidene portion against $C. albicans$ and $A. Niger$.

3. Presence of electron releasing group (o-OCH$_3$, Compound $S_4$, and m-OCH$_3$ and p-OH, Compound $S_6$) in benzylidene portion showed the significant increase in antioxidant activity.

4. Anticorrosion activity was also found to be improved because of the presence of electron releasing group (m-OCH$_3$ and p-OCH$_3$, Compound $S_7$).

5. Anticancer activity of the synthesized compound was found to be increased against human lung cancer cell

| Compound | M. formula | M. Wt. | m. p. (°C) | $R_f$ valuea | % Yield |
|----------|------------|--------|------------|--------------|---------|
| $S_1$    | C$_{18}$H$_{17}$Cl$_2$NO$_2$S | 382.30 | 96–97      | 0.53         | 84.71   |
| $S_2$    | C$_{18}$H$_{18}$BrNO$_2$S | 392.31 | 122–124    | 0.47         | 75.00   |
| $S_3$    | C$_{18}$H$_{18}$N$_2$O$_4$S | 358.41 | 87–89      | 0.62         | 82.22   |
| $S_4$    | C$_{18}$H$_{18}$N$_2$O$_4$S | 343.44 | 95–98      | 0.38         | 72.67   |
| $S_5$    | C$_{18}$H$_{18}$N$_2$O$_4$S | 384.53 | 98–100     | 0.39         | 78.86   |
| $S_6$    | C$_{18}$H$_{18}$N$_2$O$_4$S | 359.44 | 95–97      | 0.66         | 82.43   |
| $S_7$    | C$_{18}$H$_{18}$N$_2$O$_4$S | 373.13 | 97–99      | 0.45         | 83.67   |
| $S_8$    | C$_{18}$H$_{18}$BrNO$_2$S | 392.31 | 94–95      | 0.42         | 87.62   |
| $S_9$    | C$_{18}$H$_{18}$N$_2$O$_4$S | 347.86 | 87–88      | 0.41         | 85.63   |
| $S_{10}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 358.41 | 94–97      | 0.61         | 79.80   |
| $S_{11}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 358.41 | 130–131    | 0.58         | 72.58   |
| $S_{12}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 313.41 | 90–93      | 0.31         | 68.72   |
| $S_{13}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 356.48 | 134–135    | 0.53         | 83.23   |
| $S_{14}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 329.41 | 110–111    | 0.68         | 73.65   |
| $S_{15}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 329.41 | 108–109    | 0.63         | 81.35   |
| $S_{16}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 329.41 | 93–94      | 0.56         | 69.87   |
| $S_{17}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 343.44 | 93–95      | 0.55         | 81.81   |
| $S_{18}$ | C$_{18}$H$_{18}$N$_2$O$_4$S | 373.13 | 97–99      | 0.62         | 76.69   |

* TLC mobile phase-benzene

| Compound | Minimum inhibitory concentration (MIC $= \mu$M/ml) |
|----------|---------------------------------------------|
|          | **Bacterial species** | **Fungal species** |
|          | $S. aureus$ | $B. subtilis$ | $E. coli$ | $S. typhi$ | $A. niger$ | $C. albicans$ |
| $S_1$    | 0.81       | 0.81       | 0.81    | 0.81    | 3.26       | 3.26       |
| $S_2$    | 3.19       | 3.19       | 1.59    | 1.59    | 3.19       | 3.19       |
| $S_3$    | 3.49       | 3.49       | 3.49    | 3.49    | 3.49       | 3.49       |
| $S_4$    | 3.64       | 3.64       | 3.64    | 3.64    | 0.91       | 0.91       |
| $S_5$    | 3.25       | 3.25       | 3.25    | 3.25    | 3.25       | 3.25       |
| $S_6$    | 3.48       | 3.48       | 3.48    | 3.48    | 1.74       | 1.74       |
| $S_7$    | 3.35       | 3.35       | 6.70    | 6.70    | 3.35       | 3.35       |
| $S_8$    | 3.19       | 3.19       | 3.19    | 3.19    | 3.19       | 3.19       |
| $S_9$    | 3.59       | 3.59       | 3.59    | 3.59    | 3.59       | 3.59       |
| $S_{10}$ | 3.49       | 3.49       | 3.49    | 3.49    | 3.49       | 3.49       |
| $S_{11}$ | 0.87       | 1.74       | 3.49    | 3.49    | 3.49       | 3.49       |
| $S_{12}$ | 3.99       | 3.99       | 3.99    | 3.99    | 3.99       | 3.99       |
| $S_{13}$ | 3.51       | 3.51       | 3.51    | 3.51    | 3.51       | 3.51       |
| $S_{14}$ | 3.79       | 3.79       | 3.79    | 3.79    | 3.79       | 3.79       |
| $S_{15}$ | 3.79       | 3.79       | 7.59    | 7.59    | 7.59       | 7.59       |
| $S_{16}$ | 3.79       | 3.79       | 3.79    | 3.79    | 3.79       | 3.79       |
| $S_{17}$ | 3.64       | 7.28       | 3.64    | 3.64    | 3.64       | 3.64       |
| $S_{18}$ | 6.70       | 3.35       | 3.35    | 3.35    | 3.35       | 3.35       |
| Cefadroxil| 1.64       | 3.28       | 1.64    | 1.64    | 2.04       | 2.04       |

Obs. minimum inhibitory concentration in each column is showed in italics.
line (A-549) by the addition of electron withdrawing group (p-Br, Compound S8).

6. From these result we may conclude that for a compound to be most effective and potent against various target it is essential to have different structural requirements.

**Experimental section**

**Materials and methods**

Reagents and solvents were of both laboratory and analytical grade which were used in the study. Hi-media Laboratories were used to obtain the Media for antimicrobial activity and all the microbial type cell cultures (MTCC) were obtained from Institute of Microbial Technology and Gene bank (IMTECH), Chandigarh. Labtech melting point equipment was used to determined melting points by an open glass capillary method. Thin layer chromatography (TLC) was used for observing the reaction steps forward making use of commercial silica gel plates (Merck), Silica gel F254 on aluminium sheets. 1H NMR spectra were screened by Bruker Avance 400 NMR spectrometer in a suitable chloroform solvent and are expressed in parts per million (δ, ppm) downfield from tetramethyl silane (internal standard). 1H NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Infrared (IR) spectra were recorded on a Bruker FTIR spectrometer using KBr pellet method and expressed in cm⁻¹. The mass spectra of derived compounds were done on Waters Micromass Q-ToF Micro instrument by mass spectrometer (Fig. 6).

**General procedure for the synthesis of (ethyl-2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate derivatives (S1–S18)**

**Step I: Synthesis of ethyl-2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate**

Sulphur (1.92 g, 0.06 mol) was added to a mixture of ethylcyanoacetate (5.32 ml, 0.05 mol) and cyclohexanone (5.2 ml, 0.05 mol) with continuous stirring at room temperature followed by the adding up of diethylamine (5.26 ml, 0.05 mol). The reaction mixture was then stirred at 40–50 °C for 2 h. Thin layer chromatography was used for the confirmation of reaction mixture. The resulting mixture was set aside for overnight at room temperature. The precipitate obtained was filtered, washed with water, dried and recrystallized from ethanol.

**Step II: Synthesis of ethyl-2-(substituted benzylideneamino)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate**

Intermediate 1 (0.05 mol) and different substituted aromatic benzaldehyde (0.05 mol) in 15 ml of dioxane and triethylamine (0.005 mol) were taken in RBF and refluxed for 2 h. The resulting mixture was then stirred at 40–50 °C for 2 h. Thin layer chromatography was used for the confirmation of reaction mixture. The resulting mixture was set aside for overnight at room temperature. The precipitate obtained was filtered, washed with water, dried and recrystallized from ethanol.

**Analytical data**

**Compound S1:** Ethyl-2-(2,4-dichlorobenzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: M. p: 96–97 °C; yield: 84.71%; IR (KBr pellets, cm⁻¹): 2977 (C–H str.), 1582 (C=C str.), 1657 (C=N str.), 1698 (C=O str., carbonyl), 1267 (C–O–C str.), 665.53 (C=S–C str., thiophene ring), 822 (C–Cl str., aromatic); 1H NMR
Table 3  Antioxidant activity of newly synthesized derivatives (S₁–S₆)

| Compound | % Inhibition | IC₅₀ 25 µg/ml | 50 µg/ml | 75 µg/ml | 100 µg/ml |
|----------|--------------|---------------|----------|----------|-----------|
| S₁       | 25.62        | 30.56         | 51.34    | 67.28    | 73.31     |
| S₂       | 32.47        | 47.61         | 64.92    | 78.52    | 53.12     |
| S₃       | 23.26        | 47.52         | 59.68    | 72.69    | 61.35     |
| S₄       | 35.68        | 52.23         | 65.38    | 78.63    | 48.45     |
| S₅       | 23.02        | 39.65         | 56.32    | 70.23    | 66.77     |
| S₆       | 36.62        | 54.63         | 67.82    | 82.63    | 45.33     |
| S₇       | 33.21        | 48.62         | 64.65    | 81.62    | 51.62     |
| S₈       | 15.63        | 32.63         | 46.65    | 66.65    | 73.66     |
| S₉       | 15.62        | 25.66         | 46.99    | 59.63    | 79.77     |
| S₁₀      | 16.53        | 25.73         | 47.25    | 72.35    | 59.01     |
| S₁₁      | 21.03        | 33.65         | 48.56    | 76.52    | 69.96     |
| S₁₂      | 15.71        | 32.56         | 44.62    | 65.54    | 76.17     |
| S₁₃      | 21.25        | 35.62         | 56.28    | 69.58    | 69.07     |
| S₁₄      | 21.68        | 38.91         | 56.74    | 70.73    | 67.11     |
| S₁₅      | 25.32        | 30.56         | 52.36    | 67.23    | 72.90     |
| S₁₆      | 21.72        | 36.39         | 58.65    | 72.66    | 66.47     |
| S₁₇      | 31.58        | 47.54         | 63.25    | 76.85    | 54.19     |
| S₁₈      | 17.63        | 23.56         | 45.63    | 75.63    | 53.05     |
| Ascorbic acid | 37.62 | 56.63 | 67.82 | 85.63 | 43.35 |

Obs. minimum inhibition concentration at 50% (IC₅₀) in last column is showed in italics

(CDC₁₃, δ ppm): 7.28–7.50 (m, 3H, Ar–H), 8.12 (s, 1H, CH=CH=N), 4.33 (q, 2H, CH₂), 1.39 (t, 3H, CH₃), 2.74 (t, 2H, CH₂ cyclo), 1.67 (q, 2H, CH₂ cyclo); ¹³C NMR (75 MHz, CDCl₃) d 165.94, 160.53, 128.07, 122.37, 106.00, 50.97, 32.04, 29.75, 29.26, 29.90; MS ES+(ToF): m/z 359.41 [M⁺+1].

**Compound S₄:** Ethyl-2-(2-methoxybenzylideneamino)-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate: M. p: 95–96 °C; yield: 72.67%; IR (KBr pellets, cm⁻¹): 2934 (C–H str.), 1600 (C–C str.), 1657 (C=N str.), 1693 (C=O str., carbonyl), 1252 (C–O–C str.), 647 (C–S–C str., thiophene ring), 2842 (O–CH₃ str., aromatic); ¹H NMR (CDCl₃, δ ppm): 6.84–7.33 (m, 3H, Ar–H), 3.76 (s, 3H, CH₃ methoxy), 9.75 (s, 1H, CH=NH), 4.36 (q, 2H, CH₂), 1.35 (t, 3H, CH₃), 2.68 (t, 2H, CH₂ cyclo), 1.84 (q, 2H, CH₂ cyclo); ¹³C NMR (101 MHz, CDCl₃) d 166.84, 161.63, 127.77, 120.37, 107.00, 50.90, 41.04, 28.75, 29.20, 28.81; MS ES+(ToF): m/z 344.4 [M⁺+1].

**Compound S₅:** Ethyl-2-(4-(diethylamino)benzylideneamino)-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate: M. p: 98–100 °C; yield: 78.86%; IR (KBr pellets, cm⁻¹): 2985 (C–H str.), 1575 (C=C str.), 1649 (C=N str.), 1736 (C=O str., carbonyl), 1274 (C–O–C str.), 639 (C=S–C str., thiophene ring), 1333 (C–N str., aromatic); ¹H NMR (CDCl₃, δ ppm): 6.60–7.38 (m, 3H, Ar–H), 3.94 (q, 2H, N-CH₃), 1.25 (t, 3H, N-CH₃), 9.05 (s, 1H, CH=NH), 4.29 (q, 2H, CH₂), 1.34 (t, 3H, CH₃), 2.53 (t, 2H, CH₂ cyclo), 1.58 (q, 2H, CH₂ cyclo); ¹³C NMR (101 MHz, CDCl₃) d 165.50, 163.45, 129.90, 106.26, 106.82, 93.93, 74.51, 50.23, 32.24, 29.48, 22.45, 20.76, 15.11; MS ES+(ToF): m/z 385.53 [M⁺+1].

**Compound S₆:** Ethyl-2-(4-hydroxy-3-methoxy benzylideneamino)-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate: M. p: 95–97 °C; yield: 82.43%; IR (KBr pellets, cm⁻¹): 2985 (C–H str.), 1579 (C=C str.), 1645 (C=N str.), 1792 (C=O str., carbonyl), 1273 (C–O–C str.), 632 (C=S–C str., thiophene ring), 3403 (O–H str., aromatic); ¹H NMR (CDCl₃, δ ppm): 7.02–7.05 (m, 3H, Ar–H), 3.76 (s, 3H, CH₃ methoxy), 5.07 (s, 1H, OH alcohol), 9.86 (s, 1H, CH=NH), 4.26 (q, 2H, CH₂), 1.34 (t, 3H, CH₃), 2.53 (t, 2H, CH₂ cyclo), 1.78 (q, 2H, CH₂ cyclo); ¹³C NMR (75 MHz, CDCl₃) d 165.90, 164.48, 131.27, 125.37, 116.11, 54.00, 39.06, 36.17, 30.82, 39.79, 20.83, 14.26; MS ES+(ToF): m/z 362 [M⁺+1].

**Compound S₇:** Ethyl-2-(3,4-dimethoxybenzylideneamino)-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate: M. p: 97–99 °C; yield: 83.67%; IR (KBr pellets, cm⁻¹): 2985 (C–H str.), 1579 (C=C str.), 1648 (C=O str., carbonyl), 1274 (C–O–C str.), 639 (C=S–C str., thiophene ring), 2854 (O–CH₃ str., aromatic); ¹H NMR (CDCl₃, δ ppm): 6.87–7.11 (m, 3H, Ar–H), 3.73 (s, 3H, CH₃ methoxy) 9.02 (s, 1H, CH=NH), 4.26 (q, 2H, CH₂), 1.49 (t, 3H, CH₃), 2.53 (t, 2H, CH₂ cyclo), 1.67 (q, 2H, CH₂ cyclo); ¹³C NMR (101 MHz, CDCl₃) d 166.48, 163.45, 129.97, 107.25, 105.80, 94.93,
Table 4  Corrosion inhibition efficiency of thiophene derivatives at 35 °C, for 12 h

| Inhibitors | Conc. Ppm | Weight before | Weight after | Weight loss | Corrosion rate (mg/cm² h) | Inhibition efficiency (%) | Surface area coverage |
|------------|-----------|---------------|--------------|-------------|---------------------------|--------------------------|------------------------|
| Blank      | 0         | 0.7296        | 0.6706       | 0.059       | 18.27                     | –                        | –                      |
| S₁         | 50        | 0.7187        | 0.7035       | 0.0152      | 4.71                      | 74.24                    | 0.74                   |
|            | 100       | 0.7106        | 0.6998       | 0.0108      | 3.34                      | 81.69                    | 0.82                   |
|            | 200       | 0.7185        | 0.7097       | 0.0088      | 2.72                      | 85.08                    | 0.85                   |
|            | 300       | 0.7189        | 0.7123       | 0.0066      | 2.04                      | 88.81                    | 0.89                   |
| S₂         | 50        | 0.7292        | 0.7165       | 0.0127      | 3.93                      | 76.04                    | 0.76                   |
|            | 100       | 0.7354        | 0.7251       | 0.0103      | 3.19                      | 80.57                    | 0.81                   |
|            | 200       | 0.7055        | 0.6995       | 0.006       | 1.86                      | 88.68                    | 0.89                   |
|            | 300       | 0.7123        | 0.7080       | 0.0043      | 1.33                      | 91.89                    | 0.92                   |
| S₃         | 50        | 0.7073        | 0.695        | 0.0123      | 3.81                      | 77.68                    | 0.78                   |
|            | 100       | 0.701         | 0.6911       | 0.0099      | 3.06                      | 82.03                    | 0.82                   |
|            | 200       | 0.7045        | 0.6973       | 0.0072      | 2.23                      | 86.93                    | 0.87                   |
|            | 300       | 0.7046        | 0.6990       | 0.0056      | 1.73                      | 89.84                    | 0.90                   |
| S₄         | 50        | 0.7144        | 0.7057       | 0.0087      | 2.69                      | 86.41                    | 0.86                   |
|            | 100       | 0.6956        | 0.6888       | 0.0068      | 2.11                      | 89.38                    | 0.89                   |
|            | 200       | 0.7098        | 0.7023       | 0.0075      | 2.32                      | 88.28                    | 0.88                   |
|            | 300       | 0.7198        | 0.7123       | 0.0075      | 2.32                      | 88.28                    | 0.88                   |
| S₅         | 50        | 0.7062        | 0.6933       | 0.0129      | 3.99                      | 80.45                    | 0.80                   |
|            | 100       | 0.7073        | 0.6941       | 0.0087      | 2.69                      | 86.82                    | 0.87                   |
|            | 200       | 0.7004        | 0.6964       | 0.004       | 1.24                      | 93.94                    | 0.94                   |
|            | 300       | 0.7045        | 0.7008       | 0.0037      | 1.15                      | 94.39                    | 0.94                   |
| S₆         | 50        | 0.7057        | 0.6932       | 0.0125      | 3.87                      | 77.27                    | 0.77                   |
|            | 100       | 0.7067        | 0.7086       | 0.0071      | 2.20                      | 87.09                    | 0.87                   |
|            | 200       | 0.7177        | 0.7128       | 0.0049      | 1.52                      | 91.09                    | 0.91                   |
|            | 300       | 0.7123        | 0.7080       | 0.0043      | 1.33                      | 92.18                    | 0.92                   |
| S₇         | 50        | 0.7208        | 0.7098       | 0.011       | 3.41                      | 83.53                    | 0.84                   |
|            | 100       | 0.7091        | 0.703        | 0.0061      | 1.89                      | 90.87                    | 0.91                   |
|            | 200       | 0.7102        | 0.7079       | 0.0023      | 0.71                      | 96.56                    | 0.97                   |
|            | 300       | 0.7103        | 0.7089       | 0.0014      | 0.43                      | 97.90                    | 0.98                   |
| S₈         | 50        | 0.7152        | 0.7023       | 0.0129      | 3.99                      | 79.84                    | 0.80                   |
|            | 100       | 0.7333        | 0.7264       | 0.0069      | 2.14                      | 89.22                    | 0.89                   |
|            | 200       | 0.7225        | 0.7187       | 0.0038      | 1.18                      | 94.06                    | 0.94                   |
|            | 300       | 0.7046        | 0.7009       | 0.0037      | 1.15                      | 94.22                    | 0.94                   |
| S₉         | 50        | 0.7155        | 0.7114       | 0.0158      | 4.89                      | 79.10                    | 0.77                   |
|            | 100       | 0.7221        | 0.712        | 0.0101      | 3.13                      | 85.36                    | 0.85                   |
|            | 200       | 0.7193        | 0.7115       | 0.0078      | 2.41                      | 88.70                    | 0.89                   |
|            | 300       | 0.7330        | 0.7260       | 0.007       | 2.17                      | 89.86                    | 0.90                   |
| S₁₀        | 50        | 0.718         | 0.7047       | 0.0133      | 4.12                      | 77.46                    | 0.77                   |
|            | 100       | 0.7382        | 0.7311       | 0.0071      | 2.20                      | 87.97                    | 0.88                   |
|            | 200       | 0.6981        | 0.6934       | 0.0047      | 1.46                      | 92.03                    | 0.92                   |
|            | 300       | 0.712         | 0.708        | 0.004       | 1.24                      | 93.22                    | 0.93                   |

Obs: the highest inhibition efficiency (%) in 7th column is showed in italics

75.51, 55.23, 36.24, 29.48, 22.35, 19.76, 14.11; MS ES+ (ToF): m/z 374 [M⁺]+1.

**Compound S₈**: Ethyl-2-(4-bromobenzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: M. p: 94–95 °C; Yield: 87.62%; IR (KBr pellets, cm⁻¹): 2985 (C–H str.), 1596 (C=C str.), 1646 (C=N str.), 1696 (C=O str., carbonyl), 1274 (C–O–C str.), 639 (C–S–C str., thiophene ring), 607 (C–Br str., aromatic); ¹H NMR
(CDCl$_3$, δppm): 7.64–7.86 (m, 3H, Ar–H), 8.34 (s, 1H, CH=NC), 4.81 (q, 2H, CH$_2$), 1.52 (t, 3H, CH$_3$), 2.51 (t, 2H, CH$_2$ cyclo), 1.88 (q, 2H, CH$_2$ cyclo); 13C NMR (75 MHz, CDCl$_3$) δ 165.80, 161.48, 127.24, 121.35, 106.08, 51.00, 32.06, 31.16, 29.95, 29.68, 29.50, 29.88, 25.83, 15.26; MS ES$^+$ (ToF): m/z 393 [M$^++$+1].

**Compound S 9**: Ethyl-2-(2-chlorobenzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: M. p: 87–88 °C; yield: 85.63%; IR (KBr pellets, cm$^{-1}$): 2977 (C–H str.), 1582 (C=C str.), 1657 (C=N str.), 1698 (C=O str., carbonyl), 1267 (C–O–C str.), 665 (C–S–C str., thiophene ring), 822 (C–Cl str., aromatic); 1H NMR (CDCl$_3$, δppm): 7.17–7.74 (m, 3H, Ar–H), 10.09 (s, 1H, CH=NC), 4.48 (q, 2H, CH$_2$), 1.32 (t, 3H, CH$_3$), 2.40 (t, 2H, CH$_2$ cyclo), 1.69 (q, 2H, CH$_2$ cyclo); 13C NMR (75 MHz, CDCl$_3$) δ 166.89, 161.47, 128.27, 122.37, 107.12, 51.01, 32.06, 31.17, 29.84, 29.80, 29.78, 29.68, 29.49, 29.08, 23.83, 14.26; MS ES$^+$ (ToF): m/z 349 [M$^++$+1].

**Compound S 10**: Ethyl-2-(2-nitrobenzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: M. p: 94–97 °C; yield: 79.80%; IR (KBr pellets, cm$^{-1}$): 2983 (C–H str.), 1592 (C=C str.), 1649 (C=N str.), 1699 (C=O str., carbonyl), 1267 (C–O–C str.), 741 (C–S–C str., thiophene ring); 1H NMR (CDCl$_3$, δppm): 7.64–8.24 (m, 3H, Ar–H), 10.27 (s, 1H, CH=NC), 4.60 (q, 2H, CH$_2$), 1.38 (t, 3H, CH$_3$), 2.51 (t, 2H, CH$_2$ cyclo), 1.63 (q, 2H, CH$_2$ cyclo); 13C NMR (75 MHz, CDCl$_3$) δ 166.90, 164.48, 131.27, 125.37, 116.11, 54.00, 34.06, 36.17, 30.82, 39.80, 20.93, 15.26; MS ES$^+$ (ToF): m/z 359 [M$^++$+1].

**Compound S 11**: Ethyl-2-(4-nitrobenzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: M. p: 130–131 °C; yield: 72.58; IR (KBr pellets, cm$^{-1}$): 2981 (C–H str.), 1577 (C=C str.), 1593 (C=N str.), 1713 (C=O str., carbonyl), 1269 (C–O–C str.), 690 (C–S–C str., thiophene ring), 1512, 1344 (N–O str., aromatic); 1H NMR (CDCl$_3$, δppm): 7.89–8.84 (m, 3H, Ar–H), 10.15 (s, 1H, CH=NC), 4.42 (q, 2H, CH$_2$), 1.38 (t, 3H, CH$_3$), 2.57 (t, 2H, CH$_2$ cyclo), 1.64 (q, 2H, CH$_2$ cyclo); 13C NMR (75 MHz, CDCl$_3$) δ 165.83, 164.50, 131.37, 125.37, 117.11, 56.00, 39.05, 36.17, 30.82, 39.79, 21.83, 14.36; MS ES$^+$ (ToF): m/z 359 [M$^++$+1].

**Compound S 12**: Ethyl-2-(benzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: M. p: 90–93 °C; yield: 68.72; IR (KBr pellets, cm$^{-1}$): 2985 (C–H str.), 1601 (C=C str.), 1651 (C=N str.), 1736 (C=O str., carbonyl), 1272 (C–O–C str.), 741 (C–S–C str., thiophene ring); 13C NMR (75 MHz, CDCl$_3$) δ 165.97, 163.48, 130.27, 122.37, 107.12, 51.01, 32.06, 31.17, 29.84, 29.80, 29.78, 29.68, 29.49, 29.08, 23.83, 14.26; MS ES$^+$ (ToF): m/z 314 [M$^++$+1].

**Compound S 13**: Ethyl-2-(4-(dimethylamino)benzylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: M. p: 134–135 °C; yield: 83.23%; IR (KBr pellets, cm$^{-1}$): 2977 (C–H str.), 1582 (C=C str.), 1657 (C=N str.), 1698 (C=O str., carbonyl), 1267 (C–O–C str.), 665.53 (C–S–C str., thiophene ring), 822 (C–Cl str., aromatic); 1H NMR (CDCl$_3$, δppm): 6.74–7.64 (m, 3H, Ar–H), 10.27 (s, 1H, CH=NC), 3.97 (q, 2H, CH$_2$), 1.38 (t, 3H, CH$_3$), 2.51 (t, 2H, CH$_2$ cyclo), 1.63 (q, 2H, CH$_2$ cyclo); 13C NMR (75 MHz, CDCl$_3$) δ 166.90, 164.48, 131.27, 125.37, 116.11, 54.00, 34.06, 36.17, 30.82, 39.80, 20.93, 15.26; MS ES$^+$ (ToF): m/z 358 [M$^++$+1].

![Graph of inhibition efficiency vs conc. of inhibitors for 12 h](image-url)

**Fig. 4** Graph of inhibition efficiency vs conc. of inhibitors for 12 h.
Table 5  Percentage control growth against human lung cancer cell line A-549

| Molar drug concentrations | Experiment 1 | Experiment 2 | Experiment 3 | Average values |
|---------------------------|--------------|--------------|--------------|----------------|
|                           | $10^{-7}$ M  | $10^{-6}$ M  | $10^{-5}$ M  | $10^{-4}$ M    | $10^{-7}$ M  | $10^{-6}$ M  | $10^{-5}$ M  | $10^{-4}$ M  |
| S6                        | 1069         | 127.4        | 126.8        | 60.4           | 134.1        | 122.0        | 144.3        | 79.4          | 131.3        | 130.6        | 131.0        | 72.2          | 124.1        | 126.6        | 134.0        | 707           |
| S7                        | 1110         | 127.1        | 127.4        | 71.5           | 147.4        | 126.6        | 136.2        | 90.1          | 130.6        | 128.7        | 127.1        | 90.5          | 129.7        | 127.5        | 130.2        | 840           |
| S8                        | 1014         | 131.3        | 126.3        | 33.1           | 134.7        | 115.2        | 127.0        | 45.7          | 130.2        | 129.1        | 132.3        | 55.7          | 122.1        | 125.2        | 128.5        | 448           |
| S9                        | 1036         | 111.0        | 126.1        | 53.8           | 117.0        | 98.5         | 125.0        | 58.4          | 124.9        | 114.8        | 136.4        | 66.3          | 115.2        | 108.1        | 129.2        | 595           |
| S10                       | 1068         | 129.6        | 123.0        | 43.1           | 140.2        | 113.2        | 122.9        | 44.9          | 128.4        | 134.1        | 130.6        | 66.7          | 125.1        | 125.6        | 125.5        | 516           |
| ADR                       | 580          | 25.8         | $-0.2$       | $-20.2$        | 9.3          | 25.4         | $-1.6$       | $-128$        | 0.7          | 26.3         | 7.1          | 0.3           | 22.6         | 25.8         | 1.8          | $-109$        |
Table 6 Anticancer activity of selected newly synthesized derivatives

| A-549 | Molar drug concentrations calculated from graph |
|-------|--------------------------------------------------|
|       | LC_{50}  | TGI  | Gl_{50} |
| S_4   | NE       | NE   | 0.048   |
| S_5   | NE       | NE   | 0.359   |
| S_9   | 19.5     | 0.126| 8E−04   |
| S_8   | NE       | 36.2 | 0.014   |
| S_{10} | 40.84   | 0.222| 0.001   |
| ADR   | 0.199    | 2E−05| 2E−09   |

Obs. the minimum drug concentration which causes 50% inhibition of cell growth (Gl_{50}) in last column is showed in italics. Where Gl_{50} value ≤ 10 μmolar (i.e. 1 μmolar) or ≤ 10 μg/ml is considered to demonstrate activity in case of pure compounds. For extracts, Gl_{50} value ≤ 20 μg/ml is found to demonstrate activity.

Fig. 5 Graph plotted between tested compound and standard drug

**Compound S_{16}**: Ethyl-2-(3-hydroxybenzylideneamino)-4,5,6,7-tetrahydrobenzo[\textit{b}]thiophene-3-carboxylate: M. p: 93–95 °C; yield: 69.87%; IR (KBr pellets, cm⁻¹): 2977 (C–H str., O str., carbonyl), 1267 (C–O–C str.), 665.53 (C–S–C str., thiophene ring), 822 (C–Cl str., aromatic); ¹³C NMR (75 MHz, CDCl₃) d 165.89, 160.47, 127.27, 110.12, 106.12, 52.01, 33.06, 31.17, 29.80, 29.81, 29.78, 29.90, 22.80, 14.29; MS ES+ (ToF): m/z 331.5 [M⁺+1].

**Compound S_{17}**: Ethyl-2-(4-methoxybenzylideneamino)-4,5,6,7-tetrahydrobenzo[\textit{b}]thiophene-3-carboxylate: M. p: 93–95 °C; yield: 81.81%; IR (KBr pellets, cm⁻¹): 2977 (C–H str.), 1582 (C=C str.), 1657 (C=N str.), 1698 (C=O str., carbonyl), 1267 (C–O–C str.), 665.53 (C–S–C str., thiophene ring), 822 (C–Cl str., aromatic); ¹³C NMR (75 MHz, CDCl₃) d 165.98, 161.40, 127.24, 121.35, 106.08, 50.00, 32.66, 33.16, 29.82, 29.68, 29.50, 29.08, 21.83, 15.26; MS ES+ (ToF): m/z 344.4 [M⁺+1].

**Compound S_{18}**: Ethyl-2-(2,4-dimethoxybenzylideneamino)-4,5,6,7-tetrahydrobenzo[\textit{b}]thiophene-3-carboxylate: M. p: 97–99 °C; yield: 76.69%; IR (KBr pellets, cm⁻¹): 2977 (C–H str.), 1582 (C=C str.), 1657 (C=N str.), 1698 (C=O str., carbonyl), 1267 (C–O–C str.), 665.53 (C–S–C str., thiophene ring), 822 (C–Cl str., aromatic); ¹³C NMR (75 MHz, CDCl₃) d 165.70, 161.57, 127.27, 121.37, 107.12, 52.01, 33.06, 31.18, 29.83, 29.81, 29.78, 29.58, 29.49, 29.09, 23.83, 14.27; MS ES+ (ToF): m/z 374 [M⁺+1].

**Evaluation of antimicrobial activity**

Newly derived derivatives were screened for in vitro antimicrobial activity against Gram positive bacteria: *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3160), Gram negative bacteria *Salmonella typhi* (MTCC 3216), *Escherichia coli* (MTCC 443) and fungal strains *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 281) by tube dilution method [18] and results were compared with fluconazole (antifungal) and cefadroxil (antibacterial) as standard drugs. The stock solutions were prepared in DMSO having 100 µg/ml concentrations for standard and test drugs. Fresh pure cultures were used to prepare the bacterial and fungal inoculums. In the test-tubes containing serial dilutions (50, 25, 12.5, 6.25 and 3.12 µg/ml) of test and standard compounds in nutrient broth and Sabouraud dextrose broth, 100 µl of inoculum was added. After that it was incubated at 37 ± 1 °C for 24 h (bacteria), at 25 ± 1 °C for 7 days (*A. niger*) and at 37 ± 1 °C for 48 h (*C. albicans*). Antimicrobial screening results were recorded in terms of lowest concentration of test substances which inhibited the growth of microorganisms i.e. MIC.
Antioxidant activity was evaluated spectrophotometrically by using free radical scavenging method i.e. DPPH assay. The DPPH is a stable free radical and it reacts with hydrogen donors showing the reduction to its corresponding hydrazine with the maximum absorption at 517 nm. Colour of DPPH changes from violet to yellow, indicating the reaction of DPPH with an antioxidant agent as it can donate hydrogen to get reduced with a considerable decrease in absorption at 517 nm. DPPH solution (3 µg/ml) was prepared in methanol. The solution of methanol and DPPH (1:1) was used for blank reference. Four dilutions of different concentrations (25 µg/
ml, 50 µg/ml, 75 µg/ml, 100 µg/ml) of each synthesized compound and standard (ascorbic acid) were prepared in the methanol and 1 ml of each concentration was added to 1 ml of DPPH solution. The solution mixture was placed in dark place for 30 min at room temperature after vigorous shaking and their absorbance was measured by UV at 517 nm [19]. Percentage (% inhibition of free radical DPPH was calculated as follows:

\[
\% \text{Inhibition} = \left( \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \right) \times 100
\]  

(1)

where, \(A_{\text{Blank}}\) = absorbance of the blank reaction, \(A_{\text{Sample}}\) = absorbance of the test compounds.

IC\(_{50}\) value was calculated from the graph plotted between % inhibition and synthesized derivatives. The antioxidant screening results indicated that few synthesized compound exhibited significant antioxidant activity while other showed good to moderate antioxidant activity. In them, compound S\(_4\) and S\(_6\) exhibited excellent antioxidant activity and found to have IC\(_{50}\) value and (%) inhibition comparable to ascorbic acid.

**Evaluation of anticorrosion activity by gravimetric method (weight loss)**

The corrosion medium was made by dilution of 1.0 M HCl of analytical grade 37% with double distilled water. The test solutions were made up by dilution of 0.5 g thiophene derivatives with 250 ml of 1.0 M HCl solution to make 200 ppm solution (stock solution). The desired concentrations (50, 100, 200, 300 ppm) of thiophene derivatives were prepared from stock solution of 200 ppm.

The gravimetric method is commonly used corrosion monitoring method. The weight loss study has been carried out in 1.0 M HCl solution. Firstly, all mild steel samples were cut into 1 × 3 cm then scrapped with emery paper of different grade (100–1000) and rinsed with distilled water, acetone and after all dried between filter paper and weighed. The weight loss study was carried out in 25 ml of 1.0 M HCl solution filled in 50 ml beaker in presence and absence of various concentration of corrosion inhibitor for 12 h at 35 °C. After the immersion of sample in test solution, specimens were put outside and washed with double distilled water and acetone, dried and weighed again. This process of weight loss study was carried out in triplicate and the average weight loss was calculated. The % corrosion inhibition efficiency and surface coverage were calculated by following equations.

\[
\eta_w = \left( \frac{W_0 - W}{W_0} \right) \times 100
\]

(2)

\[
\theta = \frac{W_0 - W}{W_0}
\]

(3)

where, \(W_0\) = weight loss value of mild steel in absence of test compound, \(W\) = weight loss value of mild steel in presence of test compound.

The equation used for calculation of corrosion rate of mild steel was given by:

\[
Cr \left( \text{mmy}^{-1} \right) = \frac{87.6 \times W}{AtD}
\]

(4)

where, \(W\) = weight loss of mild steel (mg), \(A\) = area of sample (cm\(^2\)), \(t\) = exposure time (h) and \(D\) = density of mild steel (g cm\(^{-3}\)).

**Evaluation of in vitro anticancer activity**

Sulfurhodamine-B (SRB) assay method was used for screening in vitro cytotoxicity of the synthesized thiophene derivative on A-549 (human lung cancer cell line) [20]. Its mechanism is focused on the capability to bind electrostatically, the protein dye sulfurhodamine B and pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Anticancer screening results were shown as GI\(_{50}\) (concentration of drug causing 50% inhibition of cell growth) and the results were matched with the standard anticancer drug i.e. adriamycin.

The entire synthesized compounds which have to be screened initially at dose (10\(^{-7}\) to 10\(^{-4}\)) were submitted at anti-cancer screening facility (ACDSF) at ACTREC Mumbai (Tata Memorial Centre).

**Conclusion**

Summarizing, we may conclude that novel thiophene derivatives were synthesized with different donor or acceptor groups on aromatic rings. Compound S\(_1\) showed the highest activity against various Gram positive and Gram negative bacterial strains. The di-halogen substituted derivatives were found to have significant antibacterial activities, particularly the compounds having two substituted chloro groups at the same ring. Compound S\(_4\) emerged as the most potent antifungal agent because of the presence of o-OCH\(_3\) group. Compound S\(_4\) and S\(_6\) exhibited excellent antioxidant activity and compound S\(_7\) showed more anticorrosion efficiency with low corrosion rate due to the presence of electron releasing groups on benzylidene portion which found to have higher basicity and electron donating ability which act by adsorption on the metal surface and block the active surface sites, thus reducing the corrosion rate. Compound S\(_8\) possessed significant cytotoxicity GI\(_{50}\)=8E−04 molar against human lung cancer cell line (A-549). The results
of anticancer screening indicated that the synthesized compounds having electron withdrawing groups (p-Br) on benzylidene portion found to possessed significant activity. So, these thiophene derivatives unquestionably grasp a greater assure in discovering as lead compound for the progress of novel therapeutic agents.

Abbreviations
ACDSF: anti-cancer screening facility; CR: corrosion rate; DPPH: 1,1-diphenyl-2-picrylhydrazyl; HCl: hydrochloric acid; IR: infrared; IMTECH: Institute of Microbial Technology and Gene bank; MS: mass spectroscopy; MTCC: microbial type cell cultures; MIC: minimum inhibitory concentration; NMR: nuclear magnetic resonance; Ppm: parts per million; SRB: sulforhodamine-B; TLC: thin layer chromatography.

Authors’ contributions
PKV designed and finalized the scheme, RS performed research work and wrote the paper. Both authors read and approved the final manuscript.

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Competing interests
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