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Efficient Synthesis of Phenothiazine-based Heterocyclic Derivatives and their Biological Studies

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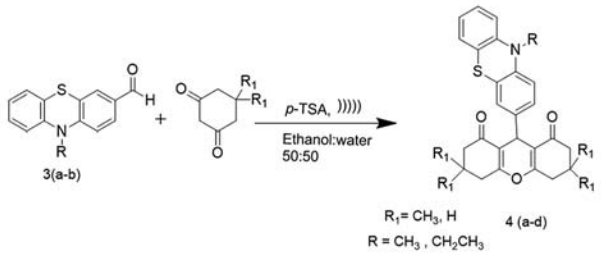
ABSTRACT Phenothiazine incorporated xanthene and 1,4-dihydropyridine derivatives were prepared by using *N*-alkyl-phenothiazine-3-carbaldehydes using one-pot reaction under reflux/ultrasonic irradiation. The synthesized compounds were characterized by using Fourier-transform infrared, ^{1}H NMR, ^{13}C NMR, and mass spectral data. Antibacterial activities of the synthesized compounds were screened against four different bacterial strains. The compound **5a** and **5b** showed good activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Klebsiella pneumoniae* whereas compounds **4a** and **4b** show good activity against *S. aureus* and *E. coli*. The free radical-scavenging activities of synthesized compounds were screened, and the results were reported.



KEYWORDS Phenothiazine, Xanthenes, 1,4–Dihydropyridine, Antioxidant, Antibacterial.

INTRODUCTION

Nitrogen and sulfur heteroatom-containing aromatic compounds are being pursued as a thrust area of research in Chemistry because of its potential applications. Phenothiazines, a tricyclic compound containing nitrogen and sulfur heteroatom in their ring, have been known for over 100 years. The parent compound, 10H-dibenzo-1, 4-thiazine, was synthesized by Bernthsen in 1883.

Phenothiazine is a bioactive heterocyclic compound which possesses biological activities such as antibacterial,[1-2] antifungal,[3] anti-inflammatory,[4] antimalarial,[5] antipsychotropic,[6] antimicrobials,[7] and antitubercular[8,9] activities. Furthermore, some of these derivatives have been reported to exhibit significant anticancer activities, which have triggered a great interest in designing and synthesizing new phenothiazine derivatives to explore their anticancer activities.[10,11]

Phenothiazine derivatives proved to act as human cholinesterase inhibitors, and on several occasions, these derivatives have been characterized as multidrug-resistant reversal agents.[12,13] In accordance with this concept, the phenothiazine ring modifications gave the derivatives [e.g., benzo[a]phenothiazines [a] phenothiazines and azaphenothiazines] with anticancer effects on various cell lines.[14,15]

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Here, in the present work, we would like to report an efficient synthesis of phenothiazine incorporated xanthene and 1,4 –dihydropyridine derivatives and characterization by using analytical and spectroscopic techniques. Antibacterial activities of the derivatives **4a**–**d** and **5a**—**d** were evaluated using agar-well diffusion method against four different bacterial strains.

RESULTS AND DISCUSSION

Phenothiazine (**1**) was treated with ethyl and methyl iodide in the presence of potassium tertiary butoxide and dry DMF. The reaction mixture was stirred at 80°C for 24 h to get N-alkyl phenothiazines[1] **(2a-b)**. The 10-alkyl-10*H*-phenothiazine-3-carbaldehydes[1] (**3a-b)** were obtained via Vilsmeier–Haack reaction of 10-alkyl phenothiazine **(2a-b)** [**Scheme 1**].

These compounds **(4a–d)** were synthesized by the reaction of dimedone with **3a–b** in the presence of *p*-TSA at 100 °C in methanol for 3 h to yield 74%–86 %. The reaction was also carried out under ultrasonic irradiation to get a higher yield of the product **[Scheme 1]**. The IR absorption band at 1664 cm^{-1} is due to the presence of α, β-unsaturated carbonyl group in compound **4a** obtained. In ^1H NMR spectrum, the singlet at δ 1.01 and 1.08 ppm which corresponds to two methyl protons of the dimedone unit and two methylene protons resonating at δ 2.45 and 2.19 ppm, which are evidence for the formation of compound **4a** obtained. The peak at δ 4.64 ppm corresponds to a CH proton present in the cyclic ring suggesting the formation of cyclized xanthene unit.

Compounds **(5a–d)** were synthesized by the treatment of 10-alkyl-3-formylphenothiazine, dimedone, and ammonium acetate with one equivalent of ethyl acetoacetate in the presence of *p*-TSA (10 mol%) under reflux and ultrasonic irradiation to yield the compounds **(5a–d)** presented in **Scheme 1**: Structures of the synthesized compounds **(5a–d)** were confirmed by recording IR, ^1H NMR, ^{13}C NMR, and mass spectra.

The IR spectrum of compound **5a** showed absorption at 3282 cm^{-1} which is due to the NH stretching, and the absorption band at 1699 and 1647 cm^{-1} is due to –CO-stretching frequency. The ^1H NMR spectrum of **5a** showed a singlet at δ 5.89 ppm corresponding to the NH proton. The singlet at δ 4.94 ppm corresponds to the C-4 hydrogen of dihydropyridine ring, which is also supported by the peak at δ 41.2 ppm in ^{13}C NMR spectrum. The spectral characterization techniques, such as IR, ^1H NMR, ^{13}C NMR, and HR-MS spectra, which are evidence the formation of synthesized phenothiazine derivatives. The physical data of all the synthesized compounds are presented in **Table 1**.

Screening of antioxidant activity

All the newly synthesized compounds were subjected to antioxidant activity using DPPH free radical-scavenging assay method.

The antioxidant studies indicate that compounds **5a, 5b, 5c,** and **5d** showed moderate activity (25.50%, 28.14%, 20.45%, and 22.59%, respectively) comparable to that of BHT and ascorbic acid (100%).

The compounds **4a, 4b, 4c,** and **4d** showed the least activity even at 100 µg mL^{-1} concentrations at 30 min of incubation time. Free radical-scavenging capacities of the synthesized phenothiazine compounds measured using DPPH assay are shown in **Figure 1**. The radical-scavenging activity (RSA) for methanolic solutions of synthesized compounds is presented in **Table 2** and compared with those of standards BHT and ascorbic acid.

Screening of antibacterial activity

The synthesized phenothiazine derivatives **(4a–d)** and **(5a–d)** were screened for *in vitro* antibacterial activity against three "Gram-positive" bacterial strains, *Staphylococcus aureus* (MTCC 3381), *Pseudomonas aeruginosa* (MTCC 2295), and *Bacillus cereus* (MTCC 8372) and two "Gram-negative" bacterial strains, *Escherichia coli* (MTCC 1302) and *Klebsiella pneumoniae* (MTCC 3384) at 25, 50 µg mL^{-1} concentrations in Mueller–Hinton agar medium using the standard procedure described in experimental section. Ampicillin was used as a standard drug for antibacterial activity. The zone of inhibition was determined for all the compounds, and the results are summarized in **Table 3**. The synthesized compounds **5a** and **5b** showed good activity against *S. aureus,*

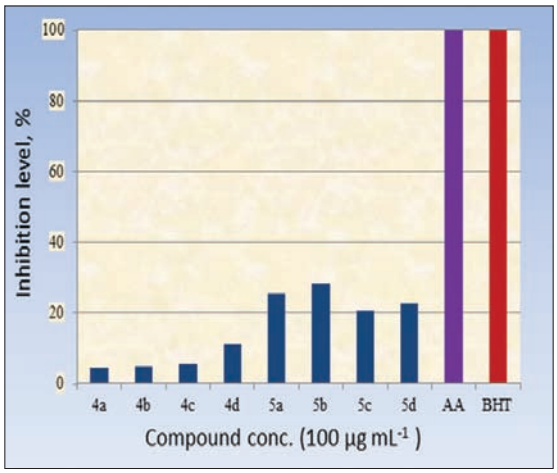
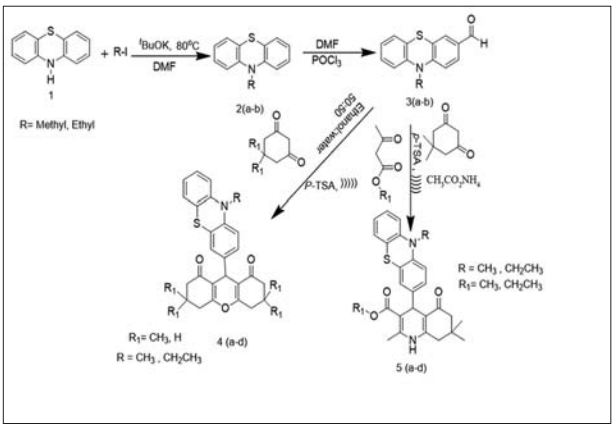


Figure 1: Antioxidant activity of synthesized compounds and BHT, ascorbic acid using DPPH free radical-scavenging method after 30 min of incubation



Scheme 1; Synthetic protocol of compound 4a–d and 5a–d

Table 1: Physical data of all the synthesized compounds 4a–d and 5a–d

Compound	R	R_1	Reaction Time, min	Yield[a, b]%	M. p. range, °C
4a	CH_3	CH_3	150/8	82/86	225–227
4b	CH_2-CH_3	CH_3	165/9	80/84	213–215
4c	CH_3	H	175/10	78/83	198–200
4d	CH_2-CH_3	H	180/10	74/79	192–194
5a	CH_2-CH_3	CH_2-CH_3	360/18	82/84	212–215
5b	CH_3	CH_2-CH_3	350/19	80/83	225–228
5c	CH_2-CH_3	CH_3	200/14	83/84	245–247
5d	CH_3	CH_3	190/15	84/86	256–258

[a]At reflux temperature/ultrasonic irradiation, [b]Isolated yields

Table 2: Antioxidant activity of synthesized compounds 4a–d and 5a–d using DPPH radical-scavenging method

Compound	Absorbance	Inhibition level, %	Compound	Absorbance	Inhibition level, %
4a	1.7655	4.50	4d	1.6458	10.98
4b	1.7575	4.93	5a	1.3772	25.50
4c	1.7465	5.53	5b	1.3285	28.14
Control*	1.8488		5c	1.4706	20.45
			5d	1.4311	22.59

*This study brings out the tested compounds (**4a**–**d** & **5a**–**d**) have moderate to poor free RSA and can be supported as a modest dosage source of antioxidants for pharmacological preparations having biological importance

E. coli, P. aeruginosa, B. cereus, and *K. pneumoniae* whereas compounds **4a** and **4b** show good activity against *S. aureus* and *E. coli* and moderate activity against *P. aeruginosa, B. cereus,* and *K. pneumoniae*. Compounds **4a, 4c, 5c,** and **5d,** showed low activity against *K. pneumoniae* and compound **4d** showed low activity against *S. aureus*.

In general, the compounds **5a**–**d** which are having a capacity of releasing protons on nitrogen atom surrounded by electron withdrawing carbonyl groups showed potent antimicrobial activity when compared to the compounds **4a**–**d** having less probability of releasing such type of ions.

CONCLUSION

In summary, xanthene **(4a–d)** and 1,4-Dihydropyridine **(5a-d)** derivatives incorporating a phenothiazine moiety has been synthesized and screened for antioxidant activity using DPPH free radical-scavenging method and are found to be moderately active. All the synthesized phenothiazine derivatives were screened for *in vitro* antibacterial activity using the agar-well diffusion method. The synthesized compounds **5a** and **5b** showed good activity *against S. aureus, E. coli, P. aeruginosa, B. cereus*, and *K. pneumoniae* whereas compounds **4a** and **4b** showed good activity against *S. aureus* and *E. coli.*

EXPERIMENTAL

General

All the chemicals were purchased from SD Fine Chemicals (India), Sigma-Aldrich (USA) and Sisco Research Laboratory. Melting points were determined using one-end open capillary tubes on a Buchi-530 melting point apparatus and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on BRUKER AV-400MHz and Bruker Spectrospin DPX 400 MHz spectrometer using $CDCl_3$ and dimethyl sulfoxide (DMSO)-d_6 as a solvent and tetramethylsilane as an internal standard. The Fourier-transform infrared (FT-IR) spectra were recorded on Perkin-Elmer model 1600 FT-IR RX1 spectrophotometer and KBr used as discs. Sonication was performed in a SONICS Vibra Cell VC 130 ultrasonic processor equipped with a 3-mm wide and 140-mm long probe, which was immersed into the reaction mixture. Mass spectra were recorded using high-resolution HRMS (JEOL 1600 HRMS) mass spectrometer, and the samples were dried under vacuum condition before the analysis.

Procedure for the preparation of 3,3,6,6-tetramethyl-9-(10-methyl-10*H*-phenothiazine-3-yl)-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione (4a–d).

Conventional method

In a 50 mL round-bottomed flask, a mixture of dimedone (2 mmol), 10-alkyl-10*H*-phenothiazine-3-carbaldehyde (1 mmol), *p*-TSA (15 mmol%), and ethanol (10 mL) was stirred at 80°C for time periods as described in T**abl**e 1. After completion, (through TLC monitoring) the reaction mixture was poured into ice-cooled water. The solid obtained was filtered off, washed with large amount of water (15 mL) and the residue was recrystallized from ethanol to get the pure product.

Ultrasonic irradiation method

In a 25 mL beaker, a mixture of 10-alkyl-10*H*-phenothiazine-3-carbaldehyde (1 mmol), dimedone (2 mmol), and p-TSA (15 mmol%) and 10 mL ethanol were taken and irradiated using the ultrasonic probe at the frequencies of 22 kHz at 60°C for the required reaction time **[Table 1]**. After the completion of the reaction (indicated by

Table 3: Antibacterial activities of the synthesized compounds 4a–d and 5a–d

Compound	Zone of bacterial Inhibition in[a]									
	E. coli		*K. pneumonia*		*B. cereus*		*P. aeruginosa*		*S. aureus*	
	25 (µg/mL)	50 (µg/mL)	25 (µg/mL)	50 (µg/mL)	25 (µg/mL)	50 (µg/mL)	25 (µg/mL)	50 (µg/mL)	25 (µg/mL)	50 (µg/mL)
4a	10	12	8	10	11	13	10	12	11	13
4b	11	12	10	11	11	12	11	12	10	12
4c	11	13	9	11	10	12	12	13	11	13
4d	10	12	10	11	8	10	10	12	9	11
5a	12	14	11	13	10	12	11	13	11	14
5b	13	15	10	12	11	12	11	13	12	13
5c	11	13	9	12	10	13	10	12	12	13
5d	10	12	9	11	11	13	10	12	10	12
(Ampicillin[b] 25 µg/mL)	19		17		16		18		16	

[a]Zone of inhibition in mm, [b]Standard antibacterial drug

TLC), the reaction mixture was poured into ice water. The precipitated was collected by filtration, washed with water and dried.

Procedure for the preparation of ethyl-7,7-dimethyl-4-(10-methyl-10*H*-phenothiazin-3-yl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5a-d)

Conventional method

A mixture of 10-alkyl-10*H*-phenothiazine-3-carbaldehyde (1 mmol), ethyl acetoacetate (1 mmol), 5,5-dimethyl-1,3-cyclohexanedione (1 mmol), ammonium acetate (10 mmol), and p-TSA (10 mmol%) was stirred in absolute ethanol (5 mL) at 80°C. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice water, and the solid separated was filtered off. The crude product was purified by column chromatography with hexane/ethyl acetate as a solvent to yield the title compound.

Ultrasonic method

Dimedone (1 mmol), ethyl acetoacetate or methyl acetoacetate (1 mmol), 10-alkyl-10H-phenothiazine-3-carbaldehyde (1 mmol), ammonium acetate (3 mmol), p-TSA (10 mol%), and 5 mL of methanol were added in a **50** mL beaker. The reaction mixture was irradiated continuously at room temperature for 13-20 min using sonic probe with a frequency of 18 kHz. The completion of the reaction was monitored by TLC using hexane: Ethyl acetate (3:2) as an eluent and the mixture was poured into ice water. The crude product was purified using column chromatography with hexane/ethyl acetate as a solvent to yield the title compound.

3,3,6,6-tetramethyl-9-(10-methyl-10H–phenothiazine-3-yl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8 (2H) -dione (4a): mp**.** 225-227°C; IR (KBr, cm^{-1}): 3178, 2958, 2872, 1664, 1622, 1577, 1465, 1359, 1328, 1199, 1165, and 1138; ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 7.18 (d, 2H, *J* 8.0, C7 & C8-Ar-H of phenothiazine ring), 6.66-7.14 (m, 5H, Ar-H of phenothiazine ring), 4.65 (s, 1H, CH), 3.29 (s, 3H, N-CH_3), 2.45 (q, 4H, *J* 16.0, 2xCH_2), 2.18 (q, 4H, J 16.2, 2xCH_2), 1.08 (s, 6H, 2xCH_3), and 1.00 (s, 6H, 2xCH_3); ^{13}C NMR (100.612 MHz, $CDCl_3$, ppm, δ): 196.5, 162.3, 145.9, 144.2, 138.7, 128.2, 127.4, 127.1, 126.6, 123.4, 122.8, 122.2, 115.4, 114.0, 113.7, 50.8, 40.9, 35.3, 32.3, 31.0, 29.2, and 27.7; HRMS (EI): *m/z* [M^+] calcd. for $C_{30}H_{31}NO_3S$: 485.2025; found: 485.2020.

9-(10-ethyl-10H-phenothiazine-3-yl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8 (2H)-dione (4b): mp**.** 213-215°C; IR (KBr, cm^{-1}): 2949, 2868, 1656, 1618, 1573, 1463, 1359, 1327, 1203, and 1138; ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 7.15 (d, 1H, *J* 8.0, C7-Ar-H of phenothiazine ring), 7.04-7.11 (m, 2H, Ar-H of phenothiazine ring), 6.91 (s, 1H, C6-Ar-H of phenothiazine ring), 6.76-6.86 (m, 3H, Ar-H of phenothiazine ring), 4.64 (s, 1H, CH), 3.84 (q, 2H, *J* 7.6, *N*-CH_2), 2.45 (q, 4H, *J* 16.0, 2xCH_2), 2.19 (q, 4H, J 17.2, 2xCH_2), 1.35 (t, 3H, *J* 6.6, CH_3), 1.08 (s, 6H, 2xCH_3), and 1.01 (s, 6H, 2xCH_3); ^{13}C NMR (100.612 MHz, $CDCl_3$, ppm, δ): 196.5, 162.3, 144.9, 143.4, 138.5, 128.0, 127.3, 127.1,

126.7, 124.3, 123.6, 122.0, 115.4, 114.9, 114.5, 50.8, 41.7, 40.9, 32.3, 30.9, 29.1, 27.7, and 13.0; HRMS (EI): *m/z* [M$^+$] calcd. for $C_{31}H_{33}NO_3S$: 499.2181; found: 499.2175.

9-(10-methyl-10H-phenothiazine-3-yl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione(4c): mp. 198-200°C; IR (KBr, cm^{-1}): 2923, 2786, 1659, 1612, 1568, 1452, 1349, 1307, 1213, and 1129; 1H NMR (400 MHz, $CDCl_3$, ppm, δ): 7.24 (d, 1H, *J* 8.4, C_7-Ar-H of phenothiazine ring), 7.09-7.12 (m, 2H, Ar-H of phenothiazine ring), 6.90 (s, 1H, Ar-H of phenothiazine ring), 6.68-6.88 (m, 3H, Ar-H of phenothiazine ring), 4.70 (s, 1H, CH), 3.30 (s, 3H, N-CH_3), 2.51-2.67 (m, 4H, 2xCH_2), 2.25-2.38 (m, 4H, 2xCH_2), and 1.99-2.01 (m, 4H, 2xCH_2); ^{13}C NMR (100.612 MHz, $CDCl_3$, ppm, δ): 196.7, 164.0, 146.0, 144.3, 139.0, 128.4, 127.4, 127.1, 126.5, 123.4, 122.9, 122.3, 116.7, 114.0, 113.7, 37.0, 36.8, 35.3, 30.9, 27.2, and 20.3; HRMS (EI): *m/z* [M$^+$] calcd. for $C_{26}H_{23}NO_3S$: 429.1399; found: 429.1392.

9-(10-ethyl-10H-phenothiazine-3-yl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (4d): mp. 192–194°C; IR (KBr, cm^{-1}): 2935, 2872, 1660, 1625, 1494, 1463, 1359, 1327, 1174, and 1130; ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 7.20 (d, 2H, *J* 8.0, C7-Ar-H of phenothiazine ring), 6.71-7.07 (m, 5H, Ar-H of phenothiazine ring), 4.69 (s, 1H, CH), 3.65 (q, 2H, *J* 7.2, *N*-CH_2), 2.51-2.66 (m, 4H, 2xCH_2), 2.30-2.38 (m, 4H, 2xCH_2), 1.72-2.20 (m, 4H, 2xCH_2), and 1.23 (t, 3H, *J* 6.8, CH_3); ^{13}C NMR (100.612 MHz, $CDCl_3$, ppm, δ): 196.8, 164.0, 145.1, 143.5, 138.7, 128.3, 127.3, 127.2, 126.6, 124.3, 123.7, 122.1, 116.7, 115.0, 114.6, 41.7, 37.0, 30.7, 27.2, 20.3, and 13.1; HRMS (EI): *m/z* [M$^+$] calcd. for $C_{27}H_{25}NO_3S$: 443.1555; found: 443.1550.

Ethyl-4-(10-ethyl-10H-phenothiazine-3-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydro quinoline-3-carboxylate (5a): mp 212-215°C; IR (KBr, cm^{-1}): 3282, 3215, 2960, 2818, 1699, 1647, 1492, 1465, 1381, 1280, 1215, and 1141; 1H NMR (400 MHz, $CDCl_3$, ppm, δ): 6.78-7.25 (m, 6H, Ar-H of phenothiazine ring), 6.69 (d, 1H, *J* 7.2, C-2, Ar-H of phenothiazine ring), 5.89 (s, 1H, NH), 4.94 (s, 1H, CH), 4.05 (d, 2H, *J* 1.6, OCH_2), 3.84 (s, 2H, *N*-CH_2), 2.35 (s, 3H, CH_3), 2.23 (d, 2H, *J* 10.8, CH_2), 2.17 (d, 2H, *J* 6.0, CH_2), 1.35 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), and 0.95 (s, 3H, CH_3); ^{13}C NMR (100.645 MHz, $CDCl_3$, ppm, δ): 195.6, 167.4, 147.9, 145.1, 143.3, 143.1, 141.5, 127.4, 127.3, 127.1, 126.7, 124.4, 123.4, 122.0, 114.9, 114.5, 112.1, 106.0, 60.0, 50.8, 41.7, 41.2, 35.7, 32.9, 29.3, 27.6, 19.7, 14.4, and 13.1; HRMS (EI): *m/z* [M$^+$] calcd. for $C_{29}H_{32}N_2O_3S$: 488.2134; found: 488.2138.

Ethyl-2,7,7-trimethyl-4-(10-methyl-10H-phenothiazine-3-yl)-5-oxo-1,4,5,6,7,8-hexahydro quinoline-3-carboxylate (5b): mp 225-228°C; IR (KBr, cm^{-1}): 3278, 3211, 3076, 2960, 2870, 1699, 1647, 1604, 1492, 1465, 1381, 1280, and 1215; ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): δ 7.08-7.26 (m, 3H, Ar-H of phenothiazine ring), 6.74-7.01 (m, 3H, Ar-H of phenothiazine ring), 6.65 (d, 1H, *J* 7.6, C-2, Ar-H of phenothiazine ring), 6.06 (s, 1H, NH), 4.95 (s, 1H, CH), 4.05 (d, 2H, *J* 6.0, OCH_2), 3.29 (s, 3H, *N*-CH_3), 2.35 (s, 3H, CH_3), 2.04–2.28 (m, 4H, 2xCH_2), 1.21 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), and 0.93 (s, 3H, CH_3); ^{13}C NMR (100.645 MHz, $CDCl_3$, ppm, δ): δ 195.6, 167.4, 148.1, 146.1, 143.9, 143.4, 141.7, 127.5, 127.4, 127.1, 126.6, 123.5, 122.6, 122.2, 113.9, 113.6, 112.1, 105.9, 60.0, 50.8, 41.2, 35.8, 35.3, 32.8, 29.3, 27.5, 19.6, and 14.4; HRMS (EI): *m/z* [M$^+$] calcd. for $C_{28}H_{30}N_2O_3S$: 474.1977; found: 474.1973.

Methyl-4-(10-ethyl-10H-phenothiazine-3-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydro quinoline-3-carboxylate (5c): mp 245-247°C; IR (KBr, cm^{-1}): 3414, 3288, 3217, 2960, 2813, 1701, 1647, 1604, 1492, 1465, 1381, 1330, 1284, and 1219; ^{1}H NMR (400 MHz, DMSO-d_6, ppm, δ): δ 9.10 (s, 1H, NH), 6.84-7.17 (m, 7H, Ar-H of phenothiazine ring), 4.76 (s, 1H, CH), 3.84 (s, 2H, *N*-CH_2), 3.53 (s, 3H, OCH_3), 2.38-2.51 (m, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.98-2.18 (m, 2H, CH_2), 1.25 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), and 0.86 (s, 3H, CH_3); ^{13}C NMR (100.645 MHz, DMSO-d_6, ppm, δ): 194.3, 167.2, 149.4, 145.2, 144.3, 142.3, 141.8, 127.5, 126.9, 126.5, 122.7, 122.1, 121.8, 115.2, 114., 109.7, 102.9, 50.19, 50.71, 40.92, 34.72, 32.1, 28.9, 26.6, 18.6, and 12.6; HRMS (EI): *m/z* [M$^+$] calcd. for $C_{28}H_{30}N_2O_3S$: 474.1977; found: 474.1974.

Methyl-2,7,7-trimethyl-4-(10-methyl-10H-phenothiazine-3-yl)-5-oxo-1,4,5,6,7,8-hexahydro quinoline-3-carboxylate (5d): mp 256-258°C; IR (KBr, cm^{-1}): 3277, 3216, 3080, 2960, 2873, 1703, 1647, 1494, 1465, 1381, 1284, 1217, and 1111; ^{1}H NMR (400 MHz, DMSO-d_6, ppm, δ): 9.11 (s, 1H, NH), 6.87-7.20 (m, 6H, Ar-H of phenothiazine ring), 6.80 (d, 1H, *J* 8.0, C-4 Ar-H of phenothiazine ring), 4.77 (s, 1H, CH), 3.5 (s, 3H, OCH_3), 3.25 (s, 3H, N-CH_3), 2.38-2.51 (m, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.97-2.18 (m, 2H, CH_2), 1.00 (s, 3H, CH_3), and 0.85 (s, 3H,CH_3); ^{13}C NMR (100.645 MHz, DMSO-d_6, ppm, δ): 194.3, 17.2, 149.4, 145.4, 145.3, 143.2, 142.1, 127.7, 126.7, 126.6, 125.5, 122.2, 121.9, 121.1, 114.4, 114.2, 109.8, 103.0, 50.7, 50.2 35.0, 34.8, 32.2, 29.0, 26.5, and 18.3; HRMS (EI): *m/z* [M$^+$] calcd. for $C_{27}H_{28}N_2O_3S$: 460.1821; found: 460.1823.

Protocol for screening of antibacterial activity

The antibacterial studies were carried out against using different bacterial strains such as *S. aureus* (MTCC 3381), *P. aeruginosa* (MTCC 2295) and *B. cereus* (MTCC 8372), *E. coli* (MTCC 1302), and *K. pneumoniae* (MTCC 3384) by using agar-well diffusion method using Muller–Hinton agar as the medium. A number of antimicrobial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. 20 mL of agar media was poured into each Petri dish. Excess of the suspension was decanted, and plates were dried by placing them in an incubator at 37°C for an hour. Using a punch, wells were made on these seeded agar plates, and different concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using DMSO as a solvent. All the plates were incubated at 37°C for 24 h. The degree of effectiveness was measured by determining the diameters of the zone of inhibition produced by the compounds. The activity of each compound was compared with the standard drug ampicillin available in the market. Minimum inhibitory concentration was calculated as the lowest concentration of the sample at which there is no visible growth of the bacteria.

Protocol for screening of antioxidant activity

The antioxidant assay is based on the measurements of the scavenging ability of compounds toward the stable radical DPPH. The disappearance of absorption of this radical is measured spectrophotometrically at 517 nm in a DMSO solution using an ultraviolet (UV)/visible-spectrophotometer (Model UV-1700(E) 23 OCE, Shimadzu, Japan) under thermostatic conditions at 25°C. 3.0 mL of a freshly prepared DPPH solution of 6.02×10^{-5} M in DMSO was placed in a test tube and 100 µL of a DMSO solution of each test compound was added. After that, the solution was kept at room temperature for 30 min in dark condition and the absorbance was measured at 517 nm. The control contained all the reagents except the synthesized compound, and the experiment was carried out in triplicate and average values are taken. DPPH has an odd electron and has absorption band at 517 nm. When this odd electron becomes paired off, the absorption decreases with respect to the number of electrons or hydrogen atoms are taken up. Hence, faster is the decrease of absorbance higher the antioxidant activity of the compound.

The DPPH scavenging activity was expressed as the inhibition percentage of free radical DPPH (I%) as described by Tepe *et al*.[16]

Inhibition level,% = [(control OD – sample OD)/control OD] × 100.

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