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Synthesis of schiff bases bearing phenothiazine nucleus and their biological activities

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ABSTRACT

In the present study a series of novel Schiff bases which containing the Phenothiazine nucleus were synthesized (PD-1 to PD-10) by the condensation of the compounds N-methyl-10H-phenothiazine-3-carbaldehyde and different acylhydrazides in methanol and glacial acetic acid. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their invitro antibacterial and antioxidant activities by agar diffusion plate method and DPPH free radical scavenging assay methods. All the synthesized compounds exhibited significant to moderate antibacterial and antioxidant activities. The results revealed that compound PD-7 exhibited significant antioxidant activity and the compounds PD-2, PD-5 and PD-8 exhibited good activity against S.aureus, E.coli, P. aeruginosa and B.subtilis and moderate activity against K. pneumonia. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the antimicrobial screening of the new compounds are reported.

Keywords: Schiff bases, Phenothiazine, DPPH, Antioxidant, Antibacterial

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INTRODUCTION

Increasing the resistance of microorganisms to currently available antimicrobial drugs is the major cause of morbidity and mortality throughout the world. Thus development of newer antimicrobial drugs is still in demand. The compounds carrying Azomethine functional group —C=N— which are known as Schiff bases have gained importance in medicinal and pharmaceutical fields due to the most versatile organic synthetic intermediates and also showing a broad range of biological activities, such as antitubercualr[1,2], anticancer[3-5], analgesic[6,7], anti-inflammatory[8,9], anticonvulsant[10,11], antibacterial[12], antifungal[13] antioxidant[14, 15], and anthelmintic[16] activities. The Schiff bases are good intermediates for the synthesis of many heterocyclic ring systems. The chemistry of Phenothiazine and its fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. In view of these and continuation of our biologically active molecules, we are hereby report the synthesis of some new Schiff bases bearing Phenothiazine ring and their *invitro* antioxidant and antibacterial studies.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC

Agilent. The results are in agreements with the structures assigned. Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyzer and the results are in agreements with the structures assigned.

Preparation of N-alkyl phenothiazine:
Phenothiazine (1.0 mmol), iodoethane or iodomethane (3.0 mmol) and DMF (30 ml) were added in a 50 ml two-necked round bottom flsak. The solution was warmed to 75^0C, treated portion-wise with potassium *tert*-butoxide (1.5 mmol) and then stirred at 80^0C for 24 h. After the reaction has proceeded to completion (as monitored through TLC), the reaction mixture was cooled to room temperature and poured into ice water and then extracted with chloroform (75 ml) and dried over Na_2SO_4. Crude gelly like material obtained by removing the solvent were purified by column chromatography hexane/ethyl acetate (4:1) to give N-alkyl phenothiazines as a white solid (yield: approx. 80%).

Preparation of N-alkyl-10H-phenothiazine-3-carbaldehyde:
Phosphorus oxychloride ($POCl_3$) (4.1 mmol) was taken in a two neck round bottom flask and to that 4.7 mmol of freshly distilled *N*,*N*-dimethylformamide was added drop wise at 0^0C under nitrogen atmosphere. A solution of (1.0 mmol) of N-alkylphenothiazine dissolved in dichloroethane 30 ml was added drop wise to $POCl_3$/DMF complex at 30^0C. The reaction mixture was stirred at 80^0C for 16h. After the reaction reached completion (monitored through TLC), the reaction mixture was cooled to room temperature and poured into ice water. The obtained mixture was neutralized with $NaHCO_3$ and then extracted with chloroform (75 ml) and dried over Na_2SO_4. The solvent was evaporated by vacuum distillation. The crude product was purified by column chromatography hexane/ethyl acetate (3.5:1.5) to yield product in the rage of 78-81%.

Preparation of N'-((10-alkyl-10H-phenothiazin-3-yl) methylene)benzohydrazide (PD-1 to PD-10): A mixture of N-alkyl-10H-phenothiazine-3-carbaldehyde (1 m.mol) and heterocyclic acetohydrazide (1 m.mol) in methanol 25 ml and 1 ml of glacial acetic acid were added in a 50 ml round bottomed flask. The resulting solution was refluxed for 3-4 hours. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, the mixture was allowed to cool to room temperature and the solid separated was filtered off and washed with excess of methanol (40 ml) and dried at room temperature.

N'-((10-methyl-10H-phenothiazin-3-yl)methylene)benzohydrazide (PD-1): An equimolar amount of compound N-methyl-10H-phenothiazine-3-carbaldehyde (1 m.mol) and benzohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 212-215^{0}C; IR (KBr) ν_{max}: 3446, 3188, 3026, 2960, 2924, 2850, 1903, 1645, 1602, 1570, 1460, 1400, 1288, 1220, 1138, 1076 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.77 (s, 1H, NH), 8.35 (s, 1H, N=CH), 7.91 (d, 2H, *J* = 7.5Hz, o & o'- Ar-H of phenyl ring), 6.99- 7.59 (m, 10H, Ar-H of phenyl and phenothiazine ring), 3.36 (s, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC = 163.4, 147.2, 147.2, 145.0, 134.0, 132.1, 129.2, 128.9, 128.4, 128.0, 127.6, 127.3, 125.2, 123.0, 121.9, 115.4, 115.1, 35.8; MS: m/z [M$^+$] calcd for $C_{21}H_{17}N_3OS$: 359.1092; found: 359.1061.

N'-((10-methyl-10H-phenothiazin-3-yl)methylene)isonicotinohydrazide (PD-2): An equimolar amount of compound N-methyl-10H-phenothiazine-3-carbaldehyde (1 m.mol) and isonicotinohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 218-220^{0}C; IR (KBr) ν_{max}: 3423, 3250, 3070, 3032, 2872, 2816, 1654, 1595, 1546, 1465, 1406, 1334, 1292, 1255, 1222, 1143, 1064 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.99 (s, 1H, NH), 8.79 (dd, 2H, *J* = 1.75 & 4.25 Hz, C_2 & C_6- Ar-H of nicotine ring), 8.36 (s, 1H, N=CH), 7.82 (dd, 2H, *J* = 2.0 & 4.5Hz, C_3 & C_5 - Ar-H of nicotine ring), 7.58 (dd, 1H, *J* = 1.75 & 8.25 Hz, C_2 - Ar-H of phenothiazine ring), 7.52 (d, 1H, *J* = 2.0 Hz, C_4 - Ar-H of phenothiazine ring), 6.98-7.24 (m, 5H, Ar-H of phenothiazine ring), 3.36 (s, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC = 161.9, 150.7, 149.9, 148.4, 147.4, 144.9, 141.3, 128.8, 128.4, 127.8, 127.3, 125.4, 123.4, 123.0, 121.9, 121.9, 115.4, 115.1, 35.8; MS: m/z [M$^+$] calcd. for $C_{20}H_{16}N_4OS$: 360.1045; found: 360.1044.

N'-((10-methyl-10H-phenothiazin-3-yl)methylene)-2-(2-oxo-2H-chromen-7-yloxy)acetohydrazide (PD-3): An equimolar amount of compound N-methyl-10H-phenothiazine-3- carbaldehyde (1 m.mol) and 2-(2-oxo-2H-chromen-7-yloxy) acetohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 210-212^{0}C; IR (KBr) ν_{max}: 3448, 3099, 2968,292, 2821, 1730, 1616, 1465, 1408, 1336, 1267, 1157, 1082 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, ppm), δH =11.55 (d, 1H, *J* = 6.5 Hz, NH), 8.21 (s, 1H, N=CH), 7.91 (s, 1H, C_4- ArH of coumarin ring), 6.97-7.72 (m, 10H, Ar-H of

coumarin and phenothiazine ring), 6.22 (d, 1H, *J* = 7.0Hz, C_3- Ar-H of coumarin ring), 4.79 (s, 2H, OCH_2), 3.34 (s, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC =168.7, 163.8, 161.2, 160.5, 155.0, 154.9, 153.8, 147.5, 147.0, 144.9, 128.3, 127.7, 126.8, 125.3, 124.8, 123.3, 122.9, 121.9, 115.3, 113.8, 112.8, 111.6, 102.0, 65.8, 35.8; MS: m/z [M^+] calcd. for $C_{25}H_{19}N_3O_4S$: 457.1096; found: 457.1095.

2-(9H-carbazol-9-yl)-N'-((10-methyl-10H-phenothiazin-3-yl)methylene)acetohydrazide (PD-4): An equimolar amount of compound N-methyl-10H-phenothiazine-3-carbaldehyde (1 m.mol) and 2-(9H-carbazol-9-yl)acetohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P.173-175^{0}C; IR (KBr) ν_{max}: 3224, 3072, 2872, 1674, 1598, 1575, 1463, 1382, 1257, 1151 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.63 (s, 1H, NH), 8.19 (s, 1H, N=CH), 8.16-8.17 (split peaks, 1H, C_5- Ar-H of carbazole ring), 7.99 (s, 1H, C_4- Ar-H of carbazole ring), 7.65 (d, 1H, *J* = 2.0Hz, C1- Ar-H of carbazole ring), 6.98-7.59 (m, 12H, Ar-H of carbazole and phenothiazine ring), 5.17 (s, 2H, NCH_2), 3.36 (s, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC =164.3, 147.2, 147.6, 146.9, 145.6, 144.9, 143.4, 141.3, 129.0, 128.9, 127.9, 127.6, 126.1, 126.0, 125.3, 123.3, 122.7, 121.9, 120.5, 119.5, 119.3, 115.4, 115.1, 115.0, 109.8, 109.8, 44.1, 35.8; MS: m/z [M^+] calcd. for $C_{28}H_{22}N_4OS$: 462.1514 found: 462.1513.

N'-((10-methyl-10H-phenothiazin-3-yl)methylene)-2-(naphthalen-2-yloxy)acetohydrazide (PD-5): An equimolar amount of compound N-methyl-10H-phenothiazine-3-carbaldehyde (1 m.mol) and 2-(naphthalen-2-yloxy) acetohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the titlecompound; M.P. 184-186^{0}C; IR (KBr) ν_{max}: 3271, 3053, 2883, 1672, 1598, 1546, 1462, 1379, 1328, 1255, 1215, 1182, 1083 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.54 (s, 1H, NH), 8.25 (s, 1H, N=CH), 7.94 (s, 1H, C_5- Ar-H of naphthalene ring), 6.99-7.89 (m, 13H, Ar-H of naphthalene and phenothiazine ring), 4.78 (s, 2H, OCH_2), 3.32 (s, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC = 169.2, 156.6, 156.1, 147.4, 147.0, 145.0, 143.3, 134.6, 134.4, 129.8, 128.9, 128.9, 127.4, 127.3, 125.3, 124.9, 124.3, 121.9, 121.9, 119.0, 115.4, 115.1, 115.0, 107.7, 107.5, 65.3, 35.8; MS: m/z [M^+] calcd. for $C_{26}H_{21}N_3OS$: 439.1354; found: 439.1353.

N'-((10-ethyl-10H-phenothiazin-3-yl)methylene)benzohydrazide (PD-6): An equimolar amount of compound N-ethyl-3-formylphenothiazine (1 mmol) and benzohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 242-244^{0}C; IR (KBr) ν_{max}: 3469, 3184, 3024, 2995, 2935, 2862, 1907, 1647, 1573, 1469, 1460, 1398, 1307, 1290, 1242, 1134, 1078 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, ppm), δH = 11.79 (s, 1H, NH), 8.32(s, 1H, N=CH), 6.95-7.91 (m, 12H, Ar-H of phenothiazine & phenyl ring), 3.96 (q, 2H, *J* = 7.2 Hz, CH_2), 1.32 (t, 3H, *J* = 6.8 Hz, CH_3); ^{13}C NMR (100.612 MHz, $CDCl_3$, ppm), δC = 160.3, 147.1, 143.7, 128.8, 128.6, 128.3, 127.5, 126.6, 123.4, 123.4, 122.4, 116.2, 115.8, 41.5, 13.0; MS: m/z [M^+] calcd. for C22H19N3OS: 373.1249; found: 373.1248.

N'-((10-ethyl-10H-phenothiazin-3-yl)methylene)isonicotinohydrazide (PD-7): An equimolar amount of compound N-ethyl-3-formylphenothiazine (1 m.mol) and isonicotinohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 203-205^{0}C; IR (KBr) ν_{max}: 3182, 2995, 2933, 2854, 1651, 1575, 1546, 1460, 1402, 1305, 1242, 1217, 1132, 1072 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.98 (s, 1H, NH), 8.78 (q, 2H, *J* = 1.5Hz, C_2 & C_6- Ar-H of nicotine ring), 8.34 (s, 1H, N=CH), 7.82 (q, 2H, *J* = 1.5Hz, C_2 & C_6- Ar-H of nicotine ring), 7.56 (dd, 2H, *J* = 2.0 & 3.5Hz, C_2 & C_4- Ar-H of phenothiazine ring), 7.49 (d, 1H, *J* = 2.0Hz, C1- Ar-H of phenothiazine ring), 6.95- 7.23 (m, 4H, Ar-H of phenothiazine ring), 3.96 (q, 2H, *J* = 7.0Hz, CH_2), 1.33 (t, 3H, *J* = 7.0Hz, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC = 161.8, 150.7, 148.4, 146.5, 143.8, 141.0, 128.7, 128.2, 127.7, 127.5, 125.6, 123.6, 123.3, 122.5, 121.9, 116.1, 115.8, 41.8, 13.0; MS: m/z [M^+] calcd. for $C_{21}H_{18}N_4OS$: 374.1201; found:374.1200.1.4.4.8

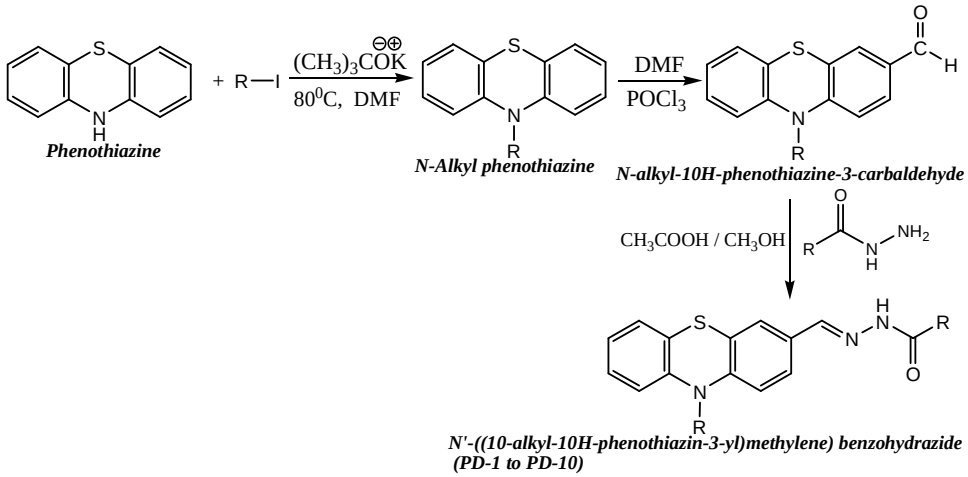
N'-((10-ethyl-10H-phenothiazin-3-yl)methylene)-2-(2-oxo-2H-chromen-7-yloxy)acetohydrazide (PD-8): An equimolar amount of compound N-ethyl-3-formylphenothiazine (1 m.mol) and 2-(2-oxo-2H-chromen-7-yloxy)acetohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 224-226^{0}C; IR (KBr) ν_{max}: 3437, 3169, 3016, 2937, 1722, 1680, 1612, 1562, 1469, 1396, 1363, 1244, 1153, 1134, 1087 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.54 (d, 1H, *J* = 5.5Hz, NH), 8.20 (s, 1H, N=CH), 7.90 (s, 1H, C4- Ar-H of coumarin ring), 6.95-7.74 (m, 10H, Ar-H of coumarin and phenothiazine ring) 6.23 (d, 1H, *J* = 7.0Hz, C_3 Ar-H of coumarin ring), 5.28, (s, 2H,

OCH_2), 3.95 (d, 2H, *J* =7.0Hz, CH_2), 1.31 (t, 3H, *J* = 6.75Hz, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC = 168.9, 161.6, 160.6, 143.9, 127.5, 126.8, 125.1, 123.7, 123.3, 116.1, 112.8, 111.7, 101.8, 65.8, 40.5, 13.0; MS: m/z [M^+] calcd. for $C_{26}H_{21}N_3O_4S$: 471.1253; found: 471.1670.

2-(9H-carbazol-9-yl)-N'-((10-ethyl-10H-phenothiazin-3-yl)methylene)acetohydrazide (PD-9): An equimolar amount of compound N-ethyl-3-formylphenothiazine (1 m.mol) and 2-(9H-carbazol-9-yl)acetohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 186-188^{0}C; IR (KBr) ν_{max}: 3219, 3062, 2964, 2914, 1681, 1597, 1546, 1485, 1460, 1325, 1244, 1211, 1155, 1080 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.62 (s, 1H, NH), 8.16, (d, 2H, *J* = 7.5Hz, N=CH & C_5- Ar-H of carbazole ring), 7.97 (s, 1H, C4- Ar-H of carbazole ring), 6.95-7.60 (m, 14H, Ar-H of phenothiazine and carbazole ring), 5.17 (s, 1H, N-CH_2), 3.95 (q, 2H, *J* = 6.5Hz, CH_2), 1.29-1.34 (m, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC = 169.1, 146.8, 146.1, 144.0, 143.4, 141.3, 141.1, 128.8, 128.2, 127.5, 127.5, 126.1, 126.0, 125.2, 123.7, 123.2, 122.7, 122.6, 120.5, 119.5, 119.3, 116.4, 115.7, 109.8, 44.1, 41.8, 13.0 ; MS: m/z [M^+] calcd. for $C_{29}H_{24}N_4OS$: 476.1671; found: 476.1671.

N'-((10-ethyl-10H-phenothiazin-3-yl)methylene)-2-(naphthalen-2-yloxy)acetohydrazide (PD-10): An equimolar amount of compound N-ethyl-3-formylphenothiazine (1 m.mol) and 2-(naphthalen-2-yloxy) acetohydrazide (1 m.mol) in methanol 10 ml and glacial acetic acid 0.5 ml were added and the reaction was carried out as per general procedure given at 1.4.4. Column chromatographic purification by eluting with hexane/ethyl acetate (3:2) provided the title compound; M.P. 194-196^{0}C; IR (KBr) ν_{max}: 3444, 3381, 3053, 2993, 2976, 2935, 2860, 1616, 1573, 1489, 1462, 1365, 1328, 1249, 1238, 1132, 1105 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, ppm), δH = 11.54 (s, 1H, NH), 8.23 (s, 1H, N =CH), 6.94-7.92 (m, 14H, Ar-H of naphthalene and phenothiazine ring), 4.78, (s, 2H, OCH_2), 3.944 (q, 2H, *J* = 7.0Hz, CH_2), 1.31 (t, 3H, *J* = 6.75 Hz, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δC = 169.2, 164.3, 156.6, 156.1, 147.4, 146.3, 146.0, 144.0, 143.9, 143.3, 134.4, 129.8, 128.8, 127.9, 126.8, 125.5, 124.3, 123.6, 122.6, 122.5, 119.0, 116.1, 115.8, 107.5, 65.3, 41.82, 13.01; MS: m/z [M^+] calcd. for $C_{27}H_{23}N_3O_2S$: 453.1511; found: 453.1510.

Scheme-1: Synthesis of Phenothiazine Derivatives (PD-1 to PD-10)



Tabel 1: Physical Data of Phenothiazine Derivatives (PD-1 to PD-10)

Comp	R	R'	Time	% Yield	M.P (^{0}C)
PD-1	Methyl	Phenyl	160 min	90	212-215
PD-2	Methyl	Pyridinyl	165 min	88	218-220
PD-3	Methyl	2-Oxochromen-7-yloxy	200 min	87	210-212
PD-4	Methyl	Carbazolyl	185 min	86	173-175
PD-5	Methyl	Naphthalen-2-yloxy	175 min	89	184-186
PD-6	Ethyl	Phenyl	162 min	90	242-244
PD-7	Ethyl	Pyridinyl	170 min	89	203-205
PD-8	Ethyl	2-Oxochromen-7-yloxy	210 min	87	224-226
PD-9	Ethyl	Carbazolyl	195 min	85	186-188
PD-10	Ethyl	Naphthalen-2-yloxy	182 min	88	194-196

BIOLOGICAL STUDIES:

***INVITRO* ANTIOXIDANT ACTIVITY:**

DPPH free radical scavenging assay [17]**:** The free radical scavenging activity of pyridine derivatives was measured by DPPH (1,1-diphenyl-2-picryl-hydrazil) employing the method described by Blois, 0.1 m M solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of 100 µg/ml of pyridine derivatives. After 30 min, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Ascorbic acid was used as a reference compound. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with drug) using the following formula.

$$\text{Percentage of inhibition} = \frac{\text{(Absorbance of control - Absorbance of Test)}}{\text{Absorbance of control}} \times 100$$

Antioxidant activities of all the newly synthesized Phenothiazine Derivatives (PD-1 to PD-10) were subjected to DPPH free radical scavenging assay method using the above standard procedure.

Table 2: Antioxidant activity of Phenothiazine Derivatives (PD-1 to PD-10) using DPPH radical scavenging method

Compd	Absorbance	% Antioxidant activity	Compd	Absorbance	% Antioxidant activity
PD-1	0.9118	39.21	**PD-6**	0.7480	50.23
PD-2	0.9069	39.54	**PD-7**	0.4955	66.96
PD-3	0.6607	55.95	**PD-8**	0.9913	33.91
PD-4	0.6281	58.12	**PD-9**	0.6247	58.35
PD-5	0.7405	50.63	**PD-10**	0.7217	51.88
Standard	1.500				

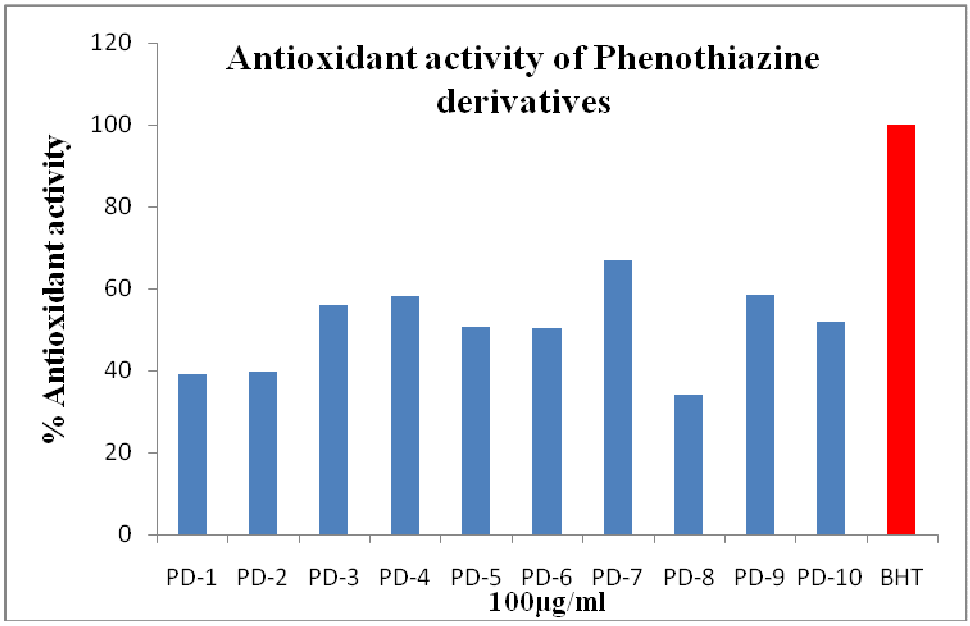


Fig 1: Comparative antioxidant activity of Phenothiazine derivatives (PD-1 to PD-10) and BHT using DPPH radical scavenging method after 30 minutes incubation at 100 µg/ml concentration

Antibacterial activity [18]**:**

Preparation of media: 37 gms of nutrient agar medium was dissolved in 1000 ml of distilled water and the pH was adjusted to 7.0. Where as in case of antifungal activity studies, potato dextrose agar, 39 gm was dissolved in 1000 ml distilled water and the pH was adjusted to 5.6. Each 20ml portion of media was distributed to test tubes and these test tubes were plugged with non-adsorbent cotton and kept in autoclave (121.1^0C) for sterilization for an hour.

Plating of media: Sterilized media was heated in a water bath thoroughly. Molten media was poured on to the Petri dish (pre-sterilized in oven for 3 hours at 110^0C in order to avoid contamination). The plated Petri dishes were kept on plain surface to avoid non-uniform solidification of medium. Micro wells (6mm diameter) were made with borepuncher at equidistance (four micro wells were made on a 4" assay-plate). All these operations were performed in "sterile room" which was equipped with a "laminar flow".

Antibacterial Screening : The synthesized compounds were tested for their antibacterial activity against namely *Bacillus subtilis* (MTCC 8372), *Staphylococcus aureus* (MTCC 3381), *Pseudomonas aeruginosa* (MTCC 2295), *Escherichia coli* (MTCC 1302) and *K. pneumonia* (MTCC 3384), at concentrations of 25 and 50 µg/ml. Nutrient agar medium was dissolved in water and pH was adjusted to 7.0. This was then disturbed in 20ml quantity in boiling tubes; they were then plugged tightly with non-absorbent cotton and sterilized in an autoclave. The bacterial culture (50µl) was then added aseptically to the agar medium maintained at 45^0C, mixed well and poured in to petriplates. Test solutions of different concentrations of compounds 26-30 were prepared in DMSO. After hardening, cups of 6mm diameter each were cut into agar and 50µl test solutions of varying concentrations (25 and 50 µg/ml) were placed in these cups. The plates were incubated at 37^0C for 24 hours and the diameter of inhibition zone was measured in mm. Solvent DMSO alone was kept as control, which did not have any inhibition zone. The activity was compared with standard antibiotic Tetracycline and the antibacterial activities inhibition zones of the compounds are measured. The result was observed after 24 hrs of incubation and given in Table 3 below.

Tabel 3: Antibacterial activity of Phenothiazine derivatives

Compd	Conc. (µg/ml)	Zone of Bacterial Inhibition in mm				
		E.coli	***K. pneumonia***	***P. aeruginosa***	***B.subtilis***	***S. aureus***
PD-1	25	10	08	10	10	9
	50	12	11	12	13	11
PD-2	25	12	10	08	11	10
	50	16	14	10	14	14
PD-3	25	11	10	10	11	12
	50	14	13	12	14	16
PD-4	25	10	11	09	10	11
	50	13	13	11	14	14
PD-5	25	13	09	10	12	10
	50	17	11	14	15	13
PD-6	25	09	09	09	10	10
	50	11	12	13	14	14
PD-7	25	14	08	10	11	11
	50	16	11	12	13	13
PD-8	25	12	10	08	10	09
	50	16	14	11	13	13
PD-9	25	10	10	10	09	11
	50	14	13	13	12	16
PD-10	25	11	10	09	10	10
	50	15	15	11	13	12
Tetracycline	25µg/ml	19	17	16	18	19

RESULTS AND DISCUSSION

In view of the biological applications of phenothiazine derivatives, the synthesis of phenothiazine analogues with hydrazones of N-alkyl-10*H*-phenothiazine-3-carbaldehyde with different substituted heterocyclic acetohydrazide. N-alkyl phenothiazine was synthesized by the reaction of phenothiazine with alkyl iodide (ethyl, methyl) in the presence of potassium tertiary butoxide and dry DMF stirred at 80^0C for 24 hours. The compound N-alkyl-10H-phenothiazine-3-arbaldehyde was obtained from N-alkyl phenothiazine via Vilsmeier Hack reaction. Then N-alkyl-10H- phenothiazine-3-arbaldehyde treated with different heterocyclic acetohydrazides in presence of catalytic amount of acetic acid in methanol under reflux and ultrasonic irradiation method to afford N'-((10-ethyl-10H-phenothiazin-3-yl)methylene)acetohydrazide (PD-1 to PD-10). All the synthesized compounds were screened for their *invitro* antioxidant and antibacterial activity studies were evaluated and reported.

The antioxidant studies revealed that compound PD-7showed good free radical scavenging activity (66.96%) as compared to BHT (Butylated hydroxyl toluene) (100%), while compounds PD-3, PD-4, PD-5, PD-6, PD-9 and PD-10 showed moderate activity and compounds PD-1, PD-2 and PD-8 showed very low activity at 100 µg/ml concentration after 30 minutes incubation time. Free radical scavenging capacities of the synthesized Phenothiazine Derivatives (PD-1 to PD-10), ascorbic acid BHT at 100 µg/ml concentration after half an hour of incubation in dark measured by DPPH assay are shown in Fig 1.

Zone of inhibition was measured for all the newly synthesized compounds and results are summarized in Table 3. The synthesized compounds PD-2, PD-5 and PD-8 showed good activity against *S.aureus, E.coli, P. aeruginosa* and *B.subtilis* and moderate activity against *K. pneumonia* whereas the compounds PD-1, PD-3, PD-4, PD-9 and PD-10 showed moderate activity against *S.aureus, E.coli, P.aeruginosa* and *B.subtilis* and very low activity against *K. pneumonia*. The compound PD-7 showed good activity against *S.aureus, E.coli* and moderate activity against *P. aeruginosa, B.subtilis* and low activity against *K. pneumonia*.

CONCLUSION

In this study, certain Schiff bases containing phenothiazine nucleus derivatives were synthesized and evaluated for their *invitro* antioxidant and antimicrobial activities. Results revealed that the compounds exhibited significant *in-vitro* antioxidant and antimicrobial activities. All the synthesized compounds are more potent to moderate antioxidant and antimicrobial activities against the test organisms. The study would be a fruitful matrix for the development of Schiff bases containing phenothiazine nucleus derivatives for further bio-evaluation.

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