Novel phenothiazine analogous: Synthesis and a new perceptivity into their antioxidant potential

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ABSTRACT

Novel series of phenothiazine derivatives obtained by the phenothiazine-aryl amines conjugates via acetyl group was described. The newly synthesized compounds were characterized by spectral and elemental analysis data. The antioxidant activity was assessed using two in vitro methods namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and inhibition on human low density lipoprotein (LDL) oxidation assay. All the conjugates exhibited good antioxidant activity whereas, compound 2e having 4-amino-2-methoxyphenol moiety exhibited potent antioxidant activities in both the assays and was found more active than the butylated hydroxy anisole (BHA), a reference antioxidant.

Keywords: Phenothiazine, 10-chloroacetylphenothiazine, aryl amines, radical scavenging activity, LDL oxidation.

INTRODUCTION

Reactive oxygen species (ROS) are major free radicals generated in many redox processes, which may induce oxidative damage to biomolecules, including carbohydrates, proteins, lipids, and DNA. Reactive oxygen species affect living cells, which mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson’s disease, Alzheimer’s disease, stroke, arthritis, chronic inflammatory diseases, cancers, and other degenerative diseases [1, 2]. The action of ROS is opposed by a balanced system of antioxidant compounds produced in vivo [3]. Therefore, the development and utilization of more effective antioxidants of natural origin are desired. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants [4, 5]. In the literature some tricyclic amines and their chemical structure shows antioxidant neuroprotective activity in vitro [6]. Nowadays, the free-radical scavenging mechanism of aromatic amines has been discussed from the view of chemical kinetics [7]. The research on free radicals provides theoretical information for the medicinal development, and supplies some in vitro methods for quick-optimizing drugs, it attracts more scientific attention from bioorganic and medicinal chemists. In addition to the traditional –OH bond type antioxidant, tricyclic amines having N–H bond functions as the antioxidant have attracted much research attention because aromatic amines (Ar2NHs) have always been the central structure in many currently used drugs [8]. Phenothiazine belongs to a class of heterocyclic compounds characterized by tricyclic aromatic ring with sulphur and nitrogen atoms. Phenothiazine structural motif has been successfully employed in the design of variety of pharmaceuticals which are clinically used for psychotropic medication, antitubercular activity [9], cholinesterase inhibitor [10], histamine H1 antagonist [11] and MDR (multiple drug resistance) reverting agent [12]. Substituted phenothiazines have also attracted interest because
of their optoelectrochemical and photophysical properties [13-16]. As the substituents on the phenothiazine rings have a great influence on their properties, efficient methods for the preparation of a diverse range of substituted phenothiazines are highly desirable. Owing to the widespread applications, synthetic and biological activity evaluation of phenothiazine and their derivatives has been subject of intense investigations. Therefore, in continuation of our interest in functionalization of tricyclic and heterocyclic compounds [17, 18] and in searching of new biologically active compounds, phenothiazine analogues were synthesized and screened for their in vitro antioxidant activity using two well documented assays.

MATERIALS AND METHODS

All chemicals used were of laboratory grade (Qualigen, Merck). The melting points were determined by open capillary method on a Campbel electronic apparatus and are uncorrected. The ultraviolet absorption spectra were determined in methanol by using a Shimadzu 1601 UV-Visible double beam spectrophotometer. The IR spectra of synthesized compounds were recorded on a Shimadzu 8400S FT-IR in potassium bromide disks. The novel synthesized compounds were recorded on a Shimadzu 8400S FT-IR in potassium bromide disks. The ultraviolet absorption spectra were determined in methanol by using a Shimadzu 1601 UV-Visible double beam spectrophotometer. The IR spectra of synthesized compounds were recorded on a Shimadzu 8400S FT-IR in potassium bromide disks. The \(^1\)H NMR was recorded in CDCl\(_3\) using a NMR Varian-Mercury 300 MHz spectrometer and chemical shifts are given in units as \(\delta\) ppm, downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on an Electron Impact mass spectrometer at 70 eV ionizing beam and using a direct insertion probe. The progress of reactions was monitored by thin layer chromatography using chloroform-methanol or chloroform-methanol-ammonia as the solvent systems and spots were visualized after exposure to iodine vapors or under ultraviolet (UV) light.

Synthesis of 10-chloroacetylphenothiazine (5)

To the well stirred solution of phenothiazine (0.4g, 2 mmol) and triethylamine (0.22g, 2.2 mmol) in 10 ml dry THF, chloro acetylchloride (0.24g, 2.2 mmol) in 5 ml dry THF was added drop by drop and the reaction mixture was stirred at room temperature for about 3 hr. Progress of the reaction was monitored by TLC using 9:1 hexane: ethyl acetate mixture as mobile phase. After the completion of reaction, the reaction mass was quenched in ice cold water evaporator at 50\(^\circ\)C. The yellow solid product was obtained by desolventation through rotary evaporator at 35\(^\circ\)C.

Yield 89%, m.p. 113-114 \(^\circ\)C. IR(KBr) cm\(^{-1}\): 3050-2832 (Ar–H), 1710 (C=O), 2972–3022 (CH\(_3\)), \(^1\)H NMR (250 MHz, CDCl\(_3\)), \(\delta\)(ppm), 6.8-7.4 (m, 8H, phenothiazine Ar-H), 4.1 (s, 2H, CH\(_2\)-N-H), \(^1\)C NMR (CDCl\(_3\), 100MHz) \(\delta\) ppm: 158.3, 138.2, 132.9, 128.3, 127.9, 126.8, 122.3, 40.3, Mass (m/z): 275.10, Anal.(%) for \(C_{14}H_{19}ClNOS\) : C, 60.98; H, 3.65; N, 5.08; Found: C, 60.96; H, 3.63; N, 5.05.

General procedure for the synthesis of novel phenothiazine analogues (5a–g)

Aryl amines (1.2 mmol) in dry THF (15 mL) was treated with K\(_2\)CO\(_3\) (600mg) in N\(_2\) atmosphere. Later the solution of 10-chloroacetylphenothiazine (0.275g, 1 mmol) in dry THF (5 mL) was added drop by drop. The reaction mixture was refluxed for 6-8 hr. The progress of the reaction mixture was monitored by TLC. The reaction mixture was then desolventized in rotary evaporator and the products were extracted in ethyl acetate. The organic layer were washed with water and dried over anhydrous Na\(_2\)SO\(_4\). The yellow solid product was obtained by desolventation through rotary evaporator at 50\(^\circ\)C. The analogues were separated and purified by column chromatography by using mixture of chloroform : methanol = 85: 15. The products were characterized by IR, mass \(^1\)H NMR and Elemental Analysis.

**2-(4-hydroxyphenylamino)-1-(10H-phenothiazin-10-yl)ethanone (5a)**

Yield 88%, m.p. 161-163 \(^\circ\)C. IR(KBr) cm\(^{-1}\): 3052-2830 (Ar–H), 1700 (C=O), 3369–3594 (phenolic-OH), 2922–3004 (CH\(_3\)), 3269.1 (N-H); \(^1\)H NMR (250 MHz, CDCl\(_3\)), \(\delta\)(ppm), 6.9-7.6 (m, 8H, phenothiazine aromatic system), 6.71-6.73 (m, 4H, aryl amine aromatics), 5.3 (s, 1H, Phenolic OH), 4.1 (s, 2H, CH\(_2\)), 4.3 (s, 1H, NH). \(^1\)C NMR (CDCl\(_3\), 100MHz) \(\delta\) ppm: 163.7, 146.2, 140.15, 138.9, 132.6, 128.3, 127.1, 126.8, 121.9, 116.5 116.9, 52.3. Mass (m/z): 348.21, Anal.(%) for \(C_{14}H_{19}NO_3\) : C, 68.94; H, 4.63; N, 7.86; Found: C, 68.93; H, 4.61; N, 7.83.

**2-(2-hydroxyphenylamino)-1-(10H-phenothiazin-10-yl)ethanone (5b)**

Yield 84%, m.p.168-170 \(^\circ\)C. IR (KBr) cm\(^{-1}\): 3054-2832 (Ar–H), 1670 (C=O), 3379-3587 (phenolic-OH), 2955-3035(CH\(_3\)), 3266.2 (N-H); \(^1\)H NMR (250 MHz, CDCl\(_3\)), \(\delta\)(ppm), 6.74-6.93 (m, 4H, aryl amine aromatics), 5.3 (s, 1H, Phenolic OH), 4.1 (s, 2H, CH\(_2\)), 4.3 (s, 1H, NH). \(^1\)C NMR (CDCl\(_3\), 100MHz) \(\delta\) ppm: 163.7, 141.15, 139.4, 138.9, 132.6, 128.3, 127.1, 126.8, 122.1, 122.4, 118.5, 116.9
The DPPH radical scavenging effect was carried out according to the method first employed by Blois [19]. Antioxidant activity was studied. Compounds at different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 µM, 50 µM, 100 µM, 200 µM and 500 µM) were taken in different test tubes, 4 ml of a 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer.

2-(3-hydroxyphenylamino)-1-(10H-phenothiazin-10-yl)ethanone (5c)

Yield 89%, m.p. 154-155 °C, IR (KBr) cm⁻¹: 3049-2827 (Ar-H), 1690 (C=O), 3355-3516 (phenolic-OH), 2972-3021(CH₃), 3291.6 (N-H); ¹H NMR (250 MHz, CDCl₃), δ(ppm), 6.9-7.5 (m, 8H, phenothiazine aromatic system), 6.14-7.03 (m, 4H, aryl amine aromatics), 5.3 (s, 1H, phenolic OH), 4.1 (s, 2H, CH₂), 4.3 (s, 1H, NH). ¹³C NMR (CDCl₃, 100MHz) δ ppm: 163.7, 159.5, 149.3, 138.9, 132.5, 130.3, 128.3, 122.7, 126.3, 122.4, 107.3, 106.5, 98.6. Mass (m/z): 348.09, Anal.(%) for C₂₀H₁₈N₂O₂S: C, 68.94; H, 4.63; N, 8.04; Found: C, 68.91; H, 4.61; N, 8.00.

2-(4-hydroxy-3-methoxyphenylamino)-1-(10H-phenothiazin-10-yl)ethanone (5e)

Yield 81%, m.p.144-146 °C, IR (KBr) cm⁻¹: 3050-2832 (Ar-H), 1694 (C=O), 3399-3561 (phenolic-OH), 2971-3027(CH₃), 3266.9 (N-H), 1640-1510 (NO₂). ¹H NMR (250 MHz, CDCl₃), δ(ppm) 6.9-7.5 (m, 8H, phenothiazine aromatic system), 6.9-7.53 (m, 3H, aryl amine aromatics), 5.3 (s, 1H, Phenolic OH), 4.1 (s, 2H, CH₂), 4.3 (s, 1H, NH). ¹³C NMR (CDCl₃, 100 MHz) δ ppm: 163.7, 152.2, 145.3, 142.6, 138.5, 132.5, 128.33, 127.1, 126.4, 122.4, 118.5, 115.8, 114.5, 112.8, 112.7, 112.0, 107.3, 52.2. Mass (m/z):393.09. Anal.(%) for C₂₆H₁₈N₂O₄S: C, 66.01; H, 3.84; N, 10.68; Found: C, 61.03; H, 3.81; N, 10.65.

2-(4-hydroxy-3-methoxyphenylamino)-1-(10H-phenothiazin-10-yl)ethanone (5d)

Yield 75%, m.p.115-117 °C, IR (KBr) cm⁻¹: 3054-2832 (Ar-H), 1694 (C=O), 3399-3561 (phenolic-OH), 2971-3027(CH₃), 3266.9 (N-H), 1640-1510 (NO₂). ¹H NMR (250 MHz, CDCl₃), δ(ppm) 6.9-7.5 (m, 8H, phenothiazine aromatic system), 5.9-6.53 (m, 3H, aryl amine aromatics), 5.3 (s, 1H, Phenolic OH), 4.1 (s, 2H, CH₂), 4.3 (s, 1H, NH), 3.8 (s, 1H, OCH₃). ¹³C NMR (CDCl₃, 100 MHz) δ ppm: 163.7, 141.5, 141.2, 138.3, 135.2, 128.4, 126.4, 127.8, 122.2, 122.7, 120.2, 107.3, 52.2. Mass (m/z):387.6. Anal.(%) for C₂₆H₁₈N₂O₄S: C, 67.21; H, 3.80; N, 10.68; Found: C, 66.63; H, 3.77; N, 7.43.

2-(4-hydroxy-3-methylphenylamino)-1-(10H-phenothiazin-10-yl)ethanone (5f)

Yield 98%, m.p.154-155 °C, IR (KBr) cm⁻¹: 3050-2832 (Ar-H), 1698 (C=O), 3396-3566 (phenolic-OH), 2972-3022 (CH₃), 3256.1 (NH), ¹H NMR (250MHz, CDCl₃), δ(ppm) 6.9-7.5 (m, 8H, Phenothiazine aromatic system), 6.34-6.53 (m, 3H, aryl amine aromatics), 5.3 (s, 1H, Phenolic OH), 4.1 (s, 2H, CH₂), 4.3 (s, 1H, NH), 2.1 (s, 3H, CH₃). ¹³C NMR (CDCl₃, 100 MHz) δ ppm: 163.2, 142.2, 140.5, 138.4, 132.2, 128.4, 127.4, 126.3, 126.8, 122.3, 116.8, 114.8, 113.2, 52.4, 15.2. Mass (m/z):362.1. Anal.(%) for C₂₅H₁₈N₂O₂S: C, 69.59; H, 5.01; N, 7.73; O, 8.83; S, 8.85; Found: C, 69.55; H, 5.04; N, 7.72; O, 8.85; S, 8.87.

2-(3-bromo-4-hydroxyphenylamino)-1-(10H-phenothiazin-10-yl)ethanone (2g)

Yield 84%, m.p.177-179 °C, IR (KBr)cm⁻¹:3050-2832 (Ar-H), 1691 (C=O), 3316-3571(phenolic-OH), 2972-3022 (CH₃), 3273.4 (N-H); ¹H NMR (250MHz, CDCl₃), δ(ppm) 6.9-7.5 (m, 8H, phenothiazine aromatic system), 6.2-6.6 (m, 3H, aryl amine aromatics), 5.3 (s, 1H, Phenolic OH), 4.1 (s, 2H, CH₂), 4.3 (s, 1H, NH). ¹³C NMR (CDCl₃, 100 MHz) δ ppm: 163.5, 145.3, 142.6, 138.5, 132.5, 128.33, 127.1, 126.4, 122.4, 118.5, 115.8, 114.5, 114.3, 52.2. Mass (m/z):427.4. Anal.(%) for C₂₅H₁₂BrN₂O₂S: C, 56.21; H, 3.54; Br, 18.70; N, 6.56; O, 7.49; S, 7.50; Found: C, 56.23; H, 3.56; Br, 18.72; N, 6.55; O, 7.48; S, 7.52.

Antioxidant activity

The newly synthesized compounds were screened for their radical scavenging activities using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and inhibition of human low-density lipoproteins (LDL) oxidation. The compounds under study were dissolved in distilled ethanol (50 mL) to prepare 1000 µM stock solution. Solutions at different concentrations (10 µM, 50 µM, 100 µM, 200 µM and 500 µM) were prepared by serial dilution and the antioxidant activity was studied.

DPPH radical scavenging assay

The DPPH radical scavenging effect was carried out according to the method first employed by Blois [19]. Compounds at different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 µM, 50 µM, 100 µM, 200 µM and 500 µM) were taken in different test tubes, 4 ml of a 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer.
and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decrease in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

Radical scavenging activity (%) = \[
\frac{(A_0 - A_t)}{A_0} \times 100
\]

Where \( A_0 \) is the absorbance of the control (blank, without compound) and \( A_t \) is the absorbance of the compound.

Human LDL oxidation assay
Fresh blood was obtained from fasting adult human volunteers and plasma was immediately separated by centrifugation at 1500 rpm for 10 min at 4 °C. LDL (0.1 mg LDL protein/mL) was isolated from freshly separated plasma by preparative ultra centrifugation using a Beckman L8-55 ultra centrifuge. The LDL was prepared from the plasma according literature method [20]. The isolated LDL was extensively dialyzed against phosphate buffered saline (PBS) pH 7.4 and sterilized by filtration (0.2 µm Millipore membrane system, USA) and stored at 4 °C under nitrogen. 1 mL of various concentrations (10 and 25 µM) of compounds were taken in test tubes, 40 µL of copper sulphate (2 mM) was added and the volume was made up to 1.5 mL with phosphate buffer (50 mM, pH 7.4). A tube without compound and with copper sulphate served as a negative control, and another tube without copper sulphate with compound served as a positive control. All of the tubes were incubated at 37 °C for 45 min. To the aliquots of 0.5 mL drawn at 2, 4 and 6 hr intervals from each tube, 0.25 mL of thiobarbituric acid (TBA, 1% in 50 mM NaOH) and 0.25 mL of trichloro acetic acid (TCA, 2.8%) were added. The tubes were incubated again at 95 °C for 45 min and cooled to room temperature and centrifuged at 2500 rpm for 15 min. A pink chromogen was extracted after the mixture was cooled to room temperature by further centrifugation at 2000 rpm for 10 min. Thiobarbituric acid reactive species in the pink chromogen were detected at 532 nm by a spectrophotometer against an appropriate blank. Data were expressed in terms of malondialdehyde (MDA) equivalent, estimated by comparison with standard graph drawn for 1,1,3,3-tetramethoxy-propane (Which was used as standard) which give the amount of oxidation and the results were expressed as protection per unit of protein concentration (0.1 mg LDL protein/mL). Using the amount of MDA, the percentage protection was calculated using the formula:

\[
\text{% inhibition of LDL oxidation} = \frac{(\text{Oxidation in control} - \text{oxidation in experimental} / \text{oxidation in control}) \times 100}{\text{Oxidation in control}}
\]

CHEMISTRY
The reaction of 2-iodoaniline with 2-bromobenzenethiol in the presence of a CuI/L-proline catalyst (10 mol% and 20 mol%, respectively) in 2-methoxyethanol at 90 °C afforded only the simple S-arylation product (3), upon heated to 110 °C for 48–72 hr affords phenothiazine (4) [21]. The key scaffold, 10 chloroacetyl phenothiazine (5) was prepared by N-acylation of phenothiazine with 3-chloro acetylchloride in the presence of triethylamine as base. Further, coupling of respective aryl amines to the scaffold by base condensation reaction affords novel phenothiazine analogues (Scheme 1).

RESULTS AND DISCUSSION
Phenothiazine (4) upon N-acylation in presence of chloro acetylchloride and triethylamine as base to produce 10-chloroacetylphenothiazine (5), a key scaffold. Further, coupling of different aryl amines was done with simple experiment protocol to obtain new phenothiazine analogues (5a-g) in good yield. Structural conformation was done using IR, 1H and 13C NMR, mass spectra and elemental analysis. The IR spectrum of compound (5) showed characteristic absorption at 1710 cm⁻¹ for carbonyl group and showed –CH₂ stretching bands at 2972–3022 cm⁻¹. The 1H NMR spectrum showed characteristic peak of –CH₂ at 4.1 ppm and the absent of N-H peak at 8.0 ppm conforms the N-acylation of phenothiazine. The IR spectra of all new phenothiazin analogues (5a-g) revealed the presence of N-H stretching band at 3256–3291 cm⁻¹ and showed broad phenolic stretching at 3316–3594 cm⁻¹. The Ar-H and C=O absorption band was appeared at the expected regions. 1H NMR spectra of all conjugated analogues (5a-g) showed N-H proton as singlet at 4.3 ppm. The signal due to phenolic OH in all analogues appeared as singlet about 9.5-10.3 ppm. In addition to phenolic OH, –OCH₃ protons present in the compounds 5e resonated as a singlet at 3.8 ppm. Other aromatic protons were observed at expected regions. Mass spectra of all newly synthesized compounds showed M⁺ peak, in agreement with their molecular formula.
Antioxidant activity

DPPH radical scavenging assay

The radical scavenging activity (RSA) of newly synthesized phenothiazine analogues were evaluated by DPPH model and some structure–activity relationships was established. The evaluation study was carried out at various concentrations and also comparative studies were done with the standard antioxidant BHA. DPPH radical scavenging activity (RSA) evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical activity of specific compounds or extracts. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radical (i.e., by providing hydrogen atoms or by electron donating, conceivable) and convert them to a colourless/ bleached product (i.e., 2,2-diphenyl-1-picrylhydrazine, or a substituted analogues hydrazine), resulting in a decrease in absorbance. Hence, more rapidly the absorbance decrease, the more potent the antioxidant activity of the compound. Percentage (%) activity of ethanolic solutions of phenothiazin-aryl amine conjugates (5a-g) were examined and compared (figure-1). Initially, 10-chloroacetylphenothiazine (5) showed negligible DPPH activity, from the figure-1 we can conclude that the coupling of different aryl amines to 10-chloroacetylphenothiazine (5) will increases the DPPH activity (Figure 1).
Figure 1: % DPPH activity of newly synthesized phenothiazine analogues. Each value represents mean ±SD

All the compounds (5a-g) showed negligible activity towards DPPH but further coupling of different aryl amines enhance the DPPH activity. Compound 5e possessing electron donating –OCH₃ in the phenol ring increased 27 fold better than the compound (5), even more than the standard BHA. The increasing order of RSA of newly synthesized analogues and standard are as follows (5e) > BHA > (5a) > (5b) > (5c) > (5d) > (5f) > (5g) > (5).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
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<tbody>
<tr>
<td>5</td>
<td>278±0.92</td>
</tr>
<tr>
<td>5a</td>
<td>12±1.72</td>
</tr>
<tr>
<td>5b</td>
<td>13±0.96</td>
</tr>
<tr>
<td>5c</td>
<td>14±0.16</td>
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<tr>
<td>5d</td>
<td>15±0.32</td>
</tr>
<tr>
<td>5e</td>
<td>10±0.11</td>
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<tr>
<td>5f</td>
<td>22±0.09</td>
</tr>
<tr>
<td>5g</td>
<td>156±0.12</td>
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<tr>
<td>BHA</td>
<td>114±0.96</td>
</tr>
</tbody>
</table>

Initially, 10-chloroacetylphenothiazine (5) showed negligible activity towards DPPH but further coupling of different aryl amines enhance the DPPH activity. Compound 5e possessing electron donating –OCH₃ in the phenol ring increased 27 fold better than the compound (5), even more than the standard BHA. The increasing order of RSA of newly synthesized analogues and standard are as follows (5e) > BHA > (5a) > (5b) > (5c) > (5d) > (5f) > (5g) > (5).

Human LDL oxidation assay
Oxidation modification is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases [22] and the dietary antioxidants that protect LDL from oxidation may therefore reduce atherogenesis and coronary heart diseases [23]. In general, oxidation of LDL follows a radical chain reaction that generates conjugated diene hydroperoxides as its initial products. It has been reported that inhibition of human LDL oxidation may arise due to free-radical scavenging [24]. The antioxidant activity of phenothiazin-aryl amine conjugates (5a-g) against human LDL oxidation with different concentrations (10 µM and 25 µM) is depicted in the figure 2.
The polyunsaturated fatty acid (PUFA) of human LDL were oxidized, and the malonaldehyde (MDA) formed have been estimated by using thiobarbituric acid (TBA) method. Initially the formation of conjugated dienes due to copper-induced LDL oxidation was unaffected by compound (5) showing less activity but phenothiazin-aryl amine conjugates (5a-g) effectively inhibited LDL oxidation showed good activity. The average induction time for copper mediated LDL oxidation was around 4 min without the addition of compounds. The compounds protected LDL from oxidation as measured by the prolongation of the induction time of the formation of conjugated dienes. Among the synthesized compounds, at the end of 2 hr after the induction of oxidation compound 5e exhibited 66.74 and 76.25% protection at 10 and 25 µM levels. Whereas, it was 87.65 and 95.25% protection at the end of 6 hr showing dominant inhibition over LDL oxidation and exhibits more activity than the standard BHA. Compound (5) exhibited 5.34 and 7.43% protection at the 10 and 25 µM levels at the end of 2 hr after the induction of oxidation. Whereas, it showed 8.87 and 12.43% protection at the end of 6 hr showing less activity. The results indicate a dose-dependent inhibition effect of compounds against LDL oxidation.
CONCLUSION

In conclusion, we synthesized aryl amine containing derivatives of phenothiazine and evaluated their antioxidant activities. Irrespective of the linkage type examined, aryl amines-containing compounds exhibited potent DPPH and inhibition of LDL oxidation activities. Initially, 10-chloroacetylphenothiazine (5) showed least activity over both the assay. Coupling of aryl amines to 2-chloro-1-(10H-phenothiazin-10-yl)ethanone enhance the antioxidant activity. Among the analogues compound 5e having electron donating -OCH$_3$ group with phenol ring revealed high antioxidant activity. These findings suggest that the introduction of the aryl amine moiety to phenothiazine may improve antioxidant activities. These effects may be useful in the treatment of pathology in which free radicals oxidation plays a fundamental role. This may warrant further in depth biological evaluations.

ABBREVIATIONS

°C = centigrade
min = minute
hr = hour
mL = milli Liter
µm = micro molar
% = percentage
IC$_{50}$ = 50 percent inhibition concentration
nm = nano meter
mM = milli molar
M = molar
RT = room temperature
BHA = Butylated hydroxyl anisole
DPPH = 2, 2-diphenyl-1-picrylhydrazyl
TLC = Thin layer chromatography
IR= Infrared
$^1$H NMR= proton nuclear magnetic resonance
MP= melting point
KBr= potassium bromide
RSA= radical scavenging activity
LDL = Low density lipoprotein

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