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RESEARCH ARTICLE

NOVEL PHENOTHIAZINE INTEGRATED CINNAMOYL AMIDE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT SCREENING

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Abstract

Novel series of phenothiazine based cinnamoyl amide derivatives, obtained by the two phenothiazine moieties connected *via* aromatic ring through Buchwald-Hartwig coupling and further derivatizations to obtain target molecules 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 2,2'-azinobios[3-ethylbenzthiazolinee]-6-sulfonic acid (ABTS) oxidation assay. All the compounds were exhibited moderate antioxidant activity, whereas compounds **6d** and **6e** exhibited good antioxidant activity in both the assays.

Keywords: Phenothiazine; Buchwald-Hartwig coupling; Cinnamoyl amide; Antioxidant property.

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 (Halliwell and Grootveld, 1987; Dermott, 2000). The action of ROS is opposed by a balanced system of antioxidant compounds produced *in vivo (*Halliwell and Gutteridge, 1999). Therefore, the development and utilization of more effective antioxidants of natural origin are desired. In the literature, some tricyclic amines and their chemical structure shows antioxidant neuroprotective activity *in vitro (*Chirtian Beh and Moosmann, 2000). Nowadays, the free-radical scavenging mechanism of aromatic amines has been discussed from the view of chemical kinetics (Lucarini et al., 1999). Like wise Phenothiazine belongs to a class of heterocyclic compounds with tricyclic aromatic ring having 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, antioxidant, antitubercular activity (Bate et al., 2007), cholinesterase inhibitor (Darvesh et al., 2008), histamine H1 antagonist (Kubota et al., 2009) and MDR (multiple drug resistance) reverting

agent (Bisi et al., 2008). In general, cinnamoyl amides are well known for their antioxidant properties due to their alpha-beta unsaturated amide.

Identification of novel scaffolds in designing new potent antioxidant agents remained a major challenge for medicinal chemistry researchers. In this view explored on the synthesis of phenothiazines incorporated cinnamoyl amide derivatives and evaluated their antioxidant activity.

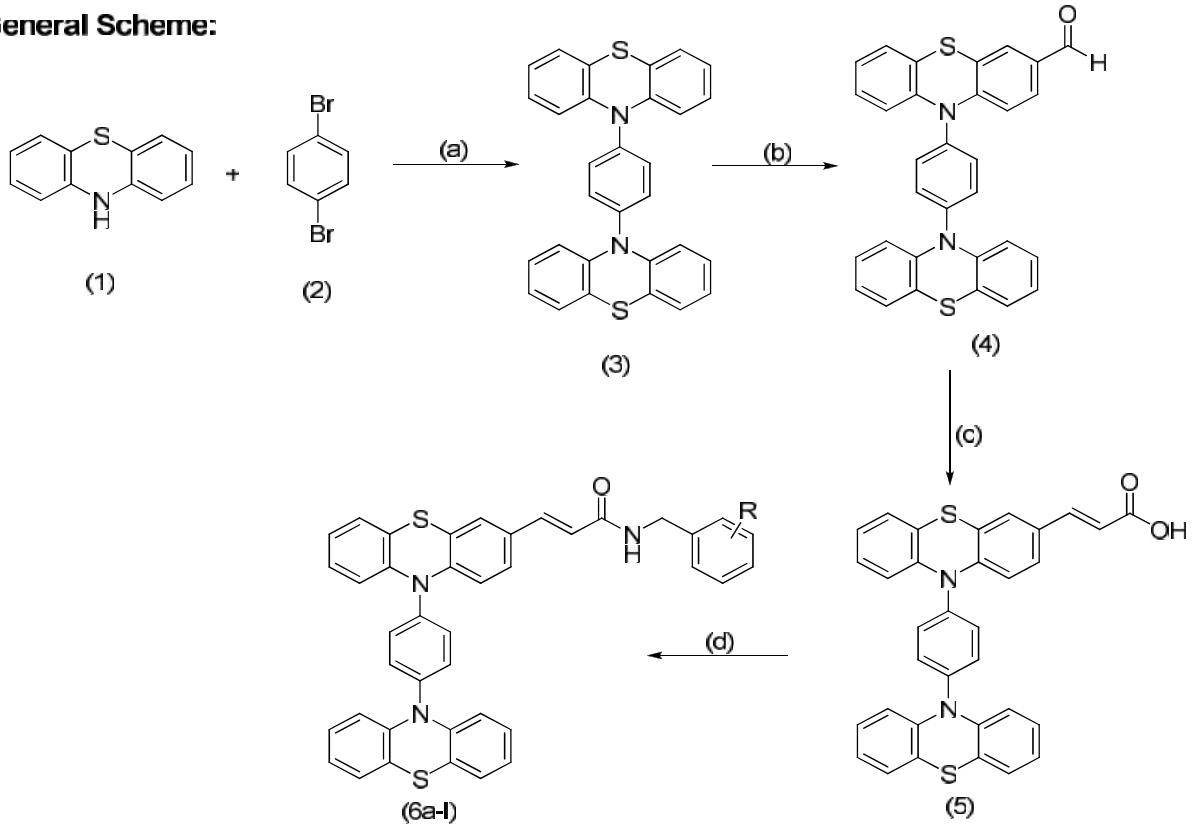
Experimental details

All the commercially available reagents and solvents were used without further purifications. All the melting points were determined on a Buchi apparatus and are uncorrected. The Infra-red spectra (KBr) were recorded on a JASCO spectrometer and frequencies are expressed in cm^{-1}. Mass spectra (GC/MS) were recorded on Agilent MSD VL mass spectrometer. ^1H NMR spectra were recorded on a Bruker Advance 400 spectrometer operating at 400 MHz. The chemical shifts are reported in ppm (δ) relative to tetra methyl silane.

Synthesis of phenothiazine cinnamoylamide

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-phenyl acrylamide derivatives (Compounds **6a-6l**) were synthesized according to the methods described in general scheme. In-total twelve new compounds were synthesized by using various (un)substituted benzyl amines. The assigned structure and Molecular formula of the newly synthesized compounds **(6a-6l)** were confirmed and supported by its IR, ^1H NMR, elemental analysis and Mass spectra. The synthesized compounds were screened for their antioxidant activity.

General Scheme:



where R = H (**6a**); 3-methyl (**6b**); 4-methyl (**6c**); 4-methoxy (**6d**); 2,4-dimethoxy (**6e**), 4-trifluorothiomethyl (**6f**); 4-fluoro (**6g**); 2-chloro-3,6-difluoro (**6h**); 4-trifluoromethyl (**6i**); 3-nitro (**6j**); 4-nitro (**6k**), 3,5-bistrifluoromethyl (**6l**).

Reagents and Conditions:

(a) $Pd_2(dba)_3$, (tBu)$_3$P.HBF_4, NaOtBu, 1,4-dioxane, 101 ^oC, 16 h; (b) DMF, $POCl_3$, 1,2-dichloroethane, 90 ^oC,18 h; (c) Malonic acid, pyridine, piperidine 90 ^oC, 3 h; (d) (un)substituted benzylamine, EDCI, HOBt, DIPEA, DMF, RT, 10 h.

Synthesis of 1.4 –di (10*H*-phenothiazin-10-yl) benzene (3)

Under inert conditions 10*H*-phenothiazine **1** (32.62 mmol), 1,4-Dibromo benzene **2** (14.83 mmol), $Pd_2(dba)_3$ (0.44 mmol), (tBu)$_3$P.HBF_4 (0.74 mmol), NaOtBu (34.1 mmol) and anhydrous 1,4-dioxane were placed in a pressure tube. The reaction mixture was stirred at 101˚C for 16 h. After cooling to rt., the solution was diluted with deionized water and saturated Na_2SO_3 solution. The aqueous phase was extracted with small portions of methylene chloride and the combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuo. The formed precipitate was triturated with ethyl acetate to furnish the product as solid. 95% yield, mp 223-225 °C; IR υmax/cm^{-1}: 3433, 2918, 1500, 1462, 1306, 743; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.51 (d, J = 8 Hz, 4H, Ar-H), 6.89-6.93 (m, 4H, Ar-H), 6.97-7.01 (m, 4H, Ar-H), 7.11-7.13 (m, 4H, Ar-H), 7.49 (bs, 4H, Ar-H); ESI mass m/z: 473.06 (M+1). Anal. Calcd for $C_{30}H_{20}N_2S_2$: C, 76.24; H, 4.27; N, 5.93; Found: C, 76.52; H, 4.32; N, 5.97.

Synthesis of 10-(4-(10*H*-phenothiazine-10-yl)-10*H*-phenothiazine-3-carbaldehyde (4)

To a solution of DMF (40.2 mmol) added drop wise $POCl_3$ (26.05 mmol) under N_2 atmosphere at 0 °C and the mixture was stirred at 0 °C for 10 min. After 10 min compound **3** (21.18 mmol) in 1,2 dichloroethane (50 mL) was added drop wise to it and heated to 90 °C and maintained for 18 h. The reaction was monitored by TLC. After completion of the reaction, reaction mass was cooled to ambient temperature, poured into ice-water, neutralized with $NaHCO_3$ and then extracted with ethyl acetate (100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography (60-120 mesh silica gel) using methanol in chloroform as an eluent. At 1 % methanol in $CHCl_3$, monoaldehyde was obtained. Yellow solid, 76% yield; mp 202-204 °C. IR υmax/cm^{-1}: 3433, 2924, 1683, 1504, 1465, 1310, 1184, 1019, 739. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.28 (d, J = 8 Hz, 1H, Ar-H), 6.37 (d, J = 8 Hz, 1H, Ar-H), 6.72 (d, J = 8 Hz, 2H, Ar-H), 6.98-7.06 (m, 4H, Ar-H), 7.11-7.14 (m, 3H, Ar-H), 7.16-7.18 (m, 2H, Ar-H), 7.51-7.61 (m, 6H, Ar-H), 9.73 (s, 1H, -CHO); MS *m/z*: 501.0 (M+1). Anal. Calcd for $C_{31}H_{20}N_2O_2S_2$: C, 74.37; H, 4.03; N, 5.60. Found: C, 74.16; H, 4.09; N, 5.49.

Synthesis of (E)-3-(10*H*-phenothiazine-10-yl)-10*H*-Phenothiazin-3-yl) acrylic acid (5)

To a stirred solution of **4** (4 mmol) in 10 mL of pyridine, malonic acid (16 mmol) and 0.1 eq of piperidine were added at 0 ^{o}C. The reaction mixture was heated to 90 ^{o}C and maintained for 3 h. The reaction was monitored by TLC. After completion of the reaction, reaction mass was cooled to ambient temperature, poured into ice-water and the formed precipitate was filtered and dried by high vacuum. Yellow solid, 93% yield; mp 252-25°C.IR υmax/cm^{-1}. 3436, 2924, 2850, 1504, 1465, 1313, 1233, 1019, 739. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.29-6.39 (m, 3H), 6.65 (d, J = 8 Hz, 2H), 6.92 (d, J = 14.76 Hz, 1H, Ar-CH=CH-C), 6.99-7.03 (m, 3H, Ar-H), 7.11-7.15 (m, 3H, Ar-H), 7.24 (d, J = 8 Hz, 2H, Ar-H), 7.31 (d, J = 8 Hz, 1H), 7.41 (d, J = 15.84 Hz, 1H, Ar-CH=CH-C), 7.47 (s, 1H, Ar-H), 7.56-7.62 (m, 4H); MS *m/z*: 618.0 (M+1). Anal. Calcd for $C_{33}H_{22}N_2O_2S_2$: C, 73.04; H, 4.09; N, 5.16. Found: C, 72.79; H, 4.21; N, 5.02.

General procedure for obtaining the Phenothiaizine based Cinnamoyl amide derivatives (6a-l)

To a solution of **5** (0.27 mmol) in dry DMF (2.5 mL), EDCI (0.611 mmol), HOBt (0.13 mmol), and DIPEA (0.833 mmol) were added and stirred at RT. After 5 min, (un)substituted benzyl amine (0.388 mmol) was added and the reaction mixture was stirred at RT for about 10 h. The reaction was monitored by TLC. After completion of the reaction, reaction mixture was poured into ice water and extracted with DCM. The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuo.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-benzylacrylamide (6a)

Yellow solid, 92% yield; mp 224 °C. IR υmax/cm^{-1}: 3423, 2921, 1652, 1617, 1466, 1310, 1251, 980, 747, 625. ^{1}HNMR (400 MHz, DMSO-d_6) δ 4.38 (d, J = 5.8 Hz, 2H, CH_2-Ar), 6.36 (d, J = 8.6 Hz, 2H, Ar-H), 6.53 (d, J = 15.7 Hz, Ar-CH=CH-C), 6.64 (d, J = 8.1 Hz, 2H, Ar-H), 6.93 (t, J = 7.5 Hz, 1H, Ar-H), 7.01-7.03 (m, 3H, Ar-H), 7.13 (t, J = 7.5 Hz, 3H, Ar-H), 7.22-7.34 (m, 10H, Ar-H), 7.56-7.61 (m, 4H, Ar-H), 8.53 (t, J = 5.8 Hz, 1H, Ar-H); MS *m/z*: 632.1 (M^{+1}). Anal calcd for $C_{40}H_{29}N_3OS_2$: C, 76.04; H, 4.63; N,6.65. Found: C, 76.13; H, 4.52; N, 6.71.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl) phenyl)-10*H*-phenothiazin-3-yl)-N-(3-methylbenzyl) acrylamide (6b)

Yellow solid, 88% yield; mp 223 °C. IR υmax/cm^{-1}: 3435, 2922, 1650, 1609, 1466, 1310, 1042, 814, 745. ^{1}HNMR (400 MHz, DMSO-d_6) *δ* 2.28 (s, 3H, CH_3), 4.34 (d, *J* = 5.8 Hz, 2H, CH_2-Ar), 6.36 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.53 (d, *J* = 15.8 Hz, -CH=CH-C), 6.64 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.01-7.15 (m, 9H, Ar-H), 7.19-7.24 (m, 4H, Ar-H), 7.29 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.56-7.61 (m, 4H, Ar-H), 8.48 (t, *J* = 5.9 Hz, 1H, Ar-H); MS *m/z*: 646.2 (M^{+1}). Anal calcd for $C_{41}H_{31}N_3OS_2$: C, 76.25; H, 4.81; N,6.51. Found: C, 76.28; H, 4.92; N, 6.58.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(4-methylbenzyl) acrylamide (6c)

Yellow solid, 86% yield; mp 214 °C. IR υmax/cm^{-1}: 3436, 2922, 1650, 1613, 1466, 1311, 1125, 743. ^{1}HNMR (400 MHz, DMSO-d_6) *δ* 2.34 (s, 3H, CH_3), 4.32 (d, *J* = 5.8 Hz, 2H, CH_2-Ar), 6.36 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.52 (d, *J* = 15.7 Hz, Ar-CH=CH-C), 6.64 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.01-7.05 (m, 3H, Ar-H), 7.11-7.21 (m, 7H, Ar-H), 7.22-7.24 (m, 3H, Ar-H), 7.28 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.56-7.61 (m, 4H, Ar-H), 8.47 (t, *J* = 5.8 Hz, 1H, Ar-H); MS *m/z*: 646.2 (M^{+1}). Anal calcd for $C_{41}H_{31}N_3OS_2$: C, 76.25; H, 4.84; N,6.51. Found: C, 76.29; H, 4.76; N, 6.57.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(4-methoxybenzyl) acrylamide (6d)

Yellow solid, 94% yield; mp 182 °C. IR υmax/cm^{-1}: 3435, 2921, 1650, 1612, 1467, 1312, 1248, 1040, 813, 743. ^{1}HNMR (400 MHz, DMSO-d_6) *δ* 3.72 (s, 3H, OCH_3), 4.30 (d, *J* =5.7 Hz, 2H, CH_2-Ar), 6.36 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.51 (d, *J* = 15.7 Hz, Ar -CH=CH-C),6.64 (d, *J* = 8.2 Hz, 2H, Ar-H), 6.87-6.95 (m, 3H, Ar-H), 6.99-7.05 (m, 3H, Ar-H), 7.12 (t, *J* = 7.6 Hz, 3H, Ar-H), 7.19-7.24 (m, 5H, Ar-H), 7.28 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.56-7.61 (m, 4H, Ar-H), 8.44 (t, *J* = 5.7 Hz, 1H, Ar-H); MS *m/z*: 662 (M^{+1}). Anal calcd for $C_{41}H_{31}N_3O_2S_2$: C, 74.41; H, 4.72; N,6.35. Found: C, 74.62; H, 4.79; N, 6.39.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(2,4-dimethoxy benzyl) acrylamide (6e)

Yellow solid, 91% yield; mp 238 °C. IR υmax/cm^{-1}: 3434, 2925, 1613, 1462, 1307, 1251, 1209, 1041, 739, 623. ^{1}HNMR (400 MHz, DMSO-d_6) *δ* 3.74 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.25 (d, *J* = 5.5 Hz, 2H, CH_2Ar), 6.36 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.46-6.49 (m, 1H, Ar-H), 6.54-6.58 (m, 2H, Ar-H), 6.64 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.00-7.06 (m, 3H, Ar-H), 7.08-7.14 (m, 4H, Ar-H), 7.20-7.26 (m, 4H, Ar-H), 7.30 (d, *J* = 4.2 Hz, 1H, Ar-H), 7.56-7.61 (m, 4H, Ar-H), 8.21 (t, *J* = 5.5 Hz, 1H, Ar-H); MS *m/z*: 662 (M^{+1}). Anal calcd for $C_{42}H_{33}N_3O_3S_2$: C, 72.91; H, 4.81; N,6.07. Found: C, 73.01; H, 4.05; N, 6.01.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(4-(trifluoro methylthio)benzyl)acrylamide (6f)

Yellow solid, 76% yield; mp 238 °C. IR υmax/cm^{-1}: 3434, 2922, 1650, 1503, 1466, 1311, 1118, 813, 745. ^{1}HNMR (400 MHz, DMSO-d_6) *δ* 4.44 (d, *J* = 5.9 Hz, 2H, CH_2-Ar), 6.36 (dd, J_1 = 8.0 Hz, J_2 = 2.2 Hz, 2H, Ar-H), 6.53 (d, *J* = 15.7 Hz, Ar-CH=CH-C), 6.64 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.05 (q, *J* = 7.6 Hz, 3H, Ar-H), 7.13 (t, *J* = 7.4 Hz, 3H, Ar-H), 7.23 (d, *J* = 7.5 Hz, 3H, Ar-H), 7.30 (s, 1H, Ar-H),), 7.34 (s, 1H, Ar-H), 7.43 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.56-7.62 (m, 4H, Ar-H), 7.68 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.63 (t, *J* = 6.0 Hz, 1H, Ar-H); MS *m/z*: 732.2 (M^{+1}). Anal calcd for $C_{41}H_{28}F_3N_3OS_3$: C, 67.28; H, 3.86; N,5.74. Found: C, 67.31; H, 3.89; N, 5.82.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(4-fluorobenzyl) acrylamide (6g)

Yellow solid, 82% yield; mp 232 °C. IR υmax/cm^{-1}: 3435, 2923, 2853, 1649, 1613, 1467, 1311, 1020, 744. ^{1}HNMR (400 MHz, DMSO-d_6) *δ* 4.35 (d, *J* = 5.9 Hz, 2H, CH_2-Ar), 6.36 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.52 (d, *J* = 15.7 Hz, Ar-CH=CH-C), 6.60 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.4 Hz, 1H, Ar-H), 6.99-7.05 (m, 3H, Ar-H), 7.14 (q, *J* = 8.5 Hz, 5H, Ar-H), 7.23 (d, *J* = 7.3 Hz, 3H, Ar-H), 7.29-7.33 (m, 4H, Ar-H), 7.56-7.61 (m, 4H, Ar-H), 8.54 (t, *J* = 5.8 Hz, 1H, Ar-H); MS *m/z*: 650.1 (M^{+1}). Anal calcd for $C_{40}H_{28}FN_3OS_2$: C, 73.93; H, 4.34; N,6.47. Found: C, 74.07; H, 4.10; N, 6.81.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(2-chloro-3,6-difluorobenzyl)acrylamide(6h)

Yellow solid, 85% yield; mp 252.8 °C. IR υmax/cm^{-1}: 3435, 2924, 1649, 1611, 1466, 1310, 1125, 812, 745. ^{1}HNMR (400 MHz, DMSO-d$_6$) *δ* 4.44 (d, *J* = 5.9 Hz, 2H, CH_2-Ar), 6.36 (dd, J_1 = 8.0 Hz, J_2 = 2.2 Hz, 2H, Ar-H), 6.53 (m, 1H, Ar-H), 6.64 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.05 (q, *J* = 7.6 Hz, 3H, Ar-H), 7.13 (t, *J* = 7.4 Hz, 3H, Ar-H), 7.23 (d, *J* = 7.5 Hz, 3H, Ar-H), 7.30 (s, 1H, Ar-H),), 7.34 (s, 1H, Ar-H), 7.43 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.59 (m, 4H, Ar-H), 7.68 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.63 (t, *J* = 6.0 Hz, 1H, Ar-H); MS *m/z*: 702.2 (M^{+1}). Anal calcd for $C_{40}H_{26}ClF_2N_3OS_2$: C, 68.41; H, 3.73; N,5.98. Found: C, 68.51; H, 3.80; N, 6.10.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(4-(trifluoromethyl) benzyl)acrylamide (6i)

Yellow solid, 87% yield; mp 231 °C. IR υmax/cm^{-1}: 3419, 3266, 3063, 1651, 1572, 1468, 1326, 1251, 1120, 814, 749. ^{1}HNMR (400 MHz, DMSO-d$_6$) *δ* 4.47 (d, *J* = 5.8 Hz, 2H, CH_2-Ar), 6.36 (dd, J_1 = 8.0 Hz, J_2 = 2.2 Hz, 2H, Ar-H), 6.53 (d, *J* = 15.7 Hz, Ar-CH=CH-C), 6.64 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.4 Hz, 1H, Ar-H), 6.99-7.03 (m, 3H, Ar-H), 7.11-7.15 (m, 3H, Ar-H), 7.22 (d, *J* = 8 Hz, 3H, Ar-H), 7.31-7.34 (m, 2H, Ar-H), 7.49 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.56-7.62 (m, 4H, Ar-H), 7.77 (d, *J* = 8.1 Hz, 2H, Ar-H), 8.65 (t, *J* = 5.9 Hz, 1H, Ar-H); MS *m/z*: 700.2 (M^{+1}). Anal calcd for $C_{41}H_{28}F_3N_3OS_2$: C, 70.37; H, 4.03; N,6.00. Found: C, 70.88; H, 4.08; N, 6.05.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(3-nitrobenzyl) acrylamide (6j)

Yellow solid, 77% yield; mp 244 °C. IR υmax/cm^{-1}: 3435, 2924, 1616, 1529, 1468, 1323, 1251, 1019, 813, 737. ^{1}HNMR (400 MHz, DMSO-d$_6$) *δ* 4.51 (d, *J* = 5.9 Hz, 2H, CH_2-Ar), 6.36 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.54 (d, *J* = 15.8 Hz, Ar-CH=CH-C), 6.65 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.02 (q, *J* = 7.6 Hz, 3H, Ar-H), 7.13 (t, *J* = 7.5 Hz, 3H, Ar-H), 7.23 (d, *J* = 6.5 Hz, 3H, Ar-H), 7.31 (s, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.56-7.65 (m, 5H, Ar-H), 7.75 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.12 (d, *J* = 11.8 Hz, 2H, Ar-H), 8.71 (t, *J* = 6.2 Hz, 1H, Ar-H); MS *m/z*: 677.1 (M^{+1}). Anal calcd for $C_{40}H_{28}N_4O_3S_2$: C, 70.98; H, 4.17; N,8.28. Found: C, 71.05; H, 3.98; N, 8.33.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(4-nitrobenzyl) acrylamide (6k)

Yellow solid, 76% yield; mp 261 °C. IR υmax/cm^{-1}: 3245, 3058, 1649, 1614, 1492, 1439, 1346, 1216, 979, 749.^{1}HNMR (400 MHz, DMSO-d$_6$) *δ* 4.51 (d, *J* = 5.9 Hz, 2H, CH_2-Ar), 6.36 (dd, J_1 = 8.1 Hz, J_2 = 2.2 Hz, 2H, Ar-H), 6.54 (d, *J* = 15.8 Hz, Ar-CH=CH-C), 6.65 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.02 (q, *J* = 7.8 Hz, 3H, Ar-H), 7.11-7.15 (m, 3H, Ar-H), 7.20 (d, *J* = 1.2 Hz, 3H, Ar-H), 7.30 (s, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.53-7.62 (m, 6H, Ar-H), 8.20 (d, *J* = 8.7 Hz, 2H, Ar-H), 8.71 (t, *J* = 6.2 Hz, 1H, Ar-H); MS *m/z*: 677.1 (M^{+1}). Anal calcd for $C_{40}H_{28}N_4O_3S_2$: C, 70.98; H, 4.17; N,8.28. Found: C, 71.05; H, 4.19; N, 8.31.

(E)-3-(10-(4-(10*H*-phenothiazin-10-yl)phenyl)-10*H*-phenothiazin-3-yl)-N-(3,5-bis (trifluoromethyl)benzyl)acrylamide (6l)

Yellow solid, 93% yield; mp 282 °C. IR υmax/cm^{-1}: 3430, 2928, 1649, 1594, 1503, 1470, 1314, 1177, 1127, 751.^{1}HNMR (400 MHz, DMSO-d$_6$) *δ* 4.56 (d, *J* = 6.0 Hz, 2H, CH_2-Ar), 6.35 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.58 (d, *J* = 15.7 Hz, Ar-CH=CH-C), 6.65 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.93 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.02 (q, *J* = 7.6 Hz, 3H, Ar-H), 7.13 (t, *J* = 7.4 Hz, 3H, Ar-H), 7.22-7.24 (m, 3H, Ar-H), 7.35-7.36 (m, 2H, Ar-H), 7.56-7.62 (m, 4H, Ar-H), 7.98-8.00 (m, 3H, Ar-H), 8.71 (t, *J* = 5.8 Hz, 1H, Ar-H); MS *m/z*: 768.2 (M^{+1}). Anal calcd for $C_{42}H_{27}F_6N_3OS_2$: C, 65.70; H, 3.54; N,5.47. Found: C, 65.98; H, 3.60; N, 5.40.

Antioxidant activity

DPPH assay

Free radical scavenging activity of compounds **6a-l** was measured by the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay method (GnanaRubaPriya et al., 2011). Briefly, 0.1mM solution of DPPH in methanol was taken and 1mL of this solution was added to sample solutions in Methanol (2 mL) at different concentrations (5-100 µg/mL). The mixture was vortexed and allowed to stand in dark at room temperature for 30 min. A DPPH blank was prepared without compound and methanol was used for the baseline correction. Ascorbic acid was used as a reference standard. Decrease in the

Table 1. Antioxidant activity of the synthesized compounds **(6a-l)**

Compound	DPPH assay (IC_{50} µm)	ABTS assay (IC_{50} µm)
6a	78.21	86.62
6b	75.83	86.53
6c	72.62	84.94
6d	**63.83**	**74.57**
6e	**61.26**	**72.32**
6f	83.18	94.52
6g	80.21	99.21
6h	82.75	93.58
6i	82.84	91.99
6j	86.87	95.79
6k	84.26	96.14
6l	85.12	102.58
Ascorbic acid	12.34	18.93

absorbance at 517 nm was measured using UV-Visible spectrophotometer and the remaining DPPH was calculated. The radical scavenging activity was expressed as the percentage inhibition and was calculated using the formula: % of Inhibition = [(Ao – A1)/Ao] X 100. Where Ao is the absorbance of the control (without compound) and A1 is the absorbance of the compound. The IC_{50} (concentration causing 50% inhibition) values of each compound was determined graphically.

ABTS assay

The antioxidant activities of synthesized compounds were measured using 2, 2´-azinobis[3-ethylbenzthiazoline]-6-sulfonicacid (ABTS) assay (Kumara Shanthamma Kavitha and Sreedharamurthy Satish, 2013; Blois, 1958).The ABTS**•+** radical was produced by the reaction between 7 mM ABTS in deionized water and 2.45 mM potassium persulfate, left to stand in the dark at room temperature for 16 h. Then, ABTS•+ solution was diluted with phosphate buffer (0.1M, pH 7.4) to give an absorbance value of ~0.700 at 734 nm. To the reaction mixture containing 1.5 ml of different concentration (5-100 µg/mL) of compounds in ethanol was added to 1mL of ABTS**•+** solution. After 30 min, the decrease in absorbance was measured at 734 nm. Ascorbic acid was used as standard (positive control). The % inhibition and the IC_{50} values were calculated as mentioned in the DPPH assay.

Results and discussion

In the present study, DPPH radical scavenging assay and ABTS radical scavenging assay were chosen to evaluate antioxidant potential of phenothiazine integrated cinnamoyl amide derivatives. The DPPH radical scavenging assay is a rapid method for screening the radical scavenging activity of specific compounds. The synthesized compounds were tested for their interaction with the stable free radical DPPH, indicates their radical scavenging activity. The IC_{50} was given in Table 1 and compared with that of standard L-ascorbic acid. The electron donating groups on the benzyl moiety induced better antioxidant activity. The compounds **6b** and **6c** with methyl groups substituted on the benzyl were exhibited moderate activity, whereas the compounds **6d** and **6e** with -OCH_3 groups showed maximum antioxidant potential. Incorporation of electron withdrawing groups like F, Cl and NO_2 on the aryl ring demonstrated decreased in the radical scavenging activity. The unsubstituted compound (**6a**) had no effects for the enhancement of activity. Nearly, all the synthesized compounds exhibited positive pattern for DPPH radical scavenging property. In ABTS radical scavenging activity, compounds **6d** and **6e** with methoxy groups on benzyl moiety were more potent than other compounds. However, all the synthesized compounds showed moderate radical scavenging activity in comparison with standard drug. These results provided an insight for essential

structural modifications that leads to compounds with favorable pharmacological properties.

Conclusion

In conclusion, we have achieved the synthesis of novel phenothiazine integrated cinnamoylamides **(6a-6l)** and investigated for their *in vitro* antioxidant activity. Among the synthesized compounds, compound **6d** and **6e** showed excellent antioxidant activity in comparison with standard drug Ascorbic acid. Further exploration on these compounds is under way.

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