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ARTICLE

Synthesis, spectral characterization and antitumor activity of phenothiazine derivatives

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Abstract

Different types of phenothiazine derivatives were synthesized by reactions of 10-alkyl-10*H*-phenothiazine-3-carbaldehydes. Structures of the prepared compounds were confirmed through spectroscopic techniques such as IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. All the compounds were studied for their antitumor activities.

1 | INTRODUCTION

The chemistry of heteroatom such as sulfur, nitrogen containing aromatic compounds is becoming a prominent area of research in synthetic organic chemistry. The combination in a molecule of two and more pharmacophores is one of the predominant approaches to design of new biologically important compounds, including natural alkaloids. Heterocyclic compounds containing sulfur and nitrogen atoms have wide a range of biological activity and occupies a special place among various heterocyclic compounds. Bernthsen first identified the parent compound, 10*H*-dibenzo-1,4-thiazine(phenothiazine) in 1883.

The phenothiazine derivatives are profoundly studied in various fields such as biological, chemical and medical research owing to their pharmaceutical activity. Many of the phenothiazine derivatives are used in analytical chemistry, especially the substitution at 3 and 7 positions (dyes), as well as the substitution at position 10 alone and positions 2 and 10. The first reagents to be successfully used for the treating psychosis were phenothiazine compounds. Phenothiazine derivatives are pharmaceutically important bioactive heterocyclic compounds with various pharmaceutical activities such as antibacterial,[1,2] antioxidant,[3–5] antifungal,[6] tranquilizers,[7] anti-inflammatory,[8] antimalarial,[9,10] antipsychotropic,[11] antitubercular[12,13] and antimicrobial.[14] In addition, a number of these derivatives have good anti-cancer activities that have given rise to an excellent interest in the preparation and synthesis of new phenothiazine compounds to investigate their anticancer activities.[15,16] Phenothiazine has been shown to be an inhibitor of human cholinesterase, and these compounds have acted as MDR (Multidrug Resistance Reversal Agents) in several instances.[17–19] Phenothiazine-based dyes mainly increase the performance of dye-sensitized solar cells.[20] Recently phenothiazine has become prominent in material science and biochemistry as marker for proteins and DNA.[21] Phenothiazine is found as the redox

active unit in donor–acceptor systems.[22] The therapeutic action of the phenothiazine drug has been reported which blocks dopamine receptors in the brain[23] with the interaction of the side chain. The substituent present in the parent compound enhances ability of phenothiazine derivatives to mimic the preferred trans-α conformation of dopamine (Data S1).

The phenothiazine derivative's donor potential is very high even in the ground state. There is practically the total transfer of an electron to an acceptor with formation of CT complexes. Consistent with the literature information, substituents attached to the tricyclic phenothiazine ring position C-2 and the alkyl bridge length linking the nitrogen atom at position 10 (N-10) of the tricyclic ring with the terminal amine in the side chain determine phenothiazine activity against cancer cells.[24,25] The activity is more closely linked to the type of substituent in the phenothiazine ring than to the nature of the attached side chain.[26]

2 | RESULTS AND DISCUSSION

N-alkyl phenothiazine compounds were prepared via reaction of alkyl iodide (ethyl, methyl), phenothiazine in the presence of KO*t*-Bu in dry DMF at 70°C-80°C for a period of 24 hours (Scheme 1). The compounds, 3a-b was obtained via Vilsmeier Hack reaction from (2a-b) 10-alkyl phenothiazines with a yield of 78%.[27]

The compounds, 3-(10-methyl-10*H*-phenothiazin-3-yl)-1-substituted phenylprop-2-en-1-one **4a-c** were prepared via Claisen-Schmidt reaction by condensation of compound 3a with different substituted acetophenones in the presence of alcoholic KOH at room temperature (Scheme 1). In their IR spectrum, the **4a-c** compounds showed bands of absorption at 1653-1657 cm^{-1} due to styril ketone, C═O stretching frequency. In ^{1}H NMR spectrum of compounds **4a-c** showed the protons attached to the carbon atoms of α, β unsaturated ketone moiety at δ 7.4 to 7.8 ppm, which were seen merged with aromatic protons.

In the presence of ammonium acetate, glacial acetic acid and benzil, phenothiazine substituted-4,5-diphenyl imidazoles **5a-b** were synthesized by refluxing with *N*-alkyl-phenothiazine-3-carbaldehyde. The synthetic pathway is represented in Scheme 2.

The IR spectra of compounds **5a-b** revealed the presence of absorption bands from 3423 to 3453 cm^{-1} for the NH group and the ^{1}H NMR spectra of compounds **5a-b** showed singlet at δ 12.5 to 12.6 ppm which also supports the formation of imidazole NH proton. Benzimidazoles substituted phenothiazine derivatives were prepared using 10-alkyl-10*H*-phenothiazine-3-carbaldehyde **3a-b**, *o*-phenylenediamine and DMF (10 mL) using sodium metabisulfite (10 mol%) catalyst and the reaction mass was stirred at 80°C for 7 hours. It created the desired product **6a-b** (Scheme 2).

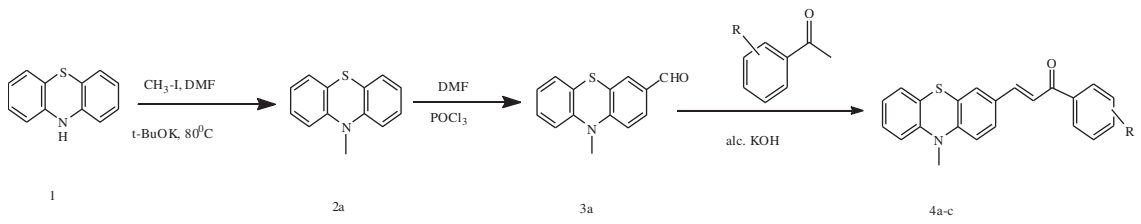
In the ^{1}H and ^{13}C NMR spectra of compounds **6a-b** the disappearance of formyl hydrogen at δ 9.77 ppm and carbonyl (C═O) peak at δ 190.67 ppm indicates the formation of benzimidazoles derivatives **6a-b**. Compounds **3a-b** were treated with 2-(9*H*-carbazol-9-yl) acetohydrazide in the presence of catalytic acetic acid in methanol at reflux temperature to provide Schiff base derivatives[27] **7a-b** (Scheme 2). The IR spectra of the compound **7a** the absorption peaks at 1645 and 1604 cm^{-1} corresponds respectively to the group of C═O & C═N. In ^{1}H NMR compound spectrum **7a** displays a singlet at 11.77 ppm indicating the NH group presence.

The singlet at 8.19 ppm due to —N═CH proton, the disappearance of NH_2 signal of compound **7a** indicates the formation of hydrazide to hydrazones **7a-b**. Yields of all the synthesized products with respect reaction time, temperature and physical properties are given in Table 1. Spectral characterization such as IR, ^{1}H NMR, ^{13}C NMR and HR-MS spectral data verified for the structures of the newly prepared phenothiazine derivatives.

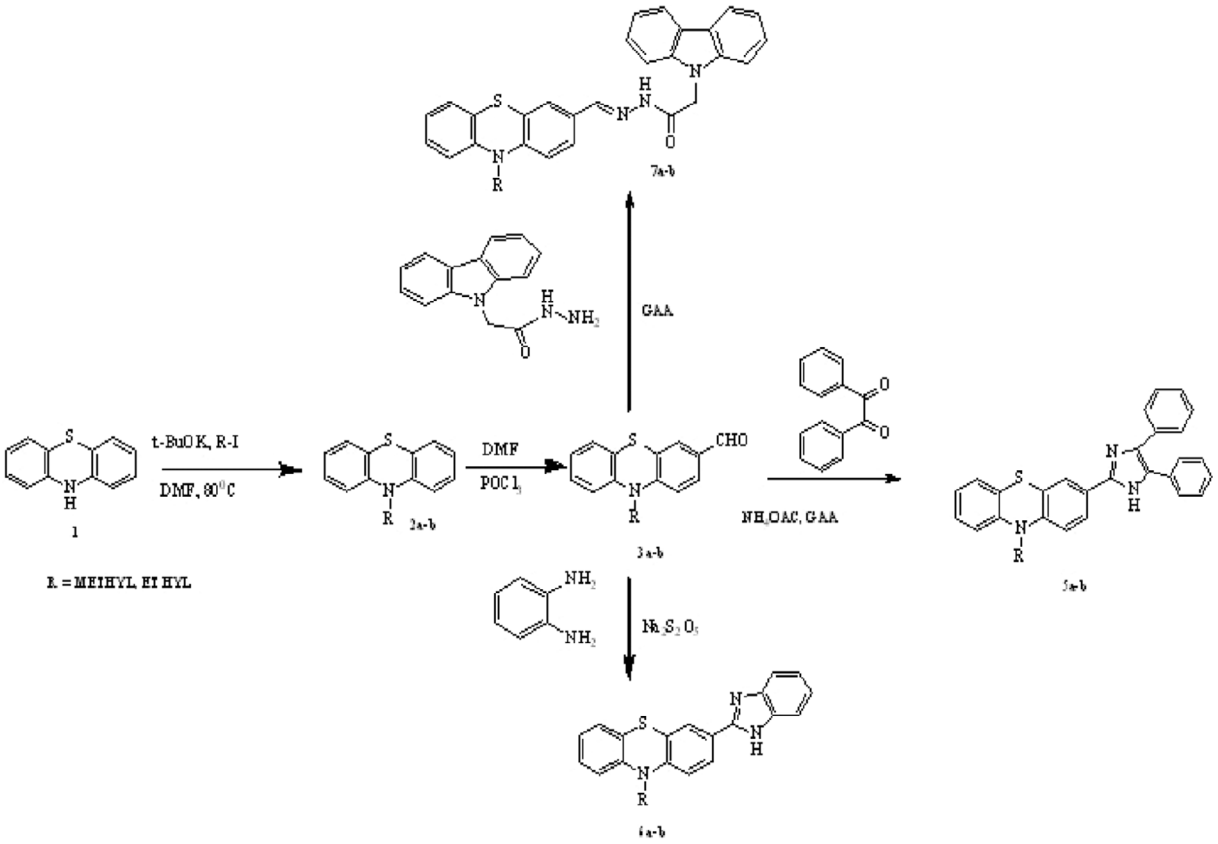
3 | ANTITUMOR ACTIVITY

3.1 | MTT assay

MTT assay was used to determine cell viability of MCF-7 cancer cells in the presence of compounds (**4a-c**, **5a-b**,



SCHEME 1 Synthetic pathway for the preparation of phenothiazine Chalcone



SCHEME 2 Synthetic pathway for the preparation of phenothiazine compounds

TABLE 1 Data of synthesized phenothiazine compounds

Compound	R	Time (min.)	Yield (%)	m.p. (°C)
4a	Methyl	160	88	161-163
4b	Methyl	165	86	142-145
4c	Methyl	170	84	135-138
5a	Methyl	320	76	252-255
5b	Ethyl	330	72	235-238
6a	Methyl	400	80	173-175
6b	Ethyl	410	82	162-165
7a	Methyl	210	86	172-175
7b	Ethyl	212	84	185-188

6a-b and **7a-b**) at different concentrations. After 24 hours of cell incubation following the addition of compounds (**4a-c**, **5a-b**, **6a-b** and **7a-b**) at 1, 10 and 1000 μg concentrations, MCF-7 cell viability decreases as the compound concentration increases as indicated in Table 2.

All the compounds showed good inhibition, but the compounds 4a and 7a required only 10 μg and 1000 μg to reach <50% inhibition compared to the remaining compounds. The inhibition rates of the compound 4a at 1, 10 and 1000 μg against MCF-7 in vitro was 75.5 ± 1.2, 42.98 ± 0.6 and 20.74 ± 3.0, respectively. A typical comparison is shown in Figure 1 for MCF-7 at different concentrations of compounds (4a-c, 5a-b, 6a-b and 7a-b).

3.2 | LDH assay

Lactate dehydrogenase (LDH) release assay was performed in MCF-7 cells to assess the cytotoxicity of compounds (**4a-c**, **5a-b**, **6a-b** and **7a-b**). This assay quantifies cell death and lysis based on measuring the release of LDH in the supernatant after the loss of membrane integrity. A dose dependent increase of LDH release was observed with compounds (4a-c, 5a-b, 6a-b and 7a-b) at 1, 10 and 1000 μg concentrations (Figure 2). Using the LDH assay, we measured the optical density (at 340 nm) of LDH release from MCF-7 cells upon the addition of different concentrations of compounds (**4a-c**, **5a-b**, **6a-b** and **7a-b)**. The LDH release increased with the concentration of compounds (**4a-c, 5a-b, 6a-b** and **7a-b**). The highest LDH release (63.69 ± 3.9 & 65.75 ± 2.0) was observed in the samples treated with compounds (4a, 4b) at 1000 μg concentration.

TABLE 2 Antitumor activity of phenothiazine derivatives by LDH and MTT methods in MCF-7 cells

Compound	Concentration (μg/well)	Percentage of viability	Percentage of released LDH
4a	1	75.55501 ± 1.2	15.92357 ± 3.0
	10	42.98482 ± 0.6	28.65605 ± 2.6
	1000	20.74763 ± 3.0	63.69 ± 3.9
4b	1	77.55468 ± 1.7	26.75159 ± 1.9
	10	70.83333 ± 1.8	48.21019 ± 3.0
	1000	58.33333 ± 2.2	65.7514 ± 2.0
4c	1	75.22037 ± 2.4	29.7835 ± 1.3
	10	68.09909 ± 0.9	35.24 ± 2.7
	1000	56.97845 ± 1.0	48.1257 ± 1.0
5a	1	76.76298 ± 0.7	24.532 ± 1.6
	10	70.77212 ± 0.1	31.3271 ± 2.9
	1000	62.25106 ± 0.9	46.1431 ± 0.9
5b	1	73.6696 ± 0.6	21.64605 ± 1.2
	10	60.74927 ± 2.2	46.1531 ± 0.8
	1000	60.71254 ± 0.9	49.0341 ± 0.5
6a	1	80.78681 ± 0.1	19.65605 ± 1.93
	10	73.84509 ± 1.7	34.94268 ± 2.7
	1000	52.78322 ± 0.5	46.237 ± 1.0
6b	1	69.40908 ± 1.0	28.02548 ± 1.2
	10	56.15736 ± 0.4	36.94268 ± 1.9
	1000	54.34215 ± 0.02	39.49045 ± 2.0
7a	1	87.0715 ± 0.7	14.023 ± 1.9
	10	64.66699 ± 2.2	31.21019 ± 1.8
	1000	47.91871 ± 1.4	47.7707 ± 1.6
7b	1	76.5875 ± 2.9	22.0951 ± 3.0
	10	73.16765 ± 2.4	33.75796 ± 2.8
	1000	68.0542 ± 0.02	42.67516 ± 3.0
Control		100	100

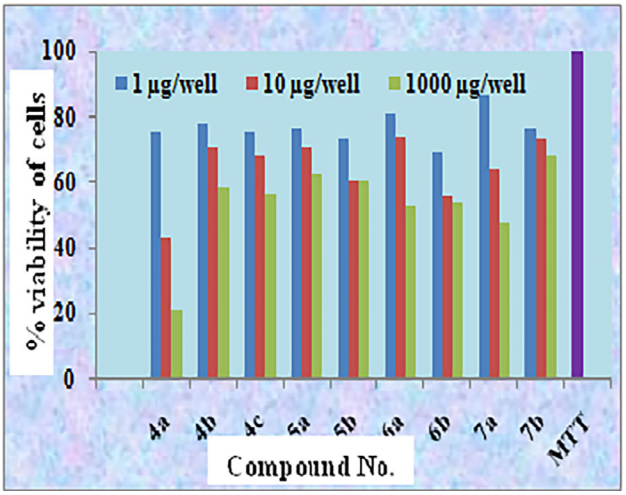


FIGURE 1 Antitumor activity of compounds **(4a-c**, **5a-b**, **6a-b** and **7a-b)** using MTT assay

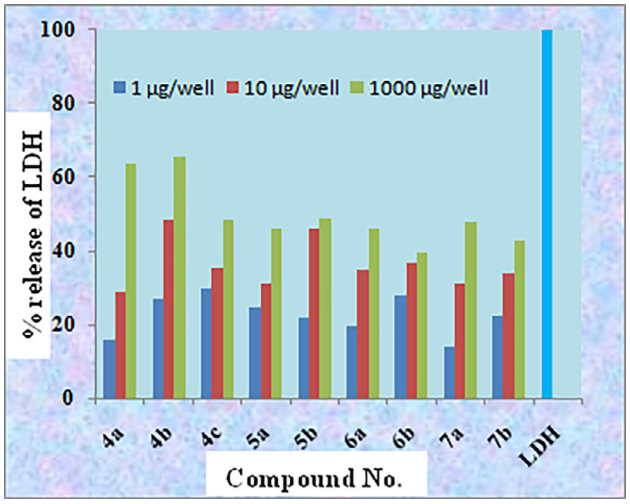


FIGURE 2 Antitumor activity of compounds (**4a-c**, **5a-b**, **6a-b** and **7a-b**) using LDH assay

4 | CONCLUSIONS

In this work, the synthesis of various heterocyclic substituted phenothiazine derivatives prepared from *N*-alkyl-phenothiazine-3-carbaldehyde using different one step reaction conditions were reported. The structures of the derivatives have been confirmed through spectral characterization such as IR, ^{1}H NMR, ^{13}C NMR and mass spectra. All the prepared compounds were checked for their antitumor activity against breast cancer cell lines (MCF-7) using MTT and LDH assay method. Among these compounds 4a and 7a showed maximum activity of cell death at 10 and 1000 μg concentrations.

5 | EXPERIMENTAL SECTION

5.1 | General

All the chemicals were purchased from SD Fine (India), Sigma-Aldrich (USA) and Sisco Research Laboratory (SRL). On a Buchi-530 melting point unit, melting points were determined using one end open capillary tubes and the results were uncorrected. In Perkin Elmer model 1600 FT-IR RX1 spectrophotometer, IR spectra were recorded using KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER AV-500 MHz and Bruker Spectrospin DPX 400 MHz spectrometers using $CDCl_3$ and DMSO-d_6 as solvent and Tetramethylsilane (TMS) as internal reference. Mass spectra were obtained using

HRMS and the samples were dried under vacuum prior to analysis.

5.2 | LDH assay

The LDH assay was performed 1 day before the assay with 1 in 10^4 cells seeded in a 96-well plate and the compounds were analyzed in triplicates in the untreated confluent culture medium, by using a commercial kit (Agappe Diagnostics, India) based on LDH pyruvate transformation at pH 7.5, in the presence of coenzyme NADH. The decrease in absorbance (A) at 340 nm, shows the NADH to NAD+ transition that correlates with the LDH activity. The change in absorbance was measured over a period of 0.5 to 4.5 minutes in the presence or absence of different doses of compounds (1/10/1000 μg/well), and the relative A/min was determined. The change in absorbance was translated to LDH international units per liter (U/L) by means of the following equations: ΔA/min·(tV·1000/EMC·l·sV), where l is path length of the light, tV is the total volume, sV is the sample volume and EMC is the NADH extinction micromolar coefficient (6.22 cm^2 μmol at 340 nm).

5.3 | Cell viability assay

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was used for determination of cell viability. In 96-well plates, the MCF-7 (1×10^4 cells/well) cells were grown in 5% CO_2 at 37°C in the RPMI medium (containing 100 μg/mL penicillin, 10% FBS and 100 μg/mL streptomycin). After overnight incubation, different concentrated compounds (1/10/1000 μg/well) replaced the RPMI medium in each well and incubated for 24 hours. Afterwards, 20 μL of MTT (5 mg/mL in PBS) was subsequently applied to each well and the cells were incubated at 37°C for an additional 4 hours. Then the supernatants were carefully removed and 100 μL of dimethyl sulfoxide (DMSO) was added to each well. The plates were shaken for another 10 minutes, and the Microplate Reader (Molecular Devices CA) recorded the absorbance values at 570 nm. The percentage of each concentration's inhibition was calculated using the following formula:

$$\text{Percentage of inhibition} = \text{Control O.D} - \text{Test O.D} \times 100/\text{Control O.D.}$$

Procedure for the preparation of 3-(10-methyl-10*H*-phenothiazin-3-yl)-1-substituted phenylprop-2-en-1-one 4 (a-c).

Equimolar quantities of 10-methyl-10*H*-phenothiazine-3-carbaldehyde (1 mmol) and substituted acetophenone (1 mmol) were dissolved in little quantity of alcohol. Sodium hydroxide solution (40%) 5 mL was added slowly and the reaction mixture stirred continuously for 3 hours until the total mixture became cloudy. The total mixture was discharged slowly into large amount of water with continuous stirring and kept in refrigerator for 1 day. The precipitate obtained was filtered, washed with water and crystallized using ethanol to get pure products.

3-(10-methyl-10*H*-phenothiazin-3-yl)-1-phenylprop-2-en-1-one (4a) mp 161-163°C; IR (KBr) νmax: 3059, 1654, 1591, 1571, 1500, 1465, 1442, 1402, 1336, 1292, 1215, 1159 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), δ_H = 8.06 (d, 2H, J = 7.2 Hz, CH and Ar—H of phenyl ring); 7.72 (s, 1H, Ar—H of phenyl ring); 7.68 (s, 1H, Ar—H of phenyl ring); 6.78-7.58 (m, 10H, CH, Ar—H of phenyl and phenothiazine ring); 3.41 (s, 3H, CH_3); ^{13}C NMR (100.645 MHz, $CDCl_3$, ppm), δ_C = 190.44, 144.86, 143.91, 138.54, 132.75, 129.43, 129.22, 129.13, 128.71, 128.55, 128.26, 127.79, 127.36, 126.38, 123.21, 122.69, 199.99, 114.55, 114.20, 35.67; HRMS (EI): m/z [M+] calcd. For $C_{22}H_{17}NOS$: 343.1031; found: 343.1031.

1-(4-methoxyphenyl)-3-(10-methyl-10*H*-phenothiazin-3-yl)prop-2-en-1-one (4b) mp 142-145°C; IR (KBr) νmax: 3145, 1653, 1589, 1463, 1400, 1330, 1257, 1184, 1124 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), δ_H = 8.02 (dd, 2H, J = 2.0 & 6.8 Hz, o, o'- Ar—H of phenyl ring); 7.71 (s, 1H, Ar—H of phenyl ring); 6.77-7.67 (m, 10H, CH, Ar—H of phenyl and phenothiazine ring); 3.88 (s, 3H, OCH_3); 3.38 (s, 3H, CH_3); ^{13}C NMR (100.645 MHz, $CDCl_3$, ppm), δ_C = 188.63, 163.45, 147.75, 144.92, 143.02, 131.46, 130.84, 129.66, 129.07, 127.77, 127.36, 126.28, 124.05, 123.15, 122.17, 119.89, 114.52, 114.18, 113.94, 55.61, 35.64; HRMS (EI): m/z [M+] calcd. For $C_{23}H_{19}NO2S$: 373.1136; found: 373.1135.

1-(2-hydroxyphenyl)-3-(10-methyl-10*H*-phenothiazin-3-yl)prop-2-en-1-one (4c) mp 135-138°C; IR (KBr) νmax: 3450, 1657, 1627, 1593, 1571, 1498, 1465, 1401, 1336, 1290 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), δ_H = 12.94 (s, 1H, OH); 7.926 (d, 1H, J = 7.6 Hz, CH); 6.81-7.84 (m, 12H, CH, Ar—H of phenyl and phenothiazine ring); 3,42 (s, 3H, CH_3); ^{13}C NMR (100.645 MHz, $CDCl_3$, ppm), δ_C = 193.61, 163.71, 148.37, 144.75, 144.60, 136.35, 129.71, 129.69, 129.15, 127.87, 127.42, 126.52, 124.22, 123.35, 122.65, 120.26, 118.94, 118.74, 117.89, 114.65, 114.25, 114.25, 35.76; HRMS (EI): m/z [M+] calcd. For $C_{22}H_{17}NO_2S$: 359.0980; found: 359.0979.

Procedure for the preparation of 3-(4,5-diphenyl-1*H*-imidazol-2-yl)-10-alkyl-10*H*-phenothiazine 5(a-b).

A mixture of 10-alkyl-10*H*-phenothiazine-3-carbaldehyde (1 mmol), ammonium acetate (10 mmol), benzil (1 mmol), and glacial acetic acid (15 mL) was refluxed for 6 hours.

The progress of reaction was checked by TLC. After reaction was completed, the reaction mass was cooled to room temperature and then discharged into cold water (200 mL) and neutralized with ammonium hydroxide. The obtained solid was filtered, washed with water and crystallized from alcohol.

3-(4,5-diphenyl-1*H*-imidazol-2-yl)-10-methyl-10*H*-phenothiazine (5a) mp. 252-255°C; IR (KBr) νmax: 3421, 3057, 2962, 1602, 1462, 1450, 1334, 1257, 1139, 1072 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, ppm), δ_H = 12.56 (s, 1H, NH); 7.92 (d, 1H, J = 8.0 Hz, Ar—H of phenyl ring); 7.87 (s, 1H, Ar—H of phenyl ring); 6.98-7.55 (m, 15H, Ar—H of phenothiazine and phenyl ring); 3.35 (s, 3H, CH_3); ^{13}C NMR (100.612 MHz, DMSO-d_6, ppm), δ_C = 145.14, 144.88, 144.78, 136.97, 135.18, 131.05, 128.62, 128.27, 123.28, 122.69, 122.30, 121.55, 114.77, 114.72, 35.26; HRMS (EI): m/z [M+] calcd. For $C_{28}H_{21}N_3S$: 431.1456; found: 431.1456.

3-(4,5-diphenyl-1*H*-imidazol-2-yl)-10-ethyl-10*H*-phenothiazine (5b) mp. 235-238°C; IR (KBr) νmax: 3421, 3132, 2987, 2935, 1604, 1462, 1382, 1328, 1251, 1136, 1072 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, ppm), δ_H = 12.61 (s, 1H, NH); 7.89 (d, 1H, J = 8.4 Hz, Ar—H of phenyl ring); 7.84 (s, 1H, o- Ar—H of phenyl ring); 6.93-7.52 (m, 15H, Ar—H of phenothiazine and phenyl ring); 3.95 (d, 2H, J = 6.0 Hz, CH_2); 1.32 (t, 3H, J = 5.4 Hz, CH_3); ^{13}C NMR (100.612 MHz, DMSO-d_6, ppm), δ_C = 144.76, 144.25, 143.83, 128.38, 127.73, 127.68, 127.08, 124.72, 124.60, 123.49, 123.00, 122.58, 122.29, 115.54, 115.41, 41.20, 12.61; HRMS (EI): m/z [M+] calcd. For $C_{29}H_{23}N_3S$: 445.1613; found: 445.1613.

Procedure for the preparation of 3-(1*H*-benzo[d] imidazol-2-yl)-10-alkyl-10*H*-phenothiazine 6(a-b).

In a 50 mL round bottom flask, a mixture of *o*-phenylenediamine (1 mmol) and 10-alkyl-10*H*-phenothiazine-3-carbaldehyde (1 mmol) in DMF (10 mL) were mixed and stirred in the presence of sodium metabisulfite ($Na_2S_2O_5$) catalyst (10 mol%) at 80°C for 7 hours. The progress of reaction mixture was monitored by TLC. After reaction was completed, the reaction mass was cooled and then discharged into ice cooled water and the solid obtained was filtered, washed with excess of H_2O and dried. Column chromatography was used to purify the crude product.

3-(1H-benzo[d]imidazol-2-yl)-10-methyl-10*H*-phenothiazine (6a) mp. 173-175°C; IR (KBr) νmax: 3419, 1629, 1581, 1460, 1340, 1259, 1087 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, ppm), δ_H = 8.04 (dd, 1H, J = 2.0 & 8.5 Hz, *p*-Ar—H of phenyl ring); 7.96 (d, 1H, J = 2.0 Hz, NH); 7.01-7.36 (m, 8H, Ar—H of phenothiazine and phenyl ring); 7.68 (q, 2H, J = 3.25 Hz, C-2, C-6 Ar—H of phenothiazine ring); 3.32 (s, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δ_C = 150.00, 148.31, 144.64, 136.25, 128.59, 127.55, 127.42, 125.52, 124.18, 123.74, 123.23, 121.56, 121.19, 115.68, 115.44, 114.81, 35.95; HRMS (EI): m/z [M+] calcd. For $C_{20}H_{15}N_3S$: 329.0987; found: 329.0987.

3-(1H-benzo[d]imidazol-2-yl)-10-ethyl-10*H*-phenothiazine (6b) mp. 162-165°C; IR (KBr) νmax: 3441, 2980, 2929, 2854, 1629, 1602, 1581, 1460, 1373, 1330, 1253, 1180, 1138 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d6, ppm), δ_H = 8.10 (dd, 2H, J = 2.0 & 8.5 Hz, p- Ar—H of phenyl ring); 7.93 (d, 1H, J = 2.0 Hz, NH); 7.67-7.70 (m, 2H, C-2, C-6 Ar—H of ptz ring); 7.36 (q, 2H, J = 4.5 Hz, Ar—H of phenyl ring); 6.97-7.48 (m, 5H, Ar—H of phenothiazine ring); 4.01 (q, 4H, J = 6.5 Hz, CH_2); 1.31 (t, 3H, J = 6.75 Hz, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δ_C = 149.86, 147.47, 143.47, 135.95, 128.48, 127.63, 127.49, 125.75, 124.32, 123.72, 123.69, 122.13, 120.68, 116.36, 116.06, 114.75, 41.96, 12.97; HRMS (EI): m/z [M+] calcd. For $C_{21}H_{17}N_3S$: 343.1143; found: 343.1142.

Procedure for the preparation of 2-(9H-carbazol-9-yl)-N′-((10-methyl-10*H*-phenothiazin-3-yl)methylene)acetohydrazide 7(a-b).

In a 50 mL round bottomed flask a mixture of 10-alkyl-10*H*-phenothiazine-3-carbaldehyde 3(a-b) (1 mmol), 2-(9H-carbazol-9-yl)acetohydrazide (1 mmol), 1 mL of glacial acetic acid and methanol 25 mL were taken. The resulting solution was refluxed for 3-4 hour. The progress of the reaction was supervised by thin layer chromatography. After reaction was completed, the reaction mass could cool at room temperature and the solid separated was filtered off and washed with large amount of methanol (40 mL) and dried at room temperature.

2-(9H-carbazol-9- yl)-N′- ([10-methyl-10*H*-phenothiazin-3-yl] methylene) acetohydrazide (7a) mp 172-175°C; IR (KBr) νmax: 3224, 3072, 2872, 1674, 1598, 1575, 1463, 1382, 1257, 1151 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, ppm), δ_H = 11.63 (s, 1H, NH), 8.19 (s, 1H, N═CH), 8.16-8.17 (split peaks, 1H, C5-Ar—H of carbazole ring), 7.99 (s, 1H, C4-Ar—H of carbazole ring), 7.65 (d, 1H, J = 2.0 Hz, 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), δ_C = 164.38, 147.27, 147.68, 146.91, 145.66, 144.99, 143.44, 141.34, 141.16, 129.01, 128.94, 128.40, 127.94, 127.60, 127.33, 126.18, 126.07, 125.39, 124.97, 123.37, 122.73, 121.98, 121.91, 120.55, 119.55, 119.34, 115.41115.17, 115.03, 109.88, 109.82, 44.15, 35.84; HRMS (EI): m/z [M+] calcd. For $C_{28}H_{22}N_4OS$: 462.1514 found: 462.1513.

2-(9H-carbazol-9-yl)-N′-([10-ethyl-10*H*-phenothiazin-3-yl]methylene)acetohydrazide(7b).

mp 185-188°C; IR (KBr) νmax: 3219, 3062, 2964, 2914, 1681, 1597, 1546, 1485, 1460, 1325, 1244, 1211, 1155, 1080 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d6, ppm), δ_H = 11.62 (s, 1H, NH), 8.16, (d, 2H, J = 7.5 Hz, N=CH & C5-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 = 6.5 Hz, CH_2), 1.29-1.34 (m, 3H, CH_3); ^{13}C NMR (125.757 MHz, DMSO-d_6, ppm), δ_C = 169.17, 146.87, 146.12, 144.02, 143.42, 141.33, 141.15, 128.86, 128.26, 127.58, 127.54, 126.18, 126.07, 125.25, 123.73, 123.28, 122.73, 122.67, 120.55, 119.55, 119.34, 116.44, 115.73, 109.87, 44.13, 41.83, 13.02; HRMS (EI): m/z [M+] calcd. For $C_{29}H_{24}N_4OS$: 476.1671; found: 476.1671.

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