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Synthesis, Spectral Characterization and Antioxidant Evaluation of Fluorinated Phenothiazines

NAVEEN GAUTAM*, MEENAKSHI SHARMA, VIBHA GAUTAM,
M.K. SHARMA and D.C. GAUTAM
Department of Chemistry, University of Rajasthan, Jaipur-302 004, India
E-mail: ngautam70@yahoo.co.in

10*H*-phenothiazines are prepared by Smiles rearrangement. These synthesized compounds have been screened for their antioxidant activity. The structures of 10*H*-phenothiazines have been established by elemental analysis, IR, ^{1}H NMR and mass spectral data.

Key Words: 10*H*-Phenothiazines, Smiles rearrangement, Antioxidant activity.

INTRODUCTION

The immense importance of 10*H*-phenothiazines are proved and it is evidenced by their large number of publications and patents registered all over the world. A slight change in substitution pattern in phenothiazine nucleus causes distinguishable difference in their biological activities[1-20]. These compounds have shown significant uses as antibacterial, antifungal, antiinflammatory, insecticides and anticancer agents, *etc*. Some of the recent publications in this area have also demonstrated their significance as antiviral, antimalarial, diuretics, analgesic, antihypertensive and anti AIDS. Research is under process to develop powerful anticancer agents. Their antioxidant activity has also been carried out.

EXPERIMENTAL

All the melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr on NICOLET-MEGNA FT-IR 550 spectrometer and the ^{1}H NMR spectra on Jeol AL-300 spectrometer (300 MHz) in DMSO-d_6 using TMS, as an internal standard (chemical shifts are measured in δ ppm). The purity of the compounds were checked by TLC using silica gel "G" as adsorbent, visualizating these by UV light or iodine chamber.

Synthesis of 2-amino-2′-nitrodiphenylsulfide (III_a): 2-Aminobenzenethiol (**I**) (0.1 mol) was dissolved in ethanol (20 mL) containing (0.1 mol) of anhydrous sodium acetate in 50 mL round bottom flask and halonitrobenzne (**II**) (0.1 mol) in 10 mL ethanol was added. The reaction mixture was refluxed for 4-5 h, concentrated and cooled in an ice bath overnight. The solid separated out was filtered, washed with 30 % ethanol and recrystallized from methanol.

Synthesis of 2-formamido-2′-nitrodiphenylsulfide (IV_a): The diphenylsulphides (III_a) (0.1 mol) obtained was refluxed for 4 h in 90 % formic acid (20 mL). The contents were then poured into a beaker containing crushed ice, a solid separated out was filtered, washed with water until the filtrate was neutralized and crystallized from benzene.

Synthesis of phenothiazine (V_a): Formyl derivatives (IV_a) (0.1 mol) in acetone (15 mL) was refluxed and an alcoholic solution of potassium hydroxide (0.2 g in 5 mL ethanol) was added. The contents were heated for 0.5 h. A second lot of potassium hydroxide (0.2 g in 5 mL ethanol) was added to the reaction mixture and refluxed for 4 h. The contents were poured into beaker containing crushed ice and filtered. The residue obtained was washed with cold water, finally with 30 % ethanol and crystallized from benzene.

Synthesis of substituted 1-nitrophenothiazine (V_{b-g}): To a stirred suspension of 2-aminobenzenthiol **I** (0.1 mol), ethanol (20 mL) and sodium hydroxide (0.1 mol) contained in round bottom flask (50 mL) fitted with a reflux condenser was added an alcoholic solution of the reactive halonitrobenzenes **II** (0.1 mol). The colour of the solution darkened immediately. The contents were refluxed for 2 h, concentrated, cooled and filtered. The solid separated out was wash well with hot water and finally with 30 % ethanol. The crystallization from methanol/acetone afforded the pure samples of 1-nitrophenothiazines.

DPPH Radical scavenging assay: Radical scavenging activity of compounds $IV_{(a-g)}$ against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were determined spectrophotometrically as described by Cuendet *et al.*[13]. A stock solution 1 mg/mL of the compound was prepared in methanol. 50 µL of compounds were added to 5 mL of a 0.004 % methanol solution of DPPH. After 0.5 h incubation in dark at room temperature, the absorbance was read against a blank at 517 nm.

The assay was carried out in triplicate and the percentage of inhibition was calculated using the following formula.

$$\text{Inhibition (\%)} = \frac{(AB - AA)}{AB} \times 100$$

where, AB = Absorption of blank; AA = Absorption of test.

ABTS Radical cation decolourization assay: The 2,2-azino*bis*(3-ethybenzothiazoline-6-sulphonic acid) radical cation (ABTS) decolourization test was also used to assess the antioxidant activity of compounds **IV**. The ABTS assay was carried out using the improved assay of Re *et al.*[14]. In brief, $ABTS^{\bullet+}$ was generated by oxidation of ABTS with potassium persulphate. For this purpose $ABTS^{\bullet+}$ was dissolved in deinoized water at a concentration of 7 mM and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12-16 h) in the dark before use; the $ABTS^{\bullet+}$ solution then was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 1 mLof the diluted ABTS solution to 10 µL of compound and mixing, absorbance readings were taken at 30 °C at intervals of exactly 1-6 min later.

RESULTS AND DISCUSSION

2-Aminobenzenethiols (**I**) on condensation with *o*-halonitrobenzene (**II**) in ethanolic sodium acetate solution gave diphenylsulphide (**III$_a$**). This diphenylsulphide (**III$_a$**) on formylation with 90 % formic acid gave its formyl derivative (**IV$_a$**) and this 2-formamido-2′-nitrodiphenylsulphide underwent Smiles rearrangement[10-13] to give various substituted 10*H*-phenothiazine (**V$_a$**). 1-Nitrophenothiazines (**V$_{b-g}$**) can be synthesized by reaction of substituted 2-aminobenzenethiols with reactive *o*-halonitrobenzenes containing one nitro and one halogen atom at *ortho* positions to the reactive halogen atom directly yielded 1-nitrophenothiazine as Smiles rearrangement and ring closure occur simultaneously *in situ*. The characterization data of the synthesized compounds are given in Table-1.

TABLE-1
CHARACTERIZATION DATA OF SUBSTITUTED PHENOTHIAZINES (**V$_{a-g}$**)

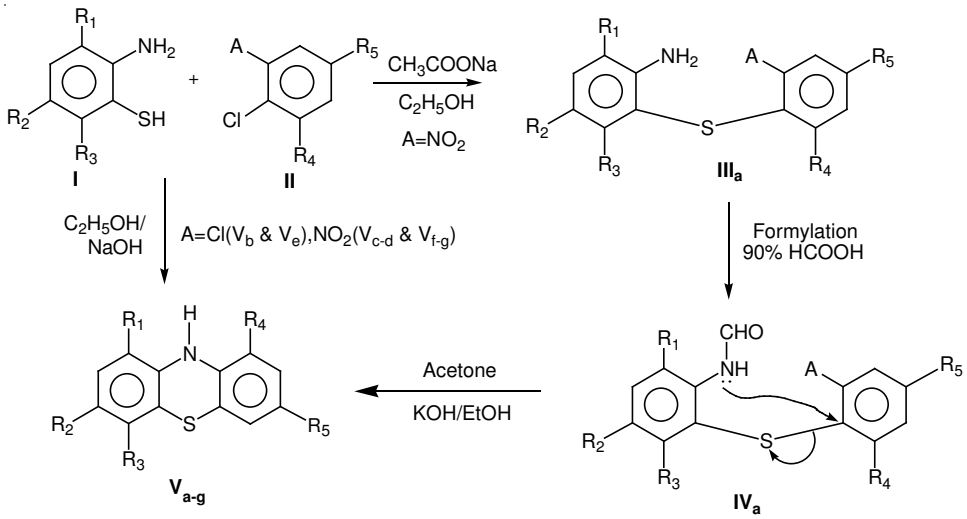
Compd.	R_1	R_2	R_3	R_4	R_5	m.f.	Yield (%)	m.p. (ºC)	Elemental analysis (%): Found (Calcd.)		
									C	H	N
V$_a$	Cl	H	CF_3	H	Cl	$C_{13}H_6NSCl_2F_3$	45	320	46.55 (46.42)	1.80 (1.78)	4.14 (4.16)
V$_b$	F	Br	H	NO_2	H	$C_{12}H_6N_2O_2SBrF$	42	200	42.35 (42.28)	1.78 (1.75)	8.19 (8.21)
V$_c$	F	Br	H	NO_2	Cl	$C_{12}H_5N_2O_2SBrClF$	45	120	38.40 (38.34)	1.35 (1.33)	7.40 (7.45)
V$_d$	F	Br	H	NO_2	COOH	$C_{13}H_6N_2O_4SBrF$	55	240	41.00 (40.52)	1.57 (1.55)	7.21 (7.27)
V$_e$	Cl	H	CF_3	NO_2	H	$C_{13}H_6N_2O_2S\,ClF_3$	50	340	45.10 (45.02)	1.79 (1.73)	8.02 (8.08)
V$_f$	Cl	H	CF_3	NO_2	Cl	$C_{13}H_5N_2O_2S\,Cl_2F_3$	55	215	40.97 (40.94)	1.35 (1.31)	7.30 (7.34)
V$_g$	Cl	H	CF_3	NO_2	CF_3	$C_{14}H_5N_2O_2S\,ClF_6$	50	250	41.55 (40.53)	1.25 (1.20)	6.70 (6.75)

The characteristic IR bands of synthesized compounds are presented in Table-2. Two peaks are observed in the region 1585-1500 cm^{-1} and 1390-1340 cm^{-1} due to asymmetric and symmetric vibrations of nitro group in compound **V$_{b-g}$**. Compounds **V$_{a-g}$** showed single peak in the region 3340-3140 cm^{-1} due to >N-H stretching vibrations.

The ^{1}H NMR data of compounds **V$_{(a-g)}$** are presented in Table-2. Compounds **V$_{(a-g)}$** showed multiplet due to aromatic protons, which is appeared in the region δ 6.70-8.35 ppm. The >NH proton show a singlet between δ 8.55- 9.21 ppm.

Mass spectra: The molecular ion peaks are in accordance with their molecular weight of synthesized compounds.

Antioxidant activity: All the synthesized compounds **V$_{(a-g)}$** were screened for their antioxidant activity by DPPH radial scavenging assay and ABTS radical cation decolourization assay.



Scheme-I

TABLE-2
^{1}H NMR AND IR SPECTRAL DATA OF SYNTHESIZED PHENOTHIAZINES ($\mathbf{V_{a-g}}$)

Compd.	^{1}H NMR (δ ppm from TMS)		IR (KBr, ν_{max}, cm^{-1})				
	>NH	Ar-H multiplet	>NH	$-NO_2$	-C-Cl	$-CF_3$ Asym., Sym.	–C-Br
$\mathbf{V_a}$	8.55	7.58-6.85	3340	-	790	1330,1140	-
$\mathbf{V_b}$	9.15	8.05-6.70	3240	1500,1360	-	-	640
$\mathbf{V_c}$	8.92	8.35-6.90	3250	1570,1375	815	-	635
$\mathbf{V_d}$	9.10	8.15-7.20	3310	1520,1370	-	-	620
$\mathbf{V_e}$	9.01	8.05-6.95	3140	1540,1390	810	1320,1150	-
$\mathbf{V_f}$	9.21	8.07-7.01	3260	1580,1340	820	1310,1140	-
$\mathbf{V_g}$	8.97	8.25-7.05	3230	1585,1360	805	1325,1120	-

TABLE-3
ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS (DPPH ASSAY)

Compound No.	DPPH (%) inhibition of 1 mg/mL of the compound
$\mathbf{V_a}$	48.15 ± 1.50
$\mathbf{V_b}$	1.50 ± 0.06
$\mathbf{V_c}$	32.12 ± 0.01
$\mathbf{V_d}$	25.68 ± 1.10
$\mathbf{V_e}$	53.01 ± 1.20
$\mathbf{V_f}$	40.12 ± 1.60
$\mathbf{V_g}$	35.89 ± 0.01

Inhibition (%) of DPPH radical scavenging activity of various compounds at particular concentration. Stock solution of crude compound was prepared as 1 mg/mL in methanol. Fifty microlitres of samples of particular concentration were added to 5 mL of 0.004 % methanol solution of $DPPH^{\bullet}$. After 0.5 h incubation in dark at room temperature, the absorbance was read against a blank at 517 nm.

The present study demonstrated that synthesized compounds showed mixed radical scavenging activity in both DPPH and $ABTS^{\bullet+}$ assays (Tables 3 and 4). (**a**) Compounds $\mathbf{V_e}$ showed strong radical scavenging activity in DPPH assay that have DPPH % inhibition > 50. (**b**) Compounds $\mathbf{V_a}$, $\mathbf{V_c}$, $\mathbf{V_f}$ and $\mathbf{V_g}$ showed moderate radical scavenging activity in DPPH assay that have DPPH % inhibition ≥ 30. (**c**) Compounds $\mathbf{V_d}$ showed mild radical scavenging activity in DPPH assay that have DPPH % inhibition < 30. (**d**) Compound $\mathbf{V_a}$ and $\mathbf{V_b}$ were found to be more active in $ABTS^{\bullet+}$ assay which showed much decline in graph.

TABLE-4
ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS ($ABTS^{\bullet+}$ ASSAY)

Compd.	$ABTS^{\bullet+}$ activity at different time intervals minutes				
	0 min	1 min	2 min	4 min	6 min
$\mathbf{V_a}$	0.733	0.110	0.108	0.108	0.107
$\mathbf{V_b}$	0.722	0.151	0.150	0.150	0.150
$\mathbf{V_c}$	0.731	0.691	0.690	0.690	0.690
$\mathbf{V_d}$	0.735	0.692	0.690	0.690	0.690
$\mathbf{V_e}$	0.732	0.287	0.286	0.286	0.286
$\mathbf{V_f}$	0.725	0.285	0.284	0.284	0.284
$\mathbf{V_g}$	0.722	0.281	0.280	0.280	0.280

The effect of time on the suppression of absorbance of ABTS by phenothiazines ($\mathbf{V_{a-g}}$). After addition of 1 mL of diluted ABTS solution (A 734 nm = 0.700 ± 0.020) to 10 µL of the compound the absorbance reading was taken at 30 ºC exactly 1 min, after initial mixing and up to 6 min. All determinations were carried out in triplicates.

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