



Synthesis and Characterization of Phenothiazine Derivatives

Kapoor D^{1*}, Gupta P²

^{1*}Dr. Dayaram Patel Pharmacy College, Sardarbaug, Station Road, Bardoli, Surat, Gujarat, India, 394601

²Senior Research Associate, ZydusCadila Pharmaceuticals, Ahemdabad, Gujarat

Abstract A series of new phenothiazine derivatives have been synthesized in which diphenylamine on treatment with sulphur give 10*H*-phenothiazine which on reaction with ethylchloro acetate yielded 2-(10*H*-phenothiazin-10-yl)acetate which on further treatment with hydrazine hydrate produced 2-(10*H*-phenothiazin-10-yl) acetohydrazide. Condensation of chief intermediate with various benzaldehyde derivatives afforded *N'*-(substitutedbenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide. All the synthesized derivatives have been characterized by FTIR, ¹H NMR, Mass, UV-visible and elemental analysis.

Keywords Phenothiazine, Ethylchloroacetate, Hydrazine hydrate, *p*-Fluorobenzaldehyde, FTIR

Introduction

Nowadays most of patients are in an advanced stage of various diseases such as cancer and various microbial infections. So there is clearly pressing need for the development of new drugs. Heterocyclic nucleuses such as indole, carbazole, phenothiazine, imidazole, thiazole etc. are a part of most of the medicinally important compounds. Among these heterocyclic, phenothiazine nucleus has found applications in drug development for the treatment of cancer, various microbial infections and mental disorders such as schizophrenia, paranoia. Phenothiazine belongs to an important class of heterocyclic compounds that is oldest, synthetic antipsychotic drugs, which do not have their precursor in the world of natural compounds [1]. The chemistry and biology of phenothiazine have attracted an increasing interest over the last few years because they have been shown to possess a broad spectrum of biological activity depending on their particular structure. Infact, its constitute the largest group of psychoactive clinically used compounds [2], phenothiazine derivatives possess several other biological activities including antibacterial, antifungal, anticancer, anti-inflammatory, antiparkinsonism, anthelmintic and antihistaminic activities [3].

A part from these biological activities phenothiazine is also known to have high binding affinities to various enzymes, neurotransmitter transporters and ion channels. In recent years, it has become increasingly clear that phenothiazine also interact with mostly uncharacterized sites at ligand gated ion channels, and it seems likely that this mechanism of action is, at least partly, responsible for their therapeutic and their side effect profiles [4].

Phenothiazine is an aromatic amine based product that was synthesized in 1883 and reported as anthelmintic agent it also exhibits broad activity as an inhibitor, antioxidant and short stopping agent in a variety of diverse applications. The product is principally utilized as an inhibitor and short stopping agent in the stabilization of acrylic acids, esters and monomers. The phenothiazine as a class and especially chlorpromazine are most widely use class of neuroleptic. The phenothiazine moiety is a frequent moiety of numerous drugs such as Chlorpromazine, Fluphenazine, Acetophenazine, Mesoridazine, Methotrimeprazine, Perphenazine, Promethazine, Prochlorperazine and Proprietary etc.

The chemical structure of phenothiazine provides an important molecular template for the development of the agent able to interact with a variety biological process. A slight change in the substitution pattern of phenothiazine nucleus causes a marked difference in their biological activities. These effects of structural change improved therapeutic effects of drug with minimum undesirable side effects. Phenothiazine avails the customer a variety of benefits. The



product is extremely active, functions at very low concentrations and will synergize with other stabilizers. The product will function in strongly acidic environments and is one of the few inhibitors that perform in either air or nitrogen environments, chemical processes in which the product is utilized, as a monomer stabilizer will exhibit improved on-stream factor, greater operational flexibility and increased production output [5]. From few last decades a considerable account of attention has been focussed on synthesis of phenothiazine derivatives and screening them for different pharmacological activities. The investigation on phenothiazine derivatives has steadily strong growth because they exhibit wide range of application. In view of these facts and in continuation of our research program on the synthesis of new phenothiazine derivatives to shed some light on their biological studies, we report herein the synthesis of some newer phenothiazine derivatives. Products and chemical processes that utilize Phenothiazine will exhibit enhanced and improved performance [6].

Material and Methods

Materials

Table 1: List of materials used and their manufacturers

S. No.	Name of material	Name and address of manufacturer
1.	Absolute ethanol	S d fine chem Limited, Mumbai
2.	Acetone	Qualigens [®] Fine chemicals, Mumbai
3.	Benzene	Qualigens [®] Fine chemicals, Mumbai
4.	<i>m</i> -Chlorobenzaldehyde	S d fine chem. Limited, Mumbai
5.	<i>o</i> -Chlorobenzaldehyde	S d fine chem Limited, Mumbai
6.	<i>p</i> -Chlorobenzaldehyde	S d fine chem Limited, Mumbai
7.	Chloroform	Qualigens [®] Fine chemicals, Mumbai
8.	Diethyl ether	S d fine Chem. Limited, Mumbai
9.	Dimethylformamide	Qualigens [®] Fine chemicals, Mumbai
10.	Dimethylsulfoxide	Central Drug House Pvt. Ltd., New Delhi
11.	Ethyl acetate	Qualigens [®] Fine chemicals, Mumbai
12.	Ethylchloro acetate	S d fine chem Limited, Mumbai.
13.	<i>p</i> -Fluorobenzaldehyde	Spectrochem Pvt. Limited, Mumbai
14.	Hydrazine hydrate (99%)	Qualigens [®] Fine chemicals, Mumbai
15.	<i>m</i> -hydroxybenzaldehyde	Spectrochem Pvt. Limited, Mumbai
16.	3,4-Hydroxy benzaldehyde	S d fine chem Limited, Mumbai.
17.	<i>p</i> -Hydroxybenzaldehyde	Central Drug House Pvt. Ltd., New Delhi.
18.	Methanol	Qualigens [®] Fine chemicals, Mumbai
19.	3,4-Methoxybenzaldehyde	S d fine chem Limited, Mumbai.
20.	<i>p</i> -Methoxybenzaldehyde	S d fine chem Limited, Mumbai.
21.	<i>o</i> -Nitro benzaldehyde	Himedia Labs Pvt. Ltd., Mumbai
22.	<i>p</i> -Nitro benzaldehyde	S d fine chem Limited, Mumbai.
23.	Phenothiazine	Hi-media laboratories Pvt. Ltd., Mumbai.
24.	Silica gel G	S d fine chem Limited, Mumbai

Methods

Experimental

Determination of melting point range

Melting point ranges of the newly synthesized compounds were determined by open capillary method, using the melting point apparatus and were uncorrected. Compounds were placed in one end sealed capillary. Thermometer was placed in the cave. The temperature at which compound starts melting and the temperature at which it completely melts was recorded as a melting point range [7].

Thin layer chromatography of compounds

Thin layer chromatographic analysis of the compounds was performed on silica gel G coated glass plates. The slurry of adsorbent silica gel G was coated to a thickness of about 0.3mm on previously cleaned TLC plates of 20×5 cm using conventional spreader. The plates were air dried for 20 min and then kept in hot air oven at 110°C for 30 min. Solution of the compounds were applied as a spot with the help of fine capillary, on the activated plate about 2 cm above from the lower edge and placed in a chamber which is pre-saturated with mobile phase. The mobile phases were selected according to the polarity of the products. Chloroform: methanol (8:2) was used as mobile phase.



When mobile phase runs $3/4^{\text{th}}$ of the plate, then spots were visualized by exposure to iodine vapors and R_f values were calculated [8].

Solubility Studies

Various solvents such as cyclohexane, benzene, diethyl ether, chloroform, ethyl acetate, acetone, methanol, ethanol, dimethylsulphoxide (DMSO) and distilled water were taken for dissolving the intermediates and final products. 10 mg of each compound was weighed and added to 10 ml of each solvent individually taken in 50 ml beaker. The observations were recorded for newly synthesized compounds.

Spectral analysis

UV Spectral analysis

10 mg compound dissolved in absolute CHCl_3 was diluted to 20 ml with CHCl_3 . 2 ml of the above solution was further diluted to 50 ml with absolute ethanol and UV spectra were recorded on a UV-Visible Spectrophotometer Pharma spec-1700 (SHIMADZU).

FTIR Spectral analysis

The IR spectrum of compounds in KBr pellets were recorded on a FTIR-8400S spectrophotometer (SHIMADZU).

Preparation of KBr pellets of compounds

100 mg of anhydrous KBr (IR grade) was accurately weighed. To this, 1.0 mg of compound was added and triturated well. The mixture was placed in an evaluable die and subjected to a pressure of 5-6 tones for 5 min. A transparent disc was produced, which was then placed in a pellet holder and IR spectra was obtained.

^1H NMR Spectral analysis

^1H -NMR spectra of the compounds were recorded on Bruker NMR spectrophotometer in CDCl_3 using TMS as internal standard. (Chemical shift measured in δ ppm).

Mass Spectral analysis

Mass spectrums of the newly synthesized compounds were obtained by MS (ESI)(SHIMADZU-2010 AT, software class VP).

Elemental analysis

Elemental analysis was carried on Elemental Vario EL III Carlo Erba 1108.

Method of Synthesis

10*H*-phenothiazine (13)

To a solution of diphenylamine (1.69gm, 0.01mole) in 50ml acetone, sulphur (0.32gm, 0.02mole), hexamine (20mg) and AlCl_3 (30mg) was added in round bottom flask. The reaction mixture was refluxed for 10 hours then it was cooled and poured into crushed ice with stirring. A precipitate appeared immediately. The precipitate was filtered, washed with water, dried and recrystallized from *n*-butanol to give compound **13**. (Yield 75.03%, m.p. 184-185°C).

Ethyl 2-(10*H*-phenothiazin-10-yl) acetate (14)

To a stirred solution of 10*H*-phenothiazine(**13**) (1gm, 0.01mole) and potassium hydroxide (25mg) in 100 ml of absolute ethanol, ethylchloroacetate (0.60gm, 0.01mole) was added drop wise until phenothiazine get dissolved. The resulting mixture was refluxed for 30 hours then it was cooled and poured into crushed ice with continuous stirring. The resulting precipitate was collected by vacuum filtration, washed with water, dried and recrystallized from ethanol to get compound **14**. (Yield 76.56%, m.p. 178-179°C).

2-(10*H*-phenothiazin-10-yl) acetohydrazide (15)

To a solution of ethyl 2-(10*H*-phenothiazin-10-yl) acetate (**14**) (2.85gm, 0.01mole) in 50 ml absolute ethanol, hydrazine hydrate (99%) (0.49gm, 0.01 mole) was added drop wise. The resulting mixture was refluxed for 20 hours then it was cooled and poured into crushed ice with continuous stirring. The resulting precipitate was collected by vacuum filtration, washed with water, dried and recrystallized from ethanol to get compound **15**. (Yield 71.0%, m.p. 173-174°C).

N'-(4-chlorobenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16a)

To a mixture of 2-(10*H*-phenothiazin-10-yl) acetohydrazide (**15**) (2.71gm, 0.01mole) and *p*-chlorobenzaldehyde (1.40gm, 0.01mole) in 50 ml ethanol, conc. hydrochloric acid (1ml) was added. The resulting mixture was refluxed for 9 hours then it was cooled and poured into crushed ice with continuous stirring. The precipitate thus obtained was collected by vacuum filtration, washed with water, dried and recrystallized from ethanol to get compound **16a**. (Yield 62.59%, m.p. 211-212°C).

N'-(4-fluorobenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16b)

To a mixture of 2-(10*H*-phenothiazin-10-yl) acetohydrazide (**15**) (2.71gm, 0.01mole) and *p*-fluorobenzaldehyde (1.24gm, 0.01mole) in 50 ml ethanol, conc. hydrochloric acid (1ml) was added. The resulting mixture was refluxed for 8 hours then it was cooled and poured into crushed ice continuous stirring. The precipitate thus obtained was



collected by vacuum filtration, washed with water, dried and recrystallized from ethanol to get compound **16b**. (Yield 66.54%, m.p. 192-193°C).

***N'*-(4-nitrobenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16c)**

To a mixture of 2-(10*H*-phenothiazin-10-yl) acetohydrazide (**15**) (2.71gm, 0.01mole) and *p*-nitro benzaldehyde (1.51gm, 0.01mole) in 50 ml ethanol, conc. hydrochloric acid (1ml) was added. The resulting mixture was refluxed for 10 hours. The reaction mixture was cooled at room temperature, the precipitate thus obtained was washed with water, filtered, dried and recrystallized from absolute ethanol to give compound **16c**. (Yield 63.75%, m.p. 219-220°C).

***N'*-(4-hydroxybenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16d)**

To a mixture of 2-(10*H*-phenothiazin-10-yl) acetohydrazide (**15**) (2.71gm, 0.01mole) and *p*-hydroxybenzaldehyde (1.22gm, 0.01mole) in 50 ml ethanol, conc. hydrochloric acid (1ml) was added. The resulting mixture was refluxed for 9 hours. The reaction mixture was cooled at room temperature; the precipitate thus obtained was washed with water, filtered, dried and recrystallized from absolute ethanol to give compound **16d**. (Yield 60.86%, m.p. 188-189°C).

***N'*-(3-methoxybenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16e)**

To a mixture of 2-(10*H*-phenothiazin-10-yl) acetohydrazide (**15**) (2.71gm, 0.01mole) and *m*-methoxy benzaldehyde (1.36gm, 0.01mole) in 50 ml ethanol, conc. hydrochloric acid (1ml) was added. The resulting mixture was refluxed for 10 hours then it was cooled and poured into crushed ice with continuous stirring. The resulting precipitate was collected by vacuum filtration, washed with water, dried and recrystallized from ethanol to get compound **16e**. (Yield 62.02%, m.p. 203-204°C).

Result and discussion

The work carried out is discussed under the following headings:

- ✓ *N'*-(4-chlorobenzylidene)-2-(10*H*-phenothiazin-10-yl)acetohydrazide (**16a**)
- ✓ *N'*-(4-fluorobenzylidene)-2-(10*H*-phenothiazin-10-yl)acetohydrazide (**16b**)
- ✓ *N'*-(4-nitrobenzylidene)-2-(10*H*-phenothiazin-10-yl)acetohydrazide (**16c**)
- ✓ *N'*-(4-hydroxybenzylidene)-2-(10*H*-phenothiazin-10-yl)acetohydrazide (**16d**)
- ✓ *N'*-(3-methoxybenzylidene)-2-(10*H*-phenothiazin-10-yl)acetohydrazide (**16e**)

***N'*-(4-chlorobenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16a)**

Compound (**16a**) was obtained by the reaction of ethyl 2-(10*H*-phenothiazin-10-yl)acetate (**15**) with hydrazine hydrate followed by the reaction of 2-(10*H*-phenothiazin-10-yl)acetohydrazide (**14**) with *p*-chlorobenzaldehyde in the presence of concentrate HCl.

FTIR spectrum analysis: A band at 3345.17cm⁻¹ was assigned to aliphatic N-H. Band for aromatic and aliphatic C-H stretching vibrations were observed at 3042.13 and 2910.17cm⁻¹ respectively. A stretching vibration at 1657.22 cm⁻¹ was assigned to C=O as well as a band at 1642.85cm⁻¹ was observed for C=N. Aromatic C=C and C-N stretching vibrations were appeared at 1572.70 and 1329.13cm⁻¹ respectively. A stretching vibration at 1037.67cm⁻¹ was appeared for C-Cl while a band at 825.37cm⁻¹ was assigned to C-H deformation vibration for *p*-disubstituted benzene. A stretching vibration at 678.18cm⁻¹ was appeared for C-S.

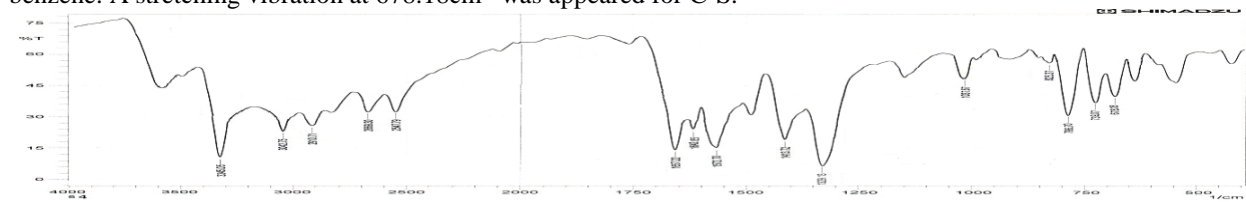


Figure 1: IR spectra of compound (16a)

¹H NMR spectrum analysis: A singlet of two protons due to alpha hydrogen to carbonyl group was appeared at δ 2.408 ppm. A singlet was observed at δ 5.993 ppm for a proton of the benzylic group. Two double triplets of four aromatic protons were appeared in range δ 6.575-6.798 ppm. A doublet of two aromatic protons was assigned between δ 7.331-7.389 ppm. Two double doublets of four aromatic protons were observed in range δ 7.510-7.762 ppm while another doublet for two aromatic protons was assigned between δ 7.893-7.954 ppm. One proton singlet at δ 8.251 ppm was assigned to the secondary amide group which was D₂O exchangeable.



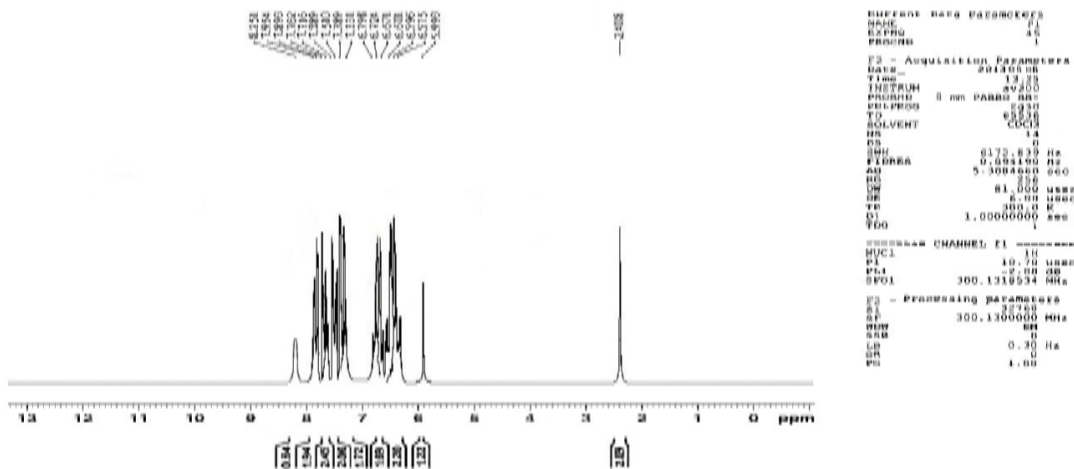


Figure 2: NMR spectra of compound (16a)

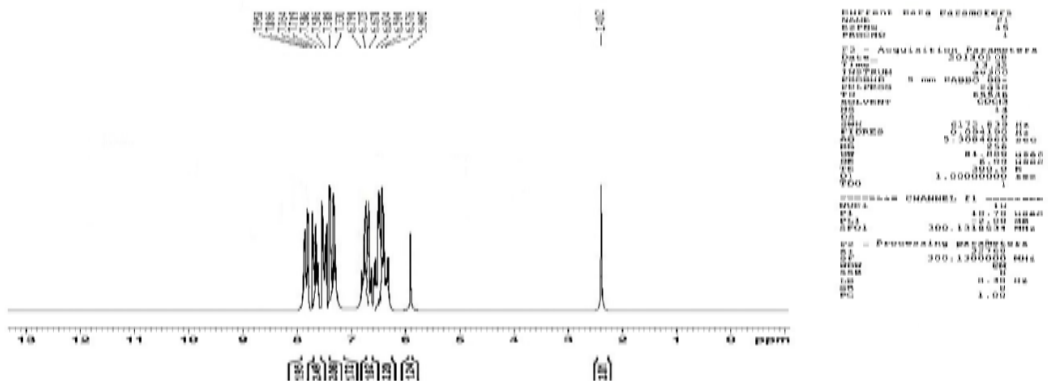


Figure 3: D₂O NMR spectra of compound (16a)

Mass spectrum analysis: The mass fragmentation pattern of compound (16a) is illustrated in Figure 4

Display Report

Analysis Info		Acquisition Date 10/06/2013 2:05 PM	
Analysis Name	D:\Data\Demo\AD3d	Operator	Sharma/Singh
Method	tune_low.m	Instrument	micrOTOF-Q II 10262
Sample Name			
Comment			

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Source

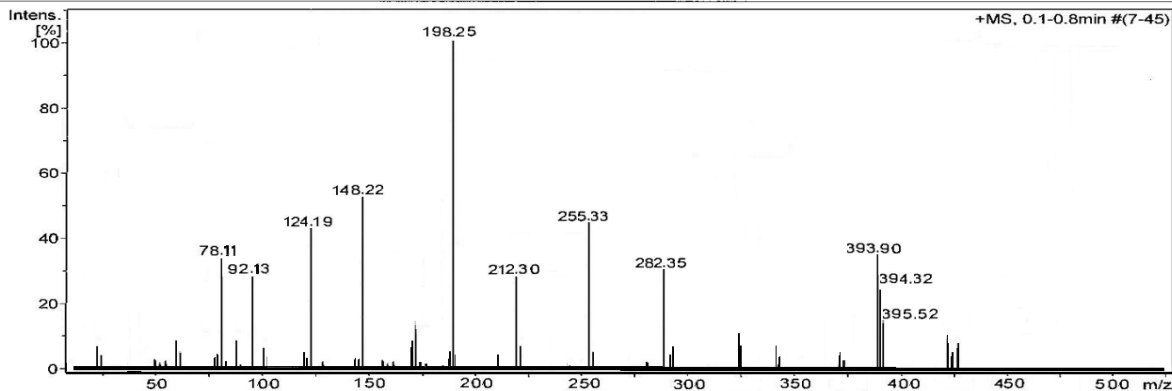


Figure 4: Mass spectra of compound (16a)



***N'*-(4-fluorobenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16b)**

Compound (16b) was obtained by the reaction of ethyl 2-(10*H*-phenothiazin-10-yl)acetate (15) with hydrazine hydrate followed by the reaction of 2-(10*H*-phenothiazin-10-yl) acetohydrazide (14) with *p*-fluorobenzaldehyde in the presence of concentrated HCl.

FTIR spectrum analysis: Peak at 3342.41cm^{-1} was assigned to N-H stretching vibration. Band for aromatic and aliphatic C-H stretching vibrations were observed at 3058.11 and 2923.88cm^{-1} respectively. A stretching vibration at 1670.82cm^{-1} was assigned to C=O as well as a band at 1648.78cm^{-1} was observed for C=N. Aromatic C=C and C-N stretching vibrations were appeared at 1587.31 and 1303.71cm^{-1} respectively. A stretching vibration at 1141.78cm^{-1} was appeared for C-F while a band at 810.05cm^{-1} was assigned to C-H deformation vibrations for *p*-disubstituted benzene. A stretching vibration at 692.40cm^{-1} was appeared for C-S.

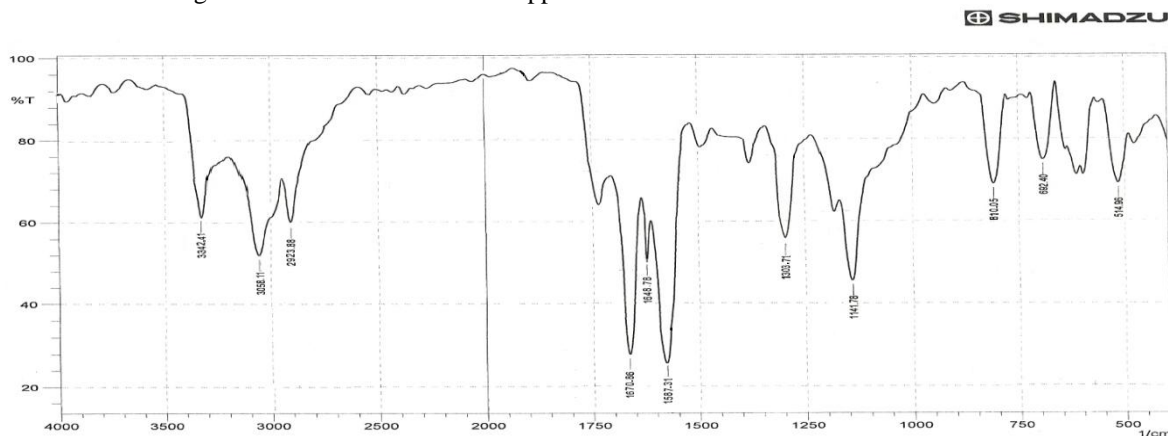


Figure 5: IR spectra of compound (16b)

^1H NMR spectrum analysis: A singlet of two protons due to alpha hydrogen to carbonyl group was appeared at δ 2.481 ppm. A singlet was observed at δ 5.712 ppm for a proton of the benzylic group. Two double triplets of four aromatic protons were appeared in range δ 6.593-7.019 ppm. A double doublet of two aromatic protons was assigned between δ 7.413--7.476 ppm as well as a doublet of two aromatic protons was observed in range δ 7.629-7.674 ppm. A double doublet for two aromatic protons was assigned between δ 7.714-7.789 ppm while another a doublet for two aromatic protons was appeared between δ 7.932-7.987 ppm. One proton singlet at δ 8.053 ppm was assigned to the secondary amide group which was D_2O exchangeable.

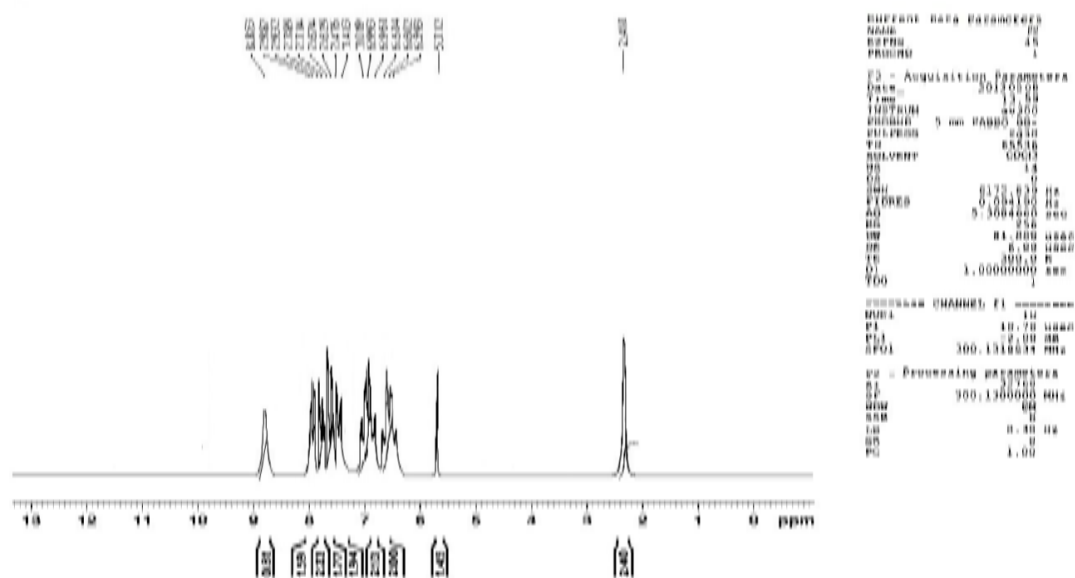
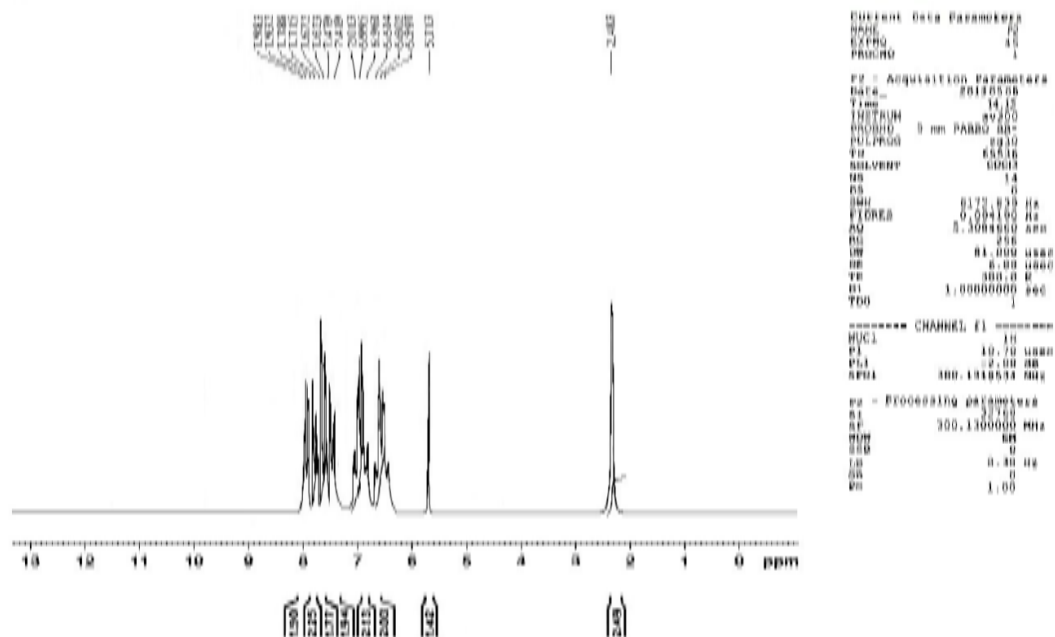


Figure 6: NMR spectra of compound (16b)



Figure 7: D_2O NMR spectra of compound (16b)

Mass spectrum analysis: The mass fragmentation pattern of compound (16b) is illustrated in Figure 8.

Display Report

Analysis Info		Acquisition Date	
Analysis Name	D:\Data\Demo\AD 3d	10/06/2013	2:25 PM
Method	tune_low.m	Operator	Sharma/Singh
Sample Name		Instrument	micrOTOF-Q II 10262
Comment			
Acquisition Parameter			
Source Type	ESI	Ion Polarity	Positive
Focus	Active	Set Capillary	4500 V
Scan Begin	50 m/z	Set End Plate Offset	-500 V
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp
		Set Nebulizer	0.3 Bar
		Set Dry Heater	180 °C
		Set Dry Gas	4.0 l/min
		Set Divert Valve	Source

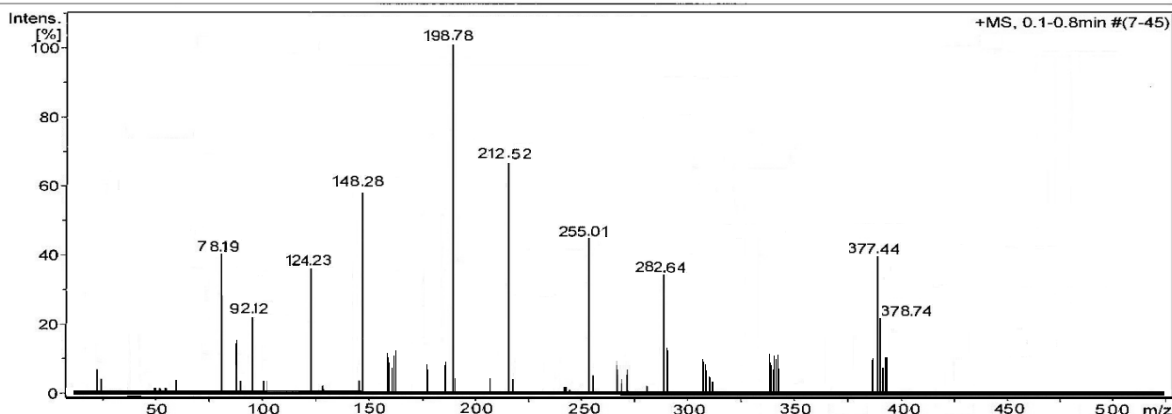


Figure 8: Mass spectra of compound (16b)

N'-(4-nitrobenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16c)

Compound (16c) was obtained by the reaction of ethyl 2-(10*H*-phenothiazin-10-yl)acetate (15) with hydrazine hydrate followed by the reaction of 2-(10*H*-phenothiazin-10-yl) acetohydrazide (14) with *p*-nitrobenzaldehyde in the presence of concentrate HCl.

FTIR spectrum analysis: A band at 3356.48cm^{-1} was assigned to aliphatic N-H. Band for aromatic and aliphatic C-H stretching vibrations were observed at 3055.96 and 2919.03cm^{-1} respectively. A stretching vibration at 1662.41



cm^{-1} was assigned to C=O as well as a band at 1649.91cm^{-1} was assigned to C=N. Peak at 1589.23cm^{-1} was assigned to C=C stretching vibration. Stretching vibrations at 1514.02 and 1341.58cm^{-1} were assigned for N-O asymmetric and symmetric respectively. Band at 1299.80cm^{-1} was assigned to C-N stretching vibration while at 854.41cm^{-1} was assigned to C-H deformation for *p*-disubstituted benzene. Peak at 649.97cm^{-1} was assigned to C-S stretching vibration.

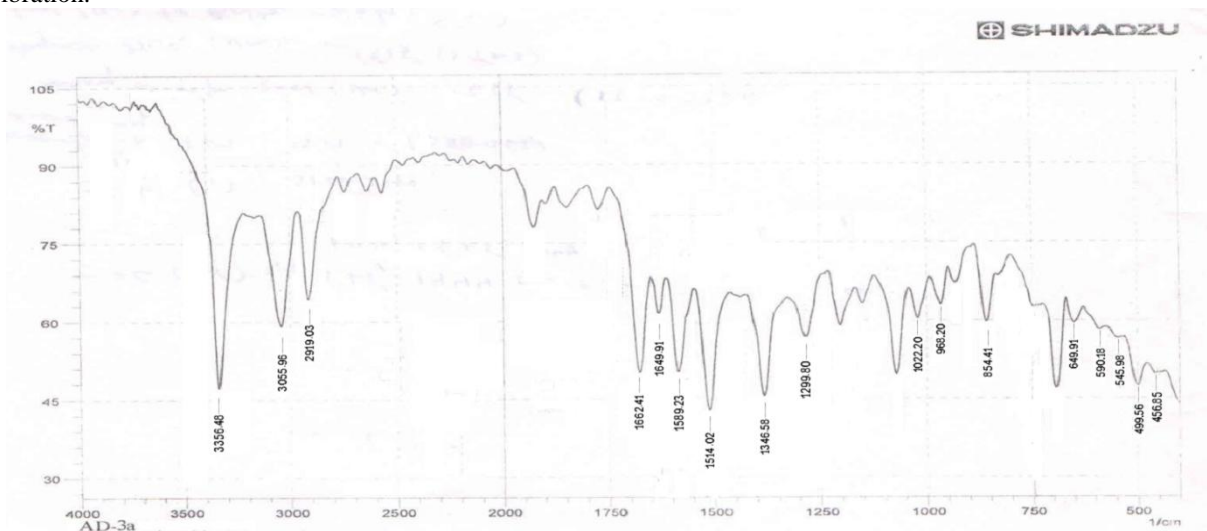


Figure 9: IR spectra of compound (16c)

^1H NMR spectrum analysis: A singlet of two protons due to alpha hydrogen to carbonyl group was appeared at δ 2.327 ppm. A singlet was observed at δ 5.621 ppm for a proton of the benzylic group. Two double triplets of four aromatic protons were appeared in range δ 6.623-7.189 ppm while two doublets for four aromatic protons were assigned between δ 7.211-7.572 ppm. Two double doublets of four aromatic protons were assigned between δ 7.608-7.804 ppm. One proton singlet at δ 8.259 ppm was assigned to the secondary amide group which was D_2O exchangeable.

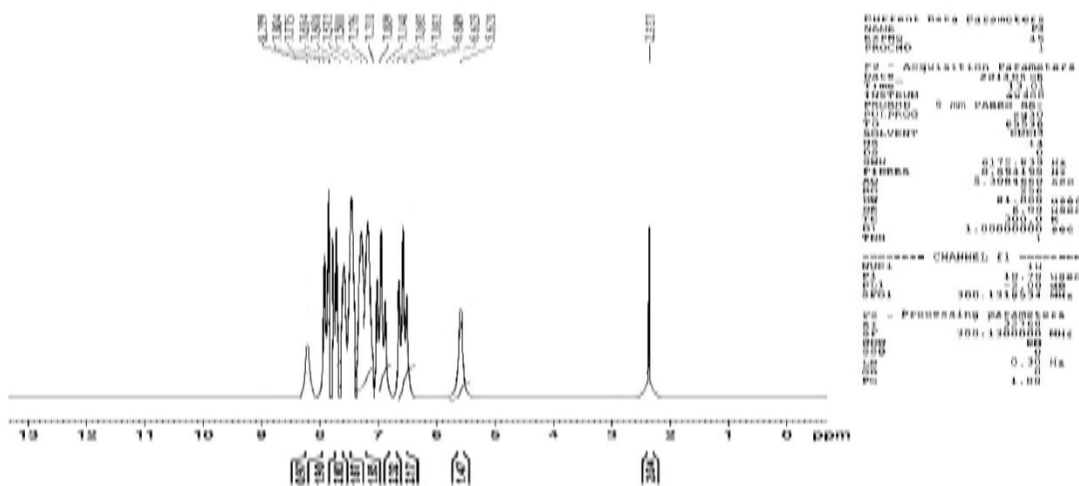


Figure 10: NMR spectra of compound (16c)



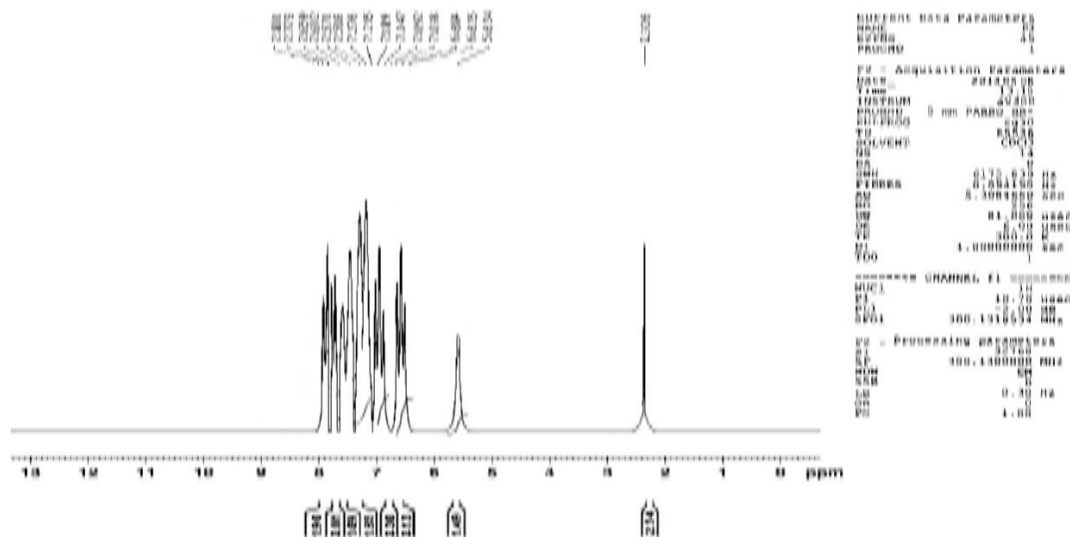


Figure 11: D_2O NMR spectra of compound (16c)

Mass spectrum analysis: The mass fragmentation pattern of compound (16c) is illustrated in Figure 12.

Display Report

Analysis Info

Analysis Name D:\Data\Demo\AD 3d
Method tune_low.m
Sample Name
Comment

Acquisition Date 10/06/2013 2:45 PM

Operator Sharma/Singh
Instrument micrOTOF-Q II 10262

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Source

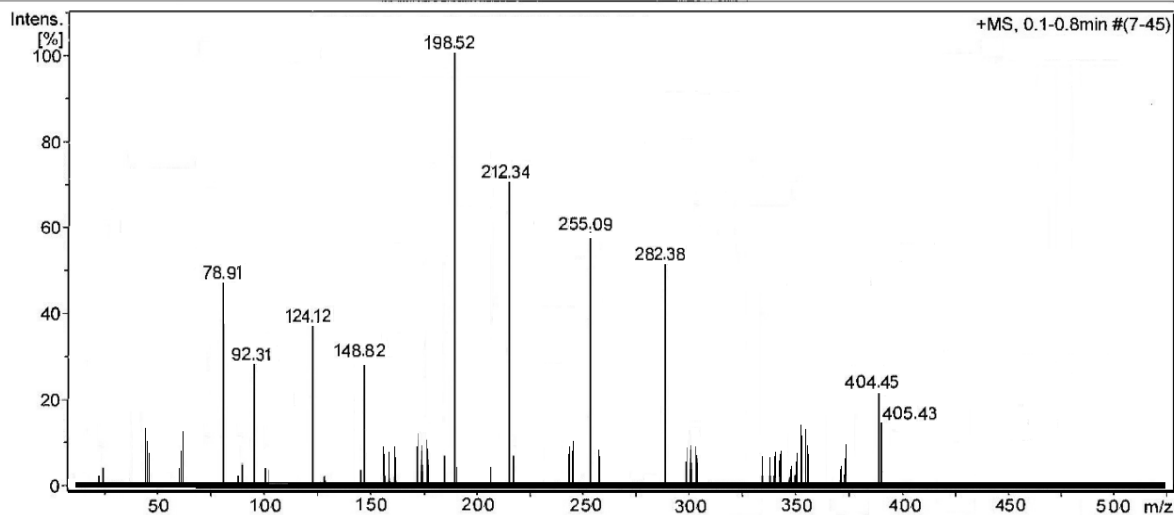


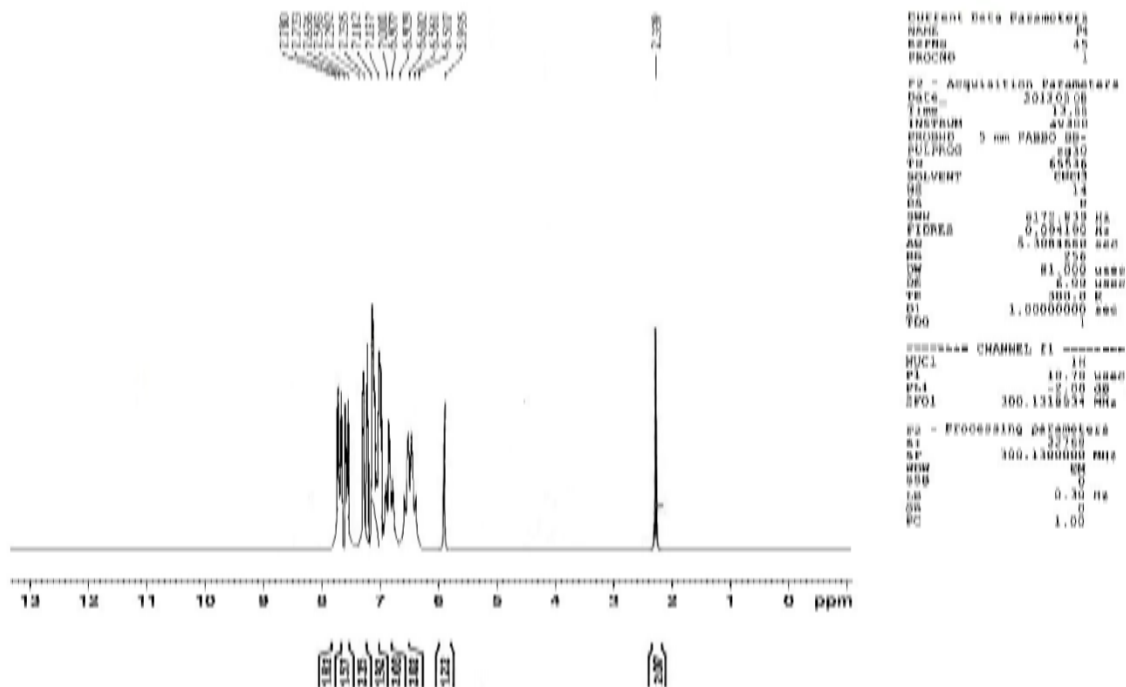
Figure 12: Mass spectra of compound (16c)

N'-(4-hydroxybenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16d)

Compound (16d) was obtained by the reaction of ethyl 2-(10*H*-phenothiazin-10-yl)acetate (15) with hydrazine hydrate followed by the reaction of 2-(10*H*-phenothiazin-10-yl)acetohydrazide (14) with *p*-hydroxybenzaldehyde in the presence of concentrate HCl.

FTIR spectrum analysis: A peak at 3622.07cm^{-1} was observed for OH stretching vibration as well as a band at 3344.34cm^{-1} was assigned to aliphatic N-H. Band for aromatic and aliphatic C-H stretching vibrations were



Figure 15: D_2O NMR spectra of compound (16d)

Mass spectrum analysis: The mass fragmentation pattern of compound (16d) is illustrated in Figure 16.

Display Report

Analysis Info		Acquisition Date		10/06/2013 2:56 PM	
Analysis Name	D:\Data\Demo\AD3d	Operator	Sharma/Singh		
Method	tune_low.m	Instrument	micrOTOF-Q II 10262		
Sample Name					
Comment					
Acquisition Parameter					
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Source

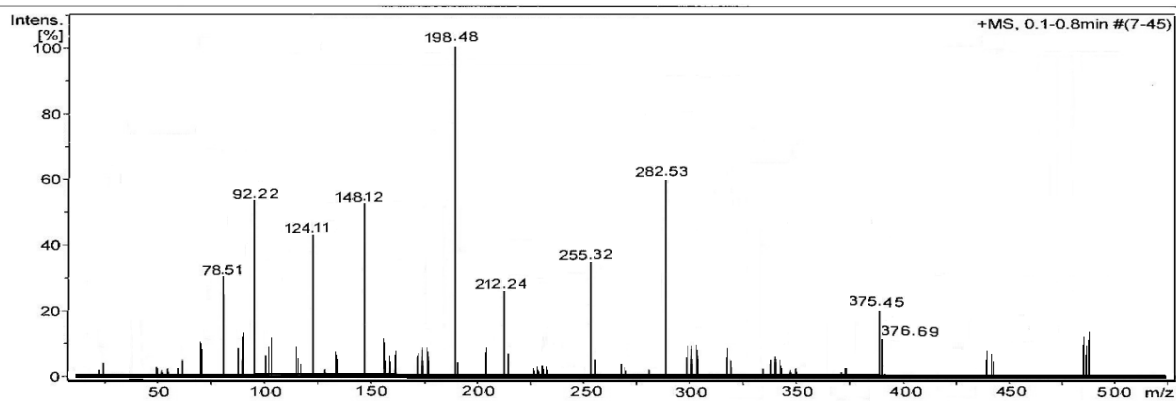


Figure 16: Mass spectra of compound (16d)

N'-(3-methoxybenzylidene)-2-(10*H*-phenothiazin-10-yl) acetohydrazide (16e)

Compound (16e) was obtained by the reaction of ethyl 2-(10*H*-phenothiazin-10-yl)acetate (15) with hydrazine hydrate followed by the reaction of 2-(10*H*-phenothiazin-10-yl)acetohydrazide (14) with *m*-methoxybenzaldehyde in the presence of concentrated HCl.

FTIR spectrum analysis: A band at 3348.19 cm^{-1} was assigned to aliphatic N-H. Band for aromatic and aliphatic C-H stretching vibrations were observed at 3061.47 and 2921.96 cm^{-1} respectively. Peak at 1679.62 cm^{-1} was assigned to C=O stretching vibration as well as a band was observed for C=N stretching vibration at 1641.99 cm^{-1} .



Aromatic C=C and C-N stretching vibrations were appeared at 1579.24 and 1317.74 cm^{-1} respectively. C-O-C asymmetric and symmetric stretching vibrations were observed at 1263.29 and 1043.42 cm^{-1} respectively. Band at 742.54 and 700.21 cm^{-1} was assigned to C-H deformation vibration for *m*-disubstituted benzene as well as a peak at 628.75 cm^{-1} was observed for C-S.

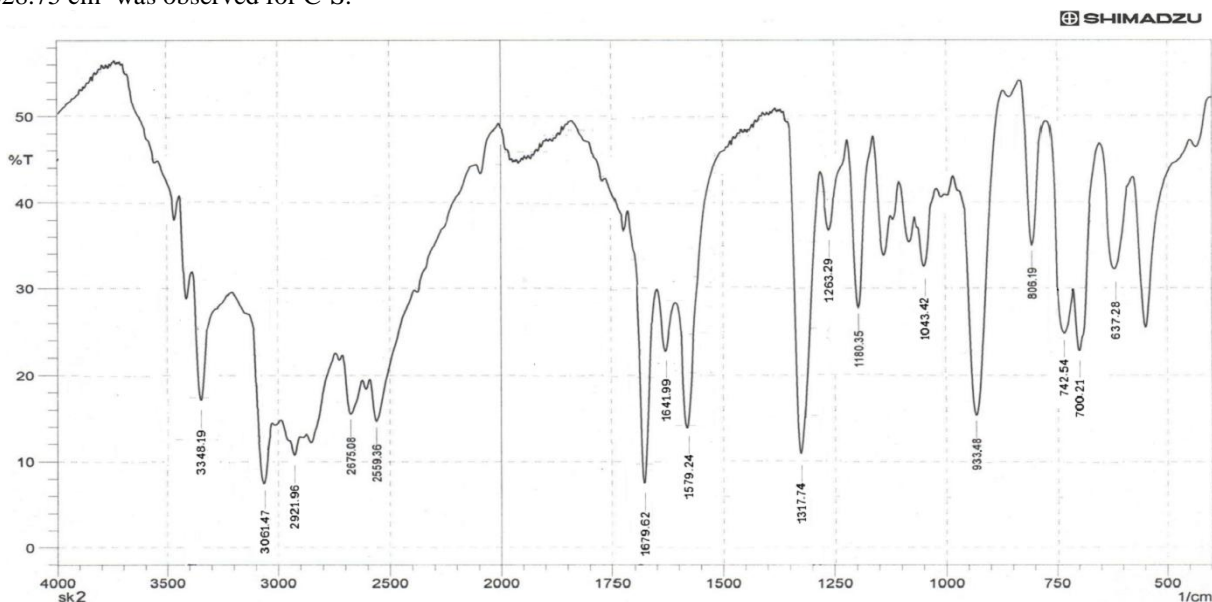


Figure 17: IR spectra of compound (16e)

^1H NMR spectrum analysis: A singlet of two protons due to alpha hydrogen to carbonyl group was appeared at δ 2.383 ppm while another singlet was observed at δ 3.762 ppm for three protons of the methoxy group. A singlet was observed at δ 5.543 ppm for a proton of the benzylic group. A double triplet for two aromatic protons was appeared in range δ 6.492-6.583 ppm while a triplet for one aromatic proton was assigned between δ 6.729-6.831 ppm. A doublet of one aromatic proton was appeared between δ 6.907-6.973 ppm. Another double triplet for two aromatic protons was appeared in range δ 7.089-7.185 ppm. A doublet of one aromatic proton was appeared between δ 7.293-7.324 ppm while a singlet was observed at δ 7.422 ppm for one aromatic proton. Two double doublets of four aromatic protons were assigned between δ 7.520-7.783 ppm. One proton singlet at δ 8.296 ppm was assigned to the secondary amide group which was D_2O exchangeable.

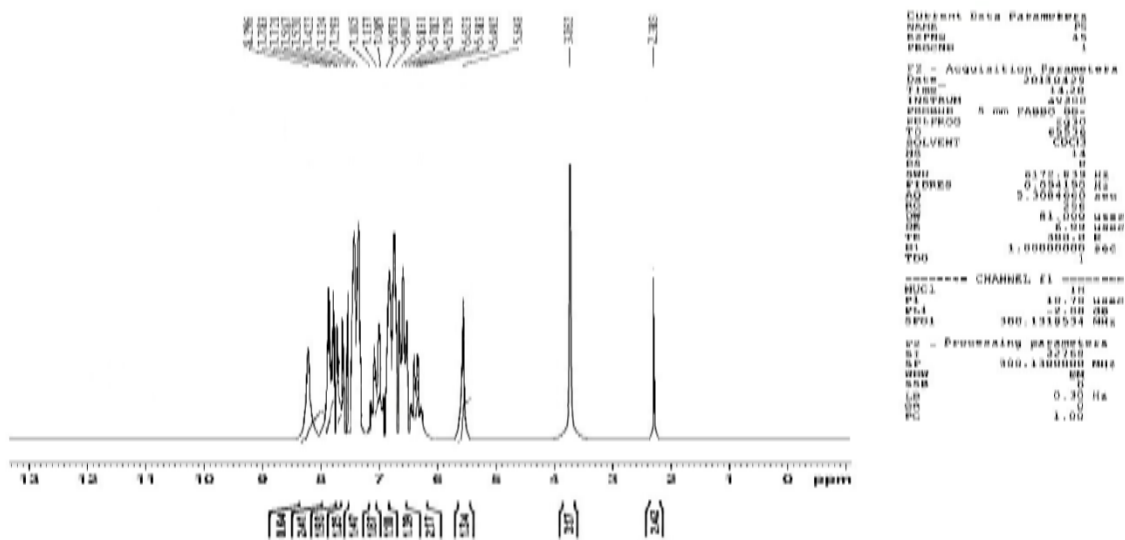


Figure 18: NMR spectra of compound (16e)



Conclusion

A series of phenothiazine derivatives was synthesized by conventional method in which diphenylamine on treatment with sulphur gave 10H-phenothiazine (**13**) which on reaction with ethylchloro acetate yielded 2-(10H-phenothiazin-10-yl)acetate (**14**) which on further treatment with hydrazine hydrate produced 2-(10H-phenothiazin-10-yl) acetohydrazide (**15**). Condensation of (**15**) with various benzaldehyde derivatives afforded *N'*-(substitutedbenzylidene)-2-(10H-phenothiazin-10-yl) acetohydrazide (**16a-16e**). Synthesized compounds were identified on the basis of physical parameters (solubility, melting point), chromatographic method (TLC), spectroscopic methods (UV, FTIR, ¹H- NMR, Mass spectral studies) and Elemental analysis. The FTIR spectra of the newly synthesized compounds (**16a-16e**) showed the presence of characteristic absorption bands in the region 3358-3342 cm⁻¹ for N-H str. and 1667-1640 cm⁻¹ for C=N str. ¹H NMR spectra of synthesized compounds showed the characteristic peaks of benzylic protons and N-H protons between δ 5.554-5.993 ppm, and δ 8.149-8.872 ppm. The results of elemental analysis were found to be in permissible limit \pm 0.04.

Acknowledgments

This work was supported by the Rajiv academy for pharmacy Mathura (India) and Tata Memorial Centre Mumbai (India).

References

1. Jaszczyszyn A, Gsiorowskik G, Wiesaw M, Cioelik B, Katarzyna PJ, Czarnik-MatusewiczBC. Chemical structure of phenothiazines and their biological activity. *Pharmacol. Rep*, 2012, 64: 16-23.
2. Ashoor A, Lorke P, Nurulain SM, Kury LA, Petroianu G, Yang KHS. Effects of phenothiazine-class antipsychotics on the function of α 7-nicotinic acetylcholine receptors. *Eur. J. of Pharmacology*, 2011, 673: 25-32.
3. ShinhaS, Pandya SN, Verma A, Yadav D. Synthesis and biological activity of phenothiazine derivatives. *Int. J. of Research Ayu*, 2011, 2(4): 1130-1137.
4. González-Muñoz GC, Arce MP, López B, Pérez C, Villarroya M, López MG, García AG, Conde S, Rodríguez-Franco MI. Old phenothiazine and dibenzothiadiazepine derivatives for tomorrow's neuroprotective therapies against neurodegenerative disease. *Eur. J. Med. Chem*, 2010, 45: 6152-6158.
5. Bisi A, Meli M, Gobbi S, Rampa A, Tolomeo M, Dusonchet I. Multidrug resistance reverting activity and antitumor profile of new phenothiazine derivatives. *Bioorg. Med. Chem*, 2008, 16: 6474.
6. Sudeshna G, Parimal K. Multiple non psychiatric effects of phenothiazines- A review. *Eur. J. Pharm. Col*, 2010, 648: 6-14.
7. Kaushik K, Kumar N, Pathak D. Synthesis of some newer carbazole derivatives and evaluation for their pharmacological activity. *Der Pharmacia Sinica*, 2012, 3 (4): 470-478.
8. Singh R, Kumar N, Yadav M, Pathak D. Microwave-Assisted, Solvent Free and Parallel Synthesis of Some Newer 2, 4-Disubstituted 1, 5- Benzodiazepines of Biological Interest. *Int. J. of Pharmaceutical Sci. and Drug Research*, 2013, 5(3):88-95.

