

## Synthesis and Evaluation of Phenothiazine Derivatives

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### ABSTRACT

**Background:** Phenothiazine is one of the prototypical pharmaceutical lead molecules in medicinal chemistry. Derivatives of phenothiazines are highly bioactive and have widespread applications including antipsychotic, antiemetic, neuroleptic action, antiproliferative effect, inhibition of P-glycoprotein transport function, urinary antiseptic, antihelminthic and insecticide

**Materials and Method:** A modified and improved method was followed for synthesis of 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide dihydrochloride and 10-(3-(4-(2-hydroxyethyl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5,5-dioxide dihydrochloride by hydrogen peroxide oxidation of 2-(4-(3-(2-(trifluoromethyl)-10H-phenothiazine-10-yl)propyl)piperazine-1-yl)ethanol dihydrochloride and 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide dihydrochloride, respectively, at room temperature followed by acidification in quantitative yields and enhanced purity. The synthesized compounds were characterised by IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopy. The synthesized compounds were also subjected to purity analysis by HPLC. The compounds were screened for antipsychotic activity using haloperidol induced catalepsy model, invitro anti-inflammatory activity using protein denaturation method and antibacterial activity using serial dilution method.

**Results:** The synthesized compounds were confirmed by IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and purity was determined by HPLC. Among the synthesized compounds, F-1 (5 mg/kg) and F-2 (5, 10 mg/kg) showed decrease in cataleptic scores, i.e., antipsychotic activity. F-1 and F-2 did not show any inhibition of protein denaturation. F-1 and F-2 showed noteworthy inhibition of growth against *S. aureus*, *P. aeruginosa*, *K. pneumoniae* stains of bacteria.

**Conclusion:** The synthesized compounds after analytic confirmation were screened for antipsychotic, anti-inflammatory and anti-bacterial activity. The synthesized compounds showed statistically significant antipsychotic activity. None of the compounds showed anti-inflammatory activity, but showed noteworthy inhibition of bacterial growth.

**Key Words:** Phenothiazine-5-oxide, Phenothiazine-5,5-dioxide, Antipsychotic, Invitro anti-inflammatory, Serial dilution antibacterial method.

### I. INTRODUCTION

Psychosis is characterized by an impaired relationship with reality. It's a symptom of serious mental disorders. People who are experiencing psychosis may have either hallucinations or delusions. Hallucinations are sensory experiences that occur within the absence of an actual stimulus. For example, a person having an auditory hallucination may hear their mother yelling at them when their mother isn't around. Or someone having a visual hallucination may see something, like a person in front of them, who isn't actually there. The person experiencing psychosis may also have thoughts that are contrary to actual evidence. These thoughts are known as delusions. Some people with psychosis may also experience loss of motivation and social withdrawal.

Symptoms of psychosis include: difficulty concentrating, depressed mood, sleeping too much or not enough, anxiety, suspiciousness, withdrawal from family and friends, delusions, hallucinations, disorganized speech, such as switching topics erratically, depression, suicidal thoughts or actions.<sup>[1]</sup>

The symptom of psychosis may be caused by serious medical illnesses such as schizophrenia or other psychiatric disorders, trauma, or other medical conditions. Psychosis may also be temporary in nature or transient and caused by medications or illicit substance use (substance-induced psychosis).

### **Normal states**

Brief hallucinations are not uncommon in those without any psychiatric disease. Causes or triggers include:

- Falling asleep and waking: hypnagogic and hypnopompic hallucinations, which are entirely normal
- Bereavement, in which hallucinations of a deceased loved one are common
- Severe sleep deprivation
- Stress

### **Trauma**

Traumatic life events have been linked with an elevated risk in developing psychotic symptoms. Childhood trauma has specifically been shown to be a predictor of adolescent and adult psychosis. Approximately 65% of individuals with psychotic symptoms have experienced childhood trauma (e.g., physical or sexual abuse, physical or emotional neglect). This suggests trauma prevention and early intervention may be an important target for decreasing the incidence of psychotic disorders and ameliorating its effects.

### **Psychiatric disorder**

From a diagnostic standpoint, subtle physical abnormalities have been found in illnesses traditionally considered functional, such as schizophrenia.

Primary psychiatric causes of psychosis include the following:

- schizophrenia and schizophrenic form disorder
- affective (mood) disorders, including major depression, and severe depression or mania in bipolar disorder (manic depression). People experiencing a psychotic episode in the context of depression may experience persecutory or self-blaming delusions or hallucinations, while people experiencing a psychotic episode in the context of mania may form grandiose delusions.
- schizoaffective disorder, involving symptoms of both schizophrenia and mood disorders
- brief psychotic disorder, or acute/transient psychotic disorder
- delusional disorder (persistent delusional disorder)
- chronic hallucinatory psychosis.<sup>[2]</sup>

Schizophrenia is a serious mental disorder in which people interpret reality abnormally. Schizophrenia may result in some combination of hallucinations, delusions, and extremely disordered thinking and behavior that impairs daily functioning, and can be disabling.

Signs and symptoms may vary, but usually involve delusions, hallucinations or disorganized speech, extremely disorganized or abnormal motor behavior and reflect an impaired ability to function.

India needs to talk about mental illness. Every sixth Indian needs mental health help. Mental problems more in 30–49 age group or over 60; low income linked to occurrence of mental disorders and urban areas to be most affected were some of the headlines in the mass media.<sup>[3]</sup>

Epidemiological studies report mental healthcare priorities need to be shifted from psychotic disorders to common mental disorders and from mental hospitals to primary health centers. Increase in invisible mental problems such as suicidal attempts, aggression and violence, widespread use of substances, increasing marital discord and divorce rates emphasize on the need to prioritize and make a paradigm shift in the strategies to promote and provide appropriate mental health services in the community.<sup>[4]</sup>

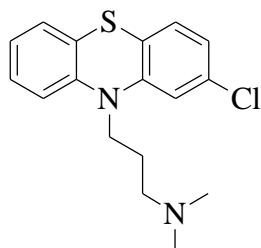
Fluphenazine is a phenothiazine antipsychotic medicine that is used to treat psychotic disorders such as schizophrenia. Common side effects may include: drowsiness, headache, blurred vision; problems with balance or muscle movement; nausea, loss of appetite, constipation; swelling, weight changes; stuffy nose, dry mouth, or drooling; increased sweating or urination; breast swelling or tenderness; or impotence, trouble having an orgasm.<sup>[5]</sup>

Phenothiazine is one of the prototypical pharmaceutical lead molecules in medicinal chemistry. These drugs have antipsychotic, antiemetic properties although they have severe side effects like extrapyramidal symptoms, hyperprolactemia and weight gain<sup>[6]</sup>. Derivatives of phenothiazines are highly bioactive and have widespread applications including neuroleptic action, antiproliferative effect, inhibition of P-glycoprotein transport function<sup>[7]</sup>, urinary antiseptic, antihelminthic and insecticide<sup>[8]</sup>.

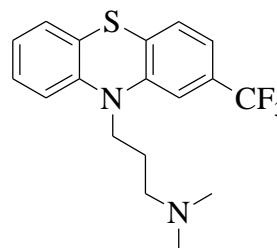
Chemistry of phenothiazine has been explained based on structure activity relationship (SAR).<sup>[9, 10, 11]</sup>

Phenothiazine (PTZ) is an organic compound with formula of  $S(C_6H_4)_2NH$  and is allied to thiazine class of heterocyclic compounds.

- Phenothiazines have tricyclic (heterocyclic) structure.
  
- Substitution of electron withdrawing group at position 2 increases antipsychotic activity. The effect of groups on antipsychotic activity can be ranked as:  $R_2 = -SO_2NR_2 > -CF_3 > -COCH_3 > -Cl$ .
- Substitution at 3<sup>rd</sup> position, can improve activity more than non-substituted compounds, but not significantly.
- Substituent at 4<sup>th</sup> position might hinder the receptor binding by sulphur atom.
- The sulphur atom at 5<sup>th</sup> position is analogous with *p*-hydroxyl group of dopamine and leads to assign receptor-binding functions.
- Nature of substituent at position 10 also influences pharmacological activity.
  - i. Compounds with aliphatic side chain (chlorpromazine and triflupromazine) are less potent, but clinically effective.



**Chlorpromazine**

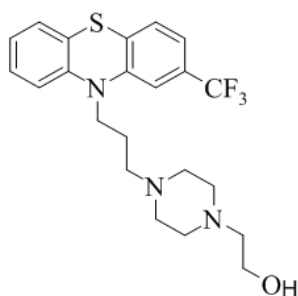


**Triflupromazine**

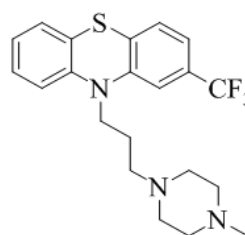
- ii. Compounds with piperidine ring in side chain (thioridazine), have somewhat low incidence of adverse EPS, possibly due to increased central antimuscarinic activity, but have depressant effects on cardiac conduction and repolarisation.

#### **Thioridazine**

- iii. Compounds with piperazine ring in side chain (fluphenazine, trifluperazine) have potent antipsychotic effects.



**Fluphenazine**



**Trifluperazine**

The strength of neuroleptic action of phenothiazines may possibly be ranked as: piperazine group > piperidine group > aliphatic chain.

- Presence of propyl connector in between the lipophilic core and tertiary amine moiety is critical for neuroleptic activity.

## II MATERIALS AND METHOD

### 2.1 General

**Chemicals:** 2-(4-(3-(2-(trifluoromethyl)-10H-phenothiazine-10-yl)propyl)piperazine-1-yl)ethanol dihydrochloride, 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide dihydrochloride, H<sub>2</sub>O<sub>2</sub>, Acetic acid, Isopropyl alcohol hydrochloride. All the chemicals were obtained from R L Fine Chemicals, Yelhanka New Town, Bengaluru-560064 Karnataka.

Completion of the reaction was checked on aluminium coated TLC plates using acetone: chloroform: methanol (1:1:1) as mobile phase and visualized under UV light. Infrared (IR) spectra were recorded using Shimadzu IR Affinity-1 FTIR spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded using Nanalysis 60 MHz spectrometer using D<sub>2</sub>O as solvent and tetramethylsilane (TMS) as internal standard.

### 2.2 Synthesis

#### **Fig 1: Synthetic pathway for 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide dihydrochloride (F-1)**

The oxidation reaction was performed in round bottom flask taking 2-(4-(3-(2-(trifluoromethyl)-10H-phenothiazine-10-yl)propyl)piperazine-1-yl)ethanol dihydrochloride (0.039 mol) **1**, acetic acid (120 mL) and H<sub>2</sub>O<sub>2</sub> (0.0702 mol) and stirred. The progress of the reaction was monitored by TLC with elution system Acetone: CHCl<sub>3</sub>: MeOH (1:1:1). After completion of the reaction, (10 h) the reaction mixture was transferred to beaker. Water was added and was basified with sodium carbonate. The resulting solution was extracted with dichloromethane. Dichloromethane layer was separated and distilled out under reduced pressure. The product **2** obtained was added with acetone (68.8 mL) and then transferred to ice bath to maintain the temperature of 0-5°C. Isopropyl alcohol hydrochloride (0.113 mol) was added and stirred. After 5hrs, the RM was then filtered using buchner funnel to obtain **3**.

Pale brown solid; Percentage yield: 74.05%; IR (KBr, cm<sup>-1</sup>): 3481-3356 (OH), 3007 (Ar C-H), 2956 (Aliphatic C-H), 1492-1454 (Ar C=C), 1247 (C-N bend), 1454 (C-H bend), 1051 (C-O), 707 (C-S); <sup>1</sup>H NMR (400MHz, δ ppm, D<sub>2</sub>O): 2.05-2.10 (t, 2H, CH<sub>2</sub> of C<sub>16</sub>), 2.97-3.10 (t, 2H, CH<sub>2</sub> of C<sub>17</sub>), 3.28-3.01 (t, 2H, CH<sub>2</sub> of C<sub>15</sub>), 3.36 (dd, 4H, CH<sub>2</sub> of C<sub>19</sub> & C<sub>23</sub>), 3.53 (dd, 4H, CH<sub>2</sub> of C<sub>20</sub> & C<sub>22</sub>), 3.80-3.82 (t, 2H, CH<sub>2</sub> of C<sub>24</sub>), 4.23-4.26 (t, 2H, CH<sub>2</sub> of C<sub>25</sub>), 7.5-7.8 (m, 7H, CH of C<sub>1</sub>, C<sub>2</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>14</sub>); <sup>13</sup>C NMR (100MHz, δ ppm, D<sub>2</sub>O): 21.7 (C<sub>16</sub>), 43 (C<sub>15</sub>), 48.4 (C<sub>17</sub>), 48.5 (C<sub>19</sub>), 53.4 (C<sub>20</sub>), 54.7 (C<sub>22</sub>), 57.9 (C<sub>23</sub>), 114.2 (C<sub>24</sub>), 117.5 (C<sub>25</sub>), 119 (C<sub>1</sub>), 121.9 (C<sub>11</sub>), 122 (C<sub>2</sub>), 123.7 (C<sub>6</sub>), 124.5 (C<sub>5</sub>), 130.9 (C<sub>12</sub>), 131.8 (C<sub>4</sub>), 134.6 (C<sub>3</sub>), 136.6 (C<sub>8</sub>), 137.3 (C<sub>9</sub>), 138 (C<sub>27</sub>); HPLC Purity: 99.056%.

#### **Fig 2: Synthetic pathway for 10-(3-(4-(2-hydroxyethyl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5,5-dioxide dihydrochloride (F-2)**

The oxidation reaction was performed in round bottom flask taking 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide dihydrochloride (0.018 mol) **3**, acetic acid (60 mL) and H<sub>2</sub>O<sub>2</sub> (0.037 mol) and stirred. The improvement in the reaction was monitored by TLC with elution system of Acetone: CHCl<sub>3</sub>: MeOH (1:1:1). After completion of the reaction, (30 h) the

reaction mixture was transferred to beaker. Water was added and basified using sodium carbonate. Then it was extracted with dichloromethane. Dichloromethane layer was separated and distilled out. The product **4** obtained was added with acetone (32.16 mL) and then transferred to ice bath to maintain the temperature of 0-5°C. Isopropyl alcohol hydrochloride (0.051 mol) was added and stirred. After 5 hrs, the reaction mixture was then filtered using buchner funnel to obtain **5**.

Colourless solid; Percentage yield: 67.29%; IR (KBr, cm<sup>-1</sup>): 3412 (OH), 3005 (Ar C-H), 2960 (Aliphatic C-H), 1494-1452 (C=C), 1452 (C-H bend), 1087-1051 (C-O), 1242 (O=S=O); <sup>1</sup>H NMR (400 MHz, δ ppm, D<sub>2</sub>O): 2.24-2.34 (t, 2H, CH<sub>2</sub> of C<sub>16</sub>), 3.58-3.61 (t, 2H, CH<sub>2</sub> of C<sub>17</sub>), 3.71-3.93 (dd, 4H, CH<sub>2</sub> of C<sub>19</sub> & C<sub>23</sub>), 4.06-4.12 (dd, 4H, CH<sub>2</sub> of C<sub>20</sub> & C<sub>23</sub>), 4.30-4.37 (t, 2H, CH<sub>2</sub> of C<sub>24</sub>), 4.39-4.78 (t, 2H, CH<sub>2</sub> of C<sub>25</sub>), 7.31-7.77 (m, 7H, CH of C<sub>1</sub>, C<sub>2</sub>, C<sub>5</sub>, C<sub>6</sub>, C<sub>11</sub>, C<sub>12</sub> & C<sub>14</sub>); <sup>13</sup>C NMR (60 MHz, δ ppm, D<sub>2</sub>O): 19.6 (C<sub>16</sub>), 43.5 (C<sub>15</sub>), 54.5 (C<sub>17</sub>), 56.8 (C<sub>19</sub>), 57.5 (C<sub>20</sub>), 65.7 (C<sub>22</sub>), 70.41 (C<sub>23</sub>), 114.7 (C<sub>24</sub>), 117.7 (C<sub>25</sub>), 119.2 (C<sub>1</sub>), 122 (C<sub>11</sub>), 122.9 (C<sub>2</sub>), 123.8 (C<sub>6</sub>), 123.9 (C<sub>5</sub>), 124.8 (C<sub>14</sub>), 131 (C<sub>12</sub>), 131.8 (C<sub>4</sub>), 134.7 (C<sub>3</sub>), 137.5 (C<sub>8</sub>), 138.1 (C<sub>9</sub>), 140.7 (C<sub>27</sub>); HPLC Purity: 86.377%.

### 2.3 Pharmacological screening

The synthesized compounds were screened for antipsychotic activity using haloperidol induced catalepsy. They were also tested for *in-vitro* anti-inflammatory activity using inhibition of protein denaturation and antibacterial activity using serial dilution method.

**Animals:** Healthy swiss albino mice (20-30 gm) of either sex were used for the experiment. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (BLDE/DPC/644/2018-19) dated 15/12/2018. All the procedures were performed in accordance with IAEC. All the animals were procured from animal house of H.S.K. College of Pharmacy, Bagalkot, Karnataka. The animals were acclimatized by keeping them in propylene cages (29x22x14) containing husk as bedding material and maintained under controlled of temperature (25±2°C), humidity (55±5%) and 12hr light and 12hr dark cycles. The animals were fed with standard pellet diet and water *ad libitum*.

**2.3.1 Haloperidol induced catalepsy:** Healthy swiss albino mice weighing 20-30 gm were used for the experiment. Animals were sorted into 6 groups, viz., control, A (haloperidol 1 mg/kg), test group-I (F-1, 5 mg/kg), test group-II (F-1, 10 mg/kg), test group-III (F-2, 5 mg/kg), test group-IV (F-2, 10 mg/kg) each containing 4 animals. The activity was carried for 7 days. Animals were treated with drugs F-1 and F-2 (5 mg/kg and 10 mg/kg). Catalepsy was induced by injecting haloperidol (1 mg/kg) intraperitoneally for all the groups except for control on Day 0 and 7, 30 minutes after administration of test drug. Then, both the front limbs of mice were placed over 4.5 cm high wooden block and time for which animals maintained the cataleptic posture was measured. Changes in cataleptic posture were observed for 3 min after 30, 60, 120 and 240 min of haloperidol injection. The end point of the catalepsy was measured when both front paws were removed from the bar.<sup>[12]</sup>

**Table no 1:** Effect of test drug on behaviour of mice in haloperidol induced catalepsy.

Treatment	Haloperidol	F-1 (5)	F-1 (10)	F-2 (5)	F-2 (10)
<b>Day 1</b>					
½	131.3±41.44*	137.0±43.00 <sup>ns</sup>	139.5±24.90 <sup>ns</sup>	131.3±16.56 <sup>ns</sup>	100.8±36.99 <sup>ns</sup>
1	60.50±11.77**	107.8±42.63 <sup>ns</sup>	142.0±22.89 <sup>ns</sup>	118.8±39.21 <sup>ns</sup>	107.3±22.53 <sup>ns</sup>
2	83.25±14.02**	139.8±25.99 <sup>ns</sup>	154.5±25.50 <sup>ns</sup>	135.0±35.71 <sup>ns</sup>	73.0±36.70 <sup>ns</sup>
4	131.8±28.66**	140.5±24.98 <sup>ns</sup>	180.0±0.00 <sup>ns</sup>	124.3±20.16 <sup>ns</sup>	136.3±18.41 <sup>ns</sup>
<b>Day 7</b>					
½	71.75±25.12*	16.50±5.80 <sup>ns</sup>	138.5±41.50 <sup>ns</sup>	31.75±8.52 <sup>ns</sup>	26.25±11.95 <sup>ns</sup>
1	150.5±29.50**	85.75±43.19 <sup>ns</sup>	141.5±25.99 <sup>ns</sup>	39.50±5.92 <sup>b</sup>	53.50±23.64 <sup>b</sup>
2	158.0±22.00***	84.50±37.29 <sup>ns</sup>	164.3±15.75 <sup>ns</sup>	75.25±36.47 <sup>ns</sup>	70.00±37.03 <sup>ns</sup>
4	165.0±15.00****	96.25±35.32 <sup>ns</sup>	163.0±17.00 <sup>ns</sup>	75.50±35.46 <sup>ns</sup>	89.25±35.12 <sup>ns</sup>

Values represent Mean ± SEM (n=6); <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>P<0.001, v/s Haloperidol Control group;

One-way ANOVA followed by multiple comparison Dennett's test and unpaired t-test; \*P<0.05,

\*\*P<0.01, \*\*\*P<0.001

**2.3.2 In-Vitro Anti-inflammatory activity by inhibition of protein denaturation:** The reaction mixture consisted of bovine serum albumin (0.45 mL) and test compound (0.05 mL) (25, 50, 100, 200 μg/mL). pH was adjusted using small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling, 2.5 mL of buffer solution was added into each test tube.

Turbidity was measured spectrophotometrically at 600nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:<sup>[13]</sup>

$$\text{Percentage Inhibition} = \frac{(\text{Abs Standard} - \text{Abs sample})}{\text{Abs Control}} \times 100$$

**Table no 2:** Effect of test drug on inhibition of protein denaturation.

Treatment	Concentration (µgm/mL)	Absorbance at 600nm	Percentage Inhibition of Protein Denaturation (%)
Blank	---	0.046 0.00057	---
Standard	25	0.077 0.00033****	41.02
	50	0.077 0.00057****	39.47
	100	0.073 0.00057****	37.83
	200	0.073 0.00088****	36.11
F-1	25	0.069 0.00057****	11.53
	50	0.071 0.00088**	3.94
	100	0.068 0.0011**	8.1
	200	0.071 0.00088	00
F-2	25	0.068 0.00066****	12.82
	50	0.067 0.00088****	11.84
	100	0.065 0.00088***	13.54
	200	0.065 0.00088**	8.33

Values represent Mean ± SEM (n=6);<sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>P<0.001, v/s Haloperidol Control group; One-way ANOVA followed by multiple comparison Dennett's test and unpaired t-test; \*P<0.05, \*\*P<0.01, \*\*\*P<0.001

2.3.3 **Antibacterial activity by serial dilution method:** Serial dilution is sequential dilution method to reduce an intense culture of cells to a more utilizable concentration. This method is used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing. 9mL of nutrient broth was taken in 5 test tubes each for cefotaxime and test compounds. 0.1mL of bacterial culture of different strains (*E.Coli*, *S. Aureus*, *P. Aeruginosa* and *K Pneumoniae*) was added to each test tube. 1m of test compound (1000µgm/mL) was added in 1<sup>st</sup> tube. 1mL of solution was pipetted from 1<sup>st</sup> tube and added to 2<sup>nd</sup> tube (100µgm/mL). The method was repeated to attain concentrations of 10µgm/mL, 1 µgm/mL and 0.1µgm/mL. The same method was followed for the cefotaxime drug. The tubes were then incubated for 24hrs and the turbidity of the solution was recorded using Nephlo-Turbidometer against McFarland standard at 100NTU. Blank contained nutrient broth and different bacterial cultures without drug.

$$\text{Turbidity} = \frac{1}{\text{Inhibition of microbial growth}}$$

**Table no 3:** Effect of test drug on inhibition of bacterial growth.

Treatment	Concentration (µgm/mL)	Percentage of Microbial growth			
		E.coli	S.aureus	P.aeriginosa	K.pneumoniae
Normal	--	58.7	55.8	72.7	64.2
Cefotaxime	1000	18.3	08.4	39.7	67.6
	100	16.6	23.0	49.0	68.2
	10	20.5	33.3	43.7	72.2
	1	19.0	31.1	54.7	62.5
	0.1	21.8	38.5	47.7	72.0

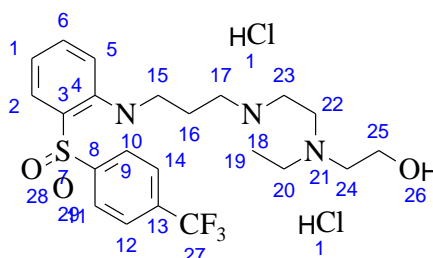
<b>F-1</b>	1000	49.0	14.0	62.3	59.3
	100	44.1	20.6	43.8	71.7
	10	44.3	29.5	54.8	63.5
	1	41.5	22.0	44.4	40.1
	0.1	43.4	29.0	47.7	63.2
<b>F-2</b>	1000	39.1	24.4	40.9	65.2
	100	40.9	17.5	13.5	60.7
	10	39.7	19.4	45.7	61.4
	1	38.4	29.4	56.0	65.0
	0.1	35.5	30.2	49.7	72.8

### III RESULTS AND DISCUSSION

We have modified and improved the process for the synthesis of 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide dihydrochloride **3** and 10-(3-(4-(2-hydroxyethyl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5,5-dioxide dihydrochloride **5**. The synthetic pathway has been illustrated in **Fig. 1 and Fig. 2**. Compound **1** on oxidation with H<sub>2</sub>O<sub>2</sub> in presence of acetic acid gave 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide **2**. Compound **2** on treatment with isopropyl alcohol hydrochloride in presence of acetone resulted in compound **3**.

The compound **3** was oxidised to 10-(3-(4-(2-hydroxyethyl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5,5-dioxide **4** by reaction with H<sub>2</sub>O<sub>2</sub> in presence of acetic acid. Compound **4** on treatment with isopropyl alcohol in presence of acetone resulted in compound **5**.

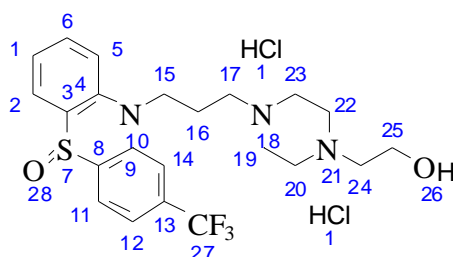
IR spectrum of the final compound **3** (**Fig. 3**) gave stretching vibrations at 3481-3356 cm<sup>-1</sup> corresponding to hydroxyl group, 3007 cm<sup>-1</sup> corresponding to Ar C-H, 2956 cm<sup>-1</sup> corresponding to aliphatic C-H and 1492-1454 cm<sup>-1</sup> corresponding to Ar C=C, 1247 cm<sup>-1</sup> corresponding to C=N bending, 1051 cm<sup>-1</sup> corresponding to C-O, 707 cm<sup>-1</sup> corresponding to C-S. <sup>1</sup>H NMR spectra of the final compound **3** showed the protons of methylene group of C<sub>16</sub> as triplet at δ=2.05-2.10 ppm, methylene groups of C<sub>17</sub> as triplet at δ=2.97-3.10 ppm, methylene group of C<sub>25</sub> as triplet at δ=3.28-3.01 ppm, methylene group of C<sub>19</sub>& C<sub>23</sub> as doublet of doublet at δ= 3.36 ppm, methylene group of C<sub>20</sub>& C<sub>22</sub> as doublet of doublet at δ= 3.53 ppm, methylene of C<sub>24</sub> as triplet at δ=3.80-3.82 ppm, methylene of C<sub>25</sub> as triplet at δ=4.23-4.26 ppm, hydrogen of phenothiazine group as multiplet at δ=7.5-7.8 ppm. While <sup>13</sup>C NMR spectra of compound **3** showed the carbon of methylene group (C<sub>16</sub>) at δ=21.7 ppm, methylene group (C<sub>15</sub>) at δ=43 ppm, methyl groups (C<sub>17</sub>) at δ=48.4 ppm, methylene group (C<sub>19</sub>) at δ=48.5 ppm, methylene group (C<sub>20</sub>) at δ=53.4 ppm, methylene group (C<sub>22</sub>) at δ=54.7 ppm, methylene group (C<sub>23</sub>) at δ=57.9 ppm, methylene group (C<sub>24</sub>) at δ=114.2 ppm, methylene group (C<sub>25</sub>) at δ=117.5 ppm, phenothiazine (C<sub>1</sub>, C<sub>11</sub>, C<sub>2</sub>, C<sub>6</sub>, C<sub>5</sub>, C<sub>12</sub>, C<sub>4</sub>, C<sub>3</sub>, C<sub>8</sub>, C<sub>9</sub>) at δ=119 ppm, 121.9 ppm, 122 ppm, 123.7 ppm, 124.5 ppm, 130.9 ppm, 131.8 ppm, 134.6 ppm, 136.6 ppm and 137 ppm respectively. Carbon of CF<sub>3</sub> at δ=138 ppm. Additionally the HPLC purity was found to be 99.056%.



**Fig 3: Atomic enumeration of compound 10-(3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5-oxide dihydrochloride.**

IR spectrum of the final compound **5** (**Fig. 4**) gave stretching vibrations at 3412 cm<sup>-1</sup> corresponding to hydroxyl group, 3005 cm<sup>-1</sup> corresponding to Ar C-H, 2960 cm<sup>-1</sup> corresponding to Aliphatic C-H, 1087-1051 cm<sup>-1</sup> corresponding to C-O, 1242 cm<sup>-1</sup> corresponding to (O=S=O). <sup>1</sup>H NMR spectra of the final compound **5** showed the protons of methylene group of C<sub>16</sub> as triplet at δ=2.24 -2.34 ppm, methylene groups of C<sub>17</sub> as triplet at δ=3.58 ppm, methylene group of C<sub>25</sub> as triplet at δ=3.61 ppm, methylene group of C<sub>19</sub>& C<sub>23</sub> as doublet of doublet at δ= 3.71 -3.93 ppm, methylene group of C<sub>20</sub>& C<sub>22</sub> as doublet of doublet at δ=4.06-4.12 ppm, methylene of C<sub>24</sub> as triplet at δ=4.30-4.37 ppm, methylene of C<sub>25</sub> as triplet at δ=4.39-4.78 ppm, hydrogen of phenothiazine group as multiplet at δ=7.31-7.77 ppm. While <sup>13</sup>C NMR spectra of compound **5** showed the carbon of methylene group (C<sub>16</sub>) at δ=19.6 ppm, methylene group (C<sub>15</sub>) at δ=43.5 ppm, methyl groups (C<sub>17</sub>) at δ=54.5 ppm, methylene group (C<sub>19</sub>) at δ=56.8 ppm, methylene group (C<sub>20</sub>) at δ=57.5 ppm, methylene group (C<sub>22</sub>)

at  $\delta=65.7$  ppm, methylene group ( $C_{23}$ ) at  $\delta=70.41$  ppm, methylene group ( $C_{24}$ ) at  $\delta=114.7$  ppm, methylene group ( $C_{25}$ ) at  $\delta=117.7$  ppm, phenothiazine ( $C_1, C_{11}, C_2, C_6, C_5, C_{14}, C_{12}, C_4, C_3, C_8, C_9$ ) at  $\delta=119.2$  ppm, 122 ppm, 122.9 ppm, 123.8 ppm, 123.9 ppm, 124.8 ppm, 131 ppm, 131.8 ppm, 134.7 ppm, 137.5 ppm and 138.1 ppm respectively. Carbon of  $CF_3$  at  $\delta=140.7$  ppm. Additionally the HPLC purity was found to be 88.377%.



**Fig 4: Atomic enumeration of compound 10-(3-(4-(2-hydroxyethyl)propyl)-2-(trifluoromethyl)-10H-phenothiazine-5,5-dioxide dihydrochloride**

The outcome of haloperidol induced catalepsy showed that the test compound possessed antipsychotic activity. Here, haloperidol was used to induce the catalepsy, therefore, no standard was used to compare with test compounds. Rather the results obtained from test compounds were compared to haloperidol control itself. From the data (**Table 1**), on Day 1, none of the test compounds show significant effect on haloperidol induced catalepsy. While on day 7, F-1 (5) showed decrease in cataleptic scores a when compared to haloperidol control and was non-statistically significant, while F-1 (10) showed increase in cataleptic scores a when compared to haloperidol control and was also non-statistically significant. F-2 (5) and F-2 (10) both showed decrease in cataleptic scores a when compared to haloperidol and was statistically significant ( $P < 0.01$ ) at 1hr.

The results obtained from invitro anti-inflammatory test showed that the test drug doesn't possess any anti-inflammatory activity (**Table 2**). It is clear that none of the test compounds showed effective inhibition of protein denaturation as compared to standard diclofenac sodium which produced 41.02% (25  $\mu$ g/mL) inhibition of protein denaturation. Denaturation of protein is well documented cause of inflammation.

The outcome of antibacterial test by serial dilution method demonstrates that the test drug possesses noteworthy inhibition of bacterial growth. The test was carried out on *E.coli*, *Staph.aureus*, *P.aureginosa* and *K.pneumoniae* strains of bacteria. From the data (**Table 3**), Cefotaxime showed 16.65% (100  $\mu$ g/mL), 08.4% (1000  $\mu$ g/mL), 39.7% (1000  $\mu$ g/mL) and 62.5% (1  $\mu$ g/mL) of bacterial growth against *E.coli*, *S.aureus*, *P.aeriginosa*, *K.pneumoniae*, respectively. Test compound F-1 and F-2 didn't show any inhibition of bacterial growth against *E.coli* when compared to standard. F-1 showed 14.0% (1000  $\mu$ g/mL), 43.8% (100  $\mu$ g/mL) and 63.5% (10  $\mu$ g/mL) against *S.aureus*, *P.aeriginosa*, *K.pneumoniae*, respectively. While, F-2 showed 17.5% (100  $\mu$ g/mL), 13.5% (100  $\mu$ g/mL) and 60.7% (100  $\mu$ g/mL) against *S.aureus*, *P.aeriginosa*, *K.pneumoniae*, respectively.

#### IV CONCLUSION

Novel compounds of phenothiazine nucleus were synthesised. All the compounds were characterized by IR,  $^1H$  NMR and  $^{13}C$  NMR and purity of final compound was determined by HPLC.

The synthesized compound was screened for in-vivo antipsychotic, invitro anti-inflammatory and antibacterial activities and the activities were compared with standard.

Compound F-1 (5 mg/kg) showed non-significant decrease in antipsychotic behaviour while F-2 (5, 10 mg/kg) showed significant ( $P < 0.01$ ) decrease in antipsychotic behaviour compared to haloperidol when tested for antipsychotic effect by Haloperidol induced catalepsy test.

Test compound did not showed effective inhibition of protein denaturation when tested for anti-inflammatory activity using inhibition of protein denaturation method.

Test compound F-1 showed noteworthy antibacterial activity against *S.aureus* and *K.pneumoniae*. F-2 showed noteworthy antibacterial activity against *S.aureus*, *P.aureginosa* and *K.pneumoniae*. Whereas, both the test compounds didn't show any effect on *E.coli* bacterial strain.



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### REFERENCES

- [1]. Psychosis: Symptoms, Causes and Risk factors: July 2020. Cited from <https://www.healthline.com/health/psychosis>.
- [2]. Introduction to Psychosis: July 2020. Cited from <https://en.wikipedia.org/wiki/Psychosis#Causes>.
- [3]. R. Srinivasa Murthy. National Mental Health Survey of India 2015–2016. *Indian Journal of Psychiatr.* Jan-Mar **2017**;59(1):21–26.
- [4]. Suresh Bada Math and Ravindra Srinivasaraju. Indian Psychiatric epidemiological studies: Learning from the past. *Indian Journal of Pyschiatry* Jan 2010;52(Suppl1):S95–S103.
- [5]. Fluphenazine-Uses, Side effects and Warnings: Aug 2020. Cited from <https://www.drugs.com/mtm/fluphenazine.html>.
- [6]. Introduction to Phenothiazine: Aug 2020. Cited from <https://en.wikipedia.org/wiki/Phenothiazine>.
- [7]. Kazimier. A. J, Piotr. S, Wieslaw. M, Katarzyna. C. B, Joanna. P, Boguslawaa.. C. M. Chemistry of phenothiazines: *Pharmacological Report* 2012;64:16-23.
- [8]. Alok. M, Sen. D, Das. J and Ghosh. P. Newer synthesis of phenothiazines: *J Pharm and Sci Inn* April 2012;1(2):41-42.
- [9]. Walode. S, Somani. R, Chandan R. S. Medicinal Chemistry – I 1<sup>st</sup> Ed. Pune: Nirali Prakashan;2019:4.25-4.27.
- [10]. Brunton. L, Lazo. J, Parker. K. Goodman & Gliman's: The Pharmacological Basics of Therapeutics 11<sup>th</sup> Ed. New York: McGraw – Hill Medical Publishing Division;2006:462-466.
- [11]. Alok. M, Sen. D, Das. J and Ghosh. P. Newer synthesis of phenothiazines: *J Pharm and Sci Inn* April 2012;1(2):41-42.
- [12]. Kamdi. A. S, Kokane. S, Bohra. P. N, Kalambe. S. M. The antipsychotic activity of alcoholic extract of *Whitiana coagulans* fruits in swiss albino mice in haloperidol induced catalepsy. *National Journal of Physiology, Pharmacy and Pharmacology* 2018;8(12):1654-1656.
- [13]. Leelaprakash. G, Dass. S. M. *In-vitro* anti-inflammatory activity of methanol extract of *Enicastermmaaxillare*. *International Journal of Drug Development and Research* 2011 July-Sept;3(3):189-196.

Hasti. Kenia, et. al. "Synthesis and Evaluation of Phenothiazine Derivatives." *IOSR Journal of Pharmacy (IOSRPHR)*, 10(9), 2020, pp. 54-62.