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Synthesis, Characterization and In-vitro Anti-Inflammatory Activity of Phenothiazine Derivatives

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ABSTRACT

Phenothiazine is an organic compound and it is related to the thiazine class of heterocyclic compounds. A novel series of Phenothiazine derivatives were prepared by the reaction of Para amino benzoic acid with 3 different substituted anilines. All the compounds were characterized by melting point, UV, TLC, IR and C,H,N elemental analysis. The synthesis of 3 derivative compounds were prepared, those are 3 derivatives of substituted phenothiazine were prepared in scheme 1 from p-chloro benzoic acid aniline derivatives. All the compounds were structurally elucidated with physical and analytical methods. All the compounds evaluated with invitro anti inflammatory activity by protein denaturation method. The synthesized derivatives were screened for invitro anti-inflammatory activity. 5C Compound showed significant effect at 200-1000 µg/ml.

Keywords: Heterocyclic compounds, Phenothiazine derivatives

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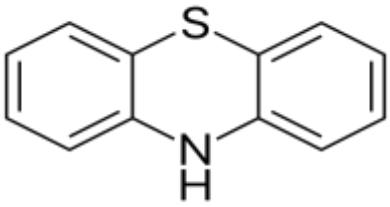
INTRODUCTION

Phenothiazine, abbreviated PTZ, is an organic compound that has the formula S $(C_6H_4)_2NH$ and is related to the thiazine-class of heterocyclic compounds. Although the parent compound has no uses, derivatives of phenothiazine are highly bioactive and have widespread use and rich history. The derivative chlorpromazine revolutionized the field of psychiatry and allergy treatment. An earlier derivative, ethylene blue, was one of the first antimalarial drugs, and derivatives are under investigation as possible anti-infective drugs. Phenothiazine is a prototypical pharmaceutical lead structure in medicinal chemistry[1-4].



Phenothiazine itself is only of theoretical interest, but its derivatives revolutionized psychiatry, other fields of medicine, and pest management. Other derivatives have been studied for possible use in advanced batteries and fuel cells.

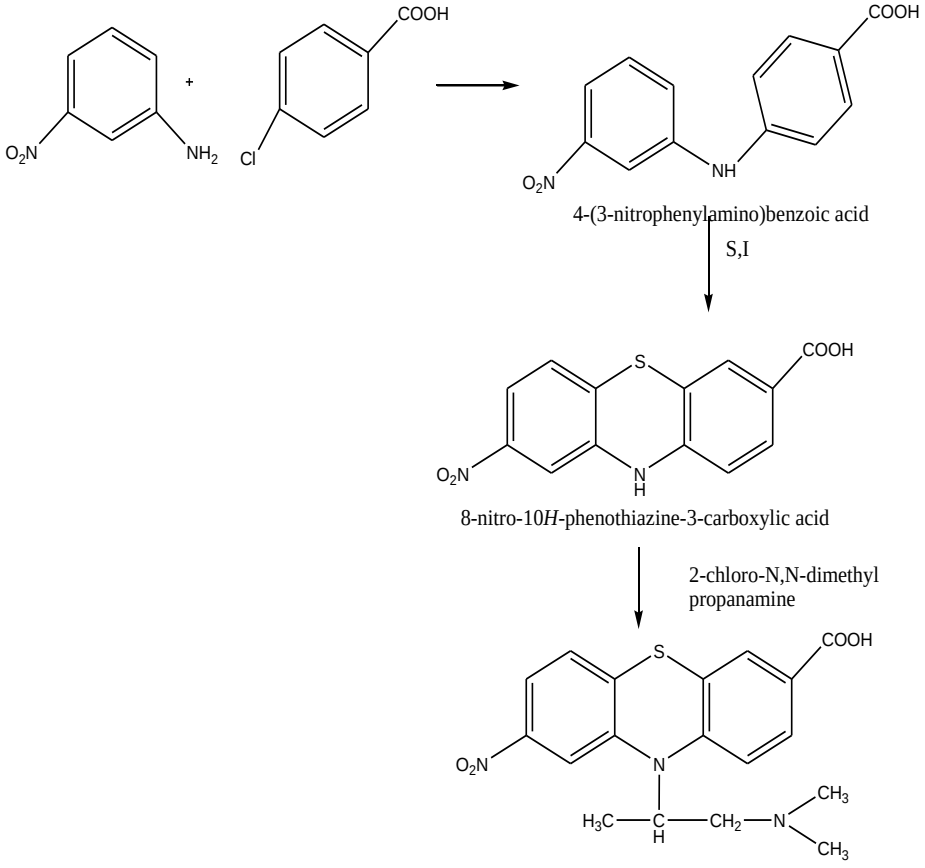
Phenothiazine derived drugs

In 1876, methylene blue, a derivative of phenothiazine, was synthesized by Heinrich Caro at BASF. The structure was deduced in 1885 by Heinrich August Bernthsen. Bernthsen synthesized phenothiazine in 1883. In the mid 1880s, Paul Ehrlich began to use methylene blue in his cell staining experiments that led to pioneering discoveries about different cell types. He was awarded a Nobel Prize based in part on that work. He became particularly interested in its use to stain bacteria and parasites such as *Plasmodiidae* – the genus that includes the malaria pathogen and found that it could be stained with methylene blue. He thought methylene blue could possibly be used in the treatment of malaria, tested it clinically, and by the 1890s methylene blue was being used for that purpose. For the next several decades, research on derivatives lapsed until phenothiazine itself came to market as an insecticide and deworming drug. In the 1940s, chemists working with Paul Charpentier at Rhone-Poulenc Laboratories in Paris (a precursor company to Sanofi), began making derivatives. This work led to promethazine which had no activity against infective organisms, but did have good antihistamine activity, with a strong sedative effect. It went to market as a drug for allergies and for anesthesia. As of 2012 it was still on the market. At the end of the 1940s the same lab produced chlorpromazine which had an even stronger sedative and soothing effect, and Jean Delay and Pierre Deniker attempted to use it on their psychiatric patients,

publishing their results in the early 1950s. The strong effects they found opened the door of the modern field of psychiatry and led to a proliferation of work on phenothiazine derivatives. The systematic research conducted by chemists to explore phenothiazine derivatives and their activity was a pioneering example of medicinal chemistry. Phenothiazine is often discussed as a prototypical example of a pharmaceutical lead structure[5-10]. The term phenothiazines describes the largest of the five main classes of antipsychotic drugs. These drugs have antipsychotic and, often, antiemetic properties, although they may also cause severe side effects such as extra pyramidal symptoms (including akathisia and tardive dyskinesia), hyperprolactinaemia, and the rare but potentially fatal neuroleptic malignant syndrome, as well as substantial weight gain. Use of phenothiazines has been associated with antiphospholipid syndrome, but no causal relationship has been established[11-15]. Phenothiazine antipsychotics are classified into three groups that differ with respect to the substituent on nitrogen: the aliphatic compounds (bearing acyclic groups), the "piperidines" (bearing piperidine-derived groups), and the piperazine[16-19].

MATERIALS AND METHODS

3-nitro aniline, p- chlorobenzoic acid, potassium carbonate, copper wire, N-dimethyl formamide.



Scheme - I

Preparation of 4-(3-nitrophenylamino) benzoic acid

A mixture of 3-nitro aniline (14.3 gm,0.1mol), p-chlorobenzoic acid (15.6gm, 0.1mol), potassium carbonate (1.37gm,0.01mol) and copper wire(0.5gm) were placed in a 30 ml of N, N-dimethyl formamide (DMF) contained round bottom flask equipped with a mechanical stirrer. The reaction mixture was stirred with a mechanical stirrer for 30 min and temperature of the mixture kept at about 25^0C. The mixture was stirred and refluxed at 80^0C for 4 h on a water bath. It was cooled to room temperature and poured into 50ml of water. The precipitated solid was separated by a vacuum filter, washed with a small portion of cold water and recrystallized from ethanol.

Preparation of 8-nitro-10H-phenothiazine-3-carboxilic acid

An ethanolic solution of 4-(3- nitro phenyl amino) benzoic acid (2.13 gm, 0.01 mol), sulphur (3.2 gm, 0.1mol) and iodine (1.26 gm, 0.01 mol) have been refluxed gently for 3 h with occasional shaking. The solution was cooled in an ice bath. The separated crude product was filtered at the pump, washed with a small portion of cold water and recrystallized from ethanol.

Preparation of 10-(2-dimethyl amino) ethyl-8-nitro-10H-phenothiazine-3-carboxylic acid

A solution of 2-chloro N,N-dimethylpropanamine (4ml) and sodium hydride (2.1 g) in DMF was added drop wise with a dropper during 15min and the reaction mixture was stirred. Then the mixture was refluxed for 3hrs on a boiling water bath with occasional shaking.

Preparation of 9-Benzoyl-10-(2-dimethyl amino-1-methyl-ethyl)-10H-phenothiazine-3-carboxylic acid

Preparation of 4-(2-benzoyl-phenylamino)-benzoic acid

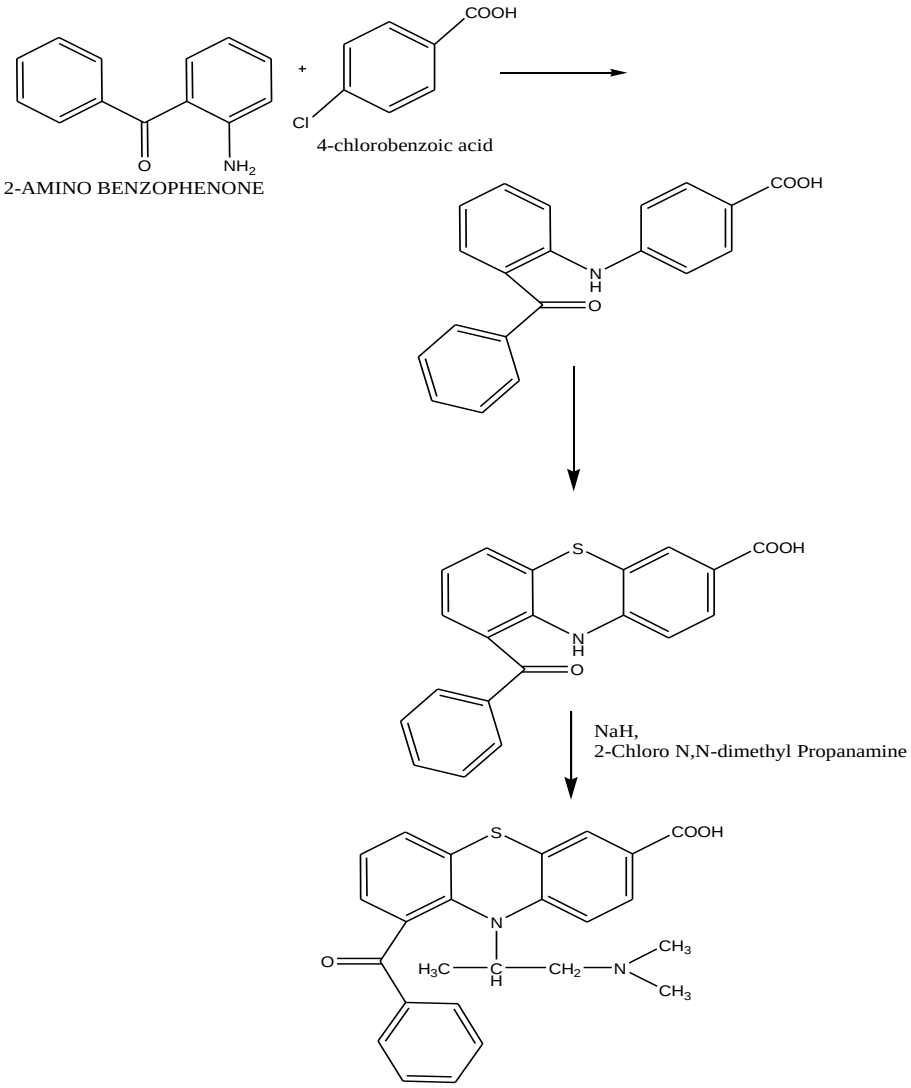
A mixture of 2-amino benzophenone (14.3 gm,0.1mol), p-chlorobenzoic acid (15.6gm, 0.1mol), potassium carbonate(1.37gm,0.01mol) and copper wire(0.5gm) were placed in a 30 ml of N, N-dimethyl formamide (DMF) contained round bottom flask equipped with a mechanical stirrer. The reaction mixture was stirred with a mechanical stirrer for 30min and temperature of the mixture kept at about 250C. The mixture was stirred and refluxed at 800C for 4 h on a water bath. It was cooled to room temperature and poured into 50ml of water. The precipitated solid was separated by a vacuum filter, washed with a small portion of cold water and recrystallized from ethanol.

Preparation of 9-Benzoyl-10H-phenothiazine – 3 - carboxylic acid

An ethanolic solution of 4-(2-phenyl keto-4- phenyl amino) benzoic acid (2.13 gm, 0.01 mol), sulphur (3.2 gm, 0.1 mol) and iodine (1.26 gm, 0.01 mol) have been refluxed gently for 3 h with occasional shaking. The solution was cooled in an ice bath. The separated crude product was filtered at the pump, washed with a small portion of coldwater and recrystallized from ethanol.

Preparation of 9-Benzoyl-10-(2-dimethyl amino-1-methyl-ethyl)-10H-phenothiazine-3-carboxylic acid.

The obtained intermediate fastly undergo reaction in the presence of sodium hydride,2 -chloro N,N-dimethyl Propamine and It was cooled to room temperature and poured into 50ml of water. The precipitated solid was separated by a vacuum filter, washed with a small portion of cold water and recrystallized from ethanol.



Scheme - II

Preparation of 4-(2-benzoyl-4-Chloro-phenylamino)-benzoic acid [18]

A mixture of (2-amino phenyl)(phenyl) methanone (14.3 gm,0.1mol), p-chlorobenzoic acid (15.6gm, 0.1mol), potassium carbonate(1.37gm,0.01mol) and copper wire(0.5gm) were placed in a 30 ml of N, N-dimethyl formamide (DMF) contained round bottom flask equipped with a mechanical stirrer. The reaction mixture was stirred with a mechanical stirrer for 30min and temperature of the mixture kept at about 250C. The mixture was stirred and refluxed at 800C for4

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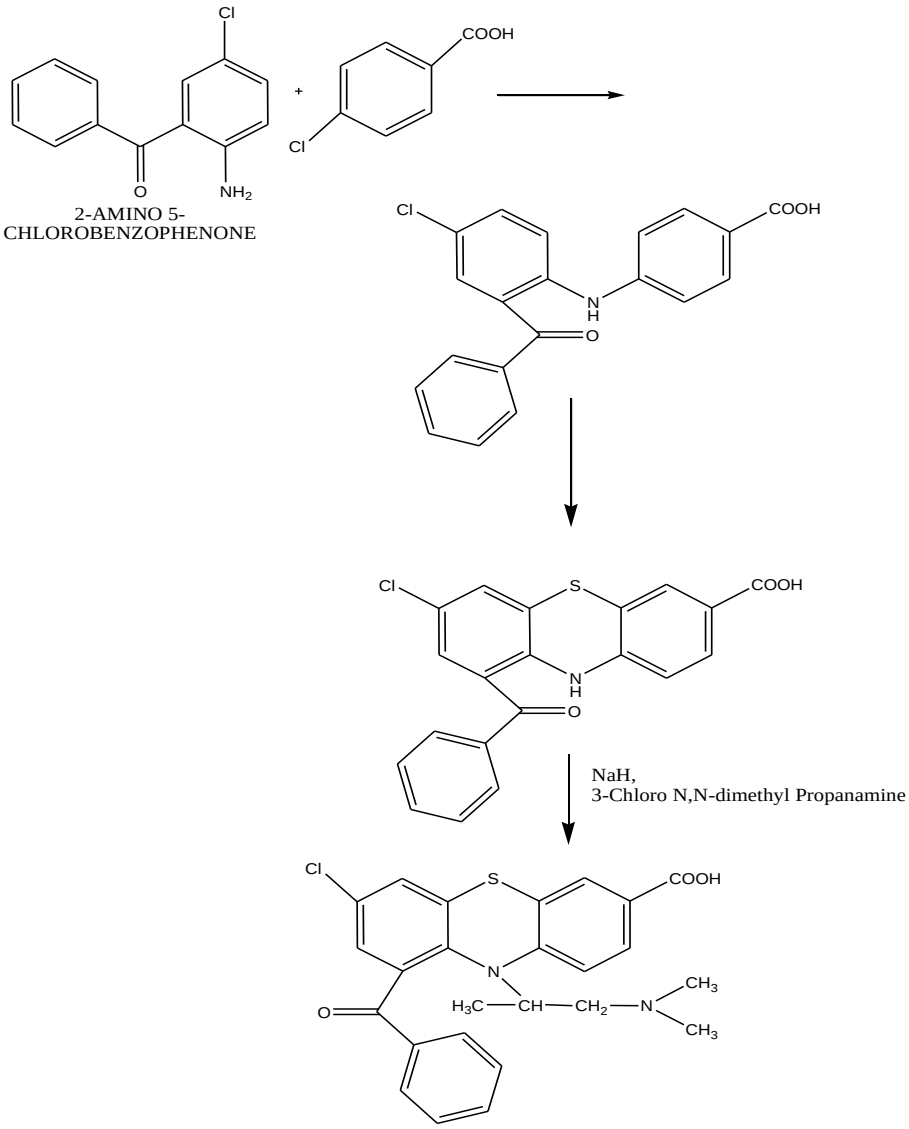

Scheme – III

Preparation of 9 -Benzoyl-7-Chloro-10H-phenothiazine-3-carboxylic acid[20,21]

An ethanolic solution of 4-(2-phenyl keto-4- phenyl amino) benzoic acid (2.13 gm, 0.01 mol), sulphur (3.2 gm, 0.1 mol) and iodine (1.26 gm,0.01 mol) have been refluxed gently for 3 h with occasional shaking. The solution was cooled in an ice bath. The separated crude product was filtered at the pump, washed with a small portion of coldwater and recrystallized from ethanol.

Preparation of 9-Benzoyl-7-Chloro-10-(2-dimethyl amino-1-methyl-ethyl)-10H-phenothiazine-3-carboxylic acid

The obtained intermediate fastly undergo reaction in the presence of sodium hydride,2 –chloro-N,N-dimethyl Propamine and it was cooled to room temperature and poured into 50ml of water. The precipitated solid was separated by a vacuum filter, washed with a small portion of cold water and recrystallized from ethanol.

RESULTS AND DISCUSSION

A total of 3 derivatives of Synthesized Phenothiazine compounds were recrystallized with appropriate solvents and all the compounds were identified and characterized by Physical and Spectral methods, the results were shown in Table 1 and Table.2**.**

Table 1: Physical Characterization Data of all the Synthesized Derivative Compounds

Compound Code	Mol. Formula	Mol. Weight (g/mole)	Melting Point(^{0}C)	% Yield w/w	R_f
3-N	$C_{18}H_{19}N_3O_4S$	373	110-115^{0}C	77.17% w/w	0.71
2-A	$C_{25}H_{24}N_2O_3S$	432	125-130^{0}C	82.72% w/w	0.96
5-C	$C_{25}H_{23}ON_2O_3SCl$	466	170-175^{0}C	72.67% w/w	0.86

***Ethyl Acetate: Benzene 4:1**

Table 2: Spectral analysis data of all synthesized compounds

Code	Mol. Formula	Spectral data's
3-N	$C_{18}H_{19}N_3O_4S$	**IR ν_{max} in ATR (cm^{-1}):** 2956.39 (Ar-CH;str),1518.72 (ArC=C ; Str), 1656(C=O;str), 1038.63(C-N; str) 729.83(Ar-CH,Bend).
2-A	$C_{25}H_{24}N_2O_3S$	**IR ν_{max} in ATR (cm^{-1}):** 2929.39(Ar-CH;str),1455.72 (ArC=C ; Str),718.83(Ar-CH,Bend), 1616(C=O;str),1372.63(C-N; str).
5-C	$C_{25}H_{23}ON_2O_3SCl$	**IR ν_{max} in ATR (cm^{-1}):** 2919.61(Ar-CH;str), 1416.78 (ArC=C ; Str),1609(C=O;str),1239.63(C-N; str).681.86(Ar-CH,Bend).

BIOLOGICAL ACTIVITY OF ALL SYNTHESISED DERIVATIVE COMPOUNDS

In vitro Anti -inflammatory activity of synthesized derivative compounds

Compounds of 3 derivatives synthesized in scheme 1 were screened for in vitro anti-inflammatory activity at concentrations 200,400,600 and 1000 µg/ml respectively, by Inhibition of Protein Denaturation test method using Diclofenac sodium as standard. All the derivative compounds were shown significant activity when compared with control and all the compounds were shown less active when compared with standard. So all the compounds were shown dose dependent activity. The IC_{50} values for *invitro* anti inflammatory activity of all compounds results were shown high than standard IC_{50} of 0.78. The results were shown in Tab.3 and Fig.1.

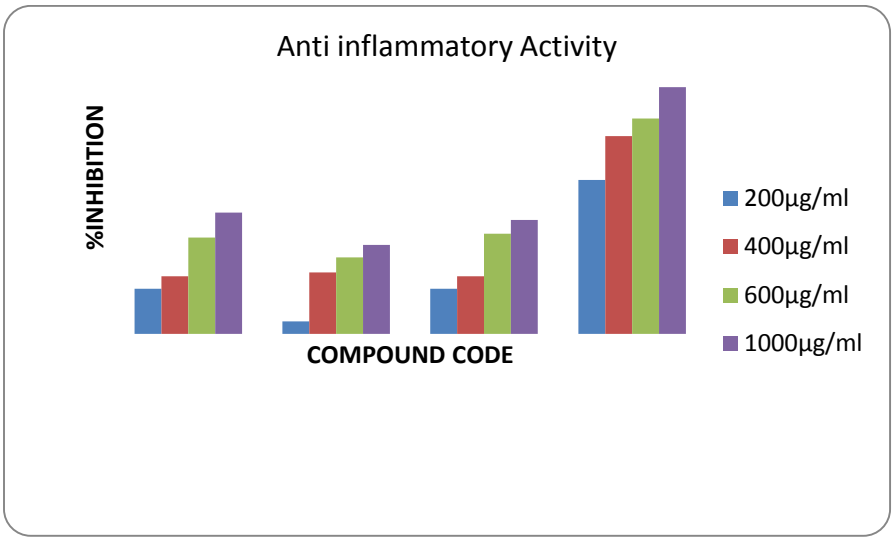

Figure 1: % Inhibition of anti-inflammatory activity of Phenothiazine derivatives

Table 3: % Inhibition values of synthesized derivatives

Compound Code	% Inhibitions 200µg/ml	500µg/ml	1000µg/ml	2000µg/ml	IC_{50}
3-N	36.92±0.27	46.76±0.34	77.26±0.52	97.78±0.45	1.81
2-A	10.67±0.76	49.21±0.78	61.76±0.23	71.63±0.22	1.79
5-C	36.87±0.39	46.73±0.45	80.27±0.70	91.23±0.62	1.74
Standard	123.38±0.72	158.06±0.56	172.5±0.37	197.5±0.67	0.78

CONCLUSION

The synthesis of 3 derivative compounds were prepared, those are 3 derivatives of substituted phenothiazine were prepared in scheme 1 from p-chloro benzoic acid aniline derivatives. All the compounds were structurally elucidated with physical and analytical methods. All the compounds evaluated with *invitro* anti inflammatory activity by protein denaturation method. In that some synthesized derivative compounds possess moderate to promising activity compared with standard and all were shown dose dependent activity. The future studies of synthesized Phenothiazine derivatives need to develop QSAR methods and to bring potential effects.

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