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

V. Jhansi Rani*, Konda Ravi Kumar¹, Y. Naga Surekha²

¹Department of Pharmaceutical Chemistry, Hindu college of Pharmacy, Amaravathi road, Guntur. A.P.
²Department of Pharmaceutics, Hindu college of Pharmacy, Amaravathi road, Guntur. A.P.

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 invtro anti inflammatory activity by protein denaturation method. The synthesized derivatives were screened for invtro anti-inflammatory activity. 5C Compound showed significant effect at 200-1000 µg/ml.

Keywords: Heterocyclic compounds, Phenothiazine derivatives

*Corresponding Author Email: jhansirani.vasa@gmail.com
Received 22 November, Accepted 13 December 2019
INTRODUCTION

Phenothiazine, abbreviated PTZ, is an organic compound that has the formula S{(C₆H₄)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 chemistry¹⁻⁴.

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\textsuperscript{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\textsuperscript{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\textsuperscript{16-19}.

**MATERIALS AND METHODS**

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

![Scheme - I](image-url)
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°C. The mixture was stirred and refluxed at 80°C 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-carboxylic 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-Benzyol-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-Benzyol-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 Propanamine and 4-chlorobenzoic acid. 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 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
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.

![Scheme III](image)

**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.

| Compound Code | Mol. Formula | Mol. Weight (g/mole) | Melting Point(°C) | % Yield w/w | Rf |
|---------------|--------------|----------------------|-------------------|-------------|----|
| 3-N           | C₁₈H₁₉N₃O₄S | 373                  | 110-115°C         | 77.17% w/w  | 0.71|
| 2-A           | C₂₅H₂₄N₂O₃S | 432                  | 125-130°C         | 82.72% w/w  | 0.96|
| 5-C           | C₂₅H₂₃ON₂O₃SCl | 466                | 170-175°C         | 72.67% w/w  | 0.86|

*Ethyl Acetate: Benzene 4:1

| Code | Mol. Formula | Spectral data’s |
|------|--------------|-----------------|
| 3-N  | C₁₈H₁₉N₃O₄S | IR $\nu_{max}$ in ATR (cm⁻¹): 2956.39 (Ar-CH;str), 1518.72 (Ar-C=O; str), 1656(C=O;str), 1038.63(C-N; str) 729.83(Ar-CH, Bend). |
| 2-A  | C₂₅H₂₄N₂O₃S | IR $\nu_{max}$ in ATR (cm⁻¹): 2929.39(Ar-CH;str), 1455.72 (Ar-C=O; Str), 718.83(Ar-CH,Bend), 1616(C=O;str), 1372.63(C-N; str). |
| 5-C  | C₂₅H₂₃ON₂O₃SCl | IR $\nu_{max}$ in ATR (cm⁻¹): 2919.61(Ar-CH;str), 1416.78 (Ar-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 IC₅₀ values for invitro anti inflammatory activity of all compounds results were shown high than standard IC₅₀ 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 | % Inhibition 200μg/ml | % Inhibition 500μg/ml | % Inhibition 1000μg/ml | % Inhibition 2000μg/ml | IC₅₀ |
|---------------|------------------------|-----------------------|------------------------|------------------------|------|
| 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.

REFERENCES

1. Tanaji N. Bansode, Jayant V. Shelke, Vaijanath G. Dongre., Synthesis and antimicrobial activity of some new N-acyl substituted phenothiazines., Vol.44 (2009) p. 5094–5098.
2. Y.S. Sadanandam, Meera M. Shetty, A. Bhaskar Rao*, Y. Rambabu., 10H-Phenothiazines: A new class of enzyme inhibitors for inflammatory diseases, vol. 44 (2009) p.197-202.
3. Krystian Pluta , Małgorzata Jeleń ; Beata Morak-Młodawska , Michał Zimecki.
4. Jolanta Artym, Maja Kocieba, Ewa Zaczyńska, Azaphenothiazines e promising phenothiazine derivatives. An insight into nomenclature, synthesis, structure elucidation and biological properties, vol. 138 (2017) p. 774-806.

5. Tanaji N. Bansode, Jayant V. Shelke, Vaijanath G. Dongre, Synthesis and antimicrobial activity of some new N-acyl substituted phenothiazines, vol. 44 (2009) p.5094–5098.

6. Pravas Kumar Baral, Mridula Swayampakula, Manoj Kumar Rout, Nat N.V. Kav, Leo Spyracopoulos, Adriano Aguzzi, and Michael N.G. James, http://dx.doi.org/10.1016/j.str.2013.11.009.

7. Dalila Belei a, Carmen Dumea a, Alexandrina Samson a, Amaury Farce b,c, Joëlle Dubois Elena Bicu a, Alina Ghinet., New farnesyltransferase inhibitors in the phenothiazine series, vol. 22 (2012) p.4517–4522.

8. Mostafa M. Ghorab, Mansour S. Alsaid, Nermin Samir, Ghada A. Abdel-Latif, Aiten M. Soliman, Fatma A. Ragab, Dalal A. Abou El Ella., Aromatase inhibitors and apoptotic inducers: Design, synthesis, anticancer activity and molecular modeling studies of novel phenothiazine derivatives carrying sulfonamide moiety as hybrid molecules., vol. 134 (2017) p.304-315.

9. Ana Marcu Uta Schurigt Klaus Müller, Heidrun Moll, R. Luise Krauth-Siegel, Helge Prinz., Inhibitory effect of phenothiazine- and phenoxazine-derived chloroacetamides on Leishmania major growth and Trypanosoma brucei trypanothione reductase, vol. 108 (2016) p. 436-443.

10. Suruchi Mahajan, Rakesh Kumar Mahajan., Interactions of phenothiazine drugs with surfactants: A detailed physicochemical overview, vol. 199–200 (2013) p.1–14.

11. Gina-Mirabela Dumitriu, Alina Ghinet, Elena Bicu, Benoit Rigo, Joëlle Dubois, Amaury Farce, Dalila Belei., Peptide chemistry applied to a new family of phenothiazine containing inhibitors of human farnesyl transferase, vol. 24 (2014) p. 3180–3185.

12. Gina-Mirabela Dumitriu, Elena Bicu, Dalila Belei, Benoît Rigo, Joëlle Dubois, Amaury Farce, Alina Ghinet, Phenothiazine-based CaaX competitive inhibitors of human farnesyltransferase bearing a cysteine, methionine, serine or valine moiety as a new family of antitumoral compounds, vol. 25 (2015) p.4447–4452.

13. Alina Ghinet, Iuliana-Monica Moise, Benoît Rigo, Germain Homerin, Amaury Farce Joëlle Dubois, Elena Bicu., Studies on phenothiazines: New microtubule-interacting compounds with phenothiazine A-ring as potent antineoplastic agents, vol. 24 (2016) p.2307–2317.
14. Sultan Darvesh, Ian R. Macdonald, Earl Martin., Selectivity of phenothiazine cholinesterase inhibitors for neurotransmitter systems, vol. 23 (2013) p.3822–3825.

15. Gabriela P. Sarmiento a,e, Roxana G. Vitale b,c,, Javier Afeltra c,d, Graciela Y. Moltrasio e, Albertina G. Moglioni., Synthesis and antifungal activity of some substituted phenothiazines and related compounds, vol. 46 (2011) p.101-105.

16. M. Hosseinnezhad a, K. Gharanjig a,c, S. Moradian b,c, S. Tafaghodi., Synthesis and application of some novel fluorescent heterocyclic disperse dyestuffs based on phenothiazine on polyester,2015.

17. Chandravadivelu Gopi , Vedula Girija Sastry , Magharla Dasaratha Dhanaraju., Synthesis, spectroscopic characterization, X-ray crystallography, structural activity relationship and antimicrobial activity of some novel 4-(5-(10-(3-N, N-dimethylamino)propyl)-10H-phenothiazine-3-yl)-1, 3, 4-thiadiazole-2-yl) Azo dye/Schiff base derivatives, vol. 3 (2017) p.79-89.

18. Katsumi Kubota, Hirotaka Kurebayashi, Hirotaka Miyachi, Masanori Tobe, Masako Onishi, Yoshiaki Isobe., Synthesis and structure–activity relationships of phenothiazine carboxylic acids having pyrimidine-dione as novel histamine H1 antagonists, vol. 19 (2009) p.2766–2771.

19. Moriz Mayer, P. Therese Lang, Sabina Gerber, Peter B. Madrid, Irene Go´mez Pinto, R. Kiplin Guy, and Thomas L. James., Synthesis and Testing of a Focused Phenothiazine Library for Binding to HIV-1 TAR RNA, vol. 13, p.993–1000.

20. B.K.Sharma Instrumental methods of Chemical Analysis, Goel publications, New Delhi 2007, 102-120.

21. Dr. Dushyanch Text book of Pharmaceutical Microbiology, B.S. Saha Prakashan publication, Ahmedabad, 2004 pp:65-78.

AJPTR is

• Peer-reviewed
• bimonthly
• Rapid publication

Submit your manuscript at: editor@ajptr.com