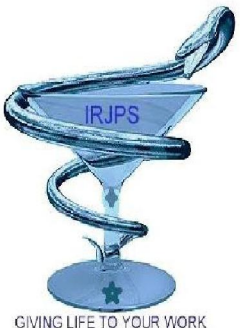
REVIEW




# RECENT PROGRESS IN BIOLOGICAL PROFILE OF SYNTHESIZED PHENOTHIAZINES

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**ABSTRACT**
This review summarizes recent medicinal chemistry investigations *in vitro* and *in vivo* in search for new phenothiazine derivatives with promising biological activities. The phenothiazines contain an interesting heterocyclic ring skeleton with two carbocyclic/ aromatic rings connected to each other via a sulfide and an imino bridge which facilitates several types of reactions, substitution at the nitrogen, electrophilic substitution on the aromatic rings, N-oxidation and photochemical reactions, etc[1-3]. Recently obtained phenothiazines exhibit promising antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, antimalarial, antifilarial, trypanocidal, anticonvulsant, analgesic, immunosuppressive and multidrug resistance reversal properties. These activities were the results of the actions of phenothiazines on biological systems via the interaction of the pharmacophoric substituent (in some cases of strict length), via the interaction of the multicyclic ring system (pep interaction, intercalation in DNA) and via the lipophilic character allowing the penetration through the biological membranes[4]. This review shows current tendency in the phenothiazine synthesis (without synthetic routes) and reveals the phenothiazine core to be very potent pharmacophoric moiety which can be a rich source of new compounds having desirable biological activities.

**KEYWORDS**: Phenothiazines, Antipsycotic, Anticancer, Antibacterial, Antifungal. Multi-drug resistance

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## 1. INTRODUCTION

Phenothiazines are heterocyclic molecules containing two benzene rings linked in a tricyclic system through nitrogen and sulfur atoms. The first known member of this class of compounds, the purple dye thionine, was synthesized by Lauth in 1876, followed by the synthesis of another dye, methylene blue, by Caro in 1877. The evidence that both thionine and methylene blue contained a phenothiazine tricycle was provided when the parent compound, 10H-dibenzo-1, 4-thiazine, was obtained the first time by Bernthsen in 1883[5,6] Since the late 19th century, many phenothiazine derivatives have been synthesized and have proven to be highly eclectic in their biological activities.[5,6] At least 100 phenothiazines were used in therapy mainly as neuroleptics. Recent reports deal with promising anticancer, antibacterial, antiplasmid, multidrug resistance (MDR) reversal activities and potential treatment in Alzheimer's and CreutzfeldteJakob diseases of classical phenothiazines[7-14]. Phenothiazines are relatively inexpensive, widely available, well tolerated and nontoxic compounds.

## 1. MODIFICATION OF PARENT COMPOUND

New derivatives of phenothiazines have been obtained by modifications of the parent phenothiazine structure in several ways by:

1. An introduction of a new substituent at the thiazine nitrogen atom (at position 10),

2. An introduction of a new substituent at the benzene ring carbon atom (at positions 1-4 and 6-9),

3. An oxidation of the sulfide function into sulfoxide and sulfone groups,

4. A substitution of one or two benzene rings with homoaromatic and heteroaromatic rings [15, 16].

The introduction of different substituents into the phenothiazine skeleton as well as the modification of the tricyclic ring system alter biological activities. Every year hundreds of new phenothiazine derivatives have been synthesized and a part of them has been biologically screened. The most significant contribution to the synthesis of phenothiazine and evaluation of their biological properties in last two decades was made by Noboru Motohashi and his international research groups that published about 60 papers and a few monograph chapters [7-11].

## 2. DEVELOPMENT OF PHENOTHIAZINES

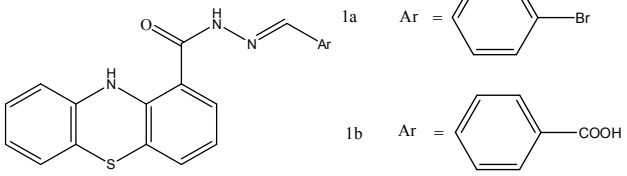
| ear | Development of Phenothiazines |
|---|---|
| 1876 | Synthesis of methylene blue by Caro. |
| 1883 | Synthesis of phenothiazine by Bernthsen. |
| 1891 | Paul Ehrlich stains plasmodia with methylene blue and explores it as a treament option for malaria. |
| 1930s | Discovery of the antifungal, insecticidal, and anthelmintic activities of phenothiazine. |
| 1940s | Global use of phenothiazine as anthelmintic in humans ("worm chocolate"). |
| 1947 | Synthesis of promethazine, discovery of its antihistaminic properties. |
| 1951 | Chlorpromazine, the first synthetic antipsychotic drug, is developed by Rhône-Poulenc laboratories. |
| 1955 | The widespread clinical use of chlorpromazine heralds a new paradigm of medicine: biological psychiatry. |
| 1990s | The antioxidant activities of certain phenothiazines are translated into biomedicine. |
| 2000s | Phenothiazines show multiple promising activites in protein aggregation disorders. |
| 2009 | Excitement about methylene blue in Alzheimer's disease. |

Timeline of the most important developments with regard to phenothiazine [17]

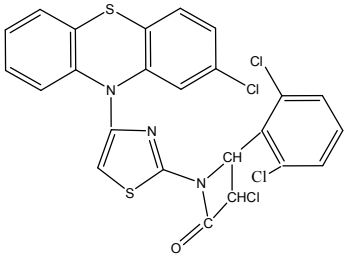
## 3. BIOLOGICAL PROFILE

**Analgesic activity**

Silva *et al*.[18] synthesised a new class of 10H-phenothiazine-1-acylhydrazone derivatives and acylhydrazide intermediates by the molecular hybridization approach between antipsychotic chlorpromazine and other heterocyclic derivatives. The change of para-substituent group of acylhydrazone framework permitted to identify hydrophilic carboxylate derivative and hydrophobic bromo derivative **(1a-b)** as two new leads of analgesics more active than dipyrone used as standard and with selective peripheral or central mechanism of action. Some new 2-chloro-10-[2-{3'-chloro-2'-oxo-4'-(substitutedphenyl)1'-azetidinyl} thiazol-4-yl] phenothiazines and 2-chloro-10-[2-{3'-chloro-2'-oxo-4'-(substitutedphenyl)1'-azetidinyl}oxazol-4-yl] phenothiazines have been synthesized from 2-chloro-10-[2-(substitutedphenyl) methylene aminothiazol-4-yl] phenothiazines and 2-chloro-10-[2-(substituted phenyl) methylene aminooxazol -4-yl] phenothiazines respectively and reported by Kushwaha *et al.*. The structures of all these compounds were established on the basis of elemental (C,H,N) and spectral (IR, $^1$HNMR and mass spectral data) analysis. Analgesic activity was performed by following the method of Berkowitz *et al*. [19]. This method is based on the property of the test compound to antagonize the phenyl quinone-induced pain syndrome in mice. Compound **(2)** 2-chloro-10-[2-{3'-chloro-2'-oxo-4'-(2, 6-dichlorophenyl) 1'-azetidinyl} thiazol-4-yl] phenothiazine showed good analgesic activity [20].




**(1a-b)**



**(2)**

**Phenothiazines showing analgesic activity**

**Antibacterial and antifungal activities**

Antibacterial and antifungal activities were found for all four types of modified phenothiazines. Most of the substituents were attached to the nitrogen atom and consist of various acyl groups and heterocycles linked directly or through alkyl chains.

A series of 2-substituted N-acylphenothiazines were synthesized by Bansode *et al* using imides, N-carboxymethyl imides and the structures of these newly synthesized compounds were confirmed by spectral and elemental analyses. All new compounds were tested for their antibacterial and antifungal activities. Some compounds **(3a-d)** showed promising antibacterial and antifungal activities against *Bacillius subtilis, E coli, Staphylicoccus aureus* (10 µg/ml) *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida albicans* respectively and found to be potent comparable to standard drug (10 µg/ml) .[21]

Several phenothiazines and related compounds were synthesized and their antifungal activity was evaluated *in vitro* by Sarmiento *et al*. With the aim to achieve a better approximation to the structure-activity relationship, some molecules with modifications at different levels of compound **(4)** were synthesized:

a. Isosteric replacement of sulfur by oxygen atom **(5)**.

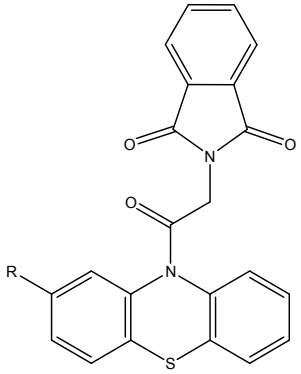
b. Opening of the phenothiazine system to obtain the corresponding dianiline or diphenylthioether **(6)**.

c. Contraction of the heterocyclic ring **(7)**.

The results obtained showed that none of these compounds inhibited the growth of the fungi tested. Replacement of the sulfur atom by oxygen or the elimination of the sulfur atom led to loss of activity. Thus concluded that the phenothiazine ring is necessary for antifungal activity. Compound (4) was found to be most potent in the antifungal activity.

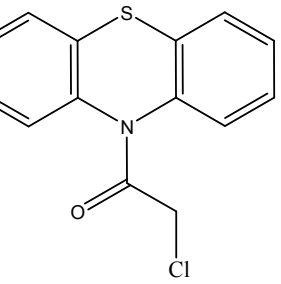
**S**ynthesis of 3, 7-Bis-[5'-aryl-isoxazol-3'-yl-acetamido]-phenothiazines was reported by Desai *et al.* . All the synthesized compounds were assayed for antimicrobial activity *B. subtillis, B. mega, E. coli, P. fluorescens* and *A. awamori* using DMF as a solvent at 50µg/mL concentration by cup-plate method. Compound **(8)** [3, 7-Bis-(5'-3, 4-dichlorobenzylisoxazol-3'-yl acetamido)-phenothiazine] was found to be most potent.

Sharma *et al*. synthesized of *N*-[2-(10*H*-phenothiazinyl)ethyl]-4-(phenyl)-3-chloro-2-oxo-1-iminoazetidine. The structures of all the newly synthesized compounds were confirmed by IR, $^1$H NMR, 13C NMR and FAB-Mass and chemical methods. All synthesized compounds were evaluated for their antibacterial, antifungal activities against *B. subtilis E. coli, S. aureus, K. pneumoniae, A. niger, A. flavus, F. oxisporium, C. albicans.* . Compounds **(9a-c)** were found to be more potent[23].

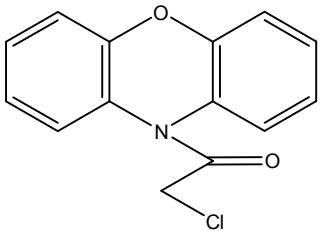


**(3a-d)**

R= a – H, b – Cl, c – $CF_3$, d – $COCH_3$



**(4)**



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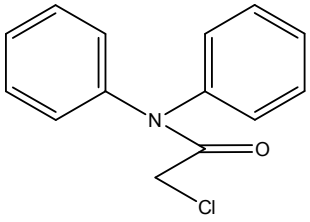

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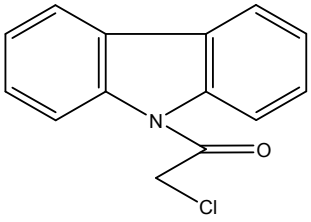

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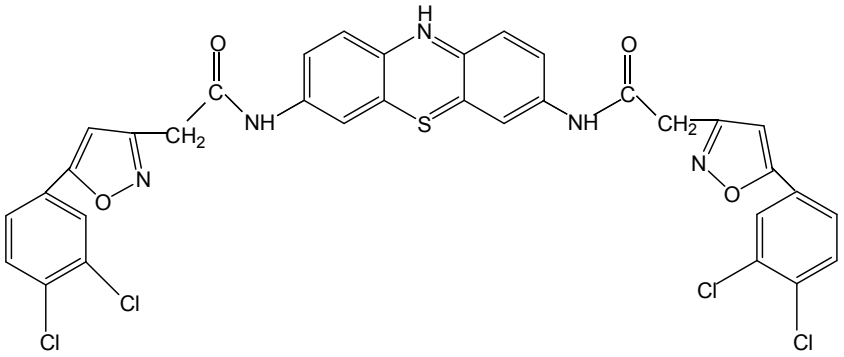
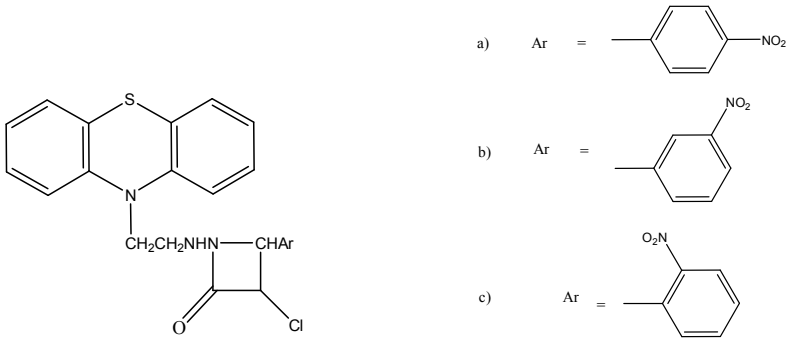


**(8)**

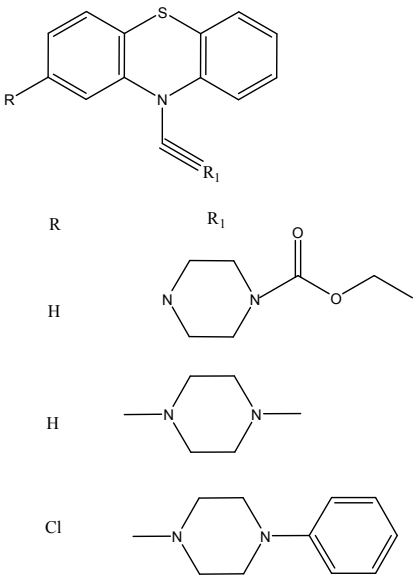


**(9a-c)**

**Phenothiazines showing antibacterial and antifungal activity**

**Anticancer activity and multidrug resistance**

A series of easily affordable phenothiazine derivatives bearing a rigid but-2-ynyl amino side chain were synthesized and tested by Bisi *et al*. to evaluate the MDR reverting activity and full antitumor profile. Some compounds endowed with remarkable MDR reverting effect were identified, and the most active one **(10a-c)** was shown to increase doxorubicin retention in multidrug resistant cells, suggesting a direct interaction with P-glycoprotein. Furthermore, a broad range of cellular activities were observed for different compounds. In particular, the ability of some derivatives to induce antiproliferative effects on resistant cell lines and to interfere with the G1 phase of the cell cycle, a phase usually not affected by classical antitumor agents, was noted. Moreover, the most cytotoxic compounds of the series were able to induce apoptosis in resistant cell lines, via an atypical pathway of caspase cascade activation, and a synergistic effect in combination with doxorubicin was also found [24].



**(10a-c)**

**Phenothiazines showing anticancer and multidrug resistance activity**

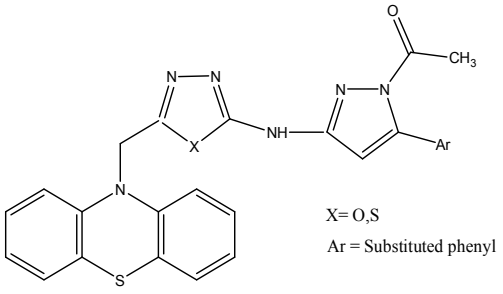
**Anti-inflammatroy activity**

Phenothiazines**(11)** with two azole (oxadiazole/thiadiazole and pyrazoline) rings in substituents in position 10 showed significant in vivo anti-inflammatory activity. The best results were found for two compounds with the o-methoxyphenyl group and X= O and S, showing not only more anti-inflammatory activity but also less ulcerogenic liability than the reference drug, phenylbutazone [25].

Some N-acyl and N-sulfonyl derivatives of 10-tetrazoloethylphenothiazines **(12)** exhibited good analgesic activity tested both by acetic writhing method and hot plate method, promising anti-inflammatory activity tested by carrageenin induced rat paw edema method. The best values were found for compounds with the p-chlorobenzoyl and p-nitrobenzoyl groups [26-27].

Benzene ring multisubstituted phenothiazines **(13)** with two methylcarbamoyl groups and substituted phenyl groups and also their 1,2-dihydro derivatives exhibited promising inhibitory activity ($IC_{50}$=13-15mM) toward regulating enzymes amplifying the inflammatory disorders such as phosphodiesterase, prostaglandine synthetase, g-glutamyltranspeptidase and superoxide dismutase, when compared to standard drug aspirin. Less lipophilic Scheme 5. Phenothiazines showing antiviral activity. compounds showed better activity [28].

A number of substituted phenothiazines were synthesized and screened for their biological activity against the regulatory enzymes involved in inflammatory diseases by Sadanandam *et al*. The newly synthesized compounds **(14a-c)** exhibited promising target specific enzyme inhibition against phosphodiesterase, prostaglandin dehydrogenase and superoxide dismutase activity depending on steric factors of the molecules [28].

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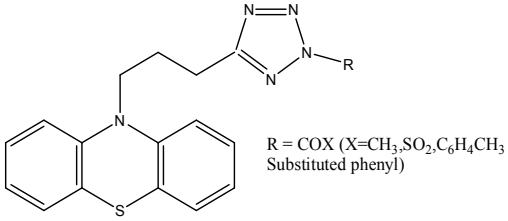


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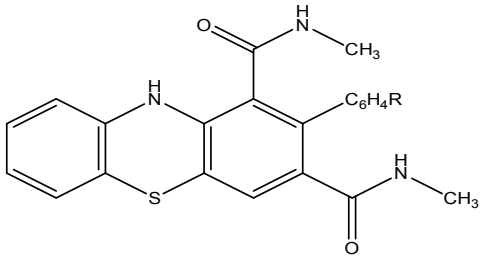
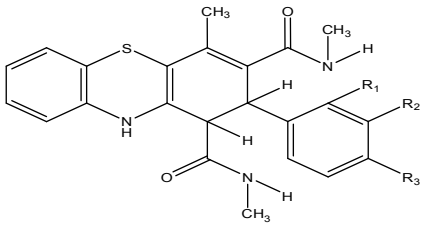



**(13)**



a- $R_1$ =$R_2$ =$R_3$=H

b- $R_1$ = $CH_3$, $R_2$ =$R_3$=H

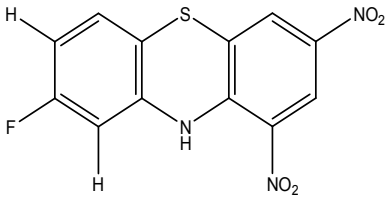
**(14a-c)**

c- $R_1$ = Cl, $R_2$ =$R_3$=H

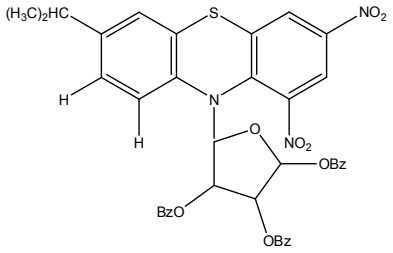
**Phenothiazines showing anti-inflammatory activity**

**Antioxidant activity**

Dixit *et al*. synthesise of some new 10H-phenothiazines, their sulfones and ribofuranosides by smiles rearrangement . These prepared phenothiazines are used as base to prepare ribofuranosides by treatment with β-Dribofuranosyl1-acetate-2,3,5-tribenzoate. 10H-Phenothiazines on refluxing with hydrogen peroxide in glacial acetic acid gave 10H-phenothiazine-5,5-dioxides. The synthesized compounds were screened for antioxidant activity [29]. Compounds **(15)** and **(16)** showed strong radical scavenging activity in DPPH assay that have DPPH% inhibition > 50.




**(15)**



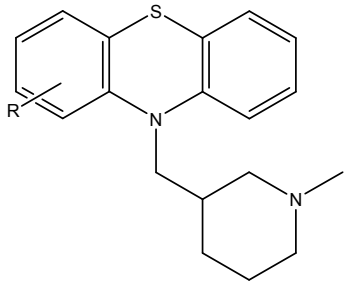

**(16)**

**Phenothiazines showing antioxidant activity**

**Antitubercular activity**

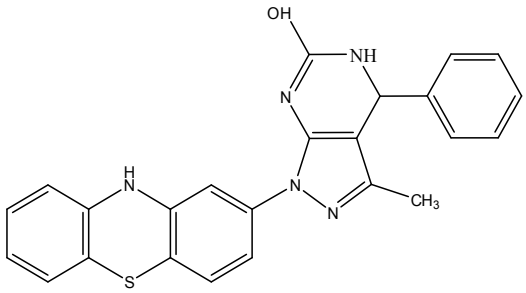
Analogs of the psychotropic phenothiazines were synthesized and examined as antitubercular agents against Mycobacterium tuberculosis H37Rv by Madrid *et al*. The microplate alamar blue assay (MABA) was used to assess the antitubercular activity of the phenothiazines, measured as the Minimum Inhibitory Concentration (MIC) against Mtb strain H37Rv. Two compounds with phenyl substitutions, **(17a)** and **(17b)**, were most potent, with MICs of 4.5 and 2.1 lg/mL, respectively[30].

Trivedi *et al.* synthesise some novel 2-heterocycle-substituted phenothiazines having a pyrazolo[3,4-*d*]pyrimidine nucleus synthesized by using the Biginelli multi-component cyclocondensation reaction. The products were evaluated for their antitubercular activity against *Mycobacterium tuberculosis* H37 Rv. Compounds **(18a-b)**were found to be more potent in antitubercular activities [31].




a) R=2-Ph, b) R=3-Ph

**(17a-b)**




a) 4-OH, b) 4-Cl

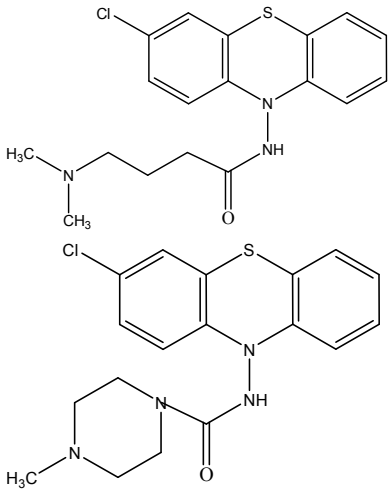
**(18a-b)**

**Phenothiazines showing antitubercular activity**

**Cholinesterases and butyryl cholinesterase Inhibitors activity**

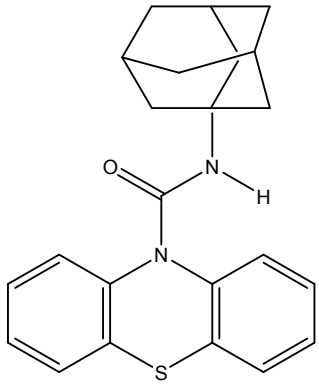
N-alkylphenothiazine, the N-acylaminophenothiazine, and the 1,4,5-dibenzo[b,f]thiadiazepine, protected human neuroblastoma cells against oxidative stress generated by both exogenous and mitochondrial free radicals. They could also penetrate the CNS, according to an in vitro blood-brain barrier model, and an N-acylaminophenothiazine derivative behaved as a selective inhibitor of butyrylcholinesterase. Due to their excellent pharmacological properties, N-acylaminophenothiazine have been selected to develop new series that are currently in progress[32]. The remarkable selectivity towards BuChE showed by the N-acylaminophenothiazines **(19)** and **(20)** could be of great importance in the development of new anti-AD therapies.

A series of N-10 urea derivatives of phenothiazine was synthesized and was evaluated for its ability to inhibit human cholinesterases by Darvesh *et al*. Most were specific inhibitors of BuChE but compound **(21)** was most potent among all the synthesized compounds. The comparative effects of aminoureas on wild-type BuChE and several BuChE mutants indicate a binding process involving salt linkage with the aspartate of the cholinesterase peripheral anionic site. The effect of such compounds on cholinesterase activity at high substrate concentration supports ionic interaction of aminoureas at the peripheral anionic site[33].



**(19)**

**(20)**



**(21)**

**Phenothiazines showing Cholinesterases and butyrylcholinesterase Inhibitors activity**

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