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Invited review

Recent progress in biological activities of synthesized phenothiazines

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ABSTRACT

This review summarizes recent medicinal chemistry investigations *in vitro* and *in vivo* in search for new phenothiazines of promising biological activities. New phenothiazine derivatives (over 50 main structures) contain dialkylaminoalkyl, cycloaminoalkyl and aminoalkyl substituents and their acyl and sulfonyl derivatives, and other substituents with varied the monocyclic (pyrazole, thiazole, oxadiazole, thiadiazole, tetrazole) and bicyclic (quinolizine, pyrazolopyrimidine, thiazolopyridine, azabicyclononane and spiro[chromanpyrimidine] heterocycles linked directly or via the alkyl chain with the thiazine nitrogen atom or with the benzene ring. The modifications of the tricyclic ring system with the bicyclic homoaromatic ring (naphthalene) and monocyclic and bicyclic azine rings (pyridine, pyrimidine, pyrazine and quinoline) led to compounds of significant biological activities. 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 (π–π interaction, intercalation in DNA) and via the lipophilic character allowing the penetration through the biological membranes. The activities were examined by using various biological systems such as cell lines, bacteria, viruses, parasites, laboratory mice, rats and rabbits, and monolayer and bilayer membranes. Some mechanisms of the actions are discussed. 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.

1. Introduction

Phenothiazines, nitrogen- and sulfur-containing tricyclic compounds, have been known for over a hundred years. The parent compound, 10*H*-dibenzo-1,4-thiazine **1**, was obtained the first time by Bernthsen in 1883. Up to now over 5000 phenothiazine derivatives have been obtained and this class of organic compounds became exceedingly important due to their varied significant biological and chemical properties. Phenothiazines mostly substituted at position 10 with the dialkylaminoalkyl groups and additionally at position 2 with small groups exhibit valuable activities such as neuroleptic, antiemetic, antihistaminic, antipuritic, analgesic and antihelmintic [1]. 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 Creutzfeldt–Jakob diseases of classical phenothiazines [2–9]. Phenothiazines are relatively inexpensive, widely available, well-tolerated and nontoxic compounds (Scheme 1).

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 [1,10].

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

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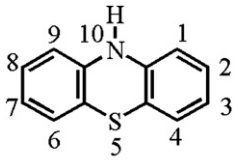


1

Scheme 1. 10*H*-phenothiazine.

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 [1–6]. To our knowledge, there is no review concerning biological activities of recently obtained phenothiazines.

This review article focuses on the biological potential of recently synthesized (during last decade) phenothiazine derivatives differing in chemical structures. We hope that this work will approach new types of phenothiazine derivatives of biological properties and will help to find the correlation between the modified chemical structure and resulted biological activity. Even a simple modification of promazine, chlorpromazine and triflupromazine by their benzylation led to compounds with better antibacterial activity against *Mycobacterium tuberculosis* than the parent compounds [11,12] but such modified compounds are not in the scope of this review. Each biological section was based on the structural features starting the discussion from phenothiazines with the alkyl, aminoalkyl and heteroalkyl substituents at the thiazine nitrogen atom, later with the substituents at the benzene ring atoms and eventually with new phenothiazine ring systems.

2. 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.

Maleates of 10-(3-aminopropyl)- and 10-(4-aminobutyl)phenothiazines **2** exhibited antibacterial and antifungal activities against *Saccharomyces cerevisiae* strains, *Candida albicans*, *Bacillus megaterium*, *Corynebacterium hoffmanni*, *Fusarium moniliforme*, *Aspergillus niger* and *Trichophyton mentagrophites*. The most significant activity was observed for 2-trifluoromethyl-10-(4-aminobutyl) phenothiazine against *S. cerevisiae* strains and *T. mentagrophites* with the minimum inhibitory concentration MIC = 0.4 and 1.5 μg/ml, respectively. These compounds interacted strongly with model phospholipid membranes (imitating bacteria membranes) as recorded by NPN fluorescence spectra and microcalorimetry. They also were also effective in inducing perturbation in the erythrocyte membrane leading to stomatocytosis and endevesiculation [13].

Various aminoalkyl derivatives of phenothiazines and 1-(or 3)-azaphenothiazines **3** and bis-phenothiazines **4** were examined as antitubercular agents against *Mycobacterium tuberculosis* H37Rv and counter-screened to a panel of dopaminergic (D1, D2 and D3) and serotonergic (5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C}) receptors. The most active compounds **3** (Y = CH, ZNR_2 = 1-methyl-3-piperidinylmethyl, CH_2, X = 2- or 3-C_6H_5; Y = CH, Z = $(CH_2)_3$, NR_2 = $NH(CH_3)CH_2OH$ and $NHCH_2C_6H_5$, X = 2-CF_3) and **4** showed MICs from 2–4.6 μg/ml. Compounds with bulkier substituents ZNR_2 were less active. The increased activity of compounds with the phenyl group suggests existing space for additional steric interaction in the receptor binding pocket. The replacement of the sulfur atom in the thiazine ring into the oxygen atom led to significant losses of activity. An increase in the steric volume of the substituent reduced binding to the dopamine and serotonin receptors [14].

Selected 10-carbamoylalkylphenothiazines **5**, possessing metal chelating groups (tertiary amines or aza-4-crown-14), showed significant activities against Gram-positive *Bacillus subtilis* with MIC's in the range of 7.8–30 μg/ml, higher than for the parent phenothiazine **1** or ampicillin. The authors suggested that the chelator-based agents were responsible for disruption of the metal-ion homeostasis in bacteria which can lead to cell stress or death [15]. Substituted 1,2,4-triazolylmethyl derivatives of phenothiazines **6** exhibited antibacterial activity against *B. subtilis*, *Escherichia coli*, *Kliebsiella pneumonia* and *Salmonella typhimurium*, some of them (when Z = 2-, 4-Cl, 3-, 4-NO_2, 3-, 4-Br) comparable with a reference drug streptomycin at 50 and 100 ppm concentrations. Those compounds showed also antifungal activity (when Z = 2-, 3-Cl, 4-NO_2, 4-Br) against *A. niger*, *A. flavus*, *Fusarium oxiporium*, *Trichoderma viride* similar to a reference drug griesofuvin at 25 and 50 ppm concentrations [16].

10-Acyl derivatives **7** and **8** (being bis-phenothiazine compound) exhibited moderate to good antibacterial activities *Bacillus subtilis*, *E. coli*, *Staphylococcus aureus*, *Pseudomonas auregenosa* and antifungal activities against *C. albicans* and *A. niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. The most active compounds were equivalent to reference drugs, ciprofloxacin, ampicillin and fluconazole with MICs = 5 and 10 μg/ml [17,18].

Substituted 1,3-thiazolidinylaminoethanoylphenothiazines **9** were evaluated against for their antimicrobial activity. Most compounds showed weak to moderate activity against *B. subtilis*, *P. mirabilis*, *K. pneumonia* and *S. typhi* in comparison with ciprofloxacin. Four compounds (R_1 = phthalimidoxy, R_2 = Cl, F, OCH_3; R_1 = succinimidoxy, R_2 = F) were more active against fungi *C. albicans* and *A. fumigatus* than a reference drug fluconazole [19].

Various benzene ring multisubstituted 10*H*-phenothiazines and their chloroacetyl and ribofuranosyl derivatives **10** and sulfones showed moderate to significant activity against bacteria *E. coli*, *S. aureus*, *Pseudomonas flueroscence* and fungi *A. niger*, *A. flavus* and *F. oxysporium* at concentration of 100 μg/disc. The ribofuranosyl derivatives exhibited also strong antioxidant in DPPH and ABTS assays. In those cases, the ribofuranosyl and chloroacetyl compounds were found to be the most active, even more than the reference drugs streptomycin, flukanozole and mycostatin [20–25].

Directly substituted phenothiazines **11** and **12** with the heteroaromatic rings, pyridine and [1,2,4]triazolo[1,5-a]pyridine, showed antimicrobial activities against variety of bacterial strains such as *S. aureus*, *Salmonella paratyphi*, *E. coli*, *Shigella flexneri*, *P. auregenosa*, *B. subtilis* and fungi such as *Cerevesae vitae*, *C. albicans* and *A. niger*. Some of them showed significant activity compared with ciprofloxacin, cloxacillin and gentamycin [26].

A building of pharmacophoric substituents in other places than the position 10 in the phenothiazine ring system is still very rare. 2-Substituted 10-chlorobenzylphenothiazines with various arylethenoyl groups **13** exhibited antimicrobial activity against *Enterococcus faecium*, *Enterococcus faecalis*, *E. coli* and *B. subtilis* bacteria and antifungal activities against *C. albicans* and *A. niger* fungi. Most of them were more active than benzyl penicillin and chloramphenicol [27].

2-Heterocycle-substituted 10*H*-phenothiazines **14** (containing the dihydropyrazolo[3,4-d]pyrimidine moiety) exhibited antitubercular activity against *Mycobacterium tuberculosis* H37Rv. strain with MIC of 6.25 μg/ml when Ar = C_6H_4Cl or C_6H_4OH [28].

Of the modified ring system of phenothiazines with heteroaromatic azines, 10*H*-pyridobenzothiazines (1-azaphenothiazines)

and their sulfoxide and ribofuranosyl derivatives **15** showed moderate activity against bacteria *E. coli*, *S. aureus* and fungi *A. niger*, *A. flavus* and *F. oxysporium* in comparison with the reference drugs, streptomycin and mycostatin at the concentration of 100 μg per disc. The best results were found for the ribofuranosyl derivatives [29].

Phenothiazines were also modified with bicyclic aromatic rings. The tetracyclic phenothiazines (modified with the naphthoquinone ring) **16** showed significant actibacterial activity against *S. aureus*, compound **16b** with the $MIC_{50} = 12.5$ μg/ml, being even better than antibacterial drug amikacin [30]. These compounds and pentacyclic phenothiazine (modified with the naphthalene and naphthoquinone rings) **17** showed weak antifungal activity against 6 fungi with $MIC_{50} \geq 50$ μg/ml. These compounds exhibited better anticancer activity (see below) [31].

The tetracyclic 12*H*-benzo[b]phenothiazines **18** substituted with the benzothiazolylazo group in position 11 were effective against *E. coli*, *Klebsiella* spp., *P. auregenosa* and *S. aureus*. Some of them ($X_1 = F$ or Cl, $X_2 = OCH_3$ and $X_1 = Cl$, $X_2 = Br$) were even more active than streptomycine and ceftazidime [32] (Scheme 2).

3. Anticancer activity

Anticancer activity of new phenothiazine derivatives was studied extensively over the last two decades by Motohashi and his research groups. They found that modified phenothiazines with the naphthalene rings instead of benzene rings led to various benzo- and dibenzophenothiazines **19**–**22** with anticancer activity toward Walker's and Erlich's cancers [33]. Later on they discovered that benzophenothiazines **19** and **20** induced monocytic differentiation and apoptotic cell death via internucleosomal DNA fragmentation in human myelogenous leukemic cell lines HL-60, ML-1, U-937 and THP-1 [34,35]. They also introduced new substituents at position 10. The most promising compounds were 10-chloroethylureidoalkylphenothiazines **23** (possessing "half-mustard type" fragment) with different cytotoxic activities against 54–60 different human tumor cell lines: leukemia, melanoma, small cell lung, colon, central nervous system, renal, breast, ovarian and prostate tumors. The nature of the substituents at position 2 and the length of alkylene group (propylene or butylene) are important for anticancer activity. Phenothiazines with the butylene linker were more effective than with the propylene linker. The highest activity of these compounds were found against colon cancer cells and leukemia. The best result was achieved for the 2-chloro-10-chloroethylureidobutyl derivative giving $GI_{50} = 1.4$ μM and 1.6 μM (50% inhibition of growth) against 4 leukemia cell lines and 7 colon cancer cell lines. This compound exhibited the most potent cytotoxic value of $LC_{50} = 5.7$ μM (the concentration required for 50% lethality of cells) against colon cancer lines. According to the authors, these compounds might act on the cancer cells through alkylurea-induced alkylation of proteins or on DNA by a particular intercalation [36,37]. Phenothiazines **23**, 10*H*- and 10-phthalimidoalkylphenothiazines **24** exhibited antitumor activity against HEp-2 and L5178Y tumor cells [38,39]. Phenothiazines **24** were transformed further into 10-aminoalkyl compounds **2** and their acyl and sulfonyl derivatives **25**. The latter compounds exhibited anticancer activity against human leukemic HL-60 and squamous carcinoma HSC-2 cells [40]. Benzophenothiazines and "half-mustard type" phenothiazines stimulated T-cell blast formation, natural killer cell activity, possibly through activation of monocytes and macrophages, and antibody-dependent cellular cytotoxicity of human peripheral blood mononuclear cells. These structures and activities were extensively discussed in the review articles [2,3] and in the chapter of the monograph [4].

10-Amino(hydroxy)propylphenothiazines **26** were tested as cell-cycle inhibitors. The most effective compound **26a** induced a marked G_2/M phase of cell-cycle arrest followed by cell death in human transformed WI38VA cells after 2-day incubation at the concentration of 5 μM. This compound triggered also complete death in HL-60 cells and remarkable cell death in HeLa cells at 10 μM. In comparison, the viability of the non-transformed WI-38 cells was about 90% after 1-day treatment. More than 90% of the mitotic cells exhibited the monoastral spindle instead of normal bipolar spindle. Compound **26a** inhibited the microtubule-activated ATPase activity by mitotic kinesin Eg5 with $IC_{50} = 1.52$ μM (the concentration of 50% inhibition) [41] (Scheme 3).

Multisubstituted phenothiazines **27** and their sulfoxide and sulfone derivatives were described in patent [42] as the anticancer agent in vitro ethoxyresorufin-*o*-deethylase study in rat hepatoma cells. Other studies prompted the authors to claim the compounds to be useful for treatment of diabetic mellitus, and as an ovulation inhibitor, an anti-obesity drug and an immunostimulant.

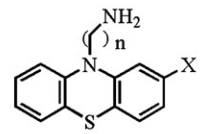
Phenothiazines **28** bearing rigid 2-butynylamino chain in position 10 showed moderate antitumor profile against sensitive human leukemia cell lines HL60 and CCRF/CEM. The most active compounds were those with the cycloamino substituents **a** and **b**, showing $IC_{50} = 10$ μM. These compounds (and two others) were even more active against resistant leukemia cells HL60R with the IC_{50} values of 1.1 and 5.0 μM. For a deeper evaluation of this cytotoxic behavior, the effect on the cell distribution in the different cell cycle phases was measured. This study indicated the ability of these compounds to recruit cells in the G1 phase of the cell cycle, a phase usually unaffected by the classical anticancer agents. More interesting results were found in the test of MDR reverting activity in combination with doxorubicin (DXR) (see below) [43].

Some new azaphenothiazines with the pyridine and quinoline rings instead of the benzene rings, 10*H*-2,7-diazaphenothiazine **29**, pentacyclic 6-aminoalkyldiquinothiazine (dibenzo-1,9-diazaphenothiazine) derivatives **30** and hexacyclic diquinothiazinium salt **31** showed significant anticancer activity against 9 types of human cancer cells (leukemia, melanoma, small cell lung, colon, central nervous system, renal, breast, ovarian and prostate tumors). The most active were found 6-chloroethylureidoethyl derivative **30** ($R = C_2H_4NHCONHC_2H_4Cl$) with the $GI_{50} = 0.088$ μM against melanoma SK-MEL-5 cells and 6-diethylaminoethyl derivative **30** [$C_2H_4N(C_2H_5)_2$] with the $GI_{50} = 0.19$ μM against ovarian cancer IGROV-1 cells [44–46].

Already mentioned above tetracyclic and pentacyclic phenothiazines **16** and **17** showed antiproliferative activity against cervical cancer (HeLa) cells. The most active was tetracyclic compound **16b** showing 77% inhibition at 20 μM concentration, while the reference drug, noscapine, showed only 50% inhibition at 34 μM concentration [31].

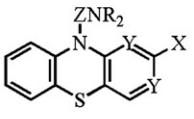
4. Anti-MDR activity

Active efflux of anticancer, antibacterial and antifungal drugs by multidrug resistance (MDR) transporters is one of the major obstacles to successful chemiotherapy of cancer and infectious diseases. Mentioned above 10-aminoalkylphenothiazines **2** (as maleates) exhibited also anti-MDR activity determined by *P*-glycoprotein inhibition. They stimulated multidrug resistance-associated protein MRP1-mediated efflux of 2′,7′-bis-(3-carboxypropyl)-5-(and -6)-carboxyfluorescein (BCPCF) out of human erythrocytes. They also stimulated ATP-dependent uptake of 2′,7′-bis-(3-carboxyethyl)-5-(and -6)-carboxyfluorescein (BCECF) into inside-out membrane vesicles prepared from erythrocity membranes and Mg^{2+}-dependent ATPase activity in erythrocyte membrane. The authors reported phenothiazines **2** to be the first compounds able to inhibit transport activity of P-glycoprotein and to stimulate MRP1 transporter and concluded that phenothiazines



2

n = 3, 4; X = H, Cl, CF_3



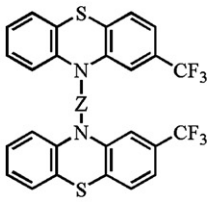
3

Z = $-CH_2-$, $-(CH_2)_2-$, $-CH_2C_6H_4CH_2-$

Y,Y = CH, CH; N, CH; CH, N,

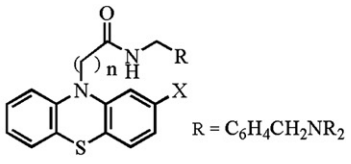
NR_2 = alkylamino, arylamino, cycloamino groups

X = H, CF_3, Cl, Ph, etc.



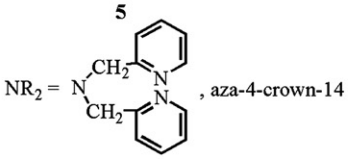
4

Z = $-CH_2$, $-(CH_2)_2-$, $-CH_2C_6H_4CH_2-$



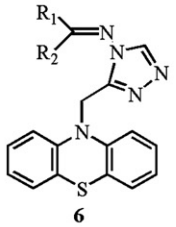
5

R = $C_6H_4CH_2NR_2$



NR_2 = , aza-4-crown-14

X = H, CF_3; n = 1, 2, 5

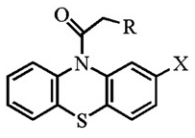


6

R_1 = H, CH_3, C_6H_5

R_2 = C_6H_4Z,

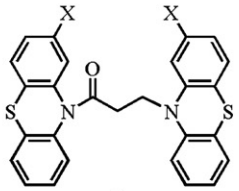
Z = Cl, NO_2, OCH_3, $N(CH_3)_2$



7

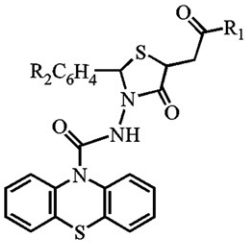
R = , ,

X = H, Cl, CF_3, $COCH_3$



8

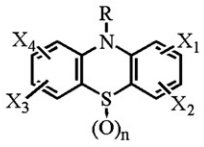
X = H, Cl, CF_3



9

R_1 = ,

R_2 = H, Cl, F, OCH_3



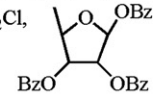
10

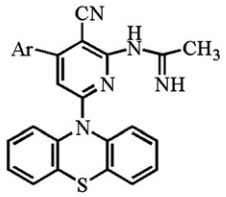
X_1, X_2, X_3, X_4 = H, Cl, Br, F, OCH_3, OC_6H_5, NO_2

R = H, $COCH_2Cl$,

n = 0, 2

Bz = COC_6H_5





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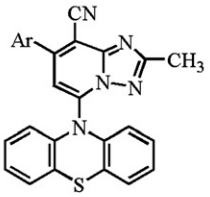
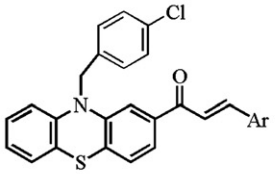


12

Ar = subst. phenyls



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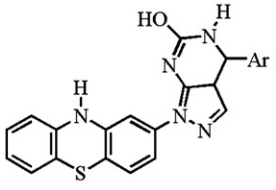

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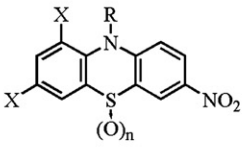


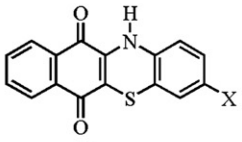
15

X= H, Cl, Br

R = H, $COCH_3$,

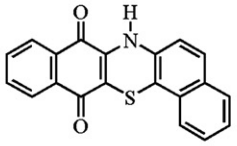
n = 0, 1

Bz = COC_6H_5

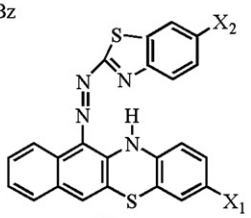


16

X = H (a), OCH_3 (b)



17



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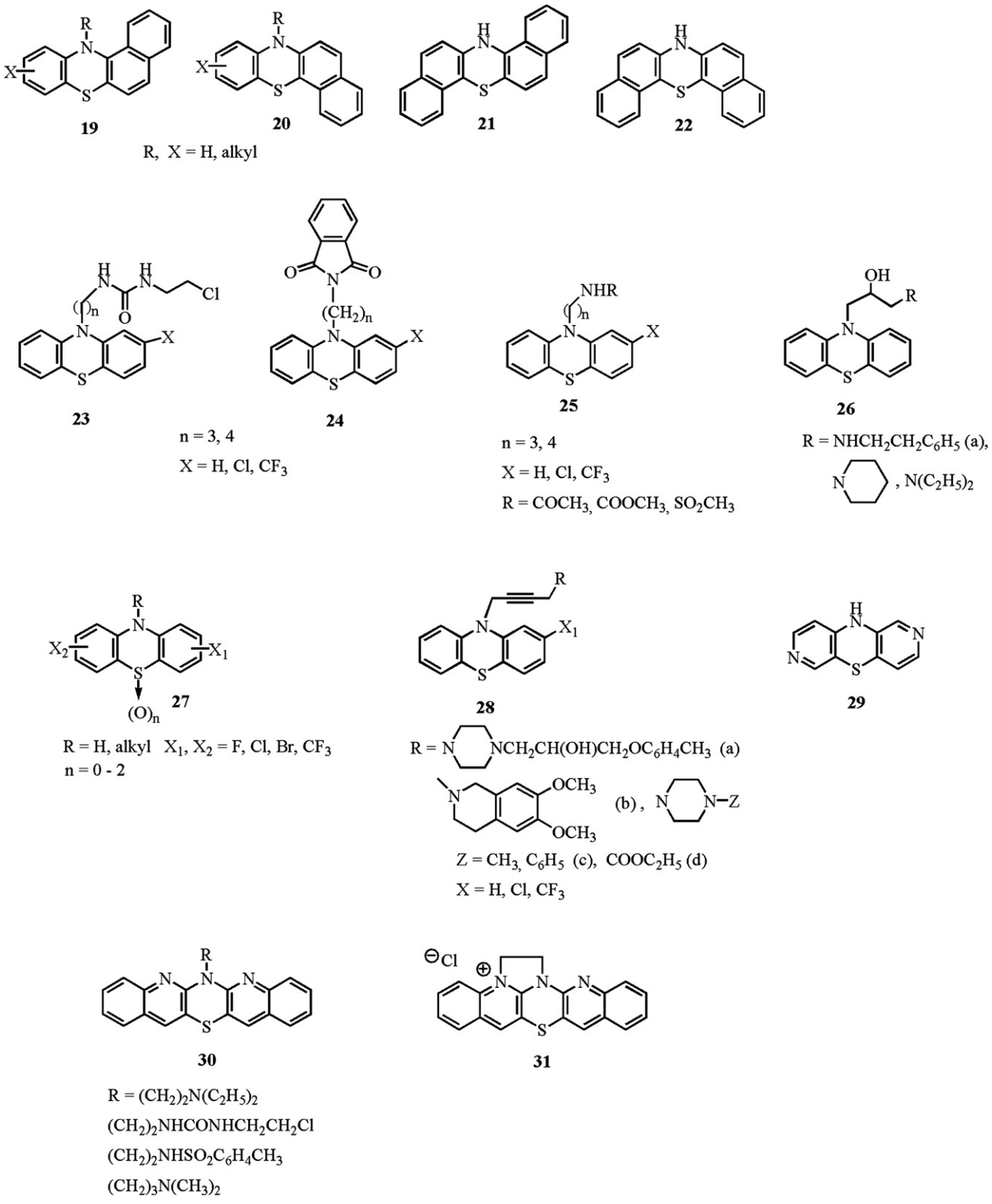
X_1 = H, Cl, F, OCH_3

X_2 = CH_3, Br, NO_2, OCH_3

Scheme 2. Phenothiazines showing antibacterial and antifungal activities.



Scheme 3. Phenothiazines showing anticancer activity.

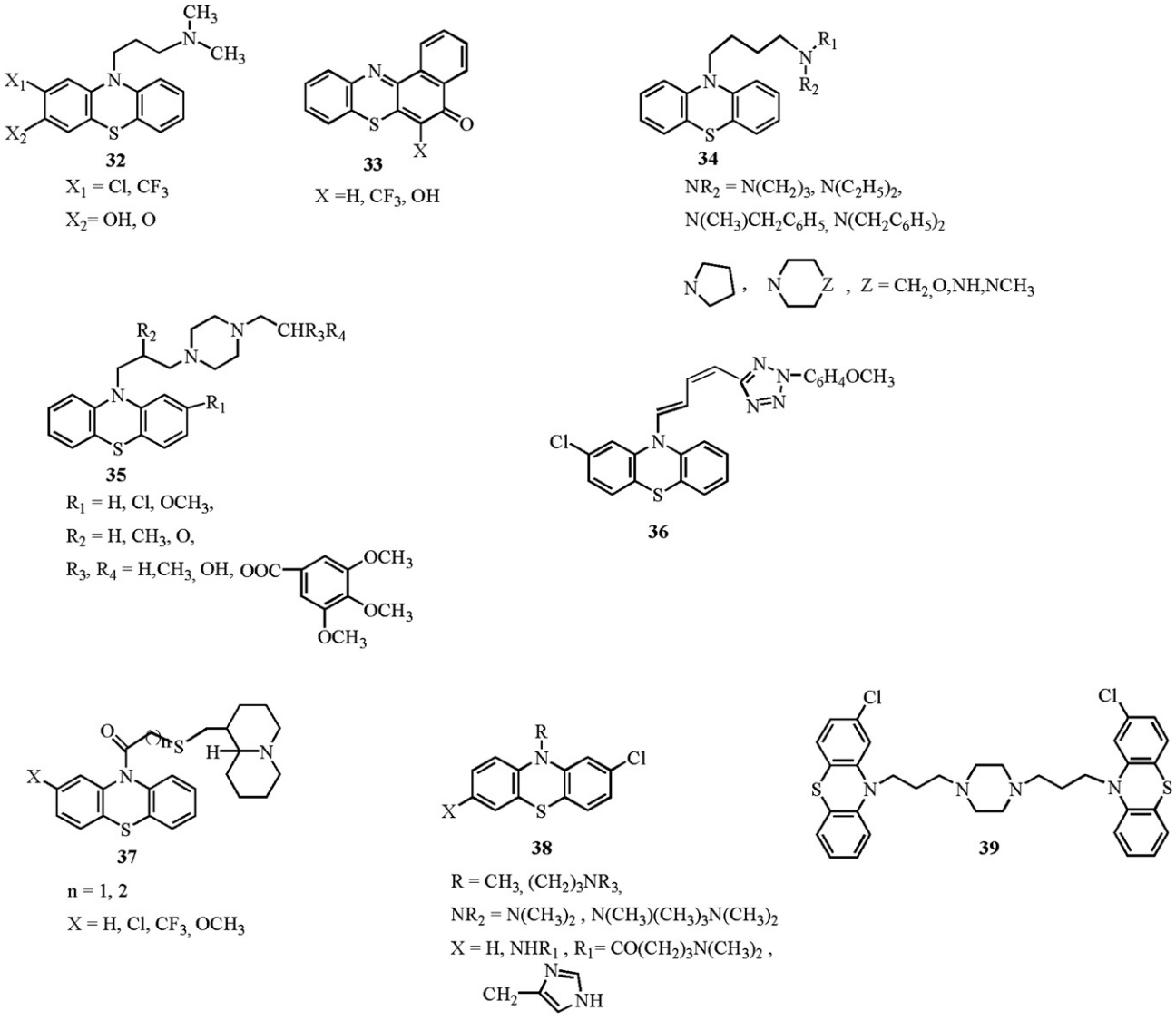
probably exerted their stimulatory effect on MRP1 by direct interaction with the protein at the site different from the substrate binding site [47]. The same authors correlated anti-MDR activity of acyl and sulfonyl derivatives **25** with calculated hydrophobicity value of logP and experimental parameters of lipid bilayers determined by calorimetry and fluorescence spectroscopy [48]. Some of those compounds (particularly **2**) exhibited the growth inhibitory and MDR modulatory effect against *S. cerevisiae* strains showing different levels of expression of MDR transporters Pdr5p, Snq2p and Yor1p. The growth inhibitory properties were increased

upon deletion of *PDR5*. Phenothiazines were synergistic with the antifungal ketoconazole at micromolar concentrations. The synergistic effect against the multidrug resistant Pdr5p overproducing strain strikingly resembles the effect of PDR5 deletion [49].

The hydroxy and oxo derivaties of phenothiazines **32** and benzophenothiazines **33** exhibited *P*-glycoprotein mediated MDR reversal activities measured in mouse T-lymphoma cell lines in comparison of parental cell line L51787Y. These compounds can serve as electron pair acceptors in charge-transfer (CT) complexes with amino acids of the first P-glycoprotein loop. For compounds **33**, a dependency of decreasing energies of lowest unoccupied orbitals (E_{LUMO}) with reduced CT binding properties to an increasing P-glycoprotein mediated MDR was found [50].

Various 10-dialkylaminobutylphenothiazines **34** with noncyclic and cyclic amino groups showed better drug-resistant reversing activity in chloroquine-resistant than in mefloquine-resistant cell lines and were more effective against chloroquine-resistant *Plasmodium falciparum* isolated from Southeast Asia than those from South America. The modulating activity was limited by steric size of the substituents on the amino group. The most active compounds ($NR_2 = N(CH_3)_2$, $N(C_2H_5)_2$, pyrrolidinyl) were more than twice as active as verapamil, one of the best-known chemosensitizers. The anti-MDR activity is the result of the interaction between phenothiazines and target protein, calmodulin, in two binding sites. The tricyclic ring system interacts with a hydrophobic pocket containing two aromatic phenylalanine residues and the positively charged nitrogen atom of side chain interacts in an electrostatic manner with a hydrophilic region composed with three glutamic acid residues. The optimal length of the alkyl chain (four carbon alkyl bridge) is dictated by the distance of the two binding sites in target protein [51]. Phenothiazines **35** with more expanded aminoalkyl substituents (containing the piperazine moiety) at position 10 were effective inhibitors of MDR1 mediated calcein-AM efflux, being more active than verapamil and one of them as active as cyclosporin A [52] (Scheme 4).

Mentioned above phenothiazines **28** with the substituents **c** (X = Cl) and **d** were shown to increase DXR retention in multidrug resistant cells HL60R and CEM/VBL300, suggesting a direct interaction with P-glycoprotein. These compounds reduced the IC_{50} values of DXR by 4-8-fold. As was mentioned in the anticancer section these phenothiazines proved to be capable of inducing cytotoxic effect in resistant cell lines by interfering with the cell cycle in the G1 phase.



Scheme 4. Phenothiazines showing anti-MDR activity.

The cytotoxic compounds triggered apoptosis through an atypical pathway of caspase activation and induced synergic effects when combined with DXR in the resistant cell lines [43].

One of tetrazolyldienyl derivatives of phenothiazines **36** exhibited promising reversal of MDR studied in human MDR1 gene transfected mouse lymphoma cells. This compound inhibited significantly the MDR efflux pump mechanism showing an increase of the rhodamine accumulation by 2.5 fold at the concentration of 4 μg/ml in vitro flow cytometric studies [53].

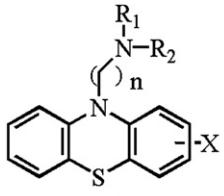
10-Acylphenothiazines **37** containing strongly basic and lipophilic quinolizidine nucleus connected by the methylenethioalkyl linkers showed MDR reversal activity against doxorubicin-resistant ovarian cancer cells A2780-DX3. All compounds appeared more effective than clomipramine in sensitizing the resistant cell to doxorubicin. The most active were 3 compounds ($n = 1$, X = Cl, CF_3, $n = 2$, X = OCH_3), which potentiated doxorubicin cytotoxicity up to 3.96 fold in A2780-DX3 cells. The bulky quinolizine ring at the end of the alkyl chain seemed to be a profitable structural feature [54].

Substituted phenothiazines **38** and bis-phenothiazine **39** with the aminoalkyl groups inhibited the growth of chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. This appreciable antimalarial activity increased with an increase in the number of basic groups in the aminoalkylamino side chain. The most active was compound **38** with R = $(CH_2)_3N[(CH_2)_3N(CH_3)_2]_2$ and X = H. The degree of antimalarial activity was correlated with the efficacy of inhibition of β-haematin formation suggesting that antimalarial activity is the result of antagonizing the sequestration of toxic haem moieties within malaria parasite [55].

5. Antiviral activity

With increasing resistance of the retrovirus HIV-1 to current drugs, there is a need for research of new compounds. Some of 2-4 substituted 10-aminoalkylphenothiazines **40** were tested for binding to HIV-1 TAR RNA by saturation transfer difference NMR (STD NMR) spectra. Both the phenothiazine ring system and the aminoalkyl substituents changed upon binding. The tightest binders were derivatives with four carbon linkers between the phenothiazine moiety and the amino group [56] (Scheme 5).

Benzophenothiazines **19** and **33** exhibited antiviral effects with acyclovir (ACV) on the multiplication of herpes simplex virus type 2 (HSV-2) in Vero cells. This combination enhances their antiviral activity probably by reduction of the mutagenic rate in the virus populations. The mechanism of the antiviral effect of benzophenothiazines is not yet known but might be due to an inhibitory action on viral DNA replication. Benzophenothiazines exhibited antimutagenic activity which may be responsible for the reduction of ACV resistant particles in the virus population or charge-transfer formation with antiviral ACV, being more effective than each compound alone [57].

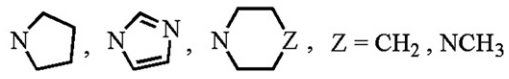


40

n = 3-5

X = H, Cl, CF_3 , OH, OCH_3, $COCH_3$

NR_2 = $N(CH_3)_2$, $N(C_2H_5)_2$, $NHCH_2CH_2OH$,



Z = CH_2 , NCH_3

Scheme 5. Phenothiazines showing antiviral activity.

6. Anti-inflammatory activity

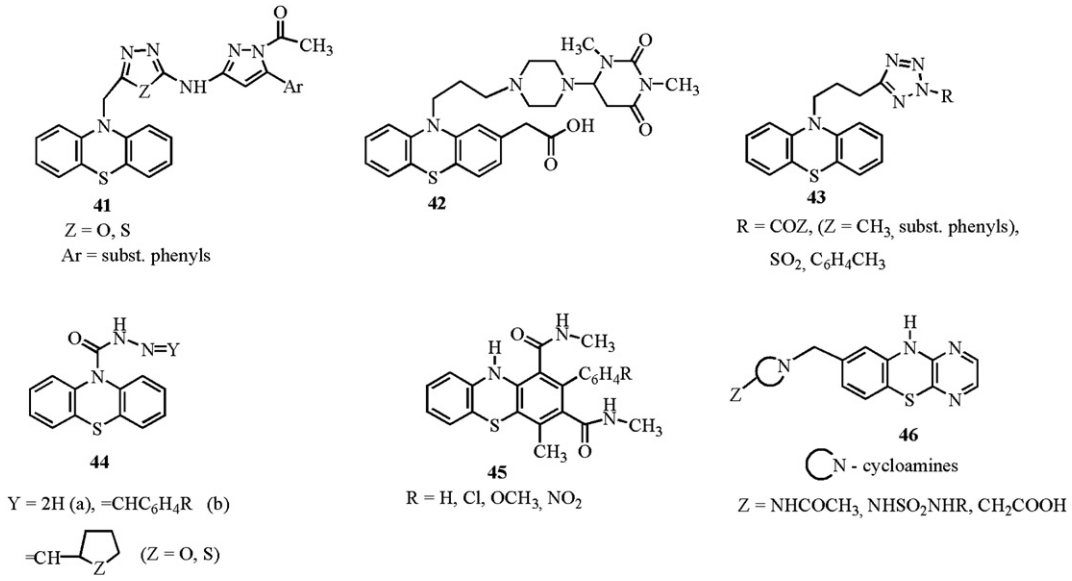
Phenothiazines **41** with two azole (oxadiazole/thiadiazole and pyrazoline) rings in substituents in position 10 showed significant in *vivo* anti-inflammatory activity. The best results were found for two compounds with the o-methoxyphenyl group and Z = O and S, showing not only more anti-inflammatory activity but also less ulcerogenic liability than the reference drug, phenylbutazone [58].

A series of phenothiazinecarboxylic acids possessing pyrimidine-2,4(1*H*,3*H*)-dione moiety linked via aminoalkyl groups to position 10 were evaluated for their affinity toward human histamine H_1 receptor and Caco-2 cell permeability and further for oral antihistaminic activity in mice and finally for anti-inflammatory potential in mice OVA-induced biphasic cutaneous reaction model. The best result was obtained for compound **42**, which exhibited not only good in vitro H_1-receptor binding affinity ($IC_{50} = 40$ nM), but also good in vivo antihistaminic activity at 3 mg/kg as well as high Caco-2 cell permeability (123 nm/s). This compound inhibited both ITR (immediate type reaction) and LTR (late type reaction) in OVA-induced biphasic cutaneous reaction model in dose dependent manner unlike known antihistamines ketotifen and prednisolon. Its potent histamine H_1-receptor antagonistic activity and remarkable anti-inflammatory activity *in vivo* model predispose this compound to be a promising next generation antihistamine [59].

Some *N*-acyl and *N*-sulfonyl derivatives of 10-tetrazoloethylphenothiazines **43** 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 [60,61].

10*H*-phenothiazine-1-acylhydrazide **44a** and its hydrazone derivatives **44b** exhibited rather moderate anti-inflammatory activity with exception of derivatives **44b** (R = 4-$N(CH_3)_2$, 4-COOH), which inhibited significantly the formation of edema in 20.5% and 51% but a little less than indomethacin. All those compounds showed significant analgesic activity in the classical acetic acid-induced mice abdominal constrictions test. Two compounds **44b** (R = 4-NO_2, 4-COOH) were more active than reference drugs, dipyrone and indomethacin. Those compounds were also screened to evaluate their effects on rabbit platelet aggregation induced by arachidomic acid (AA), collagen and adenosine 5-diphosphate (ADP). All the compounds inhibited by 100% the collagen- and AA-induced platelet aggregation, without interference with the ADP-induced aggregation. The IC_{50} values showed that the selected compounds were more potent for the inhibition of AA- than collagen-induced aggregation with the best result for compound **44b** (R = H, $IC_{50} = 2.3$ μM), which acted in arachidonic acid pathway probably by inhibition of platelet cyclooxygenase-1 (COX-1). The good central analgesic profile of some compounds could be due to their lipophilic character that favors the blood-brain barrier crossing promoting a possible COX-3 enzyme inhibition [62].

Benzene ring multisubstituted phenothiazines **45** with two methylcarbamoyl groups and substituted phenyl groups and also their 1,2-dihydro derivatives exhibited promising inhibitory activity ($IC_{50} = 13–15$ μM) toward regulating enzymes amplifying the inflammatory disorders such as phosphodiesterase, prostaglandine synthetase, γ-glutamyltranspeptidase and superoxide dismutase, when compared to standard drug aspirin. Less lipophilic compounds showed better activity [63] (Scheme 6).



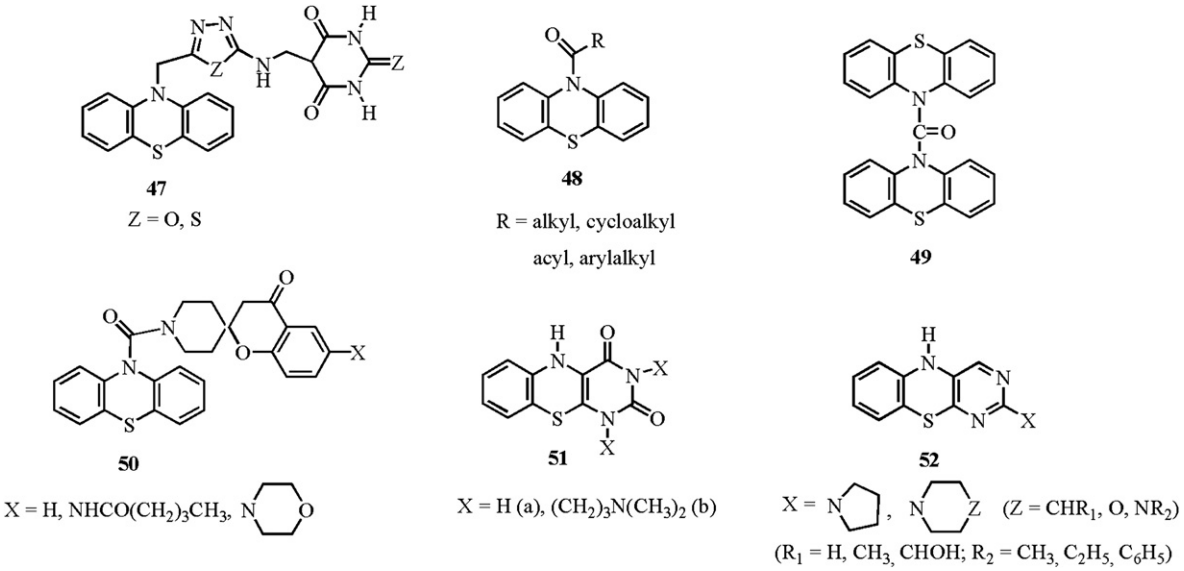
Scheme 6. Phenothiazines showing anti-inflammatory activity.

8-Substituted 10*H*-pyrazinobenzothiazines (1,4-diazaphenothiazines) **46** containing an cycloamine unit exhibited potent oral inhibitory activities against neutrophil migration in a murine interleukin-1 induced paw inflammation model using mice and leukocyte accumulation in a carrageenan pleurisy model in the rat. They showed also significant therapeutic effect in type II collagen-induced arthritis in rats. The most active were compounds possessing azabicyclononane and piperidine rings in the substituents and are expected to be promising drug candidates for treatment of autoimmune inflammatory diseases such as rheumatoid arthritis [64,65].

7. Other activities

10-Oxadiazolomethyl- and thiadiazolomethylphenothiazines **47** containing additional heteroaromatic ring, pyrimidinone, showed potential anticonvulsant activity (80-90% inhibition of seizures in rats) even more potent (for Z = S) than the reference drug, phenytoin sodium. All these compounds exhibited low toxic with ALD_{50} (acute lethal dose) over 1000 mg/kg [66].

About fifty various 10-acyl derivatives **48** and bis-phenothiazine **49** were examined for their ability to inhibit cholinesterases. This study described two distinct mechanisms for binding those compounds to butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE). Phenothiazines inhibited BuChE in a mechanism involving $\pi-\pi$ interaction between the phenothiazine ring system and aromatic residues in active site gorge. Some phenothiazines inhibited also AChE via a mechanism concerning the amide carbonyl group interaction with the active site gorge. In some aromatic derivatives, BuChE inhibition constants in the nanomolar range were achieved. Molecular volumes, steric and electronic factors were found to be important parameters for BuChE-specific inhibition. Such a specific and potent inhibitors of BuChE may have a potential for use in the treatment of dementias such as Alzheimer's disease [67,68].



Scheme 7. Phenothiazines showing other activities.

The spiro[chroman-2-4′-piperidine]carbonyl derivatives **50** were tested as acetyl-CoA carboxylase inhibitors showing 16–64% inhibition at 10 μM concentration, being, however, less active than appropriate phenoxazines [69].

Mentioned above azaphenothiazines **29**–**31** were tested in the immunological assays such as the proliferative response of human peripheral blood mononuclear cells induced by phytohemagglutin A or anti-CD3 antibodies, lipopolysaccharide-induced cytokine production by human PBMC; the secondary, humoral immune response in mice to sheep erythrocytes (*in vitro*) and delayed-type hypersensitivity in mice to ovalbumin (*in vivo*). The compounds exhibited differential inhibitory activities in the proliferation tests, with 10H-2,7-diazaphenothiazine **29** and 6-(3-dimethylaminopropyl)diquinothiazine **30** [$R = (CH_2)_3N(CH_3)_2$] being most suppressive. Compound **29** was strongly suppressive in the humoral immune response even at low concentrations (1 μg/ml) and also inhibited the delayed-type hypersensitivity lipopolysaccharide-induced production of tumor necrosis factor and interleukin-6 in cultures of human blood cells [45] (Scheme 7).

10*H*-pyrimidobenzothiazines (2,4-diazaphenothiazines) **51** were evaluated for their potential as trypanothione reductase (TryR) inhibitors showing moderate and strong inhibition (36 and 60%) of recombinant *Trypanosoma cruzi* TryR. Both compounds were inactive against promastigotes of *Leishmania mexicana amazonensis* [70].

3-Cycloaminosubstitituted pyrimidobenzothiazines (2,4-diazaphenothiazines) **52** showed broad range of inhibitory activity of 15-lipogenase (15-LO) which is implicated in the progression of certain cancers, atherosclerosis and chronic obstructive pulmonary disease. The best results found for the piperazine derivatives ($X = CH_3$, C_2H_5). The docking studies of the optimal binding conformations of phenothiazines within the 15-LO active site revealed the sulfur atom to be oriented toward the iron atom in the catalytic site. The authors suggest that catalytic iron site links to an oxygen molecule forming hydroperoxide and subsequently the sulfur atom undergoes oxidation to exert its inhibitory potency upon 15-LO [71].

8. Conclusions

We reviewed over 50 main structures of recently synthesized phenothiazines (a few hundreds of compounds) with broad valuable activities studied *in vitro* and *in vivo.* Not only well known 10-dialkylaminoethyl- and 10-dialkylaminopropylphenothiazines exhibit valuable biological activities but also new derivatives of phenothiazines with other dialkylaminoalkyl, cycloaminoalkyl and aminoalkyl substituents and their acyl and sulfonyl derivatives. Many substituents contained various monocyclic (pyrazole, thiazole, oxadiazole, thiadiazole, tetrazole) and bicyclic (quinolizine, pyrazolopyrimidine, thiazolopyridine, azabicyclononane and spiro [chromanpyrimidine] heterocycles linked directly or via the alkyl chain with the phenothiazine moiety. In some cases phenothiazines with simple alkyl or arylalkyl substituents showed biological activity. The most explored was the introduction of the substituents at the thiazine nitrogen atom which is not surprising from the synthetic point of view. Also modification of the tricyclic ring system with the bicyclic homoaromatic ring (naphthalene) and monocyclic and bicyclic azine rings (pyridine, pyrimidine, pyrazine and quinoline) led to compounds of significant biological activities. Only the modification by oxidation of the sulfur atom in phenothiazines led to the sulfoxide and sulfone derivatives with less or at most the same activities as the initial phenothiazines.

New 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 (π–π interaction, intercalation in DNA) and via the lipophilic character allowing the penetration through the biological membranes. The activities were examined by using various biological systems such as cell lines, bacteria, viruses, parasites, laboratory mice, rats and rabbits, and monolayer and bilayer membranes. As one can expect the mechanisms of the actions of new phenothiazines are not fully recognized. Phenothiazines exhibited antibacterial activities higher than the activities of many various reference drugs with the postulated interaction with the model membranes imitating bacteria membranes and the disruption of the metal-ion homeostasis leading to the cell death. Anticancer activities were displayed in many ways by stimulating T-cell blast formation, natural killer cell activity, possibly through activation of monocytes and macrophages, and antibody-dependent cellular cytotoxicity of human peripheral blood mononuclear cells. Some phenothiazines induced monocytic differentiation and the cell death by apoptosis or the cell-cycle arrest, and possible acted on cancer cells by induced alkylation of proteins or on DNA by intercalation. The anti-MDR activity of phenothiazines is mainly based on the inhibition of the transport activity of P-glycoprotein thereby the MDR efflux pump mechanism.

This review shows current tendency in the phenothiazine synthesis and reveals the phenothiazine core to be one of the most potent pharmacophoric moieties which can be a rich source of new compounds having desirable biological activities.

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