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Review

Chemical structure of phenothiazines and their biological activity

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Abstract:
Phenothiazines belong to the oldest, synthetic antipsychotic drugs, which do not have their precursor in the world of natural compounds. Apart from their fundamental neuroleptic action connected with the dopaminergic receptors blockade, phenothiazine derivatives also exert diverse biological activities, which account for their cancer chemopreventive-effect, as: calmodulin- and protein kinase C inhibitory-actions, anti-proliferative effect, inhibition of P-glycoprotein transport function and reversion of multidrug resistance. According to literature data on relations between chemical structure of phenothiazines and their biological effects, the main directions for further chemical modifications have been established. They are provided and discussed in this review paper.

Key words:
phenothiazine derivatives, antipsychotics, calmodulin, protein kinase C, P-glycoprotein, multidrug resistance

Abbreviations: CaM – calmodulin, cAMP – adenylate cyclase, cGMP – guanylate cyclase, CNS – central nervous system, CPZ – chlorpromazine, DPPC – dipalmitoylphosphatidylcholine, FPh – fluphenazine, MDR – multidrug resistance, Pgp – P-glycoprotein, Phts – phenothiazine derivatives, PKC – protein kinase C, TPZ – trifluoperazine

Introduction

Drugs from phenothiazine family exhibit a wide range of biological effects. Together with their main neuroleptic action, other biological activities of importance to their cancer chemopreventive effect were documented (anti-CaM activity, inhibition of the PKC activity, decrease of cell proliferation, and inhibition of the Pgp transport function) [14, 22, 33]. The mechanisms of these activities have been already well recognized and linked to chemical structure of compounds from the phenothiazine family.

General chemical formula of the phenothiazines is given in Figure 1.

According to the literature data, substituents attached to the position C-2 of the tricyclic phenothiazine ring and the length of the alkyl bridge connect-

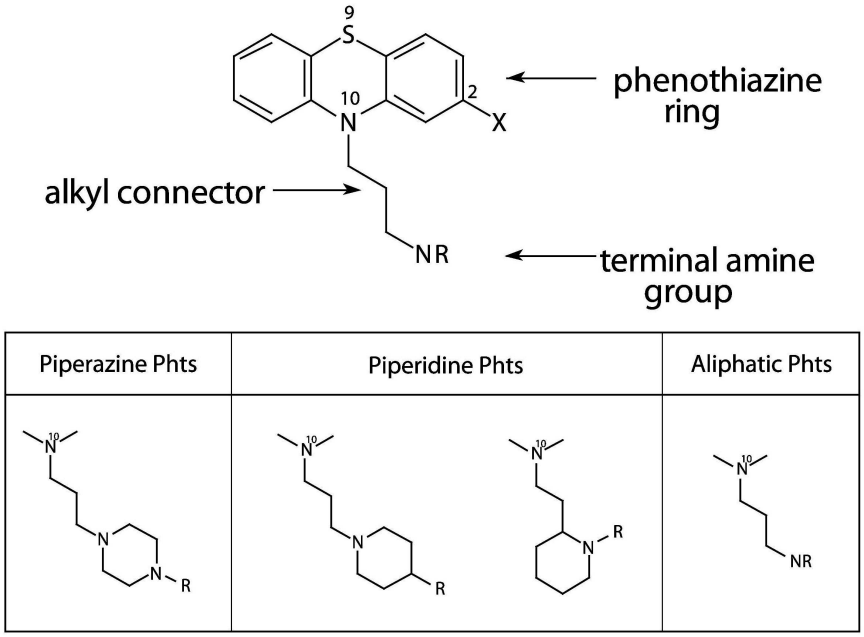

Fig. 1. General chemical structure of Phts

ing the nitrogen atom at position 10 (N-10) of the tricyclic ring, with the terminal amine group in the side chain, determine activity of Phts against cancer cells [14, 30, 31, 34], and the activity is more strongly bound to the type of substituents in the phenothiazine ring than by the nature of the side chain [20]. In accord with this concept, the phenothiazine ring modifications gave the derivatives (e.g., benzo[*a*]phenothiazines, azaphenothiazines) with marked anticancer effects on various cell lines *in vitro* [27, 28, 33].

This review paper provides the literature data on relations between the chemical, three-dimensional structure of Phts and their biological activity. To our knowledge, the subject of multidirectional biological activity of Phts regarding their chemical structure has not been documented.

Chemical structure of Phts and their antipsychotic activity

Phts are amphiphilic compounds, which acquire positive charge in physiological pH values. They are used as antipsychotic drugs, interact with various receptors in the CNS, especially strongly block the dopaminergic receptors [44]. Phts also inhibit other receptors on neurons in the CNS, including α-adrenergic, serotonin, histamine, muscarinic or GABA-ergic receptors, however, the affinity for dopaminergic receptors is the strongest [29, 32, 37, 53].

The affinity of Phts to dopaminergic receptors is explained by the fact that the three-dimensional configuration of Phts resembles (to some extent) the dopamine structure [51], as is presented in Figure 2.

Phts applied as neuroleptic drugs easily cross the blood-brain barrier, since they exhibit a strong affinity to lipid bilayers of the cell membranes in neurons and other lipid-rich tissues since the phenothiazine ring possesses a high degree of lipophilicity [41]. In order to obtain an active neuroleptic derivatives, the hydrogen atoms attached to carbon C-2 and nitrogen N-10 atoms were substituted by different chemical groups, and structures of various Phts given in the literature contained at the N-10 position: piperazine, piperidine, or aliphatic side chain [6] (Fig. 1).

Depending on the structure of substituents in the side chain, the intensity of neuroleptic action of Phts could be ranked as follows: piperazine group > piperidine group > aliphatic chain [10]. The piperazine Phts demonstrate the strongest antipsychotic action, but they also induce the central side effects (including dyskinesia and extrapyramidal disorders) [10].

It was well concluded that the presence of trifluoromethyl substituent (-CF_3) attached to carbon C-2 atom, as well as the propyl connector between the lipophilic core and the tertiary amine moiety were

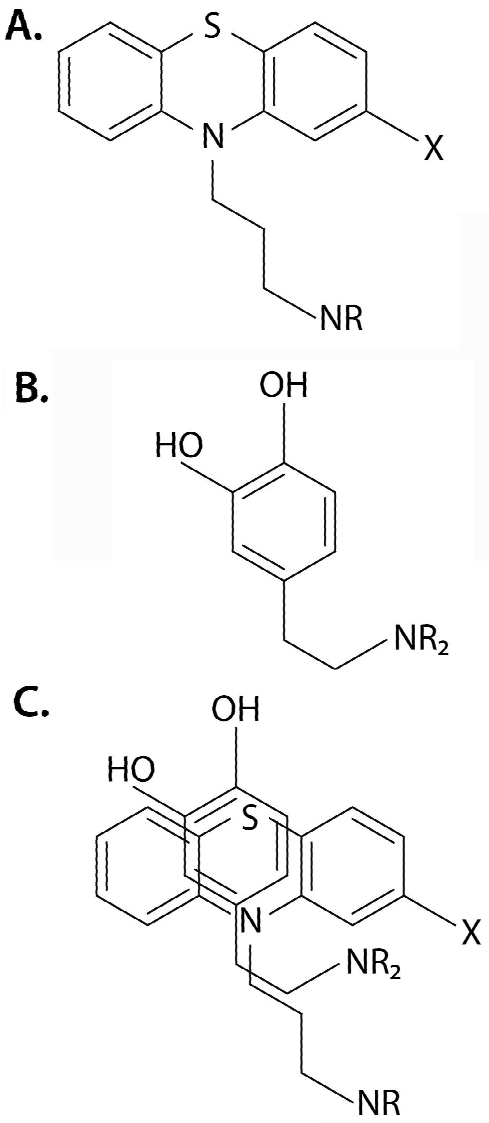

Fig. 2. The structural complementarity between the phenothiazines and dopamine. **A.** – the structure of phenotiazines, **B.** – the structure of dopamine, **C.** – the superposition of phenothiazines and dopamine structures [10]

crucial for the neuroleptic activity of Phts, and the occurrence of electronegative atoms/groups attached to carbon C-2 atom of the phenothiazine ring intensified their antipsychotic activity in the following order: X = $-SO_2NR_2$ > $-CF_3$ > -CO-CH_3 > -Cl [10]. The propyl connector between the phenothiazine ring and the final amine determined the function of Phts as dopaminergic receptor antagonists, and their antipsychotic activity. It was also established that shortening the length of this alkyl linker to two carbon atoms caused a change in the affinity for the receptors [10].

Chemical structure of Phts and their inhibition of the CaM and the PKC activities

The CaM is the multifunctional, widespread protein, which binds four calcium cations [35]. The protein is one of the major, calcium-dependent regulator of biochemical processes and plays a vital role in cell physiology. The blocking effect of Phts on the CaM activity could explain pleiotropic effects of these drugs on cell physiology, among them the inhibition of cells proliferation and the decrease of activity of many CaM-dependent enzymes in various intracellular biochemical pathways [50]. The CaM-dependent enzymes known as being inhibited by phenothiazines and the roles of these enzymes in cell physiology are listed in Table 1.

According to the literature data, the CaM-activated enzymes participate in the Pgp phosphorylation process, which is an important step in the transport function of the protein and its ATPase activity [12, 13, 16].

Cells have developed various mechanisms of protection against foreign compounds; one of the effective mechanism is connected with the Pgp activity. Physiological function of Pgp is fulfilled by transport of endogenous substrates, as well as structurally dissimilar xenobiotics (including drugs) outside a cell [46]. The Pgp overactivity reduces cell membrane permeability for various drugs, also for cytostatic drugs, and thereby decreases the effect of cancer chemotherapy [47].

Tab. 1. Selected enzymes activated by CaM, which are inhibited by Phts, and the role of those enzymes in cellular physiology [8, 50]

ENZYME	ROLE
1. cAMP	Synthesis of cyclic adenylic acid
2. cGMP	Synthesis of cyclic guanylic acid
3. CaM-dependent protein kinase	Phosphorylation of various proteins
4. CaM-dependent protein phosphatase (calcineurin)	Dephosophorylation of various proteins
5. Nicotinamide adenine dinucleotide kinase	Synthesis of nicotinamide adenine dinucleotide phosphate
6. Phosphorylase kinase	Glycogen degradation
7. Inositol triphosphate kinase	Phosphoinositol metabolism
8. Myosin light-chain kinase	Contractility and motility
9. Ca^{2+}-dependent phosphodiesterase (PDE1)	Hydrolysis of cyclic adenylic acid and cyclic guanylic acid

Tab. 2. Suggested elements of Phts' chemical structure which determine their biological/chemopreventive activity

The target of biological activity of Phts	The elements of chemical structure of Phts		
	Substituent at the C-2 position	The length of the alkyl linker	Terminal amine type in the side chain
CaM	Lipophilic substituents $-CF_3$, -Cl	n* ≥ 3	Tertiary amine group or 4-substituted piperazine
PKC	Lipophilic substituents $-CF_3$, -Cl	n ≥ *3*	Primary or secondary-(4-unsubstituted piperazine) amine group
Pgp	Hydrophilic substituents $-COCH_3$, $-COC_2H_5$, $-SO_{1-2}(CH_3)$ $-SO_2N(CH_3)_2$, $-OCH_3$	n = 2–4	Tertiary amine group or 4-substituted piperazine

* n – the amount of carbon atoms in the alkyl connector

It was established that induced by Phts an inactivation of the CaM-dependent enzymes, as well as inhibition of the PKC, lead to a decrease of Pgp phosphorylation and inhibited its transport function [2–4, 48]. The potency of Phts in decreasing the CaM and the PKC activities is related to their chemical structure. The presence of at least a propyl alkyl connector, which bound the terminal amine to the core, determined the PKC inhibitory activity by Phts, and the primary amines, as well as unsubstituted piperazines in the aminoalkyl side chain important for that activity [1]. Also a type of substituent being attached to the carbon C-2 atom of the phenothiazine ring played a crucial role in the inhibition of PKC activity; derivatives with -Cl atom exhibited the greatest potential, while the presence of $-CF_3$ group diminished the activity of phenothiazine compounds against PKC [1].

The three-dimensional structure and chemical properties of Phts determine interaction with the CaM. In general, the CaM inhibitors are amphiphatic amines with positive charge in neutral pH of the solution [50]. It was established that the presence of aromatic ring and cationic fragment in their molecule is crucial for this activity – aromatic ring showed a strong affinity for the hydrophobic surface on the CaM after its activation in the presence of calcium, whereas the cationic group interacted with anionic side chain of the CaM [35]. The substituents which increased the lipophilic properties of the ring system (for instance: $-CF_3$) led to the elevation of the anti-CaM activity, whereas the substitution of rings by hydrophilic groups (-OH) caused the reverse effect, it decreased the Phts potency in the CaM inhibition [35, 50].

The capacity of Phts to inhibit the CaM activity also depends on length of the alkyl bridge, which binds the pheniothiazine ring with the amine side group – the alkyl bridge should be structured by three carbon atoms, and the strongest inhibitors of the CaM have had a propyl chain in their structure [50]. Also a type of an amine in the side chain was of great importance, and the presence of an amine in the cyclic system e.g., the piperazine group, was typical for the CaM antagonists with high activity [50].

Anti-proliferative and anti-MDR activities of Phts

Phts exhibit anti-proliferative effect on neoplastic and genotoxically damaged cells [7, 17, 36]. It was proved that the capacity of Phts for inhibition of a cell cycle was directly proportional to their activity as the CaM inhibitors. In the aspect of their anti-proliferative activity Phts could be ranged as follows: TFP = FPh > CPZ [18].

The important biological activity of Phts is their ability to reverse the MDR of neoplastic cells to cytostatic drugs. Phts could be included in the group of compounds which increase cellular sensitivity to cytostatic drugs (they restore the drug sensitivity of neoplastic cells), mainly by a strong inhibition of the Pgp-dependent mechanism of the MDR [14, 15].

Among the possible mechanisms of the MDR reversion by Phts, we evaluated the unspecific reactions with cell membrane lipids, because of high degree of lipophilicity exhibited by the tested Phts [9]. In the case of FPh, a representative drug from the phenothiazine family, our previous results revealed that the phase transition temperatures of FPh/DPPC mixed

liposomes decreased in proportion to increased concentration of FPh [9]. Fluidization of the structure of lipid membrane, which took place in the presence of FPh, was probably one of the key mechanisms of inhibiting Pgp activity and the essential element of increase of cells' chemosensitivity [9, 45]. The other described mechanisms of the MDR reversion by Phts depend on their inhibiting influence on the CaM and the PKC function, and on direct interaction with ligand binding sites within the Pgp [26, 31, 38].

Clear relations were established between the molecular structure of Phts and their ability to inhibit proliferation and reverse of the MDR [16]. Phts, like the majority of the MDR modulators, are lipophilic, heterocyclic compounds [47], which possess at least two aromatic rings in their chemical structure. It was found that the single-ring compounds were less active than those with a tricyclic ring system [34]. Some authors assumed that the presence of a charge-assigned nitrogen atom was a very important feature of the Pgp modulators [2, 52]. However, progesterone, a strong inhibitor of the Pgp, does not contain nitrogen in its chemical structure [49]. The research proved that nitrogen atom was an important component of chemical structure of the Pgp inhibitors, although it was not decisive for that activity [11].

It was also stated that the substituents in the phenothiazine ring, which increased lipophilicity, intensified anti-proliferative action of derivatives, while the anti-MDR activity of Phts, depending on the substituent in the C-2 position, increased in the following order: -H < -Cl < $-CF_3$ [20]. Importantly, experimental data showed that the decrease of lipophilic properties of phenothiazine compounds, as by introducing the -OH group to the basic phenothiazine system, markedly lowered their effect on the MDR inhibition [14].

The lipophilic nature of Phts enables them to easily penetrate and overcome the cell membrane. In the physiological pH the cationic properties of Phts explain well the interaction of the drugs group with anionic cell membrane lipids [5, 47].

Some authors noticed, however, that the process of reversing the MDR could be influenced by the structure of a substituent at position C-2 rather than by its lipophilicity [34]. It was also established that the presence of an ether group $-OCH_3$ attached to carbon C-2 atom of Phts, and especially, a carbonyl group in the form of acetyl and propionyl group [$-COCH_3$, $-COC_2H_5$)] or a sulfinyl, sulfonyl or sulfonamide group [$-SO_{1\text{-}2}(CH_3)$, $-SO_2N(CH_3)_2$], significantly increased anti-MDR activity of the analogues [34].

Another way to increase the anti-cancer activity of Phts could be a modification of an aminoalkyl side chain length and a change of the type of terminal amine. Phts which exert a substantial anti-proliferative potential and which restore cell sensitivity to cytostatics, have had a four-carbon atoms alkyl bridge and piperazine group in the side chain; piperazine and piperidine amines are established more active in these actions in comparison with noncyclic amine groups [25, 34].

Some authors implied that Phts with a four-carbon atoms bridge in the side chain and piperazine structure substituted in position 4 by methyl group would possess a relatively higher activity in reversing the MDR than those with a di- and tricarbon atoms linker or with a noncyclic amine [34]. The same authors also suggested that the presence of tertiary amine in the side chain could enhance an anti-MDR activity of Phts when compared to derivatives containing primary or secondary amines [34]. Furthermore, it was observed that the carbonyl substituent, regardless of the position (whether in the ring structure or in the side chain), increased the chemosensitive activity of Phts only in the presence of secondary or tertiary amine [34]. Compounds devoided of such amine groups did not inhibit the MDR, regardless of the presence of carbonyl group [34]. It is likely that the carbonyl group participates in creating the intra- and intermolecular hydrogen bonds with the Pgp [34].

The capacity to create hydrogen bonds is a vital nature of compounds interacting with the Pgp [11, 38]. The presence of many elements of proton-donor capacity were identified within the intramembrane fragments of the Pgp; they probably participate in creating hydrogen bonds with acceptor substrate groups [38, 40]. It is assumed that the more hydrogen bonds the compounds create, the more intensive interaction with Pgp they exert [38], and, accordingly, the derivatives with the greatest capacity to create hydrogen bonds were the most effective inhibitors of the protein [42].

Spatial and chemical structure analysis of various compounds able to interact with the Pgp have led to the classification of Phts into separate types of compounds: those which contain two electron donor groups at the distance of 2.5 ± 0.3 Å are classified as type I pattern, while compounds with two electron donor groups at the distance of 4.6 ± 0.6 Å from each other or three electron donor groups (a spatial distance of 4.6 ± 0.6 Å between two external groups is

required) belong to the type II pattern. It was stated that compounds are able to bound to the Pgp if they contain at least one motive of the type I pattern, or one motive of the type II pattern. At least two systems of electron donor groups of the I type, or one of the I type and one of the II type, were required for the compound to be transported by the Pgp. The presence of suitably spatially located acceptor groups or electron donors (e.g., carbonyl, ether group, tertiary amine, and so forth), participated in creating hydrogen bonds with the Pgp [38, 39].

It was experimentally verified for trifluoperazine that Phts with electron donor groups creating type I patterns directly interacted with the Pgp [23, 24, 38]. The direct interaction between Phts and Pgp is considered as a crucial mechanism necessary for inhibition of the transport function of the protein and an increase of cellular chemosensitivity [19, 21].

Conclusion

Phts exert strong inhibitory activity on the CaM, the PKC and the Pgp, which collectively account for their cancer chemopreventive and also MDR-reversing effects. According to the literature and also to our previous results, several directions for future chemical modification of Phts were presented and discussed in this review.

To revolve around a cancer chemopreventive activity of Phts, the following chemical structure conditions should be perceived as determinative: 1) the presence of carbonyl group and, to a lesser extent, also of an ether group, attached to the carbon C-2 atom, 2) the presence of a four-carbon atoms alkyl bridge and piperazine group in a form of piperazine and pyrimidine amines in the side chain, 3) the occurrence of terminal tertiary amine, or 4-substituted piperazine.

These main features of chemical structure have a marked influence on the anti-MDR activity of Phts. It should be also notified that the presence of acceptor groups or electron donors, able to create hydrogen bonds with the intramembrane fragments of the Pgp (as carbonyl and ether groups, tertiary amines) are crucial requirement for effective inhibitors of the MDR.

An introduction of new drugs able to increase the effect of cytostatic drugs on cancer cells is a major challenge for pharmacy today, and it could provide a marked improvement of cancer chemotherapy. The search for compounds able to increase accumulation of cytostatic drugs and their effect on cancer cells (i.e., chemosensitizing, anti-MDR drugs) are currently being carried on by many pharmaceutical teams. Among the candidates for effective anti-MDR drugs, Phts are worth further studying, since they are strong inhibitors of the Pgp transport function and exhibit several cancer chemopreventive actions.

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