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Quantitative Structure-Trypanocidal Activity Relationship Analysis of Phenothiazine Derivatives

KEYWORDS: 2D-QSAR, Computational chemistry, Trypanosoma, Molecular descriptors, Phenothiazine derivatives

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ABSTRACT *A semi-empirical Austin Model 1 (AM1) molecular orbital method was performed on nine Phenothiazine derivatives that have trypanocidal activity, among them are the well-known antipsychotic drugs: promazine, chlorpromazine, triflupromazine and acetopromazine, to investigate the correlation between their molecular structures and the corresponding inhibition efficiency (IC50). Quantum chemical parameters such as: energy of the highest occupied molecular orbital (EHOMO), energy of the lowest unoccupied molecular orbital (ELUMO), energy gap (ΔE), dipole moment (DM), total negative charge (TNC), molar volume (MV), electronegativity (χ), hardness (η), softness (σ) and the maximum amount of electronic charge (ΔNmax) acquired from the donor (the enzyme) by the inhibitor (acceptor), have been calculated. A significant correlation between the theoretical data and the experimental results was found*

Introduction:

Trypanosomiasis is a major third-world disease, with many millions of new infections presenting annually that is caused by Trypanosoma. Trypanosoma is a genus of kinetoplastids (class Kinetoplastida), a monophyletic [1]Trypanosoma cruzi and Leishmania major have been sequenced, but the phylogenetic relationships of these three protozoa remain uncertain. We have constructed trypanosomatid phylogenies based on genes for glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH group of unicellular parasitic flagellate protozoa. All trypanosomes are heteroxenous (requiring more than one obligatory host to complete life cycle) and the most are transmitted via a vector. Trypanosomes infect a variety of hosts and cause various diseases, including the fatal human diseases sleeping sickness, caused by Trypanosoma brucei, and Chagas disease, caused by Trypanosoma cruzi.

Present chemotherapies are inadequate, toxic, or both with current drugs including the arsenicals, nifurtimox, and pentamidine [2].Nifurtimox and benznidazole are the two major drugs available for Chagas' disease prevalent in South America. A metabolic difference between the pathogen and the mammalian host, recently discovered [2], may provide a means of developing a selective antiparasitic drug. Moreover, it may even prove possible to combat all three diseases, African trypanosomiasis, Chagas' disease, and leishmaniasis, with a single agent. Trypanothione reductase is an essential component of the anti-oxidant defenses of parasitic trypanosomes which differs markedly from the equivalent host enzyme, glutathione reductase, in the binding site for the disulphide substrate. Molecular modeling of this region suggested that certain tricyclic compounds might bind selectively to trypanothione reductase without inhibiting host glutathione reductase. Glutathione is responsible for many cellular protection activities including those against free radicals and oxygen-derived species. In the course of this action glutathione disulfide is formed, it is a substrate of glutathione reductase (GR). Trypanosomes do not contain GR but rather an analogous enzyme, trypanothione reductase (TR), and its substrate is trypanothione disulfide [3–6]. This mutual substrate exclusivity indicated that selective ligand design should be possible, making TR an important potential target for drug design against parasitic diseases involving trypanosomiasis and/or leishmaniasis [2, 6, 7].

A major function of trypanothione is in the defense against oxidative stress. Here, trypanothione-dependent enzymes such as tryparedoxin reductase (TryR) reduce peroxides using electrons donated from trypanothione [8]such as trypanosomes and leishmania, some of which are the causative agents of several tropical diseases. The dithiol is kept reduced by the flavoenzyme trypanothione reductase and the trypanothione system replaces in these parasites the nearly ubiquitous glutathione/glutathione reductase couple. Trypanothione is a reductant of thioredoxin and tryparedoxin, small dithiol proteins, which in turn deliver reducing equivalents for the synthesis of deoxyribonucleotides as well as for the detoxification of hydroperoxides by different peroxidases. Depending on the individual organism and the developmental state, the parasites also contain significant amounts of glutathione, mono-glutathionylspermidine and ovothiol, whereby all four low molecular mass thiols are directly (trypanothione and mono-glutathionylspermidine, this suggests that a good inhibitor would be a bad electron donor.

In 1992, phenothiazines were first suggested as trypanocidal agents that exclusively inhibit trypanothione reductase and not affecting glutathione reductase [9]. Also, it is worth mentioning that chlorpromazine (inhibitor 3) which is a well-known CNS drug, is the most potent trypanothione reductase inhibitor among the examined promazines [10].Moreover, the potential metabolites of chlorpromazine 3, which are inhibitors 8, 9 (metabolic N-demethylation products) and chlorpromazine sulfoxide (metabolic sulfoxidation product) are also trypanothione reductase inhibitors with inhibition potency of 0.254, 0.28 and 0.462, respectively, compared with chlorpromazine 3. Finally, chlorpromazine and its potential metabolites were shown not to inhibit human erythrocyte glutathione reductase [10], which strongly recommends chlorpromazine as a potential trypanocidal drug awaiting in vivo studies before announcing it as a new effective trypanocidal drug.

Quantitative structure–activity relationship (QSAR) tries to investigate the relationship between molecular descriptors that describe the unique physicochemical properties of the set of compounds of interest with their respective biological activity or chemical property [11, 12].

The aim of this paper is to find a correlation between mo-

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lecular and electronic structures of nine investigated phenothiazines (Fig. 1) which were found to have trypanocidal activity through inhibiting trypanothione reductase as their inhibition efficiency IC_{50} was reported [10]. Molecular orbital calculations were performed looking for good theoretical parameters to characterize the inhibition property of inhibitors which will be helpful to gain insight into the mechanism of inhibition.

Computational details:
The structures of the nine compounds were drawn using ChemDraw Ultra 10.0 and then were transformed to 3D structures using Chem3DUltra 10.0 [13].Complete geometrical optimization of the investigated molecules was performed on the 3D structures using Gaussian 03 program package [14] where the semi-empirical AM1 method [15] was chosen for structure optimization.

The IC_{50}values and the calculated descriptors for the investigated compounds are shown in Table I. The following descriptors were used: total energy of the molecule(E) dipole moment (DM), electron affinity (EA), ionization potential (IP), hydrophobicity index (log P), energy of the highest occupied molecular orbital (E_{HOMO}), energy of lowest unoccupied molecular orbital (E_{LUMO}), the separation energy ΔE (E_{LUMO}- E_{HOMO}), chemical potential (μ), absolute hardness (η), absolute softness (σ), absolute electron negativity (χ), total negative charge (TNC), electrophilicity index (ω), molar volume (MV) and ΔN_{max}.

Molecular descriptors were estimated following the Koopman's theorem [16, 17] which relates the descriptors to the energy of the HOMO and the LUMO. E_{HOMO} and E_{LUMO} of the inhibitor molecule are important in governing the molecular reactivity and properties and can be related to the ionization potential (IP) and the electron affinity (EA) respectively, by the following relations:

$$IP = -E_{HOMO}$$

$$EA = -E_{LUMO}$$

Electron polarizability, also called chemical softness (σ), describes the capacity of an atom or group of atoms to receive electrons [17, 18] is given by:

$$\sigma = (\mathrm{IP} - \mathrm{EA})/2$$

Absolute electronegativity (χ) is given by:

$$\chi = (\mathrm{IP} + \mathrm{EA})/2$$

Electronegativity is the power of an atom in a molecule to attract electrons to itself and is a very useful concept for the explanation or understanding of chemical reactivity.

Chemical hardness (η) measures the resistance of an atom to a charge transfer and is given by the following equation [17, 18]:

$$\eta = 1/\sigma$$

Global electrophilicity index (ω) is estimated by using the electronegativity and chemical hardness parameters through the equation [17, 18]:

$$\omega = \chi^2/2$$

A high value of electrophilicity describes a good electrophile while a small value of electrophilicity describes a good nucleophile.

The maximum number of electrons transferred (ΔN_{max}) in a chemical reaction is given by the equation [17, 18]:

$$\Delta N_{max} = -\mu/\eta$$

Results and discussion:
It was shown from experimental results that the inhibitor 3 with R1 substituent, Cl group, has the highest inhibition efficiency among the investigated inhibitors while inhibitor 5 with R1 substituent, carboxylic group, has the lowest efficiency. According to the mechanism of action of trypanothione reductase, it was suggested earlier that a good inhibitor would be a bad electron donor; this in turn suggests that a good inhibitor will probably have a high separation energy ΔE, high hardness η and low softness σ.

The calculated quantum chemical parameters showed that the 2-carboxylic acid substituent decreases the LUMO energy by about 0.015 au and increases the HOMO energy by about 0.003 au and accordingly decreases the separation energy ΔE by about 0.019 au. This could facilitate the charge transfer from the enzyme to inhibitor 5 comparable with inhibitor 3, which probably leads to a decrease in the inhibition activity of inhibitor 5 comparable with inhibitor 3.

The calculations also showed that EA increases (from 0.0112 to 0.0266 a.u respectively), which means that inhibitor **5** is more able to form hydrogen bond interaction with the enzyme comparable with inhibitor **3.** Electronegativity χ and chemical potential μ are also increased by about 0.006 au. This leads to a decrease in electron releasing ability of inhibitor **5** comparable with inhibitor **3**. Meanwhile, IP decreases (from 0.2856 to 0.2823 a.u, respectively) which probably lead to an increase in electron donating ability of inhibitor **5** comparable with inhibitor **3**. Chemical hardness η decreases (from 0.1372 to 0.1279 a.u respectively) which leads to a decrease in the resistance of inhibitor **5** to charge transfer comparable with inhibitor **3**, and accordingly decreases the inhibition activity of inhibitor **5** comparable with inhibitor **3**. But, chemical softness σ increases (from 7.2892 to 7.8211 $a.u^{-1}$ respectively) which increases the capacity of inhibitor **5** to receive electrons comparable with inhibitor **3**. Accordingly, inhibitor **5** has a lower biological activity than inhibitor 3. The calculations showed that the electrophilicity index ω increases (from 0.0803 to 0.0933 a.u, respectively); this increases the ability of inhibitor **5** to be stabilized when it acquires an additional charge ΔN from the enzyme comparable with inhibitor **3**. Also, ΔN_{max} increases (from 1.0816 to 1.2079 e); this means that inhibitor **5** has higher ability to accept electrons comparable with inhibitor **3**. The dipole moment decreases (from 1.6540 to 0.7802 D respectively); this means that inhibitor **5** is much less polar comparable with inhibitor **3,** and accordingly inhibitor **5** is much less able to make dipole-dipole interactions with the enzyme comparable with inhibitor **3**; this probably leads to decreasing inhibition activity of inhibitor **5** comparable with inhibitor **3**.

This could indicate that inhibitor 3 has higher ability than that of inhibitor 5 to block the active site of the enzyme without subsequent chemical change of inhibitor 3, just like the case of the enzyme succinate dehydrogenase which is blocked by malonate without undergoing dehydrogenation [19]. So, the ability of inhibitor 3 to block the active site of the enzyme without subsequent chemical change of inhibitor 3 probably increases the biological activity of inhibitor 3 comparable with inhibitor 5. In the meanwhile, inhibitor 5 probably accepts the charge (an electron) from NADPH and passes it to the enzyme; this could explain the highly decreased activity of inhibitor 5.

The log IC50 values and the calculated descriptors for 9 promazines drugs are shown in Table 1. The correlation between log IC50 and some of the descriptors is shown in Figure 2, where R2 (coefficient of correlation) was calculated for each plot. The correlation coefficient, R2, is a statistical measure of how well the regression line approximates the real data points.

The inhibitor 1 has an H-atom (R1) at C2-atom of phenothiazine (Figure 1). The effect of substitution of an H-atom (R1) by an electron withdrawing substituent in the case of inhibitors 2-5 and 7, on the inhibition efficiency is investigated. Also, the replacing of R1 by $-CONH_2$ group, inhibitor 6, affects the quantum chemical descriptors (Table 1). Meanwhile, the quantum chemical parameters of inhibitors 8and 9 are

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also calculated in Table 1. It was found that there is a good linear relationship between log IC_{50} and the separation energy (ΔE), the absolute hardness (η) and softness (σ) with R2 values, 0.728, 0.728 and 0.724, respectively, (Figure 3,a-c). It was shown that the inhibition efficiency decreases as the separation energy (ΔE), and absolute hardness, η, increases. On the contrary, log IC_{50} decreases as softness decreases. This means that the higher (ΔE), the higher (η) and the lower (σ) are required for a more potent promazine inhibitor of trypanothione reductase enzyme.

When log IC_{50} was plotted against the total energy (E) of the molecule, a parabolic curve was produced with a maximum log IC_{50} of 3.4 and total energy equals -0.02a.u. (Figure 2, d), which indicate a highly significant correlation between log IC_{50} and the total energy of the molecule (E) with R^2, 0.89, suggesting that the total energy descriptor can be used for the estimation of the trypanocidal activity of promazine derivatives.

When log IC_{50} was plotted against log P, E_{LUMO}, ΔN_{max} , EA and TNC parameters, parabolic curves were produced with a maximum trypanothione reductase inhibition activity, (Figure 2,e-i).

It was found the there was no significant correlation between log IC_{50} and electrophilicity (ω), chemical potential (μ), energy of the highest occupied molecular orbital (E_{HOMO}), electronegativity (χ), ionization potential (IP), molecular volume (MV) and dipole moment (DM).

Conclusion:

The present study showed the effect of substituent, R1,R2 and R3, of phenothiazine on the quantum chemical parameters and on their inhibition efficiencies. The quantum chemical descriptors could explain the lower inhibition activity of inhibitor 5 comparable to inhibitor 3 according to decreasing the energy of LUMO, ΔE,η, IP and DM, and increasing the HOMO energy, EA, σ, χ, μ, ω and ΔNmax. This could facilitate the charge transfer from the enzyme to inhibitor 5, and then from inhibitor 5 back to the enzyme, which probably decreases the inhibition activity of inhibitor 5 comparable with inhibitor 3.

It was shown that trypanocidal activity of promazine derivatives has a strong linear correlation with the separation energy (ΔE), the chemical hardness (η) and the chemical softness, and a highly significant quadratic correlation with the total energy, which show the importance of those descriptors for more potent inhibitors. These four descriptors can be used for the estimation of the trypanocidal activity of promazine derivatives. Parabolic curves were found between log IC_{50} and the descriptors E, Log P, TNC, E_{LUMO}, EA and ΔN_{max}.

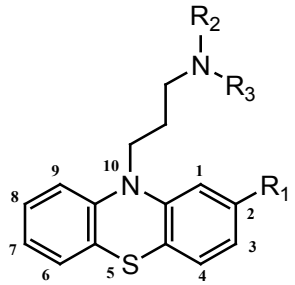

Fig.1.Chemical structures of the investigated phenothiazine derivatives.

Compound #	R_1	R_2	R_3
1	H	CH_2	CH_3
2	CF_3	CH_2	CH_3
3	Cl	CH_2	CH_3
4	$COCH_3$	CH_2	CH_3
5	COOH	CH_2	CH_3
6	CH_2OH	CH_2	CH_3
7	$CONH_2$	CH_2	CH_3
8	Cl	H	H
9	Cl	H	CH_3

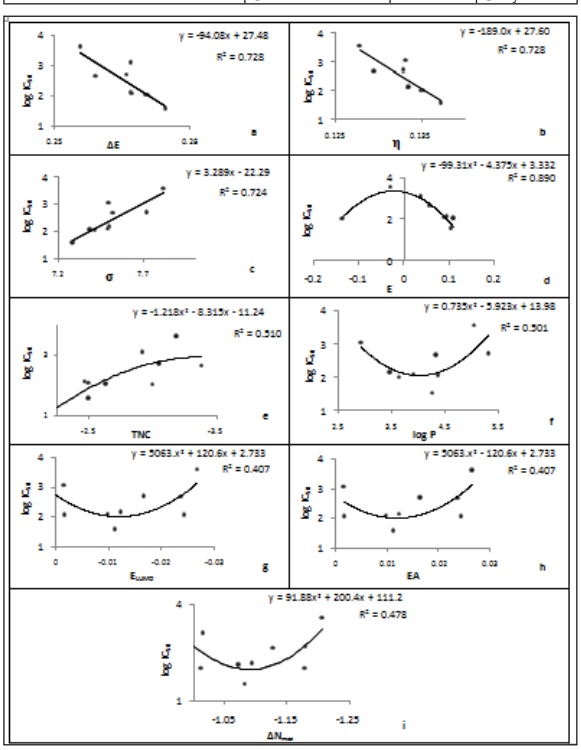

Fig.2.The correlation between log IC_{50} and the calculated descriptors.

Table 1.The trypanocidal activity (expressed in log IC_{50}) and the calculated descriptors for phenothiazine derivatives.

#	Log IC_{50}	E (a.u.)	DM (D)	E_{LUMO} (a.u.)	E_{HOMO} (a.u.)	ΔE (a.u.)	IP (a.u.)	EA (a.u.)	χ (a.u.)	η (a.u.)	σ ($a.u.^{-1}$)	TNC (e)	ω	μ (a.u.)	ΔN_{max} (e)	MV ($Å^3$)	Log P
1	2.03	0.1108	2.3355	-0.0017	-0.2724	0.2707	0.2724	0.0017	0.1370	0.1353	7.3896	-2.6370	0.0694	-0.1370	1.0126	861.07	3.64
2	2.04	-0.1354	3.9911	-0.0243	-0.2941	0.2699	0.2941	0.0243	0.1592	0.1349	7.4115	-2.9909	0.0939	-0.1592	1.1799	963.06	4.37
3	1.55	0.1019	1.6540	-0.0112	-0.2856	0.2744	0.2856	0.0112	0.1484	0.1372	7.2892	-2.5025	0.0803	-0.1484	1.0816	900.35	4.26
4	2.70	0.0544	3.5448	-0.0238	-0.2895	0.2658	0.2895	0.0238	0.1566	0.1329	7.5259	-3.0551	0.0923	-0.1566	1.1788	958.92	5.31
5	3.59	-0.0322	0.7802	-0.0266	-0.2823	0.2557	0.2823	0.0266	0.1544	0.1279	7.8211	-3.1902	0.0933	-0.1544	1.2079	934.52	5.04
6	3.07	0.0346	3.6280	-0.0018	-0.2684	0.2667	0.2684	0.0018	0.1351	0.1333	7.4996	-2.9115	0.0684	-0.1351	1.0132	927.76	2.95

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7	2.66	0.0544	2.1944	-0.0166	-0.2759	0.2593	0.2759	0.0166	0.1463	0.1296	7.7140	-3.3770	0.0825	-0.1463	1.1284	939.33	4.35
8	2.14	0.0863	1.5533	-0.0123	-0.2791	0.2668	0.2791	0.0123	0.1457	0.1334	7.4974	-2.4605	0.0796	-0.1457	1.0925	801.74	3.49
9	2.10	0.0933	2.8166	-0.0098	-0.2769	0.2672	0.2769	0.0098	0.1433	0.1336	7.4864	-2.5020	0.0769	-0.1433	1.0731	853.96	3.9

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