Bioorganic & Medicinal Chemistry 17 (2009) 2673–2679

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Molecular modeling studies and in vitro bioactivity evaluation of a set of novel 5-nitro-heterocyclic derivatives as anti-*T. cruzi* agents

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ARTICLE INFO

Article history:
Received 24 November 2008
Revised 26 February 2009
Accepted 27 February 2009
Available online 5 March 2009

Keywords:
Nitrocompounds
Molecular modeling
Bioactivity assay
Chagas disease

ABSTRACT

In this study, in vitro anti-*T. cruzi* activity assays of nifuroxazide (NX) analogues, such as 5-nitro-2-furfuryliden and 5-nitro-2-theniliden derivatives, were performed. A molecular modeling approach was also carried out to relate the lipophilicity potential (LP) property and biological activity data. The majority of the NX derivatives showed increased anti-*T. cruzi* activity in comparison to the reference drug, benznidazole (BZN). Additionally, the 5-nitro-2-furfuryliden derivatives presented better pharmacological profile than the 5-nitro-2-theniliden analogues. The LP maps and corresponding *Clog P* values indicate that there is an optimum lipophilicity value, which must be observed in the design of new potential anti-*T. cruzi* agents.

1. Introduction

Chagas disease, also known as American trypanosomiasis or South American trypanosomiasis, is a protozoal disease caused by the parasite *Trypanosoma cruzi*.[1] It is a chronic and debilitating parasitic infection that affects millions of people in Mexico, Central America, and South America. Approximately 25% of the population of Latin America is at a risk for acquiring the infection. Additionally, as a result of reactivation among immigrant populations, Chagas disease is increasingly being reported in non-endemic settings as in several countries in Europe and various parts of the United States becoming a global problem. An estimated 11 million people are infected worldwide; of these, 15–30% have clinical symptoms.[2]

The 'burden of disease' is the gap between an ideal situation where every individual in a population lives into old age in full health and the current health of a population affected by the disease. As in all neglected diseases, Chagas disease creates financial and social burdens to the individual, his or her household and country.[3–5] The early mortality and substantial disability caused by this disease, which often occurs in the most productive population, young adults, results in a devastating economic loss in the Americas. More recent data demonstrate that globally, this disease is associated with 14,000 deaths per year and 0.7 million disability-adjusted life-years (DALYs), constituting the sixth most important neglected tropical disease worldwide.[6]

There are currently two drugs in the treatment of Chagas disease, benznidazole (BZN) (available in the Latin countries) and nifurtimox (available in the United States, under an Investigational New Drug protocol from the CDC Drug Service), which are used in the acute phase and in reactivation in immunosupressed patients.[2,7,8] Chagas endemic countries generally use one of two drugs to treat disease victims. Chemotherapy can shorten the acute phase and achieve parasitological cure in 60% of the cases, but because of the adverse effects associated with the two drugs, patients being treated require careful monitoring.[7,8] In addition, these drugs do not eliminate the parasites, and resistance has been reported.[2,8] Considering that, it is still necessary to search for new potential antichagasic agents more selective and presenting less toxicity.

Molecular structure modifications might alter physicochemical properties in order to improve drug-receptor interaction and also provide information to elucidate the bioactivity mechanism. Several compounds presenting significant antichagasic activity have been reported,[9–13] but nifuroxazide (NX) [4-hidroxy-*N*′-(5-nitro-2-furfurylidene) benzhydrazide (Fig. 1)], which has a broad antibacterial spectrum, deserves particularly attention regarding the antiprotozoal activity.[9]

Successful studies have been accomplished based on molecular structure modifications of NX, structure–activity relationships, and also considering the evaluation of its derivatives activity against wild and multi-resistant strains of *Staphylococcus*[14–17] as well as *Mycobacterium* sp.[18]

Our research group have designed and synthesized a set of NX analogues. The 5-nitro-2-furfuryliden and 5-nitro-2-theniliden

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doi:10.1016/j.bmc.2009.02.056

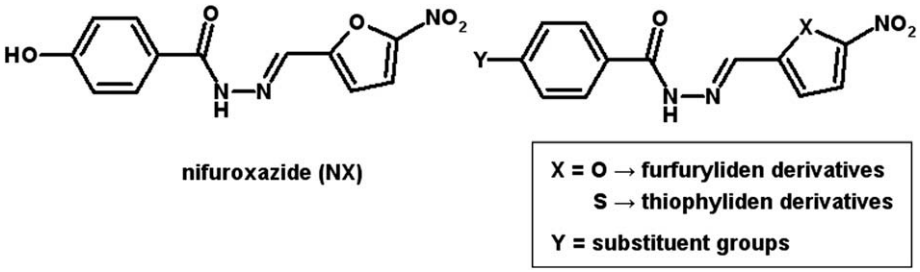


Figure 1. Chemical structures of nifuroxazide (NX) and 5-nitro heterocyclic derivatives as potential anti-*T. cruzi* derivatives.

4-**Y**-benzhydrazide derivatives (see Fig. 1) seem to be extremely promising as antibacterial agents, specially against MRSA and VISA strains.[17,18]

In this study, a set of novel NX derivatives (5-nitro-heterocyclics) were evaluated regarding their anti-*T. cruzi* bioactivity. The antibacterial, antifungal, and antimycobacterial activities of this set of compounds were already reported.[14,15,18,19]

In addition, a molecular modeling approach was performed to verify possible qualitative property-activity relationships, considering the lipophilicity potential (LP) maps of the investigated compounds, associated to the respective calculated log *P* (Clog *P*) values, and their biological data. The lipophilicity or hydrophobic property is quite important to the diffusion of those derivatives through the parasite biological system.

2. Material and methods

2.1. The set of investigated compounds

The 10 compounds tested in this study were the following: 5-nitro-2-theniliden 4-**Y**-benzhydrazide derivatives, where **Y** = H, Cl, CF_3, OCH_3, $N(CH_3)_2$, SO_2NH_2, OH, CN; and 5-nitro-2-furfuryliden 4-**Y**-benzhydrazide derivatives, where **Y** = H and Cl (Fig. 1). These compounds were previously designed and synthesized,[14–18] and they were available to be evaluated in the present work.

2.2. Anti-*T. cruzi* activity assays

In vitro anti-*T. cruzi* activity assays were performed against the epimastigote form of Y strain *T. cruzi* isolated by Silva and Nussenzweig in 1953.[20] The strain was kindly provided by Professor Maria Júlia Manso Alves of the Laboratory of Biochemical Parasitology, Department of Biochemistry, Institute of Chemistry, University of São Paulo (USP).

2.3. Medium and cultivation conditions

The parasite was cultivated in liver infusion tryptose (LIT) medium composed by 0.4% NaCl, 0.04% KCl, 0.8% Na_2HPO_4, 0.2% glucose, 0.5% tryptose, and 0.5% liver infusion. The percentage of solute in the total amount of solution was expressed as mass/volume. The medium was enriched with 10% fetal bovine serum, FBS (v/v). The pH value was adjusted to 7.2 using HCl. The incubation was at 68 °C for 1 h. When the medium reached the environmental temperature, a bovine hemoglobin solution was added up to obtain the desired medium concentration (2%, v/v). The sterilization of the culture medium was carried out by filtration using a 0.22 mm Millipore membrane. Then, the medium was fractioned in glass vessels and stored at 4 °C until the moment of use.[21,22] The cultivation of culture medium after seeded the epimastigote forms (from a culture medium with 72 h of incubation) presented approximately 50×10^6 parasites per mL (24 h of incubation).

2.4. Anti-*T. cruzi* susceptibility assays

The nitroderivatives and BZN, the reference drug, were separately dissolved in DMSO and diluted in the LIT culture medium to obtain a concentration of 1.0–20.0 μg/mL. An aliquot of the seeded medium (inocule), considering the viability of parasites, was added to Falcon tube containing the investigated compounds. The initial concentration 10^6 parasites/mL was defined as standard to develop all assays. The Falcon tubes containing the parasite suspension plus the nitrocompounds were incubated at 28 °C for 24 h.[13] This procedure was also followed for preparing the control suspension.

Biological activity of the nitroderivatives was evaluated in triplicate against *T. cruzi* applying a method to count parasites (epimastigote forms) in a Neubauer chamber considering the zero time and 24 h of incubation. Then, the equation 1 was employed to determine the percentage of inhibition of parasite growth:

$$\mathrm{PG}\ (\%) = (L_{24} - L_0) \times 100/L_0 \qquad (1)$$

where PG (%) is the percentage of parasite growth in 24 h; L_0 is the number of parasites in zero time; L_{24} is the number of parasites in 24 h incubation.

2.5. Molecular modeling methodology

The three-dimensional structures of each of the 10 nitrocompounds derivatives or ligands in their neutral forms were constructed using the HYPERCHEM 7 software.[23] The Cartesian coordinates of NX crystallized structure were retrieved from Cambridge Structural Database (CSD)[24] (entry code LEQTAC (*R*-factor 0.11)[25] and used as a geometry reference in the building up of all ligands. Each structure was energy-minimized using the following methods: MM+ force field (derived from MM2),[26] AM1 semiempirical method[27] in HYPERCHEM 7,[23] and ab initio Hartree–Fock (basis set 6-31G*) method in GAUSSIAN 03W, v.6,[28] without any restriction. Electrostatic partial atomic charges (Chelpg) were computed employing HF/6-31G* method, also implemented in the GAUSSIAN 03W program. NX and BZN models were also submitted to the same energy-minimized protocol. Crystallographic information of a BZN conformation was retrieved from the Ref. 29 and used as starting geometry for drug model.

The structures modeled as described above were taken as the initial structures for computing the LP property onto a Connolly molecular surface,[30,31] using a sphere probe of 1.4 Å radii (SYBYL 8.0 package).[32] The LP property and the Clog *P* values were calculated employing the Ghose and co-workers method[33] and SYBYL Line Notation (SLN) (SYBYL 8.0 package).[32] Properties that are applied to a surface can be a useful analytic tool in visually identifying areas of interest on the surface. The resulting LP maps were analyzed according to the color ramps, which range from brown (highest lipophilic area of the molecule) to blue (highest hydrophilic area). The color scheme is easy to interpret if the blue is associated with water and the brown as oil/fat.

3. Results

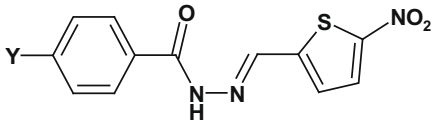
The in vitro anti-*T. cruzi* susceptibility assays of nitrocompounds were carried out considering the following concentrations: 1.0, 10.0, and 20.0 μg/mL, respectively. Those concentrations were set based on the solubility of the investigated compounds in the culture medium. This assay was also performed to BZN, which was chosen as reference because is the only drug available for treating Chagas disease in Brazil.

The nitrocompounds were dissolved in DMSO (0.1%) and then mixed in the LIT medium to obtain those desired concentrations. The presence of the DMSO solvent up to 10% did not present any interference in the biological assay. It is noteworthy that the results found for the DMSO toxicity assay against the hemoparasite were in agreement to those described by Dias and Tavares (2005).[13]

However, the precipitation of the investigated compounds was observed when the nitrocompounds concentrations increased to values higher than 20.0 μg/mL (in LIT culture medium and DMSO 1.0%).

The nitrocompounds activity was evaluated regarding the inhibition of the *T. cruzi* epimastigote forms (Y strain) growth in axenic culture medium (LIT). The epimastigote forms were chosen because they are considered as 'not-infectant'. In addition, the use of *T. cruzi* epimastigote forms is relevant in a preliminary evaluation of the antiproliferative effect of potential antichagasic agents, since those forms are present in mammal's cells.[12,34–38] The results of the biological assay, obtained using the method of inhibiting the proliferation of protozoal parasite, are presented in Tables 1 and 2. Data are expressed as the percentage of parasite growth in 24 h.

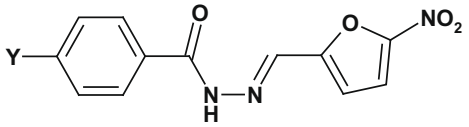
Table 1
5-Nitro-2-theniliden 4-**Y**-benzhydrazide derivatives and benznidazole in vitro activity against Y strain *T. cruzi*



Compound	Substituent group (**Y**)	Concentration (μg/mL)	Zero (h)[a]	24 (h)[a]	PG[b] (%)	Anti-*T. cruzi* activity
I	H	1.0	21.4	24.2	13.0	(−)
		10.0	15.7	24.7	57.3	(−)
		20.0	29.8	29.7	0.0	(−)
II	Cl	1.0	29.2	30.3	4.0	(−)
		10.0	38.3	30.3	0.0	(+)
		20.0	45.0	7.0	0.0	(+)
III	CF_3	1.0	48.5	62.3	28.4	(−)
		10.0	45.7	5.7	0.0	(+)
		20.0	37.0	2.0	0.0	(+)
IV	OCH_3	1.0	24.0	49.0	100.0	(−)
		10.0	40.7	34.3	0.0	(+)
		20.0	35.3	34.0	0.0	(+)
V	$N(CH_3)_2$	1.0	32.3	35.3	10.0	(−)
		10.0	28.3	40.6	43.6	(−)
		20.0	40.0	31.2	0.0	(+)
VI	SO_2NH_2	1.0	22.0	25.6	16.0	(−)
		10.0	24.1	28.49	18.2	(−)
		20.0	35.7	42.5	19.0	(−)
VII	CN[c]	10.0	22.5	15.5	0.0	(+)
VIII	OH[c]	10.0	20.7	32.3	56.6	(−)
Control suspension		—	35.8	50.7	41.7	—
benznidazole		10.0	35.0	50.0	30.0	(−)
		20.0	44.3	45.7	3.0	(−)

[a] Number of counted cells (10^6), in triplicate.
[b] Percentage of parasite growth.
[c] Ref. 10.

Table 2
5-Nitro-2-furfuryliden 4-**Y**-benzhydrazide derivatives and benznidazole in vitro activity against Y strain *T. cruzi*



Compound	Substituent group (**Y**)	Concentration (μg/mL)	Zero (h)[a]	24 (h)[a]	PG[b] (%)	Anti-*T. cruzi* activity
IX	H	1.0	44.9	43.6	0.0	(+)
		10.0	31.7	31.0	0.0	(+)
		20.0	38.0	25.6	0.0	(+)
X	Cl	1.0	48.3	44.0	0.0	(+)
		10.0	30.3	30.0	0.0	(+)
		20.0	48.0	24.3	0.0	(+)
Control suspension		—	35.8	50.7	41.7	—
benznidazole		10.0	35.0	50.0	30.0	(−)
		20.0	44.3	45.7	3.0	(−)

[a] Number of counted cells (10^6), in triplicate.
[b] Percentage of parasite growth.

The parasite growth in the presence of nitrocompounds was considered as inexistent when the number of counted cells after 24 h of incubation was the same or lower than the number found for the zero time. The percentage of inhibition of proliferation was discarded because the investigated compounds can inhibit the parasite growth as well as kill part of the inoculated parasites. Preliminary results indicated that the control suspension, which contains parasites incubated (24 h) in LIT medium and 1.0% DMSO, presented a parasite growth of approximately 40%.

Regarding Tables 1 and 2, the compounds assayed in 1.0 μg/mL concentration presented lower percentage of parasite growth than the control suspension, except compound **IV**. Compounds **II**, **III**, **IV**, **IX**, and **X** presented 100% of inhibition of parasite growth when used in 10.0 and 20.0 μg/mL, but compound **V** only when used in 20.0 μg/mL. These six compounds had better inhibition parasite growth performance in comparison to BZN, corroborating the assumption that they are promising anti-*T. cruzi* agents.

The overall hydrophobicity or lipophilicity of a molecule can be measured by its partition coefficient (log*P*) in polar/apolar heterogeneous reference systems. A comprehensive study of partition experiments in the octanol/water system leads to the definition of hydrophobic contributions of single atoms in their specific structural environment. These partial atomic values can be regarded as fragmental increments (f_i) to the total lipophilicity given by log*P*, which corresponds to the summation of all f_i values. The *C*log*P* values found for the 5-nitro heterocyclic derivatives, NX, and BZN are presented in Table 3.

Table 3
*C*log*P* values of 5-nitro heterocyclic derivatives, nifuroxazide, and benznidazole, using Ghose et al. method (1998)

Compounds-Y	*C*log*P* values
I-H	1.25
II-Cl	1.92
III-CF_3	2.20
IV-OCH_3	1.24
V-$N(CH_3)_2$	1.42
VI-SO_2NH_2	0.48
VII-CN	1.13
VIII-OH	0.99
IX-H	0.69
X-Cl	1.36
Nifuroxazide	0.43
Benznidazole	−0.59

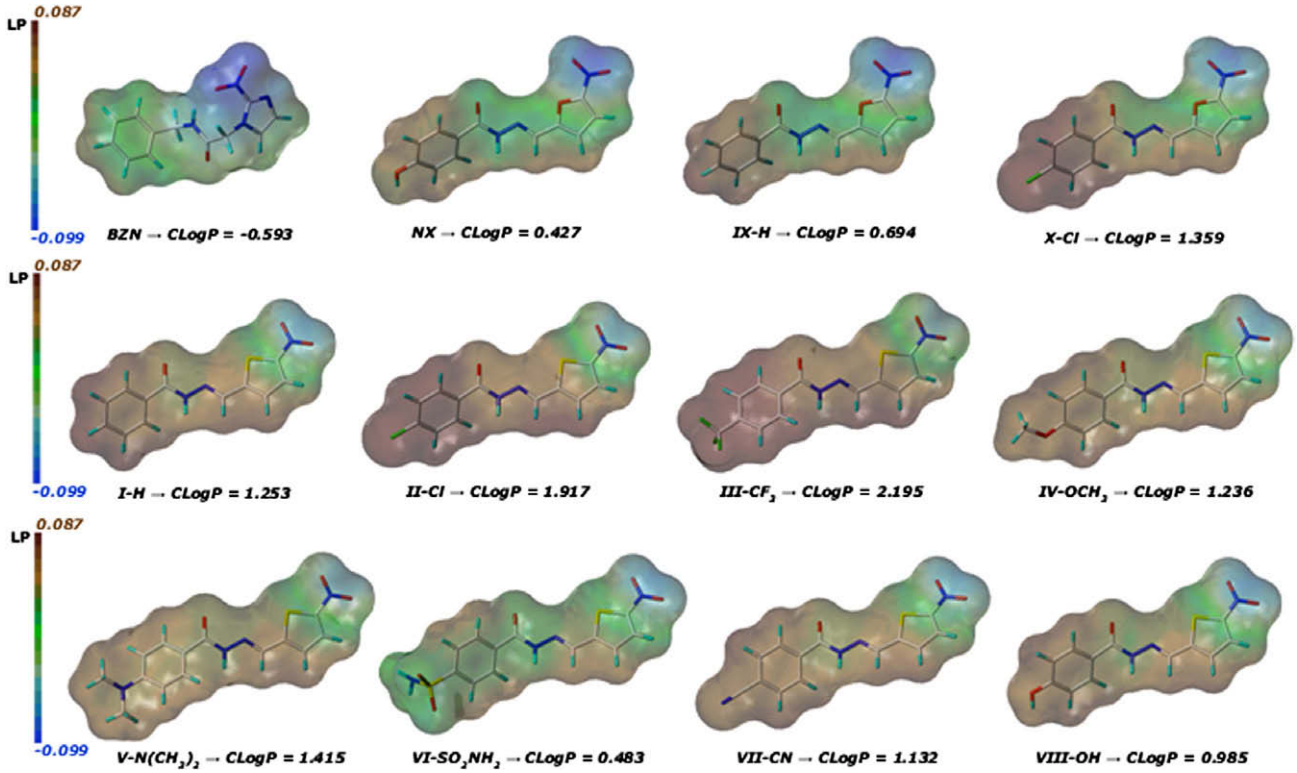


Figure 2. LP translucid colored maps of 5-nitro heterocyclic derivatives, nifurozaxide (NX) and benznidazole (BZN), using SYBYL 8.0 (Tripos, Inc., 2007).[32] Brown color indicates hydrophobic regions and blue color denotes hydrophilic areas. The molecules are displayed in stick capped model (carbon atoms are in light gray color, oxygen in red, nitrogen in blue, sulphur in yellow, chlorine in dark gray, and hydrogen atoms are presented in cyan).

Considering the 5-nitro-2-theniliden 4-**Y**-benzhydrazide derivatives, the highest *Clog P* values were given when **Y** corresponds to the CF_3 (2.20) and Cl (1.92) substituent groups, respectively. On the other hand, the 5-nitro-2-furfuryliden 4-Cl-benzhydrazide derivative presented higher *Clog P* value than the corresponding unsubstituted derivative as well as than NX. Additionally, the reference drug, BZN, presented the lowest *Clog P* value (−0.59), according to the methodology employed in this study.

On the basis of the partial atomic lipophilicity values (f_i), a distance dependent function has been defined for a LP (d_i = distance of a certain point in space from atom i).[38] The applicability of this potential has been proven for small molecules.[39] The LP maps calculated onto a Connolly molecular surface for the investigated compounds, NX, and BZN, are displayed in Figure 2. The color range applied for all molecules was −0.10 (blue) to 0.09 (brown), which is the color range found for BZN. As already mentioned, the brown color indicates highest LP values (hydrophobic regions) whereas blue color denotes lowest LP values (hydrophilic areas). The LP maps are intimately related to the *Clog P* values found for the set of compounds investigated.

The decreasing of parasite growth related to the control suspension was taken into account to evaluate the activity of the nitrocompounds. Then, compounds that reduced the number of counted cells (after 24 h of incubation) to lower values than those found for the zero time were considered as having anti-*T. cruzi* activity (+) (see Tables 1 and 2). According to that observation, compounds **II**, **III**, **V**, **IX**, and **X**, in 20.0 μg/mL concentration presented anti-*T. cruzi* activity. Furthermore, the compound **III** was the most active in the investigated set. Considering the lipophilicity effect, the CF_3 substituent group (**Y**) of compound III provides the highest *Clog P* value (Fig. 3), indicating that the molecule partitioning is a relevant property for the biological activity.

4. Discussion

It is noteworthy that BNZ and nifurtimox function as prodrugs and must be activated within the parasite to have trypanocidal effects. Despite of more than forty years of research, the mechanism(s) of action and resistance have remained elusive. Wilkinson and co-workers (2008)[40] reported that in trypanosomes, both drugs are activated by a NADH-dependent, mitochondrially localized, bacterial-like, type I nitroreductase (NTR), and that down-regulation of this explains how resistance may emerge. Loss of a single copy of this gene in *T. cruzi*, either through in vitro drug selection or by targeted gene delection, is sufficient to cause significant cross-resistance to a wide range of nitro-heterocyclic drugs.

An essential requisite for the triggering of biological activity in the nitro-heterocyclic compounds has been identified as the bioreduction of the nitro-group. That results in a disturbance of the physiological electron flow and thus inhibits enzymes associated to the cell's energy generation process, vital for the microorganism's biochemical maintenance.[41–45]

Considering the EPS partial atomic charges, some electronic differences were noticed in the ring in which the nitro-group was bound. When **X** is a sulfur (thieno-ring), the carbon atom bound to the nitrogen of nitro-group presented a more neutral charge (0.000–0.009) whereas in the furan ring (**X** = O) the same carbon had a more negative charge (−0.227 to −0.237), including the NX. Otherwise, to the reference drug, BNZ, the carbon bound to nitro-group of the imidazole ring presented a positive charge (0.219). Thus, the electron-withdrawing effect of the nitro-group on the carbon atom of the five-membered ring was more relevant to the drug BNZ and theniliden derivatives. Anyway, just the evaluation of the partial atomic charges is not sufficient to establish a possible mode of action regarding the bioreduction of nitro-group. More studies using a set containing a larger number of compounds and

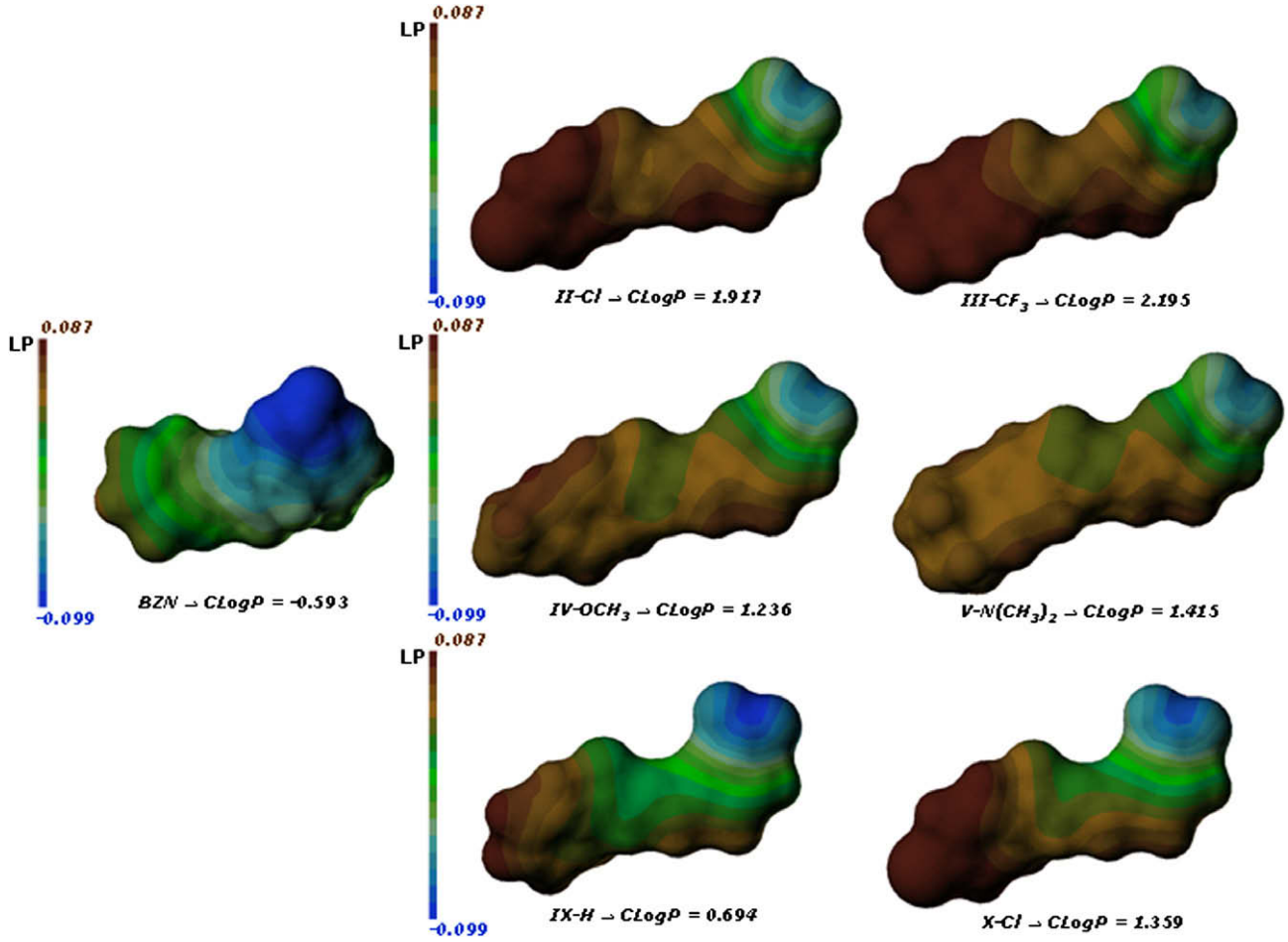


Figure 3. Comparison of LP texture maps of II, III, IV, V, IX, and X compounds to the reference drug, benznidazole (BZN), using SYBYL 8.0 (Tripos, Inc., 2007).[32] Brown color indicates hydrophobic regions and blue color denotes hydrophilic areas. Compounds **II**–**V** are theniliden derivatives while **IX** and **X** are furfuryliden derivatives.

considering more electronic parameters are ongoing to explain the electronic behavior of these nitro-heterocyclic compounds.

Tavares and co-workers[46,47] observed that hydrophobicity is the physicochemical property that has the greatest influence on the biological activity in series of 5-nitrofuran derivatives with structure analogous to that of NX.

It is generally found that increasing the hydrophobicity of a lead compound results in an increase in biological activity. This reflects the fact that drugs have to cross hydrophobic barriers such as cell membranes in order to reach their target. Even no barriers are to be crossed (in in vitro studies, for example), the drug has to interact with a target system such as an enzyme or receptor where the binding site is usually hydrophobic. The partition coefficient is a property that expresses the hydrophobic character of a drug or compound, and can be calculated by knowing the contribution that various substituents make to hydrophobicity. This contribution is known as the substituent hydrophobicity constant (π)[48] and is a measure of how hydrophobic is a substituent related to hydrogen. A positive value of π indicates that the substituent is more hydrophobic than hydrogen whereas a negative value indicates that the substituent is less hydrophobic. It is worth to remembering that in aromatic systems the substituent π and f_i values are equally employed whereas in aliphatic systems just the f_i values are recommended.[49]

Anyway, if the partition coefficient is the only factor influencing biological activity, the property-activity relationships are expressed as a parabolic curve, and the log*P* value at the maximum value of activity represents the optimum partition coefficient for biological activity [log(1/*C*)][50] (see Fig. 4). Beyond that point, an increase in log*P* results in a decrease in biological activity. The drug may become so hydrophobic that it is poorly soluble in the aqueous phase, for example, or, alternatively, it may be 'trapped' in fat depots and never reach the intended interaction site. Also, hydrophobic drugs are often more susceptible to metabolism and subsequent elimination.

The substituents at the benzene ring moiety (**Y**) in the nitro-heterocyclic compounds evaluated in this study exerted a distinct influence in the calculated hydrophobicity of the whole molecule

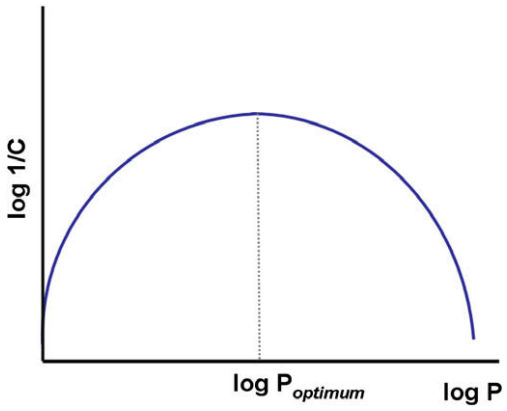


Figure 4. Parabolic curve of log(1/*C*) versus log*P*.

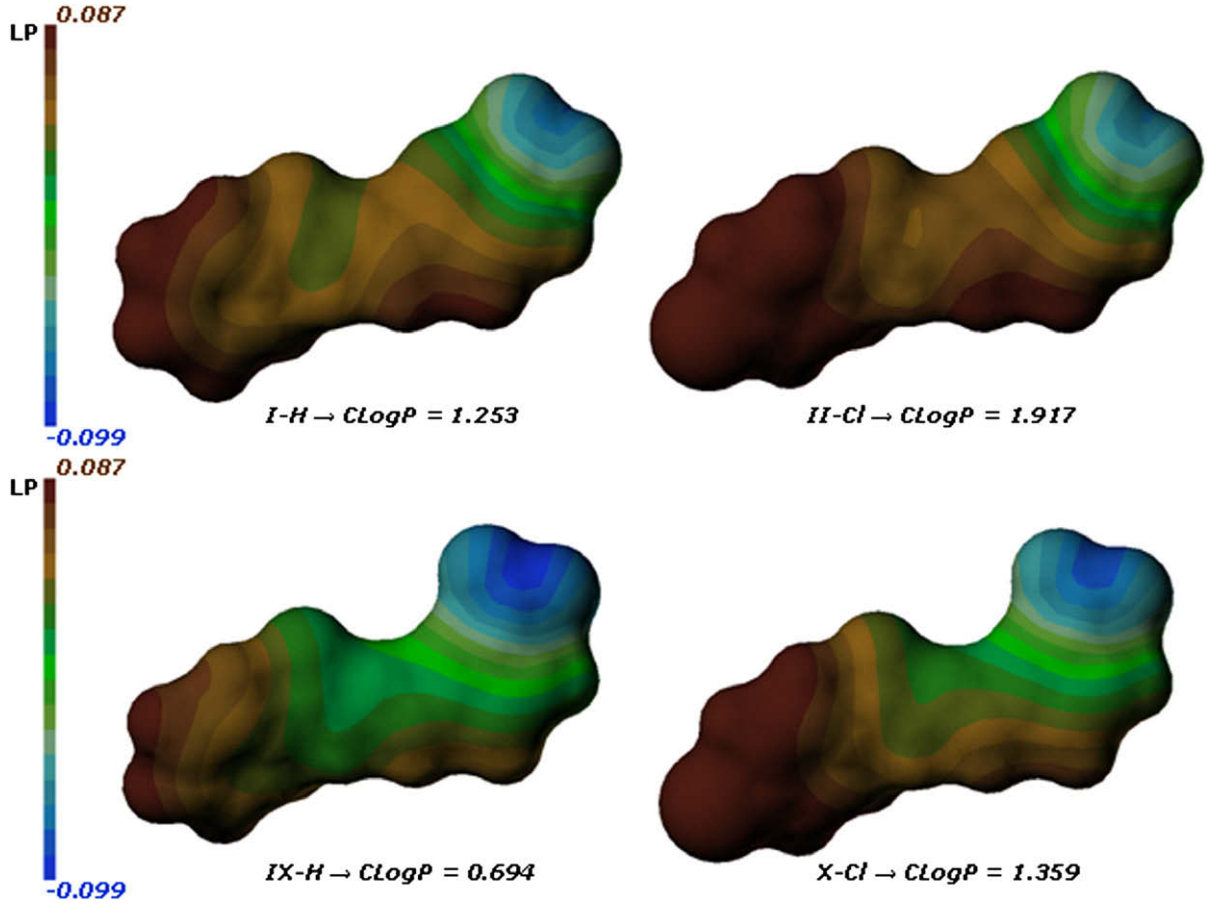


Figure 5. Comparison of LP texture maps of the compounds **I** and **II** (theniliden derivatives) to the compounds **IX** and **X** (furfuryliden derivatives), using SYBYL 8.0 (Tripos, Inc., 2007).[32] Brown color indicates hydrophobic regions and blue color denotes hydrophilic areas.

(*C*log*P* values), which can be visualized in the colored LP maps. The most hydrophobic substituents evaluated were CF_3 ($\pi = 1.16$) and Cl ($\pi = 0.71$) (dark brown color in the LP maps). The hydrophobic gradient of the **Y**-groups, according to their π values, is the following: $CF_3 > Cl > N(CH_3)_2 > H > OCH_3 > CN > OH > SO_2(NH_2)$.[51] Among the more hydrophilic groups tested are OH and SO_2NH_2. Thus, regarding the conditions applied here, the substituent that conferred the optimum calculated partition coefficient to the anti-*T. cruzi* activity was the chlorine.

Additionally, the comparison of percentage of parasite growth data between the compounds **I** and **II** to compounds **IX** and **X**, respectively, using a concentration of 1.0 μg/mL suggests that the furfuryliden derivatives (**X** = O) are more active than the theniliden (**X** = S). These compounds have **Y** = H and Cl ($\pi = 0$ and $\pi = 0.71$, respectively) at the benzene ring moiety.

The LP texture maps and the respective *C*log*P* values found for the compounds **I**, **II**, IX and **X** (Fig. 5) indicate that there is an optimum lipophilicity value, which must be observed in the design of new potential anti-*T. cruzi* agents. It seems that the two moieties in the investigated compounds must be considered, the **Y**-groups at the benzene ring moiety as well as the **X** atom in the five-membered ring moiety in which is attached the nitro-group. Compound **X** (**Clog***P* = 1.36; **Y** = Cl, and $\pi = 0.71$; **X** = O) probably should take as the lead because it presented anti-*T. cruzi* activity in all concentrations tested.

5. Conclusions

The majority part of the NX analogues showed increased anti-*T. cruzi* activity in comparison to the reference drug, benznidazole (BZN). The 5-nitro-2-furfuryliden derivatives presented better pharmacological profile than the 5-nitro-2-theniliden analogues.

The LP maps and corresponding *C*log*P* values indicated that there is an optimum lipophilicity value, represented by compound **X**, which is a furfuryliden derivative having a chloride atom as substituent at benzene ring moiety.

Acknowledgements

The authors are grateful to CNPq and FAPESP for the financial support, and to Professor Márcia Miguel Castro Ferreira, coordinator of the Laboratory for Theoretical and Applied Chemometrics, Institute of Chemistry—UNICAMP, for allowing the access to CSD.

References and notes

1. Pinto Dias, J. C. *Ann. Int. Med.* **2006**, *144*, 772.
2. Urbina, J. A.; Docampo, R. *Trends Parasitol.* **2003**, *19*, 495.
3. Moncayo, A. *World Health Stat. Q.* **1997**, *50*, 195.
4. Schofeld, C. J.; Dias, J. C. *Adv. Parasitol.* **1999**, *42*, 1.
5. Moncayo, A. *Mem. Inst. Owaldo Cruz* **2003**, *98*, 577.
6. Hotez, P.; Molyneux, D. H.; Fenwick, A.; Ottessen, E.; Ehrlich Sachs, S.; Sachs, J. D. *PLoS Med.* **2006**, *3*, e102.
7. Andrade, A. L. S.; Zicker, F.; Oliveira, R. M.; Almeida Silva, S.; Luquetti, A.; Travassos, L. R., et al *Lancet* **1996**, *348*, 1407.
8. Urbina, J. A. *Mem. Inst. Oswaldo Cruz* **1999**, *94*, 349.
9. Blumenstiel, K.; Schöneck, R.; Yardley, V.; Croft, S. L.; Kraut-Siegel, R. L. *Biochem. Pharmacol.* **1999**, *58*, 1791.
10. Coura, J. R.; de Castro, S. L. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 3.
11. Silva, C. H. T. P.; Sanches, S. M.; Taft, C. A. *J. Mol. Graphics Modell.* **2004**, *23*, 89.
12. Arán, J. V.; Ochoa, C.; Boiani, L.; Buccino, P.; Cerecetto, H.; Gerpe, A.; Gonzáles, M.; Montero, D.; Nogal, J. J.; Gómez-Barrio, A.; Azqueta, A.; Ceráin, A. L.; Piro, O. E.; Castellano, E. E. *Bioorg. Med. Chem.* **2005**, *13*, 3197.

13. Dias, M. M. A. L., Tavares, L.C. *Pró-Fármacos de derivados 5-nitro-2-tiofilidênicos: Planejamento, síntese e determinação da atividade antichagásica dos ligantes*. Dissertação de Mestrado, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, 2005.
14. Tavares, L. C.; Penna, T. C. V.; Amaral, A. T. *Boll. Chim. Farm.* **1997**, *136*, 244.
15. Tavares, L. C.; Chiste, J. J.; Santos, M. G. B.; Penna, T. C. V. *Boll. Chim. Farm.* **1999**, *138*, 432.
16. Furlanetto, M.; Santos, M. G. B.; Tavares, L. C. *Braz. J. Pharm. Sci.* **2001**, *37*, 62.
17. Masunari, A.; Tavares, L. C. *Bioorg. Med. Chem.* **2007**, *15*, 4229.
18. Rando, D. C.; Sato, D. N.; Siqueira, L.; Malvezzi, A.; Leite, C. Q. F.; do_Amaral, A. T.; Ferreira, E. I.; Tavares, L. C. *Bioorg. Med. Chem.* **2002**, *10*, 557.
19. Munro, A. W.; McLean, K. J.; Marshall, K. R.; Warman, A. J.; Lewis, G.; Roitel, O.; Sutcliffe, M. J.; Kemp, C. A.; Modi, S.; Scrutton, N. S.; Leys, D. *Biochem. Soc. Trans.* **2003**, *31*, 625.
20. Silva, L. H. P.; Nussenszweig, V. *Folia Clin. Biol.* **1953**, *20*, 191.
21. Camargo, E. P. *Rev. Inst. Med. Trop. São Paulo* **1964**, *6*, 93.
22. Gonçalves, M.F. *Fosfodiesterase do AMP cíclico de formas epimastigotas de Trypanosoma cruzi*, Dissertação de Mestrado, Instituto de Química, Universidade de São Paulo, São Paulo, SP, 1978.
23. HYPERCHEM *Program Release 7 for Windows*; Hypercube, Inc.: Gainesville, FL, 2002.
24. Allen, F. H. *Acta Crystallogr., Sect. B* **2002**, *58*, 380.
25. Pniewska, B.; Januchowski, M. *Pol. J. Chem.* **1998**, *72*, 2629.
26. Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127.
27. Dewar, M. J. S. E.; Zoebisch, G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
28. GAUSSIAN *03W for Windows*, version 6; Gaussian Inc.: Pittsburgh, PA, 1995–2003.
29. Soares-Sobrinho, J. L.; Cunha-Filho, M. S. S.; Rolim Neto, P. J.; Torres-Labandeira, J. J.; Dacunha-Marinho, B. *Acta. Crystallogr., Sect. E* **2008**, *64*, o634.
30. Connolly, M. L. *Science* **1983**, *221*, 709.
31. Connolly, M. L. *J. Appl. Crystallogr.* **1983**, *16*, 548.
32. SYBYL *8.0 for Linux*; Tripos, Inc.: 2007. 1699 South Hanley Rd., St. Louis, MO 63144-2917, USA.
33. Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Phys. Chem. A* **1998**, *102*, 3762.
34. Faucher, J. F.; Baltz, T.; Petry, K. G. *Parasitol. Res.* **1995**, *81*, 441.
35. Almeida-de-Faria, M.; Freymuller, E.; Colli, W.; Alves, M. J. M. *Exp. Parasitol.* **1999**, *31*, 263.
36. Araújo-Jorge, T. C.; Pirmez, C. Protocolos e métodos de trabalho em doença de Chagas, Normas de segurança para o trabalho com *Trypanosoma cruzi*. In Araújo-Jorge, T.C., de Castro, S.L., Ed., Doença de Chagas: *Manual para Experimentação Animal*, 1ª Edição, Fiocruz, Rio de Janeiro, 1999, pp 125–134.
37. Tyler, K. M.; Engman, D. M. *Int. J. Parasitol.* **2001**, *31*, 472.
38. Audry, E.; Dubost, J. P.; Colleter, J. C.; Dallet, P. *Eur. J. Med. Chem.—Chim. Ther.* **1986**, *21*, 71.
39. Furet, P.; Sele, A.; Cohen, N. C. *J. Mol. Graphics* **1988**, *6*, 182.
40. Wilkinson, S. R.; Taylor, M. C.; Horn, D.; Kelly, J. M.; Cheeseman, I. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5022.
41. Viodé, C.; Bettache, N.; Cenas, N.; Krauth-Siegel, R. L.; Chaviére, G.; Balakara, N.; Périe *Biochem. Pharmacol.* **1999**, *57*, 549.
42. Tocher, J. H. *Gen. Pharmacol.* **1997**, *28*, 485.
43. Murray, R. J.; Lim, T. T.; Pearson, J. C.; Grubb, W. B.; Lum, G. D. *Int. J. Infect. Dis.* **2004**, *8*, 275.
44. Ulmar, T. M.; Mitchard, M. *Biochem. Pharmacol.* **1968**, *17*, 2057.
45. Asnis, R. E.; Gots, J. S. *Arch. Biochem.* **1951**, *30*, 25.
46. Tavares, L. C.; Amaral, A. T. *Abstracts of Papers*, 3rd Workshop on Chemical Structure-Biological Activity, Brazil, São Paulo, Nov 08–10, 1991; São Paulo: IQ/USP, p 8.
47. Tavares, L. C.; Vessoni-Penna, T. C.; Amaral, A. T. *Abstracts of Papers*, 3rd Workshop on Chemical Structure-Biological Activity, Brazil, São Paulo, Nov 08–10, 1991; São Paulo: IQ/USP, p 2.
48. Hansch, C.; Leo, A. *Substituent constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.
49. Tavares, L. C. *Quim. Nova* **2004**, *27*, 631.
50. Hansch, C.; Leo, A. *Exploring QSAR: fundamentals and applications in chemistry and biology*; ACS: Washington, 1995.
51. Craig, P. N. *J. Med. Chem.* **1971**, *14*, 680.