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Application of Molecular Topology to Predict the Inhibition of *Trypanosoma cruzi* Cruzain by Thiosemicarbazones

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Abstract

Motivation. The main goal of the present work is finding a classification mathematical model of the inhibitory activity against *T. cruzi* cruzain through molecular topology. This is particularly interesting since the finding of new therapeutic alternatives for Chagas disease continues to be a very difficult task as demonstrated by the low number of lead drugs approved by the international agencies in the later years in this field.

Method. Molecular topology, a formalism based on describing the molecules as hydrogen–depleted graphs, as well as linear discriminant analysis, a statistical tool capable to distinguish between two or more categories or objects, have been used to the search of new active compounds by virtual screening throughout databases.

Results. Linear discriminant analysis has been developed with a group of thiosemicarbazone and semicarbazone derivates. A mathematical model comprised of one discriminant function has been selected. The model is able to classify correctly 92.8% of the compounds from the training set. We have built up a virtual library with several hundreds of thiosemicarbazone derivatives for virtually seeking and optimizing the inhibitory activity against *T. cruzi* cruzain.

Conclusions. Molecular topology has been successfully used to find a QSAR model classification the inhibitory activity against *T. cruzi* cruzain of a group of thiosimicarbazone and semicarbazone derivates.

Keywords. Linear discriminant analysis; *T. cruzi*; molecular topology; Chagas disease; quantitative structure–activity relationships; QSAR.

Abbreviations and notations

QSPR, quantitative structure–property relationships	QSAR, quantitative structure–activity relationships
LDA, linear discriminant analysis	

1 INTRODUCTION

Chagas disease or American trypanosomiasis is a widespread infection caused by the haemoflagellate protozoan *Trypanosoma cruzi*, which is transmitted to humans and other animals by hematophagous hemipterans of the subfamily Triatominae. Disease transmission occurs by the

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deposition in the skin of metacyclic trypomastigotes forms of the parasite, which are eliminated by the Triatomines through faeces and urine. Other routes of transmission include blood transfusion, organ transplantation, and oral feeding [1].

Almost a century after its discovery it is still a challenge to international public health: in all over the world there are between 16 and 18 million carriers of *T. cruzi*, and 50,000 of them die every year. In Latin America, an estimated 12 to 14 million people infected and 60 million of people at risk of transmission, being more prevalent in poor rural regions.

There are three stages of Chagas disease, namely acute, chronic and unspecific chronic phase. Human infection can be very serious, with significant mortality in children in the acute phase and with high incidence of cardiac arrest and/or gastrointestinal in chronic patients, being a major socio–economic and health problem [2–3]. The disease can be fatal for 10% of severe cases, the vast majority with meningoencephalitis and almost always fatal in children under two years.

The only two drugs available for treatment of Chagas disease are Nifurtimox, developed in 1960 by Bayer, and Benznidazol, developed in 1974 by Roche, which only shows efficacy in the acute phase of infection, being ineffective in the chronic phase of the disease. They also present significant problems, because they require administration over extended periods of time, and also due to the presence of parasite strains resistant to both drugs and lack of pediatric formulations [4].

One of the fronts of research on this disease is based on the fact that the parasite *Trypanosoma cruzi* contains high amounts of a cysteine–protease similar to cathepsin L, which has been called cruzipain (also known as gp51/57 or cruzain, in its recombinant form), which is responsible for most of the proteolytic activity of this parasite in all stages of their life cycle. Selective inhibitors of this protease are able of blocking the proliferation of both the extracellular form (epimastigotes) and the intracellular amastigotes, as well as prevent metaciclogenesis (transformation of epimastigotes to metacyclic trypomastigotes), indicating that the protein has essential functions in the life cycle of the parasite.

Molecules have been described that inhibit cruzipain with potent and selective anti–*T. cruzi in vitro*, among them are non–peptide inhibitors of the enzyme based on the structure of the thiosemicarbazone. These compounds are active against the amastigotes of *T. cruzi* at intracellular nanomolar concentrations *in vitro*, and its simplicity and low cost makes them ideal as a synthetic starting point for the development of new trypanocidal agents.

In this way, Xiaohui Du *et al*. [5] have identified a novel series of potent thiosemicarbazone small–molecule inhibitors of the *Trypanosoma cruzi* cysteine protease cruzain. Some of these inhibitors have been shown to be trypanocidal. Thus, cruzain is an appealing target for new antitrypanosomal chemotherapy [6].

The prediction of biological properties of organic compounds is one of the main objectives of the quantitative structure–activity relationships (QSAR) and quantitative structure–property relationships (QSPR) methods. The success of these methods is closely depending of an appropriate characterization of the molecular structure as well as an adequate selection of the molecular descriptors to be correlated.

Molecular topology has widely demonstrated its ability for a straightforward and efficient characterization of molecular structure by means of the so–called topological indices, TIs [7–9]. When these indices are adequately selected, it is possible to obtain a precise mathematical characterization of each chemical compound, which allows the development of QSAR and QSPR models [10,11].

This way, TIs have demonstrated their usefulness in the prediction of diverse physical, chemical and biological properties for different types of compounds [12–16], as well as in the selection and design of new active compounds [17–22]. In some cases, the predicted structures can be regarded as new lead drugs [23].

In the present study, the cruzain inhibitory activities of 55 thiosemicarbazone and semicarbazone derivate compounds were considered to obtain predictive models using molecular topology combined with linear discriminant analysis. Furthermore, a molecular screening was performed to select new compounds with theoretical higher bioactivity.

2 MATERIALS AND METHODS

2.1 Analysed Compounds

In this study we have selected a group of 55 thiosemicarbazone and semicarbazone derivates, Figure 1, as inhibitors of the trypanosomal cysteine protease cruzain that have been selected from the literature [5,6]. The structures and corresponding IC_{50} values for the whole set of inhibitors are included in Tables 1 and 2.

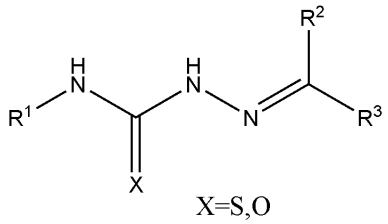
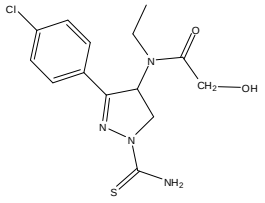


Figure 1. General scaffold of thiosemicarbazone and semicarbazone derivatives.

Table 1. Structures of Thiosemicarbazone and Semicarbazone Studied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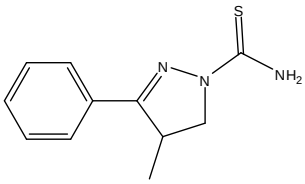
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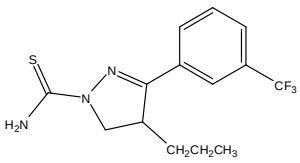
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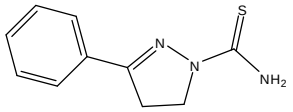
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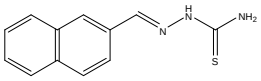
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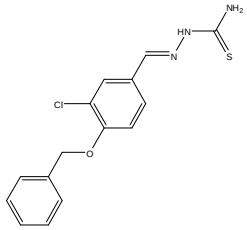
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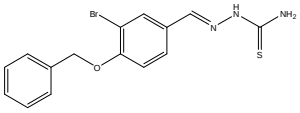
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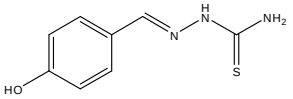
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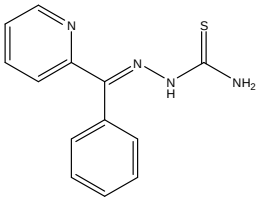
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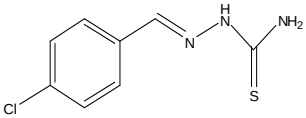
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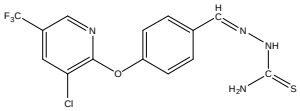
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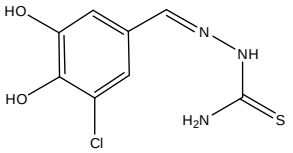
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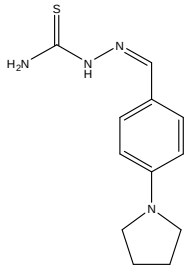
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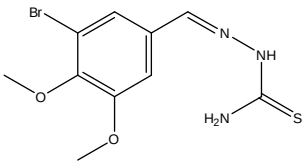
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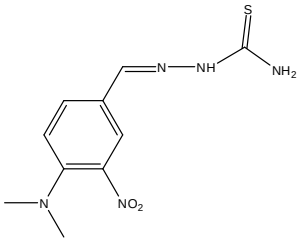
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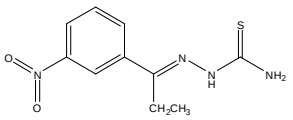
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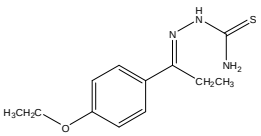
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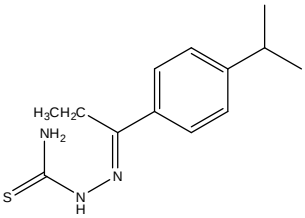
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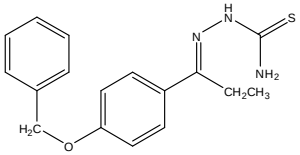
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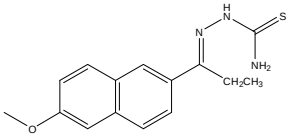
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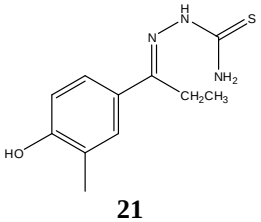
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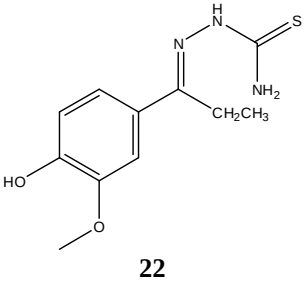
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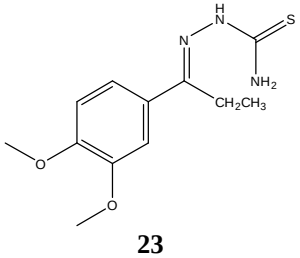
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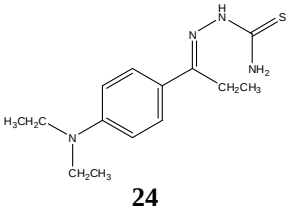
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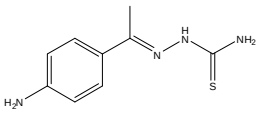
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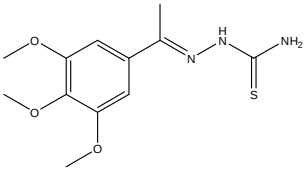
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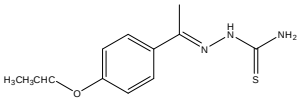
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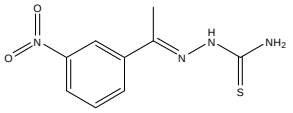
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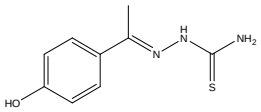

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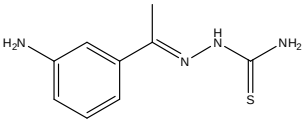

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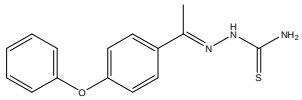

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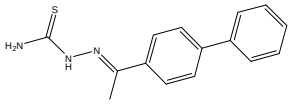

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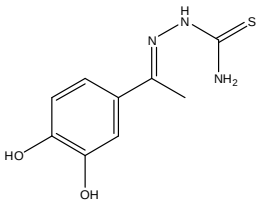

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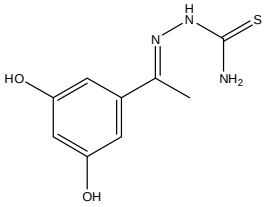

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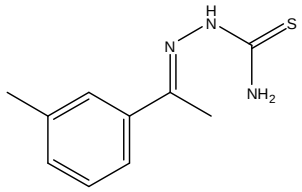

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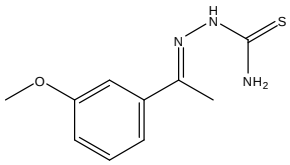

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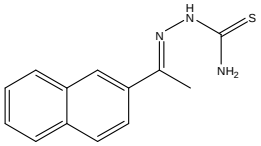

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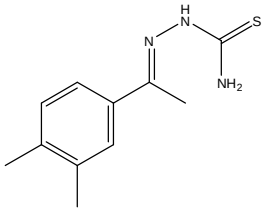

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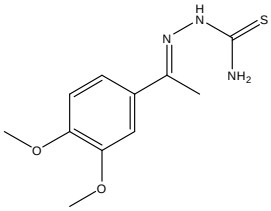

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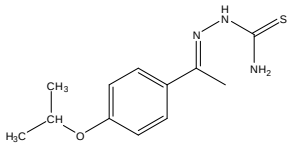

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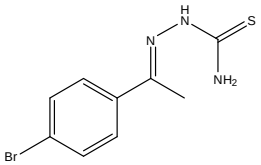

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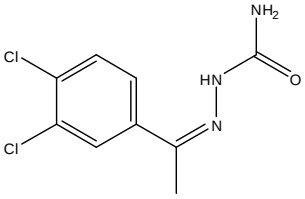

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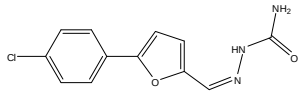

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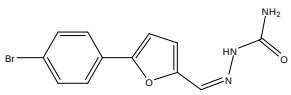

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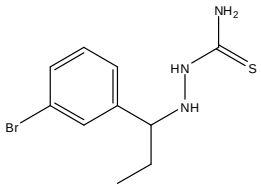

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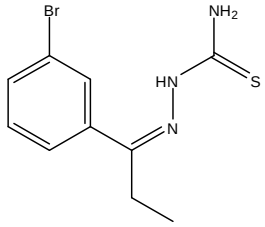

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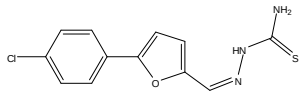

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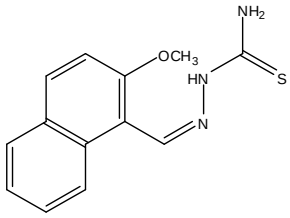

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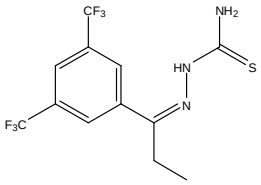

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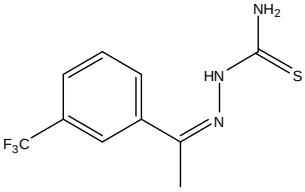

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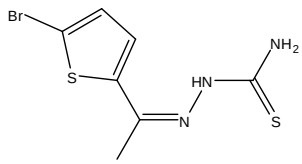

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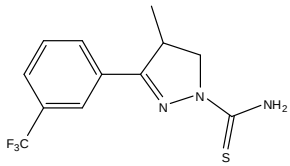

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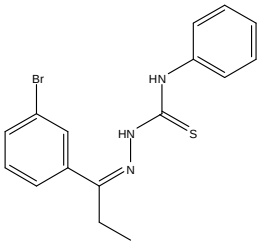

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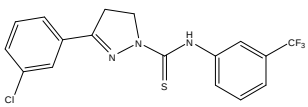

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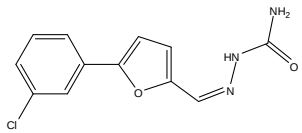


55

2.2 Molecular Descriptors

A set of well–known topological descriptors was used in this work: Subgraph Randić–Kier–Hall like indices up to the fourth order ($^{m}\chi_t$, $^{m}\chi_t^{v}$) [24,25], topological charge indices, TCI, up to the fifth order, (J_m, G_m, J_m^{v}, G_m^{v}) [26], quotients and differences between valence and non–valence connectivity indices ($^{m}C_t = {}^{m}\chi_t / {}^{m}\chi_t^{v}$ and $^{m}D_t = {}^{m}\chi_t - {}^{m}\chi_t^{v}$), PRn (number of pairs of ramifications at topological distance *n*, with *n* ranging from 0 to 4), Vn (number of vertices with topological valence *n*, with *n* being 3 or 4), and other graph–theoretical descriptors (not outlined here, as they were not selected for the final model). Each compound was characterized by a set of 62 descriptors. All descriptors used in this work were obtained with the aid of the Desmol11 program (available by e–mail request) [27].

2.3 QSAR Algorithms: Linear Discriminant Analysis

The objective of the linear discriminant analysis (LDA), which is considered as a heuristic algorithm able to distinguish between two or more categories or objects, is to find a linear function capable to discriminate between the active and inactive compounds as for their different descriptor values. Two sets of compounds, the first with a proven inhibitory activity (in our case, all the compounds with $IC_{50} \leq 1\mu M$) and the second comprised of inactive compounds ($IC_{50} > 1\mu M$) were considered for the analysis. The discriminant ability was tested by the percentage of correct classifications into each group. LDA was performed by using the BMDP 7M package [28]. The selection of the descriptors was based on the F–Snedecor parameter, and the classification criteria was the shortest Mahalanobis distance (distance of each case to the mean of all cases used in the regression equation).

From the selected discriminant function, the corresponding distribution diagram of inhibitory of *T. cruzi* cruzain activity, PDD, was drawn. This diagram was pictured just to establish the intervals of the discriminant function in which the expectancy, E, of finding active compounds is maximum. PDDs are histogram–like plots of connectivity functions in which expectancies appear on the ordinate axis. For an arbitrary interval of values of a given function, we define the expectancy of activity as: $Ea = a/(i+1)$; where *a* is the number of active compounds in the interval divided by the total number of active compounds, and *i* is the number of inactive compounds in the interval divided by the total number of inactive compounds. The expectancy of inactivity is defined in a symmetrical way, as $Ei = i/(a+1)$. This representation provides a good visualization of the regions of minimum overlap, and allows the selection of regions in which the probability of finding active compounds reaches a maximum [29].

3 RESULTS AND DISCUSSION

To obtain the discriminant function, we apply the LDA to a training group comprised of 55 compounds distributed in two subgroups: an active group (compounds with values of $IC_{50} \leq 1\mu M$) and an inactive group (compounds with $IC_{50} > 1\mu M$).

$$DF = 22.34 - 26.18 {}^{V}J_1 - 80.64 J_4 - 37.3 {}^{2}C + 19.58 {}^{4}C_{PC} - 0.023 W + 1.993 PR2 + 7.266 V_4 \quad (1)$$
$$N = 55 \qquad \lambda \text{ (Wilks' lambda)} = 0.394 \qquad F = 10.3$$

From here, a given compound will be selected as a potential inhibitor *T. cruzi* cruzain if DF > 0, otherwise it is classified as "inactive". The classification matrix is very significantly for the training set (92.6% of correct prediction for the active group, 25 out of 27 correctly classified, and 92.9% for the inactive group, 26 correct out of 28 (see Table 2).

In Eq. 1 appear topological descriptors that evaluate, on the one hand, the topological aspects of each compound as for instance the molecular branching, ${}^{4}C_{PC}$, PR2, V_4 and, on the other, the distributions of the intramolecular charge through the charge indices ${}^{V}J_1$ and J_4.

Figure 2 depicts the inhibitory activity distribution diagram obtained with the function DF (white and black bars represent inactive and active groups, respectively). It is apparent that the regions with minimum overlap for the compounds with theoretical inhibitory activity occur for DF > 0 and DF < 8, so the highest expectation of activity occurs in these intervals.

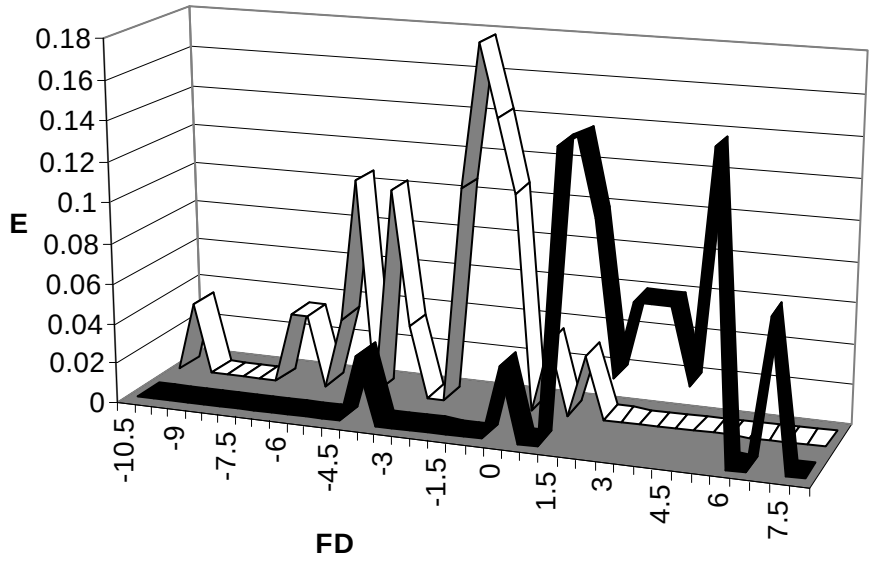


Figure 2. Pharmacological distribution diagram for inhibitory activity against *T. cruzi* cruzain by plotting expectancy (E) versus DF (Eq. (1)) function (the black bars represent the compounds with $IC_{50} < 1\mu M$ and the white bars, the compounds with $IC_{50} > 1\mu M$).

Table 2. LDA results for the thiosemicarbazone and semicarbazone derivatives analyzed

Compound	IC_{50}[a] (μM)	Clas(exp)	Prob[b]. (active)	FD	Clas(calc)[c]
		Active group training (IC_{50}<1μM)			
11	0.02	A	0.865	1.85	A
49	0.02	A	0.991	4.69	A
52	0.04	A	0.788	1.31	A
10	0.05	A	0.966	3.34	A
22	0.05	A	0.891	2.10	A
20	0.06	A	0.986	4.26	A
28	0.06	A	0.976	3.69	A
29	0.07	A	0.999	6.52	A
25	0.08	A	0.999	6.74	A
30	0.08	A	0.778	1.25	A
46	0.10	A	0.889	2.07	A
3	0.14	A	0.982	3.98	A
50	0.17	A	0.937	2.69	A
19	0.20	A	0.920	2.44	A
24	0.20	A	0.987	4.36	A
9	0.22	A	0.786	1.30	A
27	0.23	A	0.995	5.39	A
26	0.27	A	0.963	3.26	A
1	0.28	A	0.449	–0.21	I
4	0.28	A	0.827	1.55	A
47	0.30	A	0.841	1.66	A
17	0.33	A	0.995	5.30	A
21	0.46	A	0.016	–4.14	I
18	0.48	A	0.993	5.00	A
14	0.56	A	0.877	1.96	A
48	0.56	A	0.815	1.47	A
37	1.00	A	0.995	5.24	A
		Inactive group training (IC_{50}>1μM)			
16	1.30	I	0.011	–4.47	I
38	1.40	I	0.000	–10.27	I
36	1.60	I	0.004	–5.65	I
54	1.70	I	0.141	–1.82	I
23	1.75	I	0.004	–5.42	I
5	2.80	I	0.015	–4.20	I
8	2.80	I	0.078	–2.47	I
51	3.80	I	0.233	–1.20	I
13	3.90	I	0.590	0.36	A
2	7.10	I	0.001	–6.66	I
6	>10	I	0.137	–1.84	I
7	>10	I	0.120	–2.00	I
12	>10	I	0.005	–5.24	I
15	>10	I	0.097	–2.24	I
31	>10	I	0.121	–1.99	I
32	>10	I	0.011	–4.5	I
33	>10	I	0.001	–7.24	I
34	>10	I	0.252	–1.09	I
35	>10	I	0.005	–5.37	I
39	>10	I	0.366	–0.56	I
40	>10	I	0.144	–1.79	I
41	>10	I	0.270	–1.00	I
42	>10	I	0.139	–1.83	I
43	>10	I	0.216	–1.29	I
44	>10	I	0.232	–1.20	I
45	>10	I	0.019	–3.96	I
53	>10	I	0.369	–0.55	I
55	>10	I	0.752	1.10	A

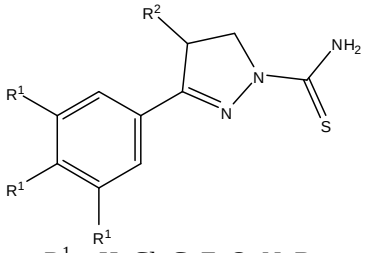
[a] from reference [6]; [b] Active probability from Eq.1; [c] Classification from Eq. 1.

Table 3. Internal validation of the LDA model

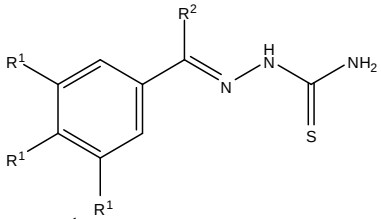
Test, nº (compounds)	λ	Training set Active	Training set inactive	Test set active	Test set inactive
	0.394	92.6%(25/27)	92.9%(26/28)	–	–
A (8,10,19,23,25,30,35,41,49,51)	0.372	90.9%(20/22)	91.3%(21/23	100%(5/5)	100%(5/5)
B (7,9,16,18,24,29,34,40,47,51)	0.326	90.9%(20/22)	91.3%(21/23)	100%(5/5)	80%(4/5)
C (4,6,15,17,22,28,33,39,44,47)	0.412	90.9%(20/22)	91.3%(21/23)	100%(5/5)	60%(3/5)
D (3,5,13,14,21,27,32,38,43,46)	0.298	95.5%(21/22)	95.7%(22/23)	80%(4/5)	100%(5/5)
E (1,2,11,12,20,26,31,36,37,42)	0.373	95.5%(21/22)	91.3%(21/23)	80%(4/5)	100%(5/5)
Average	0.356	92.70%	92.20%	92%	88%

The validation of the selected discriminant function was carried out by dividing the training set into five subsets (A–E), containing each subset the 20% of the compounds analyzed (five active and five inactive compounds). Four of five subsets (A, B, C and D), (A, B, C and E), (A, B, D and E), (A, C, D and E) and (B, C, D and E) consist the training set with the remaining subset corresponding to the test set. The discriminant equation obtained for each of the training sets using the same descriptors, was used to predict values for the corresponding test sets. Table 3 shows the values of λ (Wilks' lambda) and classification matrix for each training and test set analyzed. The variability of λ is little for each subset and the average λ (λ = 0.356), is similar to obtained with the selected model (λ = 0.372). Based upon the selected topological model, we carried out a virtual screening with the Scifinder Scholar database, with the aim of selecting new thiosemicarbazone derivatives showing a better activity. Table 4 shows the base structures (scaffold) used and some of the compounds selected by the model.

Table 4. Computational screening applied to thiosemicarbazone analogues obtained from Scifinder Scholar database and using the inhibitory *T. cruzi* cruzain topological model selected by linear discriminant analysis



R^1 = H, Cl, C, F, O, N, Br
R^2=C, H



R^1 = H, Cl, C, F, O, N, Br
R^2=C, H

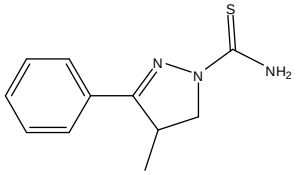
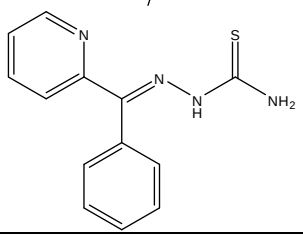
Compound: CAS Nº	Structure	Prob. (active)	DF	Class
888955–68–8		0.800	1.35	A
870457–71–9		0.914	2.32	A

Table 4. (Continued)

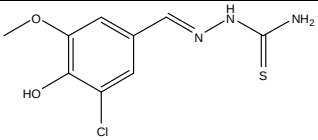
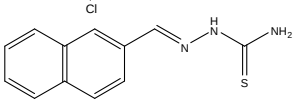
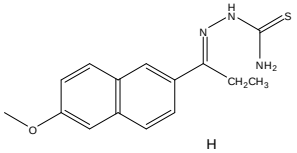
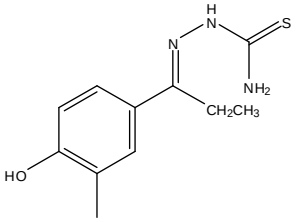
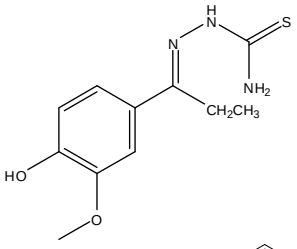
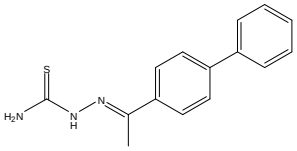
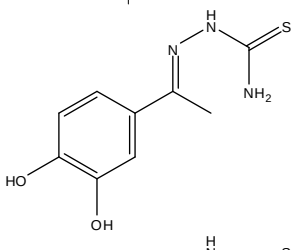
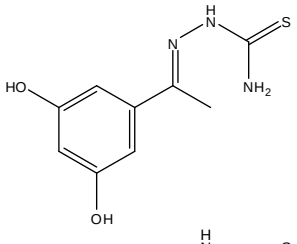
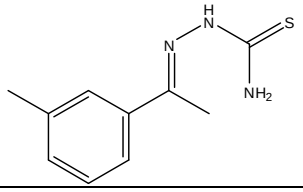
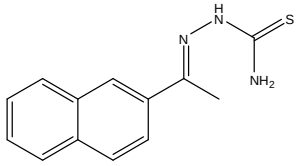
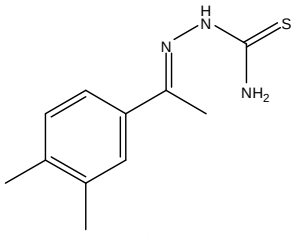
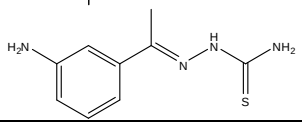
Compound: CAS Nº	Structure	Prob. (active)	DF	Class
447451–99–2		0.986	4.19	A
1081852–79–0		0.934	2.61	A
443144–55–6		0.967	3.34	A
393131–14–1		0.999	7.28	A
352641–91–9		0.700	0.80	A
712349–33–2		0.984	4.07	A
546112–31–6		1.000	8.64	A
546112–30–5		0.997	5.62	A
546112–25–8		0.913	2.31	A

Table 4. (Continued)

Compound: CAS Nº	Structure	Prob. (active)	DF	Class
133477–39–1		1.000	8.19	A
99988–14–4		0.994	5.13	A
712349–53–6		0.807	1.39	A

Some interesting comments can be stated from results in Table 4:

- Compound #º888955–68–8, selected as active, shows a chemical structure similar to # **25**, **27**, **29** and **52**, all of them known to be active. The only difference is the lack of halogens on the aromatic ring, which may enhance its pharmacological profile.

- The substitution of the methoxy group on the aromatic ring of inactive compound #**12** by a methyl (comp. # º546112–25–8) or an amine group (comp. #º712349–53–6) increases the activity.

- From the analyzed compounds, only #**48** contains the naphthalene ring. Other similar compounds have also been selected as active, namely #º1081852–79–0, 133477–39–1 and 443144–55–6. The last has been registered and patented as an inhibitor of cysteine protease cruzain and to prevent and treat protozoan infections such as trypanosomiasis, malaria and leishmaniasis [30].

Some of them, for example # 888955–68–8 showed antimicrobial activity [31], others such as # 712349–33–2 and # 712349–53–6 have antiprotozoal activity against *Plasmodium falciparum, T. brucei*, and *T. cruzi*. [32]. The compound Nº99988–14–4 has herbicidal activity [33]. The incorporation of activity against *T. cruzi* to these compounds would be really interesting as it would enlarge their therapeutic field and use.

These suggestive results need to be corroborated with the corresponding inhibitory cysteine protease cruzain activity assays, which should allow the validation or evaluation of the model proposed and serve as an useful tool for the search of novel compounds with a higher activity against *T. cruzi*.

4 CONCLUSIONS

Molecular topology has been successfully used to find a QSAR model classification the inhibitory activity against *T. cruzi* cruzain of a group of thiosimicarbazone and semicarbazone derivates. All the molecular descriptors used are graph–theoretical ones. The mathematical model employed in this work retains the main structural features that involve the correlated property, IC_{50}, and, therefore, can be applicable to the search of new active compounds by virtual screening throughout databases. We have built up a virtual library with several hundreds of thiosemicarbazone derivatives for virtually seeking and optimizing the inhibitory activity against *T. cruzi* cruzain. Interesting improvements in the activity have been obtained.

Acknowledgment

We thank the Master Internacional en Enfermedades Parasitarias Tropicales, Universitat de Valencia, for support of this work.

5 REFERENCES

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