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# QSAR study and conformational analysis of 4-arylthiazolylhydrazones derived from 1-indanones with anti-*Trypanosoma cruzi* activity

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ABSTRACT

A set of 4-arylthiazolylhydrazones derived from 1-indanones (TZHs) previously synthesized and assayed against *Trypanosoma cruzi*, the causative agent of Chagas disease, were explored in terms of conformational analysis. We found that TZHs can adopt four minimum energy conformations: *cis* (A, B and C) and *trans*. The possible bioactive conformation was selected by a 3D-QSAR model. Different molecular parameters were calculated to produce QSAR second-generation models. These QSAR results are discussed in conjunction with conformational analysis from molecular modeling studies. The main factor to determine the activity of the compounds was the partial charge at the N(3) atom ($q_{N3}$). The predictive ability of the QSAR equations proposed was experimentally validated. The QSAR models developed in this study will be helpful to design novel potent TZHs.



## 1. Introduction

Chagas disease, also called American trypanosomiasis, is a vector-transmitted parasitic disease caused by the flagellated protozoan microorganism *Trypanosoma cruzi* (*T. cruzi*). It is the third largest disease burden in Latin America after malaria and schistosomiasis, all considered as neglected diseases (WHO, 2008). The World Health Organization estimates that Chagas disease is responsible for the death of over 10,000 people per year (DNDI, 2008). At present, only two nitro derivative drugs, introduced in the 1960s and 1970s, are available for the treatment of chagasic patients: nifurtimox and benznidazole. These drugs are effective for acute infections, but their undesirable side effects and controversial use for chronic patients have been forcing the abandonment of the treatment (Urbina and Docampo, 2003; McKerrow et al., 2009). Moreover, no effective vaccines are available and their development in the near future seems to be out of reach (Vazquez-Chagoyan et al., 2011). Consequently, more efficient drugs are needed.

Recently, in continuation of our search for bioactive molecules, we envisaged that the thiazolylhydrazone moiety would generate novel templates which are likely to exhibit anti-*T. cruzi* activity. Thus, we prepared seventeen 4-arylthiazolylhydrazones derived from 1-indanones (TZHs) in an efficient and simple manner. Most of these new derivatives exhibited promising activity against the different forms of *T. cruzi* and were more potent and selective than the reference drug benznidazole (Caputto et al., 2012). A preliminary analysis of the structure–activity relationship suggested that better trypanocidal activity may be attained when $R_1$, $R_2$ or $R_3$ is a methyl group (Fig. 1).

Quantitative structure–activity relationship (QSAR) is one of the most important areas in chemometrics, and is a valuable tool extensively used in drug design and medicinal chemistry. Thus, in this work, we performed a QSAR study with the aim to explore the controlling factors governing the observed pharmacological properties of TZHs and to predict the biological activities of new compounds. Furthermore, an exhaustive conformational analysis was performed to obtain more precise representation of the biological active molecules at the atomic level. Also, three new TZHs are described.

## 2. Materials and methods

### 2.1. Chemistry

Melting points (uncorrected) were determined on a Thomas Hoover apparatus. Thin layer chromatography (TLC) was used to

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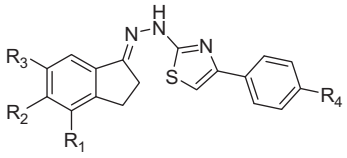

**Fig. 1.** General structure of 4-arylthiazolylhydrazones derived from 1-indanones (TZHs).

monitor reactions. Reactions were carried out in a Microwave Synthesis Reactor Microwave 300 Anton Paar. IR spectra were recorded as KBr pellets using a Perkin Elmer Spectrum One FT-IR spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker 500 MHz spectrometer. High resolution mass spectra were acquired on a Bruker micrOTOF-Q II spectrometer. 4-Methoxy-phenacyl bromide and 4-nitro-phenacyl bromide were purchased from Sigma–Aldrich and used as received. 4-Fluor-phenacyl chloride and thiosemicarbazones derived from 1-indanones were prepared according to the protocols previously described (Caputto et al., 2012, 2011; Finkielsztein et al., 2008).

*2.1.1. General procedure for the synthesis of 4-arylthiazolylhydrazones derived from 1-indanones*

In a typical procedure a mixture of thiosemicarbazone derived from 1-indanone (0.010 mmol), phenacyl chloride or bromide (0.013 mmol) and DMF (0.15 mL) in a borosilicate boiling tube, was placed in a microwave synthesizer at 80 °C. After completion of the reaction (monitored by TLC), the mixture was suspended in water, filtered, and washed with EtOH and hexane. All synthesized TZHs were crystallized from EtOH.

*2.1.1.1. 4-(4-fluorophenyl)-2-(2-(6-methyl-2,3-dihydro-1H-inden-1-ylidene)hydrazinyl)thiazole (18).* Yield: 73%. Mp: 216-217 °C. IR $\nu/cm^{-1}$ (KBr): 2615 ($NH^{+}$), 1621 and 1598 (C=N). $^{1}H$ NMR (DMSO-$d_6$): δ: 2.34 (3H, s, $CH_3$), 2.87 (2H, m, $CH_2$), 3.02 (2H, m, $CH_2$), 7.17 (1H, d, $J$ = 7.8 Hz, H-Ar), 7.23 (2H, t, $J$ = 8.9 Hz, H-Ar), 7.25 (1H, d, $J$ = 7.8 Hz, H-Ar), 7.28 (1H, s, H-thiazole), 7.42 (1H, s, H-Ar), 7.89 (2H, dd, $J_1$ = 5.5 Hz, $J_2$ = 8.9 Hz, H-Ar), 11.13 (1H, s, NH). $^{13}C$ NMR (DMSO-$d_6$): 20.8, 27.7, 27.9, 103.5, 115.3, 115.5 ($J$ = 21.8 Hz), 120.8, 125.4, 127.4, 127.6 ($J$ = 8.2 Hz), 131.1, 131.4, 136.2, 137.8, 145.2, 149.8, 156.4, 160.6, 162.6 ($J$ = 244.3 Hz), 169.5. HRMS (ESI) $m/z$ $(M+H)^{+}$ calcd for $C_{19}H_{17}FN_3S$ 338.11217, found 338.11035.

*2.1.1.2. 4-(4-methoxyphenyl)-2-(2-(5-methyl-2,3-dihydro-1H-inden-1-ylidene)hydrazinyl)thiazole (19).* Yield: 99%. Mp: descompose before melting. IR $\nu/cm^{-1}$ (KBr): 2765 ($NH^{+}$), 1624 and 1583 (C=N). $^{1}H$ NMR (DMSO-$d_6$): δ: 2.33 (3H, s, $CH_3$), 2.86 (2H, m, $CH_2$), 3.03 (2H, m, $CH_2$), 3.77 (3H, s, $OCH_3$), 6.96 (2H, d, $J$ = 8.8 Hz, H-Ar), 7.11 (1H, s, H-thiazole), 7.12 (1H, d, $J$ = 7.8 Hz, H-Ar), 7.18 (1H, s, H-Ar), 7.50 (1H, d, $J$ = 7.8 Hz, H-Ar), 7.78 (2H, d, $J$ = 8.8 Hz, H-Ar), 11.05 (1H, s, NH). $^{13}C$ NMR (DMSO-$d_6$): 21.2, 27.7, 28.0, 55.1, 101.5, 114.0, 120.5, 126.1, 126.5, 126.9, 128.0, 135.1, 135.6, 139.9, 148.2, 158.8, 159.2, 169.5. HRMS (ESI) $m/z$ $(M+H)^{+}$ calcd for $C_{20}H_{20}N_3OS$ 350.13216, found 350.13162.

*2.1.1.3. 2-(2-(5-methyl-2,3-dihydro-1H-inden-1-ylidene)hydrazinyl)-4-(4-nitrophenyl)thiazole (20).* Yield: 92%. Mp: 241-242 °C. IR $\nu/cm^{-1}$ (KBr): 2742 ($NH^{+}$), 1597 (C=N), 1566 and 1335 ($NO_2$). $^{1}H$ NMR (DMSO-$d_6$): δ: 2.33 (3H, s, $CH_3$), 2.86 (2H, m, $CH_2$), 3.04 (2H, m, $CH_2$), 7.12 (1H, d, $J$ = 7.9 Hz, H-Ar), 7.18 (1H, s, H-Ar), 7.50 (1H, d, $J$ = 7.9 Hz, H-Ar), 7.69 (1H, s, H-thiazole), 8.11 (2H, d, $J$ = 8.9 Hz, H-Ar), 8.27 (2H, d, $J$ = 8.9 Hz, H-Ar), 11.16 (1H, s, NH). $^{13}C$ NMR (DMSO-$d_6$): 21.2, 27.7, 28.0, 108.5, 120.5, 124.1, 126.1, 126.3, 128.0, 135.1, 139.9, 140.9, 146.1, 148.3, 148.6, 156.7, 169.9. HRMS (ESI) $m/z$ $(M+H)^{+}$ calcd for $C_{19}H_{17}N_4O_2S$ 365.10667, found 365.10675.

## 2.2. Biology

### 2.2.1. Parasites

*T. cruzi* epimastigotes (Tulahuen strain, Tul 2 stock) were grown at 28 °C in a liquid medium containing 0.3% yeast extract, 0.9% tryptose, 0.4% dextrose, 1% disodium phosphate 2-hydrate, 0.36% sodium chloride, 0.04% potassium chloride, 0.15% powered beef liver, 0.5% brain heart infusion and 0.5–1.0 mg/100 mL hemin.

### 2.2.2. In vitro trypanocidal activity assay

To evaluate the growth inhibition of *T. cruzi* epimastigote, parasites from a 5 days-old culture were inoculated into fresh culture medium to reach an initial concentration of 1.5–2.5 × $10^6$ cells/mL. Cells were cultured with different concentrations of compounds (usually of 1.50–15 μM or 0.5–2.5 μM) for 4 days. Benznidazole (2.50–15 μM) was used as the reference trypanocidal drug (positive control). The compounds ability to inhibit growth of the parasite (antiproliferative activity) was evaluated, in triplicate, in comparison to the control without drug. Cells growth was followed by counting the number of cells per mL of culture using a Neubauer chamber and was expressed as cellular density (CD). For the count only the cells showing motility (parasites without motility were dead as demonstrated by positive staining with trypan blue) were taking into account. The percentage of inhibition (%I) was calculated as: $\%I = \{1-[(CD_{5t} - CD_{0t})/(CD_{5c} - CD_{0c})]\} \times 100$, where $CD_{5t}$ is cellular density of treated parasites at day 5; $CD_{0t}$ is cellular density of treated parasites just immediately after adding the drug (day 0); $CD_{5c}$ is cellular density of untreated parasites (control) at day 5; and $CD_{0c}$ is cellular density of untreated parasites at day 0. The $IC_{50}$ (50% inhibitory concentration on epimastigote forms) was estimated by lineal regression analysis from the %I values and the decimal logarithm (log) of drug concentration.

## 2.3. Computational methods

### 2.3.1. Molecular modeling methods

The initial conformations (IC) of the compounds were drawn by means of the "Model Build" modulus of the HyperChem 8.0.7 (Hypercube, 2009). The molecular structures were pre-optimized with the MM+ procedure included in the HyperChem software. The resulting geometries were refined by means of the AM1 semiempirical Method from the Molecular Orbitals Theory, setting the calculation of the Self Consistent Field (SCF) with a convergence limit equal to 1 × $10^{-7}$ and iteration limit equal to 1000, using the Polak–Ribiere algorithm and a RMS gradient norm limit of 0.001 kcal $Å^{-1}$ mol. The lowest energy conformer of each compound was corroborated by vibrational analysis. Full geometry optimization of minimum energy conformations was subsequently carried out at the HF/6-31G(d) *ab initio* level using Gaussian 09 (Gaussian Inc, 2009). The study of the structural conformers was performed using Balloon 1.3.1.983 software (Vainio and Johnson, 2007). This software generates conformational ensembles (CEs) employing the multiobjective genetic algorithm (GA) together with the MMFF94 force field and was configured with the following options: *fullforce* (optimization of the found post-GA conformations), *nconfs* = 90 (initial population size), *nGenerations* = 500 (maximum number of generations) and *keepinitial* (output file including the IC). Molecular descriptors were calculated with the PaDEL-descriptor 2.11 software (Yap, 2011).

### 2.3.2. QSAR settings

The construction of QSAR equations was performed by McQSAR software (Vainio and Johnson, 2005) using the GA to create