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# Anti-Trypanosoma Activity of Some Natural Stilbenoids and Synthetic Related Heterocyclic Compounds

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**Abstract**—We report the anti-Chagasic activity of the natural dihydrostilbenoid isonotholaenic acid and several simple derivatives, as well as that of some representative compounds of related synthetic series, with basic structures of benzalphthalides, dihydrostilbamides, isoindoles, phthalazin-1-ones, imidazo[2,1-*a*]isoindoles and pyrimido[2,1-*a*]isoindoles. The evaluation was performed in vitro on cultures of epimastigote and trypomastigote forms of *Trypanosoma cruzi*. Some of the tested compounds resulted to be as potent as benznidazole (epimastigotes), and others were shown to be more active than gentian violet (trypomastigotes), used as reference drugs. 

Chagas' disease, or South American trypanosomiasis, is caused by *Trypanosoma cruzi*, a flagellated protozoan, and transmitted by a blood-sucking insect of the genus *Triatoma*. In Latin America, about 10 million people are infected[1] and currently around 600,000 people suffer the symptoms characteristic for the chronic stage that produce about 17,000 deaths per year.[2] Only two drugs are commercially available for the treatment of this disease: nifurtimox and benznidazole. Unfortunately, they are not consistently effective and have serious side effects, including cardiac and/or renal toxicity. This explains the need for discovering new effective chemotherapeutic and chemoprophylactic agents against *T. cruzi*.

Blood transfusion is also considered as the second most frequent route of Chagas disease transmission in endemic countries,[3] because *T. cruzi* may survive in whole blood stored for more than 21 days at 4 °C and the techniques of detection are not strictly applied. Gentian violet is the only trypanocidal agent available in blood banks and there are some restrictions on its use. Thus, agents that are able to clear the parasites from donated blood would also be valuable.

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In preliminary assays looking for new bioactive natural products, we found that isonotholaenic acid (**1a**), the main component of dichloromethane extracts from *Notholaena nivea*, gave promising trypanocidal (epimastigotes) results, with $IC_{50}$ values similar to those found for benznidazole. This fact induced us to prepare some simple derivatives of the natural product and to extend the bioactivity exploration to several series of synthetic analogues, containing the basic carbon skeleton of **1a**. We report here the anti-Chagasic activity of these series of compounds, tested against epimastigote and trypomastigote forms of *T. cruzi*.

## Chemistry

Isonotholaenic acid (**1a**), as mentioned above, was isolated from dichoromethane extracts of the Andean fern, *Notholaena nivea* var. *nivea*.[4] Compounds **1b–e** were obtained through simple chemical transformation as has been reported recently.[5]

The condensation of phthalic anhydride with several substituted phenylacetic acids gave the corresponding benzalphthalide intermediates **2**, which were subsequently transformed into compounds **3** to **8**. By treatment with piperidine, hydrazine derivatives or diamines,

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the piperidides (**3** and **4**), the phthalazinones (**5** and **6**) or imidazo- and pyrimido-isoindole (**7** and **8**, respectively) were obtained, respectively (Scheme 1). The main isonotholaenic acid (**1a**) stilbenoid skeleton is maintained in all synthetic compounds. In addition some of the compounds contain fragments as trimethoxyphenyl or a masked amidine moiety, which are present in clinically used antimicrobial and antiprotozoal agents (trimethoprim, pentamidine). Synthetic procedure details, physicochemical and spectroscopic data and structure assignments for these compounds will be reported in a complete paper.

## Biological Assays

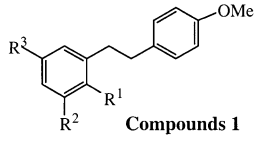
*Epimastigote* forms of *T. cruzi*, Tulahuen strain, were cultivated at 26 °C in liver infusion tryptose medium (LIT), supplemented (5%) with heat inactivated (56 °C for 30 min) foetal calf serum (technically modified from Chataing et al.[6]). Parasites in logarithmic growth phase were distributed in 96-well flat bottomed plates at a concentration of $5\times10^4$/mL. Each well was exposed to increased concentration of the extract from 10 μg/mL up to 100 μg/mL, for 72 h. The activity was measured by optic counting with an inverted microscope and comparison with control wells. Benznidazole was used as the reference drug for this assay. All assays were carried out in triplicate.

*Trypomastigote* forms of *T. cruzi*, Y strain, were maintained by passage every week in Swiss mice.[7] Parasitized whole blood was collected by heart puncture, diluted in LIT medium to a final parasite density of 300,000 to 400,000 trypomastigotes/well, mixed with chelators dissolved in DMSO plus LIT medium, and incubated at 4 °C in 96-well culture plates. After 24 h, the suspensions were examined microscopically and the motility of the parasites was assessed. Gentian violet (100 to 400 μg/mL or 0.25 to 1 μmol/mL) was used as the positive control drug.

## Results and Discussion

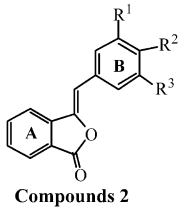
The results of activity assays, along with the comparative potency index relative to the reference drugs, are summarised in Table 1. Isonotholaenic acid (**1a**) proved to be active against epimastigotes of *T. cruzi*, but to a lesser extent than benznidazole. Interestingly, its transformation into the piperidide **1e** doubled its potency in relation to this reference drug. Moreover, the compound **1e** was found to be active against the trypomastigote form, with a potency 1 order of magnitude higher than the drug of reference, gentian violet, while isonotholaenic acid was devoided of such capacity. Other small modifications of the original isonotholaenic structure, compounds **1b–d,** acetate, methyl ester and decarboxylated analogues, gave practically inactive compounds, thus revealing the importance of the phenolic hydroxyl, the carboxyl and, particularly, the piperidide groups for the trypanocidal activity.

Of the benzalphthalide series, only compound **2a** showed activity against *T. cruzi* epimastigotes, but was not as effective as the control, thus meaning that the presence of substituents on the aromatic ring B is not relevant for the activity in this type of compounds. The opening of the lactone ring by piperidine, followed or not by autoxidation, gave the mono- and dioxygenated dihydrostilbenoids **3** and **4**, which in general were inactive. Only the trimethoxy derivatives **3e** and **4e**

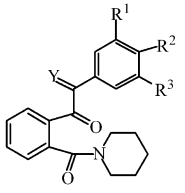



Compounds 1

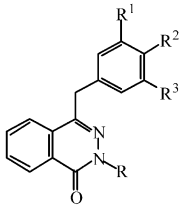
| | 1a | 1b | 1c | 1d | 1e |
|---|---|---|---|---|---|
| R1 | COOH | COOH | COOMe | H | CO N |
| R2 | OMe | OMe | OMe | OMe | OMe |
| R3 | OH | OAc | OH | OH | OH |



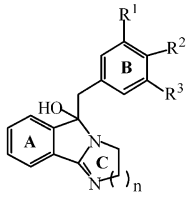

Compounds 2




Compds 3, Y = H,H
Compds 4, Y = O




Compds 5, R = H
Compds 6, R = Me




Compds 7, n = 1
Compds 8, n = 2

| | R1 | R2 | R3 |
|---|---|---|---|
| a | H | H | H |
| b | H | OMe | H |
| c | OMe | OMe | H |
| d | O-$CH_2$- O | | H |
| e | OMe | OMe | OMe |
| f | H | Cl | H |

**Scheme 1.** Natural, semisynthetic and synthetic compounds tested against *T. Cruzi*.

**Table 1.** In vitro activity of dihydrostilbenoids and analogues on *Trypanosoma cruzi*

| | Epimastigotes (Tulahuen) | | | Trypomastigotes (Y strain) | | |
|---|---|---|---|---|---|---|
| Compound | $IC_{50}$ (μg/mL) | $IC_{50}$ (μM) | Index | $IC_{50}$ (μg/ml) | $IC_{50}$ (μM) | Index |
| **1a** | 50 | 165.6 | 5.5 | I | | |
| **1b–1e** | I | | | I | | |
| **1e** | 30 | 81.3 | 2.7 | 12 | 33.0 | 0.13 |
| **2a** | 45 | 202.7 | 6.7 | I | | |
| **2b–2f** | I | | | I | | |
| **3a–3d** | I | | | I | | |
| **3e** | 65 | 163.7 | 5.4 | I | | |
| **3f** | I | | | I | | |
| **4a** | I | | | I | | |
| **4b** | I | | | I | | |
| **$4b_1$, ethylamine** | I | | | I | | |
| **$4b_2$, pyrrolidine** | I | | | I | | |
| **4c, 4d** | I | | | I | | |
| **4e** | 70 | 146.0 | 4.8 | I | | |
| **4f** | I | | | I | | |
| **5a–f, 6a–f** | I | | | I | | |
| **7a** | 60 | 227.3 | 7.5 | I | | |
| **7b** | 40 | 136.7 | 4.5 | I | | |
| **7c–e** | I | | | I | | |
| **7f** | 10 | 33.6 | 1.1 | 2.3 | 7.9 | 0.03 |
| **8a** | 45 | 161.8 | 5.4 | I | | |
| **8b–e** | I | | | I | | |
| **8f** | 20 | 64.1 | 2.1 | I | | |
| **Benznidazole** | 7.4 | 30 | 1.0 | | | |
| **Gentian violet** | | | | 100 | 245.1 | 1.0 |

I = $IC_{50}$ > 100 μg/mL (inactive). Index = $IC_{50}$ μM compound/$IC_{50}$ μM reference drug. Values under 1 correspond to higher potencies than that of the reference.

displayed some activity, with similar potency, though slightly higher for **4e**. Contrarily to the case of the natural series **1**, in the cases of the synthetic piperidides, without additional substituents on ring A, the presence of trimethoxy substituents on ring B seems to enhance the potency. It is also interesting to note that the substitution of the piperidine fragment by the closely related secondary amines pirrolidine (**$4b_1$**) or diethylamine (**$4b_2$**) led to inactive compounds.

None of the phthalazinone derivatives (**5a–5f, 6a–6f**) showed appreciable activity at concentrations of 100 μg/mL. In contrast, imidazo-isoindoles and pyrimido-isoindoles (**7** and **8**) were found to be active and, in some cases, potent trypanocides. From the data in Table 1, it can easily be deduced that the presence of multiple substituents on ring B will reduce the potency, while their absence (**7a** and **8a**) or the presence of only one *p*-substituent (**7b**, **7f**, **8f**) will increase the trypanocidal potency against epimastigotes. In both types of derivatives **7** and **8**, among the substituents considered, the *p*-chloro group promoted the largest activity enhancements and, most interestingly, in the case of the fused-imidazoline **7f**, induced a great activity against the human infective trypomastigote form, attaining potency levels almost two orders of magnitude higher than gentian violet.

It can be concluded that we have discovered two novel anti-Chagasic leads, represented by the dihydrostilbenamide **1e** and the imidazo-isoindole **7f**, which are active against both forms of *T. cruzi*, especially the trypomastigote form. On the basis of other assays, we also know that these compounds are not cytotoxic to normal human cells (data not shown); therefore, they represent very promising structures to take into consideration in the development of new agents for Chagas' disease. In fact, studies to design and synthesise new analogues with improved antiparasitic profiles, as well as schedules for in vivo trypanocidal and toxicity determinations, are currently in progress.

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