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Short communication

Trypanocidal activity of 5,6-dihydropyran-2-ones against free trypomastigotes forms of *Trypanosoma cruzi*

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Abstract

Sixteen 5,6-dihydro-2*H*-pyran-2-ones were evaluated in in vitro assay against trypomastigotes forms of *Trypanosoma cruzi*, the causative agent of Chagas' disease. A structure–activity relationship study (SAR) allowed us to establish the relevant structural features for the trypanocidal activity of goniothalamin analogues against *T. cruzi*. In fact, non-natural form of goniothalamin (*ent*-**1**) was threefold more potent than the natural one (**1**). In addition, we have identified analogues **9** and **10** (both displaying *S* configuration) as the highest potent compounds against *T. cruzi* with $IC_{50} = 0.12$ and 0.09 mM (IC_{50} value for crystal violet was 0.08 mM) whereas significantly lower toxicities were observed when these compounds were evaluated under LLC-MK_2 lineage cells (1.38 and 4.89 mM, respectively). In addition, epoxides derivatives **12** and *ent*-**12** were shown to be more potent than the corresponding stereoisomers **2** and *ent*-**2** and non-natural argentilactone (*ent*-**3**, $IC_{50} = 0.47$ mM) was twofold more potent than natural argentilactone (**3**, $IC_{50} = 0.94$ mM).

Keywords: 5,6-Dihydropyran-2-ones; Goniothalamin Analogues; Trypanocidal Activity

1. Introduction

Trypanosoma cruzi, a hemoflagellate protozoa (family Trypanosomatidae, order Kinetoplastida) [1], comprises the causative agent of South American Chagas' disease. Chagas' disease is endemic in Latin America, affecting 16–28 million people, with more than 100 million exposed to the risk of infection, and causing the death of approximately 400 000 people per year [2,3]. In Brazil about 5–6 million people are infected with 300 000 of them located in São Paulo state [4]. Due to the high socio-economic impact associated with Chagas' disease, efforts have been addressed by several research groups to find more efficient and safe agents for the treatment of this disease [5–9]. Nowadays, [Nifurtimox (a 5-nitrofuran derivative) and Benzonidazole (a 2-nitroimidazole acetamide)] are used in the therapy against *T. cruzi*. However, these compounds present severe side effects, and its efficacy depends on the susceptibility of different parasite populations. In fact, current chemotherapics against all forms of trypanosomiasis are very limited and unsatisfactory and the search for new lead compounds is worth to pursue [10].

Natural products play an important role in the development of drugs and mankind has always taken advantage of nature as a pharmacy: approximately 40% of the drugs that have been approved over the last years are either natural products or derivatives and analogues thereof [11–13]. The 5,6-dihydro-2*H*-pyran-2-one moiety is present in a large number of biologically active natural products such as goniothalamin (**1**), goniothalamin oxide (**2**) and argentilactone (**3**) (Fig. 1).

Goniothalamin (**1**) is a styryl lactone isolated from various species of the genus *Goniothalamus* [14]. This compound bears the (*R*)-configuration in its natural form and displays in vitro cytotoxic effect especially by inducing apoptosis on different cancer cell lines [15,16], antimicrobial and larvicidal activities [17,18], and anti-inflammatory activity [19].

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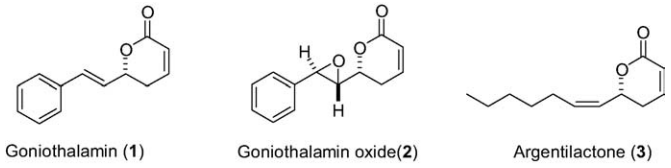

Fig. 1. Structures of natural 5,6-dihydro-*2H*-pyran-2-one: goniothalamin (**1**), goniothalamin oxide (**2**) and argentilactone (**3**).

Goniothalamin oxide (**2**) is also a member of styryl lactones and was isolated from *G. macrophyllus* [20], *G. amuyon* [21] and *G. dolichocarpus* [22]. The (6*R*,7*R*,8*R*)-absolute configuration of natural goniothalamin oxide (**2**) was established by X-ray diffraction studies on the minor diastereoisomer obtained from the *m*-MCPAB epoxidation of natural goniothalamin (**1**) [22]. Goniothalamin oxide (**2**) showed toxicity against the larvae of *Aedes aegypti* requiring concentration lower than 100 ppm [22]. Moreover, goniothalamin (**1**) and goniothalamin oxide (**2**) have been identified as the active embryotoxic and teratogenic components from *G. macrophyllus* [20]. Argentilactone (**3**) also bears the (*R*)-configuration in its natural form and it has been isolated from *Aristolochia argentina* (Aristolochiaceae) [23], *Chorisia crispflora* (Bombaceae) [24] and *Annona haematantha* (Annonaceae) [25]. This natural pyranone was shown to have in vitro antiprotozoa activity against *Plasmodium falciparum* [26], *Leishmania panamensis* [26], and *Leishmania amazonensis* [25], as well as cytotoxic activity against leukemia cells (P-388) [24]. In spite of biological activities exhibited by goniothalamin (**1**), goniothalamin oxide (**2**) and argentilactone (**3**), studies regarding their trypanocidal activity have not been reported so far.

Herein, we report our results concerning the trypanocidal activity of goniothalamin (**1**), its enantiomer (*ent*-**1**) and eight analogues (**4**–**11**), natural goniothalamin oxide (**2**) and its stereoisomers *ent*-**2**, isogoniothalamin (**12**) and *ent*-**12**, as well as argentilactone (**3**) and its enantiomer (*ent*-**3**) (Fig. 2).

2. Results and discussion

Goniothalamin (**1**), its enantiomer *ent*-**1**, analogues **4**–**11**, argentilactone (**3**) and its enantiomer *ent*-**3** were obtained as previously described [16,27–30]. Goniothalamin oxide (**2**), isogoniothalamin oxide (**12**) and their respective enantiomers (*ent*-**2** and *ent*-**12**) were obtained according to Sam et al. [20] and Goh et al. [22].

First of all, we evaluated natural goniothalamin (**1**) and its enantiomer *ent*-**1** against blood-stream forms of *Trypanosoma cruzi* (Table 1). According to Table 1, non-natural form of goniothalamin (*ent*-**1**) was threefold more potent than natural one (**1**). The same behavior was observed when we compared natural argentilactone (**3**) and its enantiomer *ent*-argentilactone (*ent*-**3**). At this point, the results clearly pointed out the importance of the absolute configuration for the trypanocidal activity, a pattern previously observed when the cytotoxic activity of this family of compounds were evaluated against human tumor cells [16]. However, *ent*-**1** and **1** showed lower activity when compared with crystal violet (IC_{50} = 0.08 mM) and we promptly evaluated analogues **4**–**11** in order to improve the trypanocidal activity and to identify the pharmacophoric groups responsible for it.

Table 1 shows that analogue **4** lacking the endocyclic double bond was almost twofold more potent than *ent*-**1** while analogue **5** without the exocyclic double bond was less potent. Surprisingly, compound **6** where both *endo* and *exo* double bonds were removed was shown to be equally potent to **4**. Taken together, these data suggest that better trypanocidal activity may be attained when the endocyclic double bond is

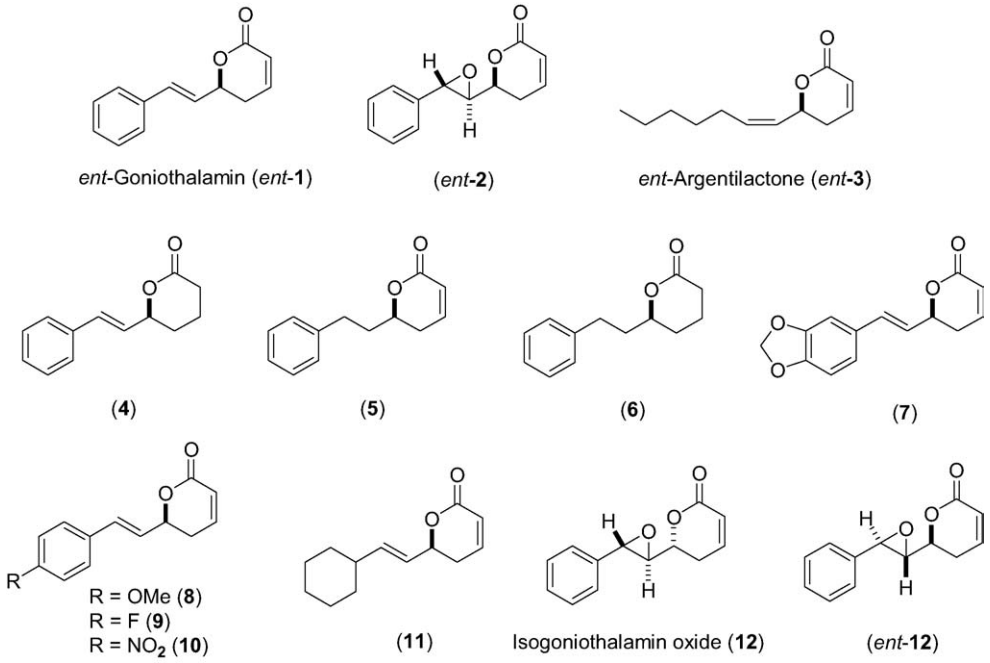

Fig. 2. Structures of the non-natural 5,6-dihydro-*2H*-pyran-2-ones.

Table 1
Trypanocidal activity of natural and non-natural 5,6-dihydro-2*H*-pyran-2-ones[a]

Compound	Concentration (μM) × lysis %			IC_{50} (mM)[b]
	8	32	128	
1	23.6 ± 0.7	32.9 ± 3.8	36.7 ± 0.1	1.30
ent-**1**	17.7 ± 5.5	23.2 ± 3.9	40.1 ± 8.1	0.35
2	12.7 ± 1.3	17.3 ± 3.2	35.4 ± 3.3	0.41
ent-**2**	12.2 ± 4.8	12.7 ± 8.9	27.0 ± 2.6	1.50
3	5.5 ± 1.9	6.3 ± 2.6	19.8 ± 1.5	0.94
ent-**3**	17.7 ± 6.3	17.3 ± 3.2	35.4 ± 3.3	0.47
4	12.7 ± 7.9	11.8 ± 2.6	41.8 ± 7.2	0.21
5	5.1 ± 1.7	12.7 ± 1.7	23.4 ± 0.9	0.91
6	12.7 ± 1.8	34.6 ± 5.1	45.0 ± 4.4	0.19
7	8.0 ± 3.9	15.6 ± 1.9	22.8 ± 3.3	2.39
8	16.4 ± 4.7	20.7 ± 1.9	22.8 ± 3.8	6.27
9	10.5 ± 1.5	40.5 ± 4.6	47.7 ± 4.4	0.12
10	12.7 ± 1.3	32.1 ± 6.2	54.8 ± 0.7	0.09
11	15.2 ± 5.5	35.8 ± 0.7	41.8 ± 6.7	0.22
12	30.0 ± 2.6	38.4 ± 5.3	45.6 ± 4.6	0.25
ent-**12**	2.5 ± 2.2	8.9 ± 1.8	31.2 ± 1.9	0.26

[a] Positive control: crystal violet at 250 μg ml^{-1} (IC_{50} = 0.08 mM); negative control: infected blood plus dimethylsulfoxide.
[b] Concentration that elicits lysing by 50% of blood-stream forms of *Trypanosoma cruzi*.

removed. While goniothalamin analogues with electron-rich aromatic rings such as **7** and **8** were significantly less potent when compared to *ent*-**1**, the presence of electron withdrawing groups in the aromatic ring significantly increased the potency analogues (**9** : (IC_{50} = 0.12 mM) and **10** (IC_{50} = 0.09 mM)) when compared to *ent*-**1** (IC_{50} = 0.35 mM). Since analogues **9** and **10** were shown to be the most active compounds against trypomastigotes forms of *T. cruzi* displaying IC_{50} values very similar to that for crystal violet used as positive control (IC_{50} = 0.08 mM), we carried out an assay with LLC-MK_2 cells in order to check for non-specific cytotoxicity of compounds **9** and **10**. Significantly lower toxicities were observed when these compounds were inoculated with *p*-fluoro and *p*-nitro analogues **9** and **10** (1.38 and 4.89 mM, respectively) thus indicating that the cytotoxicity observed is specific for the parasites forms investigated.

Moreover, the percentage of lyses observed for the trypomastigote forms of *T. cruzi* treated with analogues **9** and **10** (Fig. 3) showed that goniothalamin derivatives bearing electron poor aromatic substituents are promising lead compounds for the development of new drugs to Chagas' disease treatment. Finally, substitution of the aromatic group for cyclohexyl group in analogue **11** as well the use of isogoniothalamin oxide (**12**) or its enantiomer *ent*-**12** provided lower IC_{50} values than *ent*-**1** but were still not as effective as derivatives **9** and **10** (Table 1). Overall, these results allowed us to identify the pharmacophoric groups in goniothalamin (**1**) (Fig. 4).

3. Conclusion

In conclusion, our studies suggest that the *S* configuration in the pyranone ring is beneficial for trypanocidal activity displayed by the 5,6-dihydro-2*H*-pyran-2-ones studied. In this way, we have identified analogues **9** (IC_{50} = 0.12 mM) and **10** (IC_{50} = 0.09 mM) as the highest potent compounds against

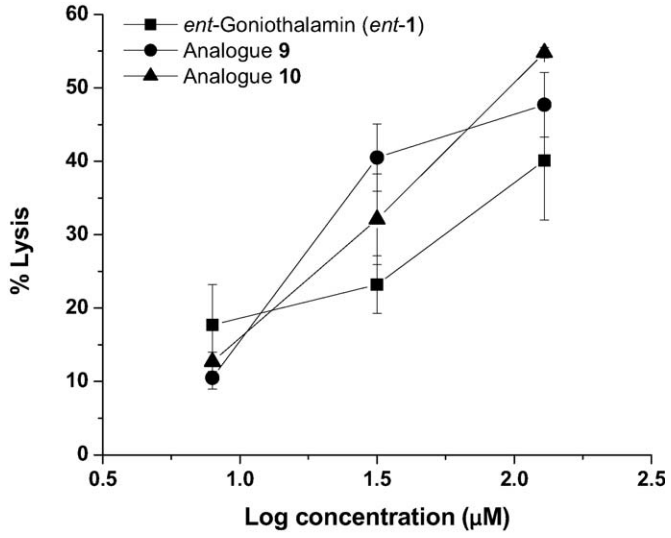

Fig. 3. Concentration–response curve for lytic activity of *ent*-goniothalamin (*ent*-**1**), analogues **9** and **10** on trypomastigotes forms of *Trypanosoma cruzi*.

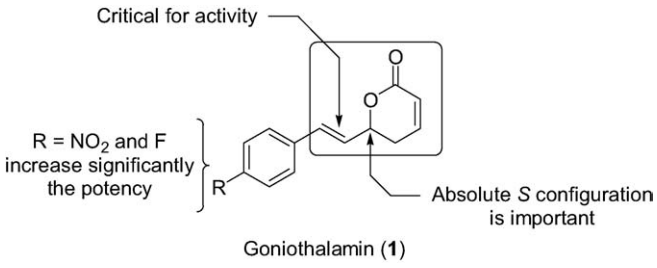

Fig. 4. Pharmacophoric groups of goniothalamin (**1**) for its trypanocidal activity against *Trypanosoma cruzi*.

T. cruzi with potencies comparable to that of crystal violet (IC_{50} = 0.08 mM). Epoxides **12** and *ent*-**12** presented higher potency than their corresponding stereoisomers **2** and *ent*-**2** while non-natural argentilactone (*ent*-**3**, IC_{50} = 0.47 mM) was twofold more potent than argentilactone (**3**, IC_{50} = 0.94 mM). Studies are underway in order to prepare and evaluate the trypanocidal activity of other goniothalamin derivatives as well as other 5,6-dihydro-2*H*-pyran-2-ones.

4. Experimental section

4.1. Chemistry

The 5,6-dihydro-2*H*-pyran-2-ones, goniothalamin (**1**), its enantiomer *ent*-**1**, analogues **4**–**11**, argentilactone (**3**) and its enantiomer *ent*-**3** were obtained as previously described [27–30]. Goniothalamin oxide (**2**), isogoniothalamin oxide (**12**) and their respective enantiomers, *ent*-**2** and *ent*-**12**, were obtained according to previous reports [20,22].

4.2. Trypanocidal assay in vitro

The bioassays were carried out using the blood of infected Swiss albino mice, which was collected by cardiac puncture at the peak of parasitemic infection (7th day of infection for Y

strain). The infected blood was diluted with the blood of healthy mice to achieve a concentration of 10^6 trypomastigote forms ml^{-1}. The 5,6-dihydro-*2H*-pyran-2-ones (**1**–**12**) solution were prepared in dimethyl sulfoxide (DMSO) and were added into the infected mouse blood to provide concentrations of 8, 32 and 128 μM, respectively. The plates were incubated at 4 °C for 24 h. Afterwards, the trypanocidal activity was evaluated by counting the trypomastigote forms of the remaining parasites, following the method described [31–33]. The bioassays were performed in triplicate on microtiter plates (96 wells), which contained 200 μl of mixture per well. Negative and positive controls containing either DMSO or crystal violet at 250 μg ml^{-1} were run in parallel.

4.3. Cytotoxicity determination

The cytotoxicity assay were carried out based in MTT-dye reduction assay as described by Mosmann [34] with some modifications. Aliquots of cell suspension (100 μl of 1×10^6 cells per ml: LLC-MK_2 cell) were seeded in 96-well microplates. Following 2 h incubation at 37 °C the cells were exposed to the analogues **9** and **10** for 24 h at 0.5, 2.0, 8.0 and 32.0 μM. After the incubation period MTT solution (5 mg ml^{-1} in PBS) was added (10 μl per well) and the plates were further incubated for 4 h at 37 °C. Thereafter the formazan crystals formed were dissolved through addition of 100 μl per well 5% hydrochloric acid in 2-propanol and the absorption of the samples was measured with an ELISA reader at 570 nm. Hundred microliters of RPMI 1640 medium, 10 μl MTT stock and 100 μl 5% hydrochloric acid in 2-propanol served as a blank solution. All assays were performed in triplicate.

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