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LEISHMANICIDAL ACTIVITY OF COMBRETASTATIN ANALOGUES AND HETEROANALOGUES

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Abstract:

We have investigated the *in vitro* leishmanicidal activity of representative members from series **II-V** of combretastatin analogues and heteroanalogues. Most of them exhibited different degrees of activity against various strains of *Leishmania spp*. The diaryl(heteroaryl)ethane system or the more complex fused heterocyclic stilbenoids, constitute useful skeletal bases to support some kind of antiparasitic activity. Particularly, the incorporation of 2-furyl substituents led to potent antileishmanial compounds, which have been selected for *in vivo* testing on murine models.

Combretastatins constitute an interesting type of natural products receiving much attention due to their antineoplastic properties[1]. Few natural combretastatins have been described, but many other derivatives and analogues have been synthesised and evaluated for antimitotic and antitumoral activities[2]. Recently we have prepared different families of combretastatin analogues, including heterocyclic analogues, which have been tested for their cytotoxic activity[3]. Several representatives of these series of compounds have been now also tested *in vitro* on parasite cultures of the main parasitic *Leishmania spp*[4]. This is the first study that describes the *in vitro* leishmanicidal activity of combretastatins or related compounds.

Leishmaniasis is an endemic disease in South America[5]. It affects 12 million people in the world and 350 million at risk, of whom some 1.5 to 2 million will be infected each year. Drugs currently in use, as pentavalent antimony, pentamidine or amphotericin B, are toxic and can produce severe complications or even threaten patient's life, with inconveniences in their administration routes and give rise to clinical resistance[6]. This situation shows the necessity of finding new therapeutic drugs for the treatment of the leishmaniasis. The present paper describes the *in vitro* antileishmanial effects of combretastatins and heterocombretastatins on three different strains of *Leishmania*.

Dedicated to the memory of Professor Joaquín de Pascual Teresa.

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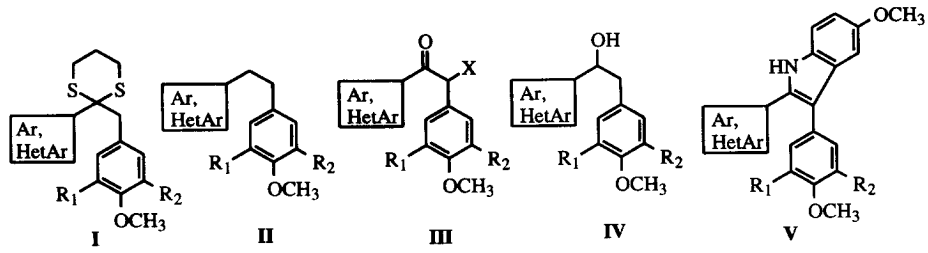

Comp.	Fam.	Ar/HetAr	Substituents	Comp.	Fam.	Ar/HetAr	Substituents
1	**I**	*N*-Me-3-indolyl	R_1=R_2=OMe	**3d**	**III**	3,4-methylene dioxyphenyl	R_1=R_2=OMe
2a	**II**	2-furyl	R_1=R_2=OMe				
2b	**II**	3-furyl	R_1=R_2=OMe	**3e**	**III**	2-furyl	R_1=H R_2=OH
2c	**II**	2-naphtyl	R_1=R_2=OMe	**3f**	**III**	2-thienyl; X=OH	R_1=R_2=OMe
2d	**II**	*N*-Me-3-indolyl	R_1=R_2=OMe	**4a**	**IV**	2-furyl	R_1=R_2=OMe
2e	**II**	2-furyl	R_1=H R_2=OH	**4b**	**IV**	3,4-methylene dioxyphenyl	R_1=R_2=OMe
2f	**II**	3,4-methylene dioxyphenyl	R_1=H R_2=OH	**5a**	**V**	2-furyl	R_1=R_2=OMe
3a	**III**	2-furyl	R_1=R_2=OMe	**5b**	**V**	3,4-methylene dioxyphenyl	R_1=R_2=OMe
3b	**III**	3-furyl	R_1=R_2=OMe				
3c	**III**	5-methyl-2-furyl	R_1=R_2=OMe	**5c**	**V**	2-naphthyl	R_1=R_2=OMe

Scheme 1. Families **I-V** and compounds evaluated as antileishmanial agents.

The assayed compounds (Scheme 1) belong to families **II-V** and their synthesis, through dithianes **I**, has been previously reported by us [3].

The isolation, cultivation and maintenance of promastigote-stage parasites have been previously detailed[7]. Promastigotes inhibition studies were performed on *L. amazonensis* (IFLA/BR/67/PH8), *L. brasiliensis* (MHOM/BR/75/M2903) and *L. donovani* (MHOM/BR/74/PP75) grown at 22°C in Schneider's *Drosophila* medium containing 20% foetal bovine serum. Promastigotes cultures in the logarithmic phase were transferred at a concentration of 10^6 cell per mL. Compounds (1mg) were dissolved in 40 µL of dimethyl sulphoxide (DMSO), and added to 1 mL of the medium from which aliquots are drawn. From this stock solution 200 µL were dissolved in 800 µL of medium. An amount of 100 µL of this second solution was mixed with 100 µL of parasite culture reaching a compound concentration of 100 µg/mL and 0.4% of DMSO placed in microtitre plates in triplicates. Other concentration as 50, 25 and 10 µg /mL were also tested. The activity of compounds was evaluated after forty-eight hours by optical observation of a drop of each culture with a microscope, by comparison with control cells and with the reference drug, pentamidine[8]. The drug concentration required to inhibit parasite growth was evaluated and at least two independent experiments, performed in triplicate with different stock drugs solutions were carried out.

Results and Discussion

In this study analogues of combretastatin were tested *in vitro* on promastigote forms of *Leishmania spp.* A total lysis of parasites was observed at 50 µg/mL when **2a**, **2d**, **3a**, **3c**, **3d** and **5a** were incubated by 48 hours with promastigotes of the three afore cited strains (Table 1). However, it is important to notice that at 10 µg/mL the lysis reaches 90% of the total promastigotes observed.

Table 1. *In vitro* activity of combretastatin analogues and heteroanalogues toward three strain of promastigotes of *Leishmania spp.*

Compounds	*L. amazonensis* (PH8) *				*L. brasiliensis* (2903) *				*L. donovani* (PP75) *			
	100	50	25	10	100	50	25	10	100	50	25	10
1a	++				++				++			
2a	+++	+++	++	++	+++	+++	++	++	++	+++	++	++
2b	++				+				+			
2c	+++	++			+++	++			+++	++		
2d	+++	+++	++	++	+++	+++	++	++	+++	+++	++	++
2e	++				++				++			
2f	++	++			++	++			+++	++		
3a	+++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	++
3b	++				++				++			
3c	+++	+++	+++	++	+++	+++	+++	++	++	+++	+++	++
3d	+++	+++	++	++	+++	++	++	++	+++	+++	++	++
3e	++				++				++			
3f	+				+				+			
4a	++				++				++			
4b	+++	++	++	++	+++	++	++	++	+++	++	++	++
5a	+++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	++
5b	+++	++			+++	++			+++	++		
5c	++				++				++			
Control	0				0				0			
Pentamidine#	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++: Total lysis of parasites; ++: 80-90% of lysis; +: < 70%; 0: no lysis. * in μg/mL

: IC_{50} (mg/mL): 1.03 (*L. mexicana*), 0.48 (*L. amazonensis*) and 0.45 (*L. donovani*) [9]

In a first analysis of data shown in Table 1, it can be observed that every series **II-V** contains representative compounds showing high antileishmanial potency. In other words, the basic diarylethane, 1-aryl-2-heteroarylethane or conformationally restricted or fused heterocyclic stilbenoids, constitute useful skeletal systems to support the antiparasitic activity.

In relation to the type of aryl or heteroaryl groups in 1,2-disubstituted ethanes (series **II**) it can be observed from the comparison between compounds **2a/2e** and **3a/3e**, that the 3,4,5-trimethoxyphenyl group seems to be better for the activity than the 3-hydroxy-4-methoxyphenyl group. Similarly, the 2-furyl group results better than the 2-naphthyl group (**2a/2c**) and close to the 3,4-methylenedioxyphenyl group (**2e/2f**).

Some other observations related to structure-activity relationships are the following: 2-furyl derivatives (**2a**, **3a**, **3c**) are more potent than 3-furyl (**2b**, **3b**) analogues and similar to the indole derivatives **2d**. Ethanones (series **III**, **3a**) are comparatively more potent than ethane (series **II**, **2a**) and ethanol (series **IV**, **4a**) derivatives. On the basis of the mentioned observations and previous cytotoxicity results found for combretastatins and

analogues, some 2,3-disusbstituted indole derivatives (series **V**) were synthesized incorporating in all the cases the trimethoxyphenyl fragment in combination with the other useful leishmanicidal groups 2-furyl, 3,4-methylenedioxyphenyl and 2-naphthyl residues (**5a**, **5b** and **5c**). Accordingly, compound **5a** resulted to be the most potent leishmanicide *in vitro* of the series.

As a consequence of the above results and considerations, compounds **II-V** are promising models for the development of new families of antileishmanial agents. Taking into account the existence of two main types of leishmaniasis: visceral, which needs the use of systemic agents and muco-cutaneous, usually treated with topical applications of drugs, compounds **3c** (less toxic for mammalian cells) and **5a** (more cytotoxic) were selected for submission to *in vivo* assays, which are currently in progress.

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