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Leishmanicidal Activity of Some Stilbenoids and Related Heterocyclic Compounds

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Dedicated to the memory of Professor Joaquín De Pascual Teresa

Abstract—We have evaluated the leishmanicidal activity of some natural and semisynthetic dihydrostilbenoids and several compounds of other series of dihydrostilbamides, isoindoles, phthalazinones, imidazoisoindoles and pyrimidoisoindoles. The evaluation was performed in vitro, on cultures of cutaneous, mucocutaneous and visceral strains of *Leishmania* spp. The most potent and selective compounds of these series were the dihydrostilbene piperidides.

Natural stilbenoids and their synthetic analogues, in addition to their formerly reported therapeutic uses, mainly based on their hormonal/antihormonal activities, are presently well known by other kinds of bioactivity, such as antitumoral and antileukemic, platelet antiaggregation,[1] anti-inflammatory and antihistaminic[2] activities. A number of recently described representative reports can be found in the literature, for example, piceatannol,[3] lavendustin A,[4] resveratrol,[5] etc. Nevertheless, a computerized literature search looking for descriptions of antiparasitic activities of stilbenoids, revealed the inexistence of reports on this subject, with the only exception of one paper published by one of us, in this journal, dealing with the in vitro leishmanicidal activity of combretastatins and some heteroanalogues.[6]

Leishmaniasis is a parasitic disease endemic to the American, African and Asian tropical countries.[7] It affects some 12 million people around the world and 350 million live on risk of becoming infected, of whom some 2 million will actually be infected each year.[8] The most common varieties of leishmaniasis, the cutaneous and mucocutaneous ones, produced by *Leishmania tropica, L. major, L. mexicana, L. braziliensis, L. peruviana, L. amazonensis* and *L. aethiopica*, are not directly lethal, but provoke multiple granulomatose or diffuse, autoinoculable and even metastatic skin ulcers, resembling leprosy lesions. On the other hand, visceral leishmaniasis, produced by *L. donovani*, localized in blood, liver, spleen and other vital organs and tissues, is fatal in more than 90% of untreated cases.[9] Drugs currently in use as the antimony derivative glucantine, the bis-amidines, pentamidine and stilbamidine or the glycomacrolide amphotericin B, display high liver and heart toxicities, develop clinical resistance after a few weeks of treatment[10] and currently contribute to increase co-infections leishmaniasis-AIDS in some countries.[11] For these reasons it becomes necessary to discover new, natural or synthetic, more potent and selective agents for treating this increasing parasitosis. This paper describes the in vitro anti-leishmanial evaluation of some natural stilbenoids and some derivatives as well as of some related condensed heterocyclic derivatives, on cutaneous, mucocutaneous and visceral strains of *Leishmania*.

Chemistry

The structural similarity of isonotholaenic acid (**1a**) with combretastatins prompted us to submit the compound to a number of tests of bioactivity and, among

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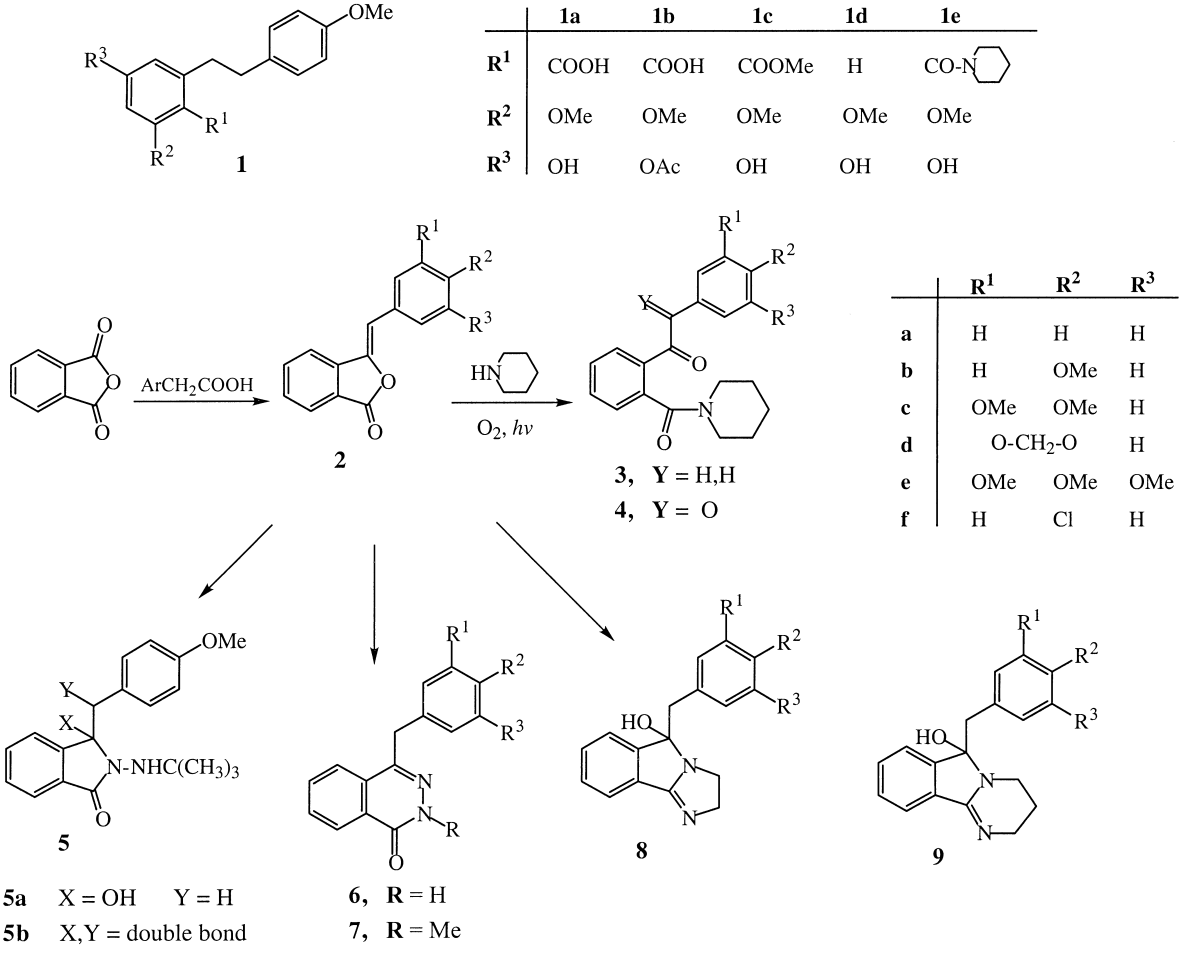
them, those on *Leishmania*, *Trypanosoma* and *Plasmodium* growth. Compound **1a** displayed some activity on *Leishmania* spp and *Plasmodium falciparum* and much less activity on *Trypanosoma cruzi*. This finding prompted us to prepare and evaluate the new substances depicted in Scheme 1, with the aim of obtaining improved antiparasitic agents.

Compounds **1** are natural products or were obtained through simple chemical transformations from isonotholaenic acid (**1a**), the main component of dichloromethane extracts from the Andean fern, *Notholaena nivea* var. *nivea*.[12] Compounds **3**–**9** were prepared by total synthesis, through condensation of phthalic anhydride with several substituted phenylacetic acids, to give the corresponding benzalphthalide intermediates **2**, which were subsequently treated with piperidine, hydrazine derivatives or diamines, to obtain, respectively, the piperidides (**3** and **4**), isoindolinones (**5**), phthalazinones (**6** and **7**), imidazo- (**8**) and pyrimidoisoindoles **9** shown above. Compounds **4** could probably be formed through the autoxidation of a piperidine enamine intermediate.[13] Indeed, the proportion **3**/**4** can be modulated by controlling the reaction conditions. All the compounds retained the main stilbenoid skeleton of isonotholaenic acid (**1a**) and incorporated some structural fragments, as the common natural trimethoxyphenyl fragment and a masked amidine moiety, whose presence in antiprotozoal and other antimicrobial agents has proven to be useful clinically. The detailed description of synthetic procedures, physicochemical and spectroscopic data and structure assignments for these compounds will be reported in a complete paper.

Biological Assays

The isolation, culture and maintenance of promastigote-stage parasites have been previously described.[14] Promastigotes inhibition studies were performed on *L. amazonensis* (IFLA/BR/67/pH8), *L. braziliensis* (MHOM/BR/75/M2903) and *L. donovani* (MHOM/BR/74/pp75) grown at 27 °C in Schneider's drosophila medium containing 10% of fetal bovine serum. Compounds were dissolved in dimethyl sulphoxide (DMSO) (final concentration of DMSO less than 0.1%) and liquid medium placed in microcells Titertek 96 (Flow Laboratories) to obtain final concentration of 100, 50, 25 and 10 μg/mL. All assays were done in triplicate. Promastigote cultures in the logarithmic phase were transferred at the level of 10^5 parasites per cell. The activity of the compounds was evaluated after



Scheme 1. Natural and synthetic derivatives tested as anti-leishmanial agents.

incubation during 72 h, by optical observation and cell counting, on a drop of each culture, with an inverted microscope and comparison with control cells without product and other containing the reference drug, pentamidine (10 μg/mL).

Results and Discussion

The results of the anti-leishmanial evaluation are shown in Table 1. Within the group of natural products and derivatives, isonotholaenic acid (**1a**) provoked the total lysis of the three strains of *Leishmania* parasites at the concentration of 50 μg/mL ($IC_{50} = 32$ μg/mL), while the masking of its phenolic group as acetate (**1b**) or the carboxylic acid as its methyl ester (**1c**), fairly decreased or fully eliminated the activity, respectively. Elimination of the carboxylic function also led to an inactive product (**1d**), while the transformation into an amide (**1e**) improved the activity of **1a** four times, attaining a potency only one order lower than that of the reference drug, pentamidine.

Benzalphthalide intermediates, **2**, were in general less active, with the exception of compounds **2a** (unsubstituted phenyl ring), **2e** (3,4,5-trimethoxyphenyl) and **2f** (*p*-chlorophenyl). It is to be noted that while **2a** showed similar potency against the three *Leishmania* strains, compounds **2e** and **2f** displayed some selectivity for one of the strains, mucocutaneous PH8 in the case of **2e** and visceral PP75 in that of **2f**.

In the case of piperidides (**3** and **4**), some main considerations can be made. The presence of two carbonyl groups at the ethylenic bridge seems to induce some enhancement of the activity, in relation with those less oxidized compounds, having only one keto group (**4a** vs **3a**, **4b**/**3b** and **4f**/**3f**), except for **3e**, the most active compound of all the series evaluated. In parallel with phthalide **2e**, the piperidide **4e** displayed certain selectivity against the PH8 strain. Compound **3e** was similarly selective against M2903 and PH8 strains, with potency close to that of pentamidine. The chloro derivative **4f** also was active against the three strains, with some selectivity for *L. amazonensis* and *L. donovani*. It must be noted that the presence of the piperidide (**1e**, **3e**, **4e**) or the *N*,*N*-diethylamide moities (**$4b_2$**) are very important for the anti-leishmanial activity, and that the other type of amide like pyrrolidide seems to be less significant.

Only a few compounds with structures **5** to **9** (Table 1) displayed some activity against *Leishmania*; namely, the chlorobenzylphthalazinone derivative **7f** and the unsubstituted benzyl derivatives of imidazo- (**8a**) and pyrimido-isoindole (**9a**). They seem do not merit further consideration.

On the basis of the results obtained for the piperidide series, a number of different electron donating or withdrawing substituents are being incorporated on the starting anhydride, and further chemical and evaluation studies, focused to enhance the selectivity and potency of these new types of anti-Leishmanial agents, will be done.

Table 1. In vitro activity of stilbenoid derivatives on *Leishmania* spp. promastigotes

Type	Compound	*L. braziliensis* (M2903)	*L. amazonensis* (PH8)	*L. donovani* (PP75)
Dihydrostilbenoid	**1a**	32	32	32
	1b	62	62	62
	1c, 1d	I	I	I
	1e	10	7	7
Benzalphthalide	**2a**	45	45	45
	2b–2d	I	I	I
	2e	I	52	I
	2f	I	I	52
Piperidide	**3a–3d**	I	I	I
	3e	7	10	20
	3f	I	I	I
	4a	75	75	75
	4b	80	I	80
(Pyrrolidide)	**$4b_1$**	I	I	I
(Diethylamide)	**$4b_2$**	75	75	75
	4c, 4d	I	I	I
	4e	52	28	52
	4f	52	25	28
Isoindolinone	**5a, 5b**	I	I	I
Phthalazinone	**6a–6f**	I	I	I
Methylphthalazinone	**7a–7e**	I	I	I
	7f	I	I	64
Imidazo-isoindole	**8a**	60	60	60
	8b–8f	I	I	I
Pyrimido-isoindole	**9a**	45	45	45
	9b–9f	I	I	I
Reference	Pentamidine	1.3	1.3	1.3

IC_{50} values (μg/mL); I ≥ 100 μg/mL (inactive).

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References and Notes

1. Ko, S. K.; Lee, S. M.; Whang, W. K. *Arch. Pharm. Res.* **1999**, *22*, 401.
2. Matsuda, H.; Hiroshi, S.; Masayuki, Y. *Bioorg. Med. Chem.* **1999**, *7*, 1445.
3. (a) Geahlen, R. L.; McLaughlin, J. L. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 241. (b) Zheng, J.; Ramirez, V. D. *Biochem. Biophys. Res. Commun.* **1999**, *261*, 499.
4. Onoda, T.; Inuma, H.; Sasaki, Y.; Hamada, M.; Isshiki, K.; Naganawa, H.; Takeguchi, T. *J. Nat. Prod.* **1989**, *52*, 1252.
5. (a) Chun, Y. J.; Kim, M. Y.; Guenagerich, F. P. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 20. (b) Jang, M.; Pezzuto, J. M. *Drugs Exp. Clin. Res. Commun.* **1999**, *25*, 65. (c) Lu, R.; Serrero, G. *J. Cell. Physiol.* **1999**, *179*, 297.
6. Del Rey, B.; Ramos, A. C.; Caballero, E.; Inchaustti, A.; Yaluff, G.; Medarde, M.; Rojas de Arias, A.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2711.
7. World Health Organization. *Tropical Disease Research. Progress 1975–1994. Highlights 1993–1994*; UNDP/World Bank/WHO: Geneva, 1995; p 137.
8. World Health Organization (OMS). *Rapport sur la Santé dans le Monde, 1998*; WHO: Genève, 1998; p 48.
9. Berkow R. *The Merck Manual*, 16th ed.; Merck Res. Lab., Rahway, 1992; p 232.
10. (a) Croft, S. L. *Trends Pharmacol. Sci.* **1988**, *9*, 376. (b) Berman, J. D. *Rev. Infect. Dis.* **1988**, *10*, 560.
11. World Health Organization (OMS). *Rapport sur la Santé dans le Monde, 1998*; WHO: Genève, 1998; p 59.
12. Wollenweber, E.; Doerr, M.; Waton, H.; Bonvin, J. F. *Phytochemistry* **1993**, *33*, 611.
13. Rappoport, Z. *The Chemistry of Enamines, Part 2*; J. Wiley & Sons: Chichester, UK, 1994; p 924.
14. (a) Mesa-Valle, C. M.; Rodríguez-Cabezas, M. N.; Moraleda-Lindez, V.; Craciunescu, D.; Sánchez-Moreno, M.; Osuna, A. *Pharmacology* **1998**, *57*, 160. (b) Canto-Cavalheiro, M. M.; Echevarría, A.; Araujo, C. A.; Bravo, M. F.; Santos, L.; Jansen, A. M.; Leon, L. L. *Microbios.* **1997**, *90*, 51.