Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2769–2772

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Imidazo[2,1-*a*]isoindole System. A New Skeletal Basis for Antiplasmodial Compounds

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Received 24 February 2003; revised 14 April 2003; accepted 9 May 2003

In the Memory of Dr. Alicia Seldes, University of Buenos Aires, Argentina.

Abstract—The in vitro antiplasmodial activity of some dihydrostilbenamides, phtalazinones, imidazo[2,1-*a*]isoindole and pyrimido[2,1-*a*]isoindole derivatives related to the natural dihydrostilbenoid isonotholaenic acid is reported. The evaluation was performed on cultures of F32 strain of *Plasmodium falciparum* and potent representative compounds were also evaluated in the ferriprotoporphyrin IX biomineralization inhibition test (FBIT). Compounds having the imidazo[2,1-*a*]isoindole skeleton were the most active and one compound of this group resulted to be as potent as chloroquine, but acting through a mechanism different that of the inhibition of heme biomineralization.

Malaria is one of the major health threats to a wide population across the third world.[1] The therapeutic arsenal of efficacious antimalarial drugs is very limited and lessened by the spread of resistance.[2] Thus, the development of new alternative agents becomes necessary. In this regard, among other natural products, we evaluated the activity of isonotholaenic acid (**1**), a dihydrostilbenoid isolated from the Andean fern *Notholaena nivea*, as well as several of its derivatives as the piperidide **1a**, against *Plasmodium falciparum* by in vitro assays. They displayed IC_{50} values under 20 μg/mL and this fact prompted us to consider this stilbenoids as a source and model for the preparation of new potential antimalarials. Thus, along with a set of isonotholaenic acid derivatives, we prepared several families of synthetic analogues including some fused heterocyclic derivatives as phthalazinones, imidazo[2,1-*a*]isoindoles and pyrimido[2,1-*a*]isoindoles, all of them retaining the main stilbenoid moiety of **1** and incorporating pyridazine, imidazole and pyrimidine related residues, which could increase their antiprotozoal potency. Some of these compounds were previously found to be active against different *Leishmania spp*[3] and *Trypanosoma cruzi*[4] strains. Here we report the results of the in vitro evaluation of these types of compounds against *Plasmodium falciparum* and, in order to get further insight into their mechanism of action, we also report the results of the evaluation of their ability to interfere the ferriprotoporphyrin (FP) biomineralization process, a crucial metabolic process for *Plasmodium*.[5]

Chemistry

Most compounds were prepared by total synthesis, as depicted in Scheme 1, through condensation of phthalic anhydride with several substituted phenylacetic acids,[6] to give the corresponding benzalphthalide intermediates **2**, which were subsequently treated with piperidine, hydrazine, ethylenediamine or 1,3-propylenediamine to give the dihydrostilbenamide (types **3** and **4**), phtalazinone (types **5** and **6**), imidazoisoindole (type **7**) and pyrimidoisoindole (type **8**) derivatives, respectively. Synthetic procedures details, physicochemical and

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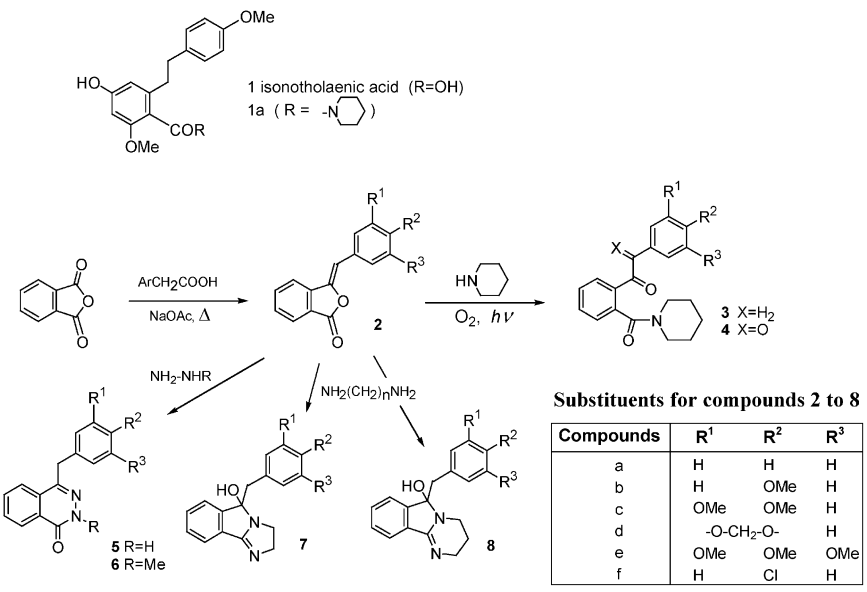
doi:10.1016/S0960-894X(03)00509-2



Substituents for compounds 2 to 8

Compounds	R^1	R^2	R^3
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. Compounds tested as antiplasmodial agents.

spectroscopic data and structure assignments for these compounds, will be reported in a complete paper.

Biological Assays

Assay against *Plasmodium falciparum*

F32-Tanzania (chloroquine sensitive) strains of *Plasmodium falciparum*[7] were cultured according to Trager and Jensen,[8] on glucose-enriched RPMI 1640 medium supplemented with 10% human serum at 37 °C in a candle jar incubator.[9] After 24 h of incubation at 37 °C, the medium was replaced by fresh medium containing the compound to be evaluated, and incubation was continued for a further 48 h period. On the third day of the test, a blood smear was taken from each well, parasitaemia counted and the inhibition percentage of parasitaemia calculated for each concentration of sample in relation with the control. IC_{50} values were determined graphically by plotting the inhibition percentage versus concentration. Each test also included an untreated control with solvent and a positive control with Chloroquine ($IC_{50} = 40$ nM).

FBIT

The procedure for testing FP biomineralization was described by Deharo et al.[5] and consisted of incubating a solution containing the compound to be evaluated (or solvent for control), haemin chloride and sodium acetate buffer. After incubation, the plate was centrifuged, the remaining pellet resuspended in DMSO to remove unreacted FP and centrifuged once again. The pellet, consisting of precipitate of β-haematin, was dissolved for direct spectroscopic quantification.[10] The data were expressed as the percentage of inhibition of FP biomineralization calculated by the equation: % inhibition = 100 × [(O.D. control − O.D. compound)/(O.D. control)]. Chloroquine showed $IC_{50} = 28\mu M$.

Results and Discussion

The results of the antiplasmodial evaluation are shown in Table 1. Data for isonotholaenic acid **1** and its piperidide **1a**, and for chloroquine are included for comparison and as that a reference drug, respectively. The intermediate benzalphthalides **2** were also evaluated, but they resulted practically inactive. In the case of phthalazinones, derivatives with other substituents at position N-2 (ethyl, allyl, butyl, phenyl, etc.) were also assayed giving results close to those found for compounds **5a–f** and **6a–f**. An index describing the antiplasmodial potency of the compounds, relative to chloroquine, has been calculated for each

Table 1. In vitro antiplasmodial activity of compounds **1**, **1a** and **3–8** (*Plasmodium falciparum,* strain F32)

Compd	IC_{50}		Index (%)	Compd	IC_{50}		Index (%)
	μg/mL	μM			μg/mL	μM	
1	16.0	53.0	<0.1	**1a**	6.0	16.3	0.25
3a	nt		—	**4a**	nt		—
3b	>100	>100	<0.1	**4b**	17	48	<0.1
3c	36	98	<0.1	**4c**	32	84	<0.1
3d	>100	>100	<0.1	**4d**	8.5	20.7	0.2
3e	100	>100	<0.1	**4e**	30	82	<0.1
3f	12	35	0.11	**4f**	12	33.8	0.12
5a	>100	>100	<0.1	**6a**	5	20	0.2
5b	1.0	3.8	1.1	**6b**	1.0	3.6	1.1
5c	95	>100	<0.1	**6c**	26	84	<0.1
5d	>100	>100	<0.01	**6d**	3.8	11	0.4
5e	>100	>100	<0.01	**6e**	32	>100	<0.1
5f	6	22	0.18	**6f**	15	52.8	<0.1
7a	2.5	9.5	0.4	**8a**	1	3.6	1.1
7b	**0.017**	**0.04**	**100**	**8b**	2.3	7.5	0.5
7c	4.9	9.3	0.4	**8c**	35	104	<0.1
7d	**0.067**	**0.19**	**21**	**8d**	38	104	<0.1
7e	**0.022**	**0.07**	**57**	**8e**	5.2	16.1	0.2
7f	**0.036**	**0.11**	**36**	**8f**	3.6	11.5	0.3
Chloroquine	**0.013**	**0.04**	**100**				

Index = IC_{50} μM chloroquine/IC_{50} μM compound (antiplasmodial potency, relative to chloroquine = 100).
nt = not tested.

Table 2. Inhibition of ferriprotoporphyrin biopolymerization (FBIT) by selected compounds

Compd	FBIT IC_{50}		Index (%)
	μg/mL	μM	
5b	1530	5752	0.5
6b	na		—
7b	na		—
7e	590	1735	1.6
7f	na		—
8a	na		—
Quinine	80	250	11
Chloroquine	8.5	28	100

Index [IC_{50} chloroquine /IC_{50} compound]; na = not active

compound displaying appreciable activity and included in Table 1.

As it can be observed in Table 1, the series of ketostilbenamides (**3**), diketostilbenamides (**4**), phtalazinones (**5**) and 2-methylphthalazinones (**6**) show discrete activity against *Plasmodium*, with representative members showing IC_{50} values within the 1–50 μM range (**4d** and **4f**; **5b** and **5f**; **6a**, **6b**, **6d** and **6f**). Their clearly lower potency compared to chloroquine, in addition to the small number of compounds evaluated do not permit to establish fair structure–activity correlations. Nevertheless, it could be noted that for these series the size and position of the substituent(s) on the phenyl group, rather than their electron-donating or withdrawing nature, would have an influence on the antiplasmodial potency.

The series of pyrimidoisoindoles (**8**) seems to be slightly more potent. Four (**8a**, **8b**, **8e** and **8f**) of six compounds of this series display IC_{50} values under 20 μM and, in an overall sense, they behave similarly to those of the other series mentioned above.

Surprisingly, the reduction in size of the fused six-member tetrahydropyrimidine ring towards the close analogue five-member imidazoline ring, promotes a considerable increase of the anti-*Plasmodium* activity in the imidazoisoindole series (**7**). Most compounds of this series, while maintaining the amidine moiety at an equivalent position and having identical substitution pattern on the phenyl group, display activities from one to more than two orders of magnitude higher than those corresponding pyrimidoisoindoles (**7b**/**8b**, **7d**/**8d**, **7e**/**8e** and **7f**/**8f**).

The *p*-methoxyphenyl group promotes the highest activity in compound **7b**, with IC_{50} value and anti-*Plasmodium* Index equivalent to those of chloroquine. Other symmetric *p*-ClPh- or 3,4,5-triMeOPh-substituents, though being three/four times less potent than **7b**, retain the order of activity. In contrast with the other series the 3,4-methylenedioxyphenyl group, present in compound **7e**, increases the antiplasmodial activity up to more than 55% of the potency of chloroquine. Nevertheless, a complete SAR analysis must wait to the results of evaluation of a larger number of substances with a greater variety of substituents.

On the light of these in vitro results, looking for correlating the activity with an established mechanism of action, some selected compounds were tested to check their ability to block the heme biomineralization process, but none of them was fairly active. The results of the FBIT are shown in Table 2. As it can be seen, only compounds **5b** and **7e** show a very low degree of inhibition, which is much far from the values observed for quinine and chloroquine. Thus, the antiplasmodial activity evidenciated here for these types of compounds must clearly proceed through another mechanism.

It can be concluded that a novel antimalarial type of compounds, based on the imidazo[2,1-*a*]isoindole system and represented principally by compound **7b**, has been discovered. In addition, it must be considered that compounds of type **7** and **8** have been evaluated as racemic mixtures and their resolution or enantioselective synthesis could give access to pure enantiomers, with probable better antiplasmodial properties for one of each pair of them; unless the stereochemical lability of the hemi-ketoaminal moiety would provoke the equilibration of each enantiomer towards the racemic form. While the synthesis and evaluation of new related compounds, their evaluation against resistant strains and the in vivo antimalarial assays with several selected substances are already in progress, cytotoxicity, acute toxicity and mechanistic studies on these compounds are scheduled.

Acknowledgements

M.G.A. thanks AECI (Spain) for a fellowship. M.I.Y. thanks the University of Tucuman (Argentina) for the support. Financial support of the chemical part came from Spanish DGICYT (SAF 98/0103). Biological evaluations were supported by the Institut de Recherche pour le Développement (IRD-France) and the OAS (OEA-Regional Flora). This collaborative work has been performed under the auspices of the Ibero-American Program of Science and Technology for Development (CYTED), Sub-Program X.

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9. DMSO (50 μL) was added to the product to be evaluated and the solution dispersed in RPMI 1640 medium with the aid of mild sonication in a sonicleaner bath (Branson Ltd), and

then diluted as required in culture medium. The final DMSO concentration was never greater than 0.1%. A total volume of 150 μL of total culture medium with the diluted products and the suspension of human red blood cells in medium (0^+ group, 5% haematocrit) with 1% parasitaemia, was placed into the wells of 96-well microtitre plates. All tests were performed in triplicate. Chemicals were obtained from Sigma Chemicals Co., USA

10. Incubation was performed in a normal flat bottom 96-well plate at 37 °C for 18–24 h. The mixture contained: 50 μL of a 10 mg/mL of compound solution (or 50 μL of solvent for control), 50 μL of 0.5 mg/mL freshly solution of haemin chloride (Sigma H 5533) in DMSO and 100 μL of 0.5 M sodium acetate buffer pH 4.4, prepared according to Deutscher.[11] The final pH of the mixture was 5–5.2. It is important to adhere to the following order of addition: first the haemin chloride solution, second the buffer, and finally the solvent or the compound solution. After incubation, the plate was centrifuged at $1600\times g$ for 5 min. The supernatant was discarded by vigorously flipping of the plate upside down the plate twice. The remaining pellet was resuspended with 200 μL of DMSO and centrifuged once again and the supernatant similarly discarded. The pellet, was dissolved in 150 μL of 0.1 M NaOH in the same plate and its absorption at 405 nm measured with a micro-ELISA reader (Tittered Multiskan MCC/340).

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