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Leishmanicidal Activity of Aurones

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This is the first report on aurones as a new class of natural products with leishmanicidal activity. A series of aurones with drug-potential for *Leishmania* infections was identified *in vitro* using both a direct cytotoxicity test against extracellular promastigotes of *Leishmania donovani*, *L. infantum*, *L. enriettii*, and *L. major*, and a test against intracellular amastigote *L. donovani* residing within murine macrophages. The compounds proved to be active at concentrations in the microgram range between 0.4 and 5.0 µg/ml. When tested against murine bone marrow-derived macrophages as a mammalian host cell control, all compounds showed only moderate cytotoxicity (EC_{50} 2.32-25.0 µg/ml).

Keywords : Aurones, *Leishmania*, Antiprotozoal, *In vitro*

INTRODUCTION

The World Health Organization (WHO) estimates that 350 million people live at risk of infection with *Leishmania* parasites with an annual incidence of about 2 million new cases [1]. Combined, the different forms of leishmaniasis currently affect some 12 million people in 88 countries, all but 16 of which are in the developing world. Recently, there has been an increase in coincidence between visceral leishmaniasis (VL) and HIV-infection due to spread of the AIDS pandemic. *Leishmania*/HIV co-infection is considered to be a genuine emerging disease, especially in southern Europe, where 25-70 % of adult VL cases are related to HIV infection, and 1.5-9.5 % of AIDS cases suffer from newly acquired or reactivated VL. The pentavalent antimonials, sodium stibogluconate and meglumine antimonate, the first line drugs for visceral and cutaneous leishmaniasis, have variable efficacy and severe side effects [2]. In traditional medicine, many plants have already provided valuable clues for potential antiparasitic compounds, especially phenols, flavonoids and related chalcones [3]. The antiprotozoal activities of plant-derived phenolics have attracted renewed attention since licochalcone A was identified as a potential drug against *Leishmania*, *Trypanosoma*, and *Plasmodium* parasites [4].

MATERIALS AND METHODS

For stock solutions, all test compounds and Pentostam® were first dissolved in DMSO at 25 mg/ml. Two-fold dilution series were prepared in RPMI 1640-medium supplemented with 10 % fetal calf serum (FCS). Promastigote cultures of *Leishmania donovani* LV9 [5] were kept in RPMI supplemented with 5 % FCS, 15 % macrophage-conditioned medium, antibiotics, 20 mM Na-pyruvate, and hemin (solution B of Hosmem II medium [6]) at 25 ℃ and 5 % CO_2. The effect of test compounds or Pentostam® on the viability extracellular promastigote *L. donovani* was assessed by monitoring the MTT-metabolism [7, 8] after a 72 h culture period in presence of serial dilutions of the respective compound. For testing leishmanicidal activity against intracellular amastigotes, C57BL/6 mice bone marrow-derived macrophages (BMMΦ) were infected *in vitro* with *L. donovani*, cultured in presence of serial dilutions of test compounds or Pentostam® for 72 h, and the intracellular parasite survival determined as described [7]. EC_{50} -values were expressed as the concentration of a com-

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pound necessary to provoke a 50 % reduction in extracellular respective intracellular viability of the parasite. General cytotoxicity for mammalian cells was assessed by incubating human cell lines (squamous carcinoma (KB), melanoma (SK-Mel), lung carcinoma (A 549), mamma carcinoma (MDA) or murine primary macrophage cultures (BMM Φ) with serial dilutions of test compounds and measuring their MTT metabolism 72 h later.

RESULTS and DISCUSSION

In their mammalian hosts, protozoa of the genus *Leishmania* are obligate intracellular parasites of the monocyte-macrophage system. In contrast to other intracellular pathogens such as *Toxoplasma*, *Leishmania* do not inhibit fusion of infected vacuoles with catabolic lysosomes. The micro-flagellated promastigote form is physiologically well adapted to high temperature and the microbicidal environment within macrophages. Intracellular localization of the pathogen may pose specific problems for drugs which first have to pass the host cell membrane or be otherwise internalized by the host cell. Intracellular efficacy of a drug depends on its pathway and rate of uptake, its resistance to intracellular degradation, intracellular trafficking, and host cell toxicity. In consequence, when screening for leishmanicidal drugs, one needs to test both against the extracellular promastigote and against the intracellular amastigote form of the parasite.

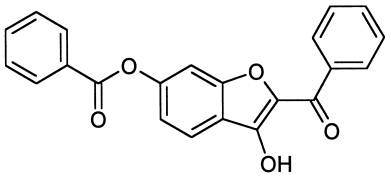
Aurones were tested *in vitro* against promastigotes of *Leishmania donovani*, *L. infantum*, *L. enriettii*, *L. major*, and against intracellular amastigotes of *Leishmania donovani* as displayed in Fig. 1. Compound (1) exhibited the highest relative toxicity for intracellularly persisting *L. donovani* parasites associated with relatively high toxicity for murine macrophages. In comparison to sodium stibogluconate, compounds (2), (3), and (4) also showed pronounced leishmanicidal effects against promastigotes. These derivatives had appreciable activity against intracellular *L. donovani* amastigotes as well and showed only minor toxicity for macrophages. Interestingly, compounds (5), (6), and (7) displayed a moderate but significant activity against *Leishmania* promastigotes as well as intracellular amastigotes, but no toxicity against macrophage host cells ($EC_{50} > 25.0\ \mu g/ml$).

Regarding molecular structure and function, these *in vitro* studies showed that the antileishmanial activity of aurones is determined by the nature of their substituents. Conspicuously, the ability of simple aurones to inhibit parasite growth apparently depends exclusively on the oxygenation pattern of the substituents. Highly active compounds like (1), (2), (3), and (4) show a limited number of oxygen substituents indicating their lipophilic nature. The increasing numbers of hydroxyl groups in compounds (6) and (7) unexpectedly reduced antiprotozoal activity. However, this may be explained by a shift to a more hydrophilic character. First results indicate, that an introduction of bulky substituents also decreases the antileishmanial activity of aurones.

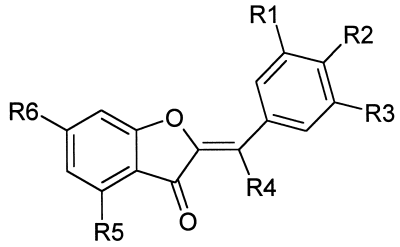
The close biosynthetic relationship between aurones and chalcones, may provide some explanation for their antiparasitic activity. Chen *et al.* [4] demonstrated strong antileishmanial and antimalarial activities for licochalcone A. While the mechanism by which licochalcone A kills these parasites is still a matter of debate, molecular modelling experiments with a panel of chalcones gave first insights [9]. Apparently, possession of electron drawing groups on the A-ring and a three-carbon linker between the two aromatic rings is essential for both antimalarial and trypanosomicidal activity.

However, since inhibition of *Leishmania* growth occurs in the acidic environment of the phagolysosome, the relative acid-stability of the α/β-unsaturated ketone linker of chalcones as well as of aurones offers a further explanation for their high intracellular activity. Aurones share certain, important similarities with chalcones. They have roughly the same size, three-carbon linkers, and similar substituents on both aromatic rings. The main difference lies in the conjugation of the three-carbon linker which in aurones is linked to the B ring, giving a two-member ring system. A planar structure is typical for all aurones and this conformation bears high similarity with the proposed optimal lead structure of chalcones discussed above. These findings suggest that both aurones and chalcones might occupy similar sites in essential parasite enzymes and thus have mechanisms of antiparasitic activity.

In conclusion, our study provides first evi-



(1)



No Compound	R1	R2	R3	R4	R5	R6
(1) 6-Benzoyl-2-[oxomethylpheny]-3-hydroxy-benzofurane	-	-	-	-	-	-
(2) 6-Methoxy-2-[phenylmethylene]-3(*2H*)-benzofuranone	H	H	H	H	H	OCH_3
(3) 6-Hydroxy-2-[phenylmethylene]-3(*2H*)-benzofuranone	H	H	H	H	H	OH
(4) 6-Methoxy-2-[phenylhydroxymethylene]-3(*2H*)-benzofuranone	H	H	H	OH	H	OCH_3
(5) 4,6-Dibenzoyl-2-[phenylhydroxymethyl]-3(*2H*)-benzofuranone	H	H	H	OH	Bz	Bz
(6) 6-Hydroxy-2-[(2,3,4-trihydroxyphenyl)-methylene]-3(*2H*)-benzofuranone-5-*β-D*-glucopyranoside (Bractein)	OH	OH	OH	H	Glc	OH
(7) 4,6-Dihydroxy-2-[(2,3-dihydroxyphenyl)-methylene]-3(*2H*)-benzofuranone (Sulfuretin)	OH	OH	H	H	H	OH

Bz: Glc: (ß-D-glucose)

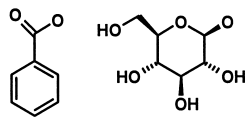


Fig. 1 Chemical structures of the aurones used in this study

dence that aurones exhibit interesting antileishmanial properties with moderate toxicity for mammalian host cells. These results possibly bear implications for other intracellular pathogens or phylogenetically related parasites such as *Trypanosoma*. Also, further structure/function analysis may contribute to the search for new lead candidates for drug development. The potent leishmanicidal activities of certain aurones described here represent an exciting advance in the search for novel antiprotozoal agents at a time when the efficacy of currently available drugs is declining.

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