Leishmaniasis is a family of parasitic diseases that affect about 12 million people in tropical and subtropical areas in the form of three clinical expressions: visceral leishmaniasis, which is fatal in the absence of treatment; cutaneous leishmaniasis; and cutaneous leishmaniasis, which is often self-curing. Classical drugs such as antimonials (Pentostam and Glucantime) are toxic, and drug resistance is increasing dangerously in the field (3). A liposomal amphotericin B formulation (AmBisome) less toxic than amphotericin B deoxycholate is gradually becoming the first-line therapy, especially in immunocompromised patients, but this drug must be administered by a parenteral route (11). Miltefosine (Impavid) was the first drug registered against visceral leishmaniasis in the last decade; however, its toxicity and the appearance of drug resistance justify the search for new chemical series in order to find an orally safe and active drug (8).

Quinolines substituted at the 2-position have shown in vitro antileishmanial activities and cytotoxicities. The 7-aroylstyrylquinoline scaffold appeared to be the most promising one, with the most interesting compound, no. 35, exhibiting a 50% inhibitory concentration (IC50) of 1.2 μM and a selectivity index value of 121.5. Compound 35 was 10-fold and 8-fold more active than miltefosine and sitamaquine, the reference compounds, with selectivity indices 607-fold and 60-fold higher, respectively.

A series of 9 quinolines and 18 styrylquinolines was evaluated for the drugs' in vitro antileishmanial activities and cytotoxicities. The 7-aroylstyrylquinoline scaffold appeared to be the most promising one, with the most interesting compound, no. 35, exhibiting a 50% inhibitory concentration (IC50) of 1.2 μM and a selectivity index value of 121.5. Compound 35 was 10-fold and 8-fold more active than miltefosine and sitamaquine, the reference compounds, with selectivity indices 607-fold and 60-fold higher, respectively.

Leishmania donovani


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In Vitro Activities of New 2-Substituted Quinolines against Leishmania donovani

A series of 9 quinolines and 18 styrylquinolines was evaluated for the drugs' in vitro antileishmanial activities and cytotoxicities. The 7-aroylstyrylquinoline scaffold appeared to be the most promising one, with the most interesting compound, no. 35, exhibiting a 50% inhibitory concentration (IC50) of 1.2 μM and a selectivity index value of 121.5. Compound 35 was 10-fold and 8-fold more active than miltefosine and sitamaquine, the reference compounds, with selectivity indices 607-fold and 60-fold higher, respectively.
units (RLU). Data were transformed into a graphic program (Excel). The 50% inhibitory concentration (IC50) for antileishmanial activity was calculated by nonlinear regression analysis of the concentration-response curve by using the four-parameter Hill equations. The in vitro Leishmania donovani intramacrophage amastigote system used to evaluate the antileishmanial activity of the compounds was the most relevant one, since it takes into account the pharmacokinetics barriers that a compound has to overcome before entering the parasite.

KB cells were used to evaluate the cytotoxicity of the compounds to mammalian cells, which allowed us to determine an in vitro selectivity index. The cell viability was determined with the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (12). Exponentially growing KB cells at a density of 1 × 10⁴ cells per ml in a 100-μl final volume were incubated in a 96-well plate with test drugs for 72 h. The test compounds were added at 3-fold dilutions for up to 7 points in complete medium starting from a 400-μM concentration and were incubated at 37°C in a humidified mixture of CO2 and 95% air in an incubator. Podophyllotoxin was used as a reference drug, and control wells containing dimethyl sulfoxide (DMSO) without drugs were also included in the experiment. Stock solutions of compounds were initially dissolved in DMSO and further diluted with fresh complete medium. After incubation, 25 μl of MTT reagent (5 mg/ml) in PBS medium, followed by syringe filtration, was added to each well and incubated at 37°C for 2 h. At the end of the incubation period, the supernatants were removed by inverting the plate completely without disturbing the cell layer, and 150 μl of pure DMSO was added to each well. After 15 min of shaking, the readings were recorded as absorbance at 544 nm on a microplate reader. The cytotoxic effects were expressed as 50% lethal dose (i.e., as the concentration of a compound which provoked a 50% reduction in cell viability compared to cells in culture medium alone). Fifty percent cytotoxic concentration (CC50) values were estimated as previously described (5, 12). The selectivity index (SI) for each compound was calculated as the ratio between cytotoxicity (CC50) and activity (IC50) against Leishmania amastigotes.

Among the simple quinolines (Table 1) and the styrylquinoline derivatives (Table 2), three compounds exhibited an IC50 for parasites of less than 10 μM (compounds 17, 18, and 20). The most interesting compound was compound 18, with an IC50 for L. donovani intramacrophage amastigotes of 4.1 μM and a selectivity index of 8.3. A clear-cut structure-activity relationship showed that the introduction of a carboxyl group at any position was responsible for both a dramatic decrease in the antileishmanial activity and a decrease in the cytotoxicity decrease. This observation was confirmed when two carboxyl groups were introduced into the same molecule, resulting in no activity and no cytotoxicity (compound 27). These results could be ascribed to an excessive hydrophilicity limiting the drug-parasite membrane interactions or a reaction between the carboxyl group with some compounds of the culture medium preventing the entry of the compound into the parasite.

Among the 7-aroylstyrylquinolines (Table 3), the most interesting compound was compound 35, which exhibited an IC50 of 1.2 μM and a selectivity index of 121.5. Compound 35 was 10-fold and 8-fold more active than miltefosine and sitamaquine, the reference compounds, with selectivity indexes 607-fold and 60-fold higher, respectively. Compound 34 had an IC50 of 2.1 μM and a selectivity index of 27.3. These compounds exhibited the best selectivity indexes in their series, despite the presence of a nitro group. The presence of the nitro group at the meta position greatly in-
have tried to select quinoline-resistant lines as promising series of antileishmanial drugs. Moreover, we have studied the selectivity index (SI) is defined as the ratio of CC_{50} on KB cells to IC_{50} on L. donovani intramacrophage amastigotes.

Recent work has confirmed the interesting antileishmanial properties of other quinoline series (2, 6, 18). In addition, quinolines have recently been found to inhibit leishmanial GDP-mannose-pyrophosphorylase, an enzyme system producing a range of mannose-rich glycoconjugates that are essential for parasite survival and virulence (7). This potential for selective action against a Leishmania-specific target makes quinolines a promising series of antileishmanial drugs. Moreover, we have tried to select quinoline-resistant L. donovani promastigotes in the lab by in vitro drug pressure and have only obtained a slight decrease in sensitivity since the IC_{50,s} were no more than twice those of the wild-type line (data not shown). This encouraging result is an additive justification for further studies of 2-substituted quinolines.

In conclusion, compound 35, due to its high in vitro antileishmanial activity and low toxicity, is the most interesting compound to emerge from more than 150 derivatives of 2-substituted quinolines that have now been synthesized and evaluated. It has now been selected as a candidate for evaluation in vivo with L. donovani mouse or hamster models via the DNDi pipeline.

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