Antimicrobial Agents and Chemotherapy

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

Philippe M. Loiseau, Suman Gupta, Aditya Verma, Saumya Srivastava, S. K. Puri, Faten Sliman, Marie Normand-Bayle and Didier Desmaele

Antimicrob. Agents Chemother. **2011, 55(4):1777. DOI: 10.1128/AAC.01299-10.**
Published Ahead of Print 10 January 2011.

Updated information and services can be found at:
http://aac.asm.org/content/55/4/1777

These include:

REFERENCES

This article cites 22 articles, 2 of which can be accessed free at:
http://aac.asm.org/content/55/4/1777#ref-list-1

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: **http://journals.asm.org/site/misc/reprints.xhtml**
To subscribe to to another ASM Journal go to: **http://journals.asm.org/site/subscriptions/**

Journals.ASM.org

Downloaded from http://aac.asm.org/ on April 13, 2014 by NATIONAL CHUNG HSING LIBRARY

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2011, p. 1777–1780 Vol. 55, No. 4
0066-4804/11/$12.00 doi:10.1128/AAC.01299-10
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

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

Philippe M. Loiseau,[1]* Suman Gupta,[2] Aditya Verma,[2] Saumya Srivastava,[2] S. K. Puri,[2] Faten Sliman,[3] Marie Normand-Bayle,[3] and Didier Desmaele[3]

Groupe Chimiothérapie Antiparasitaire, UMR 8076 CNRS BioCis, Faculté de Pharmacie, Univ Paris-Sud, 92290-Châtenay-Malabry, France[1]; Division of Parasitology, Central Drug Research Institute (CSIR), Lucknow, India[2]; and Laboratoire de Synthèse Organique et Pharmacochimie, UMR 8076 CNRS BioCis, Faculté de Pharmacie, Université Paris-Sud, 92290-Châtenay-Malabry, France[3]

Received 23 September 2010/Returned for modification 8 November 2010/Accepted 2 January 2011

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 (IC_{50}) 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 indexes 607-fold and 60-fold higher, respectively.

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; muco-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 (Impavido) 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 vivo* activities against *Leishmania donovani*, and many compounds have been synthesized over the last decade (14). The Drug for Neglected Diseases Initiative (DNDi) has been considering this series for evaluation in preclinical development for about a year and a half. However, although promising, the series still requires improvements, and here we report the *in vitro* antileishmanial evaluation of new quinoline derivatives, including 2-[2-aryl(ethenyl)]-substituted quinoline (2-styrylquinolines) bearing additional aroyl/acyl groups at the C-7 position. In addition, some compounds within this series were recently shown to display substantial antiviral activity in HIV-infected cells (13, 22).

The synthesis of most of the compounds has been previously reported. Briefly, Kolbe carbonation of 8-hydroxyquinaldine afforded the pivotal hydroxyacid compound 1 (9), which was further elaborated into the 5-iodoquinaldine compound 12 and amide compound 17 (13). Similarly, bromination of the C-5 position and protection of the salicylic moiety provided 5-bromoquinaldine compound 2, which was engaged in a modified Suzuki cross-coupling reaction to give 5-arylated derivatives 14 to 16 (19). Styrylquinoline compounds 19 to 27 were prepared from the corresponding quinaldine compound 3 by Perkin-type condensation in refluxing acetic anhydride, followed by hydrolysis in a pyridine-water mixture (10, 16, 21, 22). Finally, the 7-aroyl-stryryquinoline derivatives 28 to 35 were obtained via the 7-bromostyrylquinoline compound 5 according to a three-step sequence involving lithiation followed by condensation with the required aldehyde, manganese dioxide oxidation, and deprotection (15) (Fig. 1).

The antileishmanial evaluation of these compounds was then performed on *Leishmania donovani* amastigotes by using the luciferase-transfected *Leishmania donovani* (strain MHOM/IN/80/Dd_8) promastigotes maintained in the laboratory of the Division of Parasitology, Central Drug Research Institute, Lucknow, India, since 2005 as described by Sunduru et al. (20). In order to assess the activity of compounds against the amastigote stage of the parasite, the mouse macrophage cell line J-774A.1, infected with promastigotes expressing the luciferase firefly reporter gene, was used. Cells were seeded in a 96-well plate at a density of 4×10^4 cells per ml in a final volume of 100 μl in RPMI 1640 containing 10% fetal calf serum, and the plates were incubated at 37°C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary-phase promastigotes (4×10^5/100 μl/well). Promastigotes were engulfed by the macrophage and transformed there into amastigotes. The test compounds were added at 2-fold dilutions in up to 7 points in fresh complete medium starting from a 100 μM concentration, and the plates were incubated at 37°C in a CO_2 incubator for 72 h. After incubation, the drug-containing medium was decanted and 50 μl phosphate-buffered saline (PBS) was added in each well and mixed with an equal volume of Steady-Glo luciferase assay substrate dissolved in Steady-Glo luciferase assay buffer. After gentle shaking for 1 to 2 min, the readings were recorded in a luminometer (1, 4, 17). The values were expressed as relative luminescence

* Corresponding author. Mailing address: Groupe Chimiothérapie Antiparasitaire, UMR 8076 CNRS BioCis, Faculté de Pharmacie, Université Paris-Sud, 92290-Châtenay-Malabry, France. Phone: 33 (0) 1 46 83 55 53. Fax: 33 (0) 1 46 83 55 57. E-mail: philippe.loiseau@u-psud.fr.
▽ Published ahead of print on 10 January 2011.

Downloaded from http://aac.asm.org/ on April 13, 2014 by NATIONAL CHUNG HSING LIBRARY

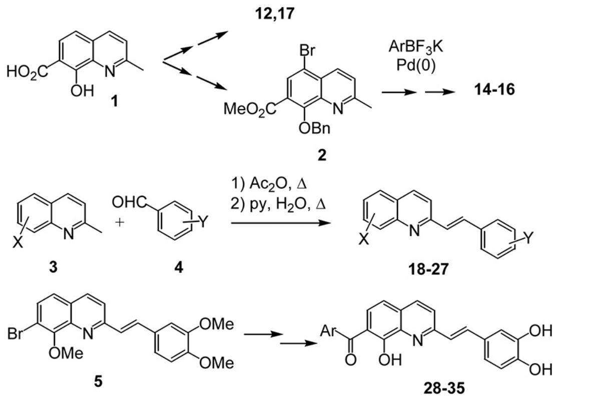


FIG. 1. General synthetic scheme for the quinoline and styrylquinoline derivatives evaluated in this study.

units (RLU). Data were transformed into a graphic program (Excel). The 50% inhibitory concentration (IC_{50}) 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.

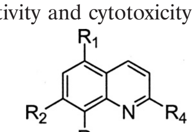
KB cells were used to evaluate the cytotoxicity of the compounds to mammalian cells, which allowed us to determine an *in vitro* selectivity index. The cell viability was determined with the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (12). Exponentially growing KB cells at a density of 1×10^5 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 CO_2 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 (CC_{50}) values were estimated as previously described (5, 12). The selectivity index (SI) for each compound was calculated as the ratio between cytotoxicity (CC_{50}) and activity (IC_{50}) against *Leishmania* amastigotes.

Among the simple quinolines (Table 1) and the styrylquinoline derivatives (Table 2), three compounds exhibited an IC_{50} for parasites of less than 10 μM (compounds 17, 18, and 20). The most interesting compound was compound 18, with an IC_{50} 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 IC_{50} 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 IC_{50} 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-

TABLE 1. *In vitro* antileishmanial activity and cytotoxicity results for compounds 6 and 10 to 17

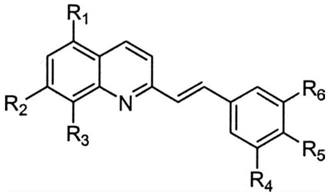


Compound	R_1	R_2	R_3	R_4	IC_{50} (μM)	CC_{50} (μM)	SI (CC_{50}/IC_{50} ratio)[a]
6	H	Br	OH	CH_3	16.1	75.4	4.6
10	H	CO_2H	OH	H	>100	335	<3.3
11	H	H	CO_2H	CH_3	>100	303.5	<3.0
12	I	CO_2H	OH	CH_3	>100	331.5	<3.3
13	H	CHO	OH	CH_3	>100	181.9	<1.8
14	Ph	CO_2H	OH	CH_3	>100	329.9	<3.2
15	4-MePh	CO_2H	OH	CH_3	>100	169.7	<1.6
16	3-Quinolinyl	CO_2H	OH	CH_3	>100	>400	
17	H	*p*-$FPhCH_2NHCO$	OH	CH_3	5.9	32.9	5.5

[a] The selectivity index (SI) is defined as the ratio of CC_{50} on KB cells to IC_{50} on *L. donovani* intramacrophage amastigotes.

Downloaded from http://aac.asm.org/ on April 13, 2014 by NATIONAL CHUNG HSING LIBRARY

TABLE 2. *In vitro* antileishmanial activity and cytotoxicity results for styrylquinolines 18 to 27



Compound	R_1	R_2	R_3	R_4	R_5	R_6	IC_{50} (μM)	CC_{50} (μM)	SI (CC_{50}/IC_{50} ratio)[a]
18	H	CN	OH	H	OH	OH	4.1	34.5	8.3
19	H	H	NO_2	H	OAc	OAc	21.3	39.7	1.8
20	H	H	OH	H	H	H	6.9	4.0	0.6
21	H	H	OAc	H	OAc	OAc	13.5	9.8	0.7
22	H	CO_2H	OH	H	NH_2	H	>100	35.6	<0.3
23	H	CO_2H	OH	H	SO_2CH_3	H	>100	263.3	<2.6
24	H	CO_2H	OH	H	F	F	>100	193.8	<1.9
25	H	CO_2H	OH	H	Cl	OH	79.6	299.8	3.7
26	H	CO_2H	OH	OH	H	OH	>100	>400	
27	CO_2H	CO_2H	OH	H	OH	OH	>100	>400	

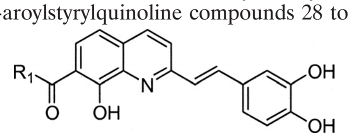
[a] The selectivity index (SI) is defined as the ratio of CC_{50} on KB cells to IC_{50} on *L. donovani* intramacrophage amastigotes.

creased the selectivity index, as evidenced by the much lower selectivity index of the parent compound, no. 28.

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.

TABLE 3. *In vitro* antileishmanial activity and cytotoxicity results for 7-aroylstyrylquinoline compounds 28 to 35



Compound	R_1	IC_{50} (μM)	CC_{50} (μM)	SI (CC_{50}/IC_{50} ratio)[a]
28	Ph	2.5	36.5	14.6
29	1-Naphthyl	20.0	1.1	0.06
30	$PhCH_2CH_2$	3.9	10.3	2.6
31	3,4-DiFPh	2.5	16.5	6.5
32	2-Pyridyl	58.7	260.5	4.4
33	3-Pyridyl	17.5	9.0	0.5
34	$2\text{-}NO_2Ph$	2.1	57.4	27.3
35	$3\text{-}NO_2Ph$	1.2	145.8	121.5
Sitamaquine		9.7	19.5	2.0
Miltefosine		13.4	3.2	0.2

[a] The selectivity index (SI) is defined as the ratio of CC_{50} on KB cells to IC_{50} on *L. donovani* intramacrophage amastigotes.

This work was supported by the Drug for Neglected Disease Initiative (DNDi).

The transgenic *L. donovani* promastigotes were originally procured from Neena Goyal, Division of Biochemistry, Central Drug Research Institute, Lucknow, India.

REFERENCES

1. **Bhandari, K., et al.** 2010. Synthesis of substituted aryloxy alkyl and aryloxy aryl alkyl imidazoles as antileishmanial agents. Bioorg. Med. Chem. Lett. **20:**291–293.
2. **Ferreira, M. E., et al.** 2010. Antileishmanial activity of furoquinolines and coumarins from Helietta apiculata. Phytomedicine **17:**375–378.
3. **Frézard, F., C. Demicheli, and R. R. Ribeiro.** 2009. Pentavalent antimonials: new perspective for old drugs. Molecules **14:**2317–2336.
4. **Gupta, L., A. Talwar, Nishi, S. Palne, S. Gupta, and P. M. Chauhan.** 2007. Synthesis of marine alkaloid: 8,9-dihydrocoscinamide B and its analogues as novel class of antileishmanial agents. Bioorg. Med. Chem. Lett. **17:**4075–4079.
5. **Huber, W., and J. C. Koella.** 1993. A comparison of three methods of estimating EC50 in studies of drug resistance of malaria parasites. Acta Trop. **55:**257–261.
6. **Isaac-Márquez, A. P., J. D. McChesney, N. P. Nanayakara, A. R. Satoskar, and C. M. Lezama-Dávila.** 2010. Leishmanicidal activity of racemic +/− 8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline. Nat. Prod. Commun. **3:**387–390.
7. **Lackovic, K., et al.** 2010. Inhibitors of Leishmania GDP-mannose pyrophosphorylase identified by high-throughput screening of small-molecule chemical library. Antimicrob. Agents Chemother. **54:**1712–1719.
8. **Maltezou, H. C.** 2010. Drug resistance in visceral leishmaniasis. J. Biomed. Biotechnol. **2010:**617521.
9. **Meek, W. H., and C. H. Fuschman.** 1969. Carboxylation of substituted phenols in *N*,*N*-dimethylamide solvents at atmospheric presssure. J. Chem. Eng. Data **14:**388–391.
10. **Mekouar, K., et al.** 1998. Styrylquinoline derivatives: a new class of potent HIV-1-integrase inhibitors that block HIV-1 replication in CEM cells. J. Med. Chem. **41:**2846–2857.
11. **Moore, E. M., and D. N. Lockwood.** 2010. Treatment of visceral leishmaniasis. J. Global Infect. Dis. **2:**151–158.
12. **Mosmann, T. J.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65:**55–63.

Downloaded from http://aac.asm.org/ on April 13, 2014 by NATIONAL CHUNG HSING LIBRARY

13. **Mouscadet, J. F., and D. Desmaële.** 2010. Chemistry and structure-activity relationship of the styrylquinoline-type HIV integrase inhibitors. Molecules **15:**3048–3078.
14. **Nakayama, H., et al.** 2005. Efficacy of orally administered 2-substituted quinolines in experimental murine cutaneous and visceral leishmaniases. Antimicrob. Agents Chemother. **49:**4950–4956.
15. **Normand-Bayle, M., et al.** 2005. New HIV-1 replication inhibitors of the styryquinoline class bearing aroyl/acyl groups at the C-7 position: synthesis and biological activity. Bioorg. Med. Chem. Lett. **15:**4019–4022.
16. **Polanski, J., et al.** 2002. Use of the Kohonen neural network for rapid screening of *ex vivo* anti-HIV activity of styrylquinolines. J. Med. Chem. **45:**4647–4654.
17. **Porwal, S., et al.** 2009. Discovery of novel antileishmanial agents in an attempt to synthesize pentamidine-aplysinopsin hybrid molecule. J. Med. Chem. **52:**5793–5802.
18. **Rizvi, S. U., et al.** 2010. Antimicrobial and antileishmanial studies of novel (2E)-3-(2-chloro-6-methyl/methoxyquinolin-3-yl)-1-(aryl)prop-2-en-1-ones. Chem. Pharm. Bull. **58:**301–306.
19. **Sliman, F., and D. Desmaële.** 2010. Synthesis of 5-aryl- and 5-heteroaryl-7-carboxyl-8-hydroxyquinaldines through Suzuki cross-coupling reaction with potassium organotrifluoroborates. Synthesis **2010:**619–630.
20. **Sunduru, N., Nishi, S. Palne, P. M. Chauhan, and S. Gupta.** 2009. Synthesis and antileishmanial activity of novel 2,4,6-trisubstituted pyrimidines and 1,3,5-triazines. Eur. J. Med. Chem. **44:**2473–2481.
21. **Zouhiri, F., et al.** 2005. HIV-1 replication inhibitors of the styrylquinoline class: introduction of an additional carboxyl group at the C-5 position of the quinoline. Tetrahedron Lett. **46:**2201–2205.
22. **Zouhiri, F., et al.** 2000. Structure-activity relationships and binding mode of styrylquinolines as potent inhibitors of HIV-1-integrase and replication of HIV-1 in cell culture. J. Med. Chem. **43:**1533–1540.

Downloaded from http://aac.asm.org/ on April 13, 2014 by NATIONAL CHUNG HSING LIBRARY