European Journal of Medicinal Chemistry 54 (2012) 75–86

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Discovery of a new antileishmanial hit in 8-nitroquinoline series

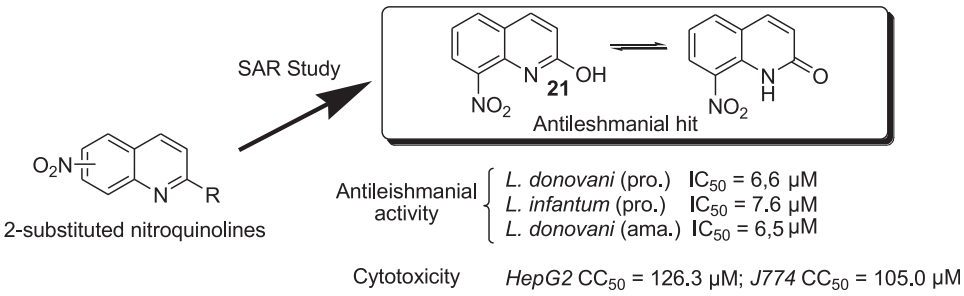
Lucie Paloque [a], Pierre Verhaeghe [b], Magali Casanova [a], Caroline Castera-Ducros [b], Aurélien Dumètre [a], Litaty Mbatchi [b], Sébastien Hutter [a], Manel Kraiem-M'Rabet [b], Michèle Laget [a], Vincent Remusat [b], Sylvain Rault [c], Pascal Rathelot [b], Nadine Azas [a,**], Patrice Vanelle [b,*]

[a] *Infections Parasitaires, Transmission, Physiopathologie et Thérapeutique, UMR MD3, Faculté de Pharmacie, Aix-Marseille Université, 27 Boulevard Jean Moulin, CS 30064, 13385 Marseille cedex 05, France*

[b] *Laboratoire de Pharmaco-Chimie Radicalaire, Faculté de Pharmacie, Aix-Marseille Université – UMR CNRS 7273, Institut de Chimie Radicalaire, 27 Boulevard Jean Moulin, CS 30064, 13385 Marseille cedex 05, France*

[c] *Centre d'Etudes et de Recherche sur le Médicament de Normandie (CERMN), UPRES EA 4258, FR CNRS 3038 INC3M, UFR des Sciences Pharmaceutiques, Université de Caen Basse Normandie, Boulevard Becquerel, 14032 Caen cedex, France*

GRAPHICAL ABSTRACT



HIGHLIGHTS

- ► A series of 2-substituted nitroquinolines was prepared and studied concerning their *in vitro* antileishmanial properties.
- ► The 2-hydroxy-8-nitroquinoline (molecule **21**) appeared as a hit molecule on both pro- and amastigotes stages of *L. donovani*.
- ► Hit molecule 21, non-toxic on 2 different cell lines also appeared as a selective anti-infectious agent among protozoa.

ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form
18 April 2012
Accepted 20 April 2012
Available online 2 May 2012

Keywords:
8-Nitroquinoline
Visceral leishmaniasis
Leishmania donovani
Leishmania infantum

ABSTRACT

A series of nitrated 2-substituted-quinolines was synthesized and evaluated *in vitro* toward *Leishmania donovani* promastigotes. In parallel, the *in vitro* cytotoxicity of these molecules was assessed on the murine J774 and human HepG2 cell lines. Thus, a very promising antileishmanial hit molecule was identified (compound **21**), displaying an IC_{50} value of 6.6 µM and CC_{50} values $\geq$ 100 µM, conferring quite good selectivity index to this molecule, in comparison with 3 drug-compounds of reference (amphotericin B, miltefosine and pentamidine). Compound **21** also appears as an efficient *in vitro* antileishmanial molecule against both *Leishmania infantum* promastigotes and the intracellular *L. donovani* amastigotes (respective IC_{50} = 7.6 and 6.5 µM). Moreover, hit quinoline **21** does not show neither significant antiplasmodial nor antitoxoplasmic *in vitro* activity and though, presents a selective antileishmanial activity. Finally, a structure–activity relationships study enabled to

* Corresponding author. Tel.: +33 491 835 578.
** Corresponding author. Tel.: +33 491 835 564.
E-mail addresses: nadine.azas@univ-amu.fr (N. Azas), patrice.vanelle@univ-amu.fr (P. Vanelle).

0223-5234/$ – see front matter
doi:10.1016/j.ejmech.2012.04.029

Promastigotes
Amastigotes
hepG2 cytotoxicity
Selectivity index

define precisely the antileishmanial pharmacophore based on this nitroquinoline scaffold: 2-hydroxy-8-nitroquinoline.

1. Introduction

Leishmaniasis is one of the most important parasitic diseases worldwide as regards of its critical impact on human health and also because of the rapid emergence and spreading of resistant parasites. It is encountered in 97 countries, mainly in tropical and sub-tropical regions, but also in southern Europe, especially around the Mediterranean area. These parasitic infections are caused by a protozoan of the *Leishmania* genus transmitted to its mammal hosts (humans, dogs, monkeys, rodents…) by the bite of an infected sandfly (*Phlebotominae*). *Leishmania* parasites present two major morphological stages: extracellular flagellated promastigotes in the digestive tract of their sandfly vector and non-motile amastigotes inside the cells of their hosts' mononuclear phagocytic system. Leishmaniasis pathologies depend on the *Leishmania* species and range from restraint cutaneous leishmaniasis to very severe visceral leishmaniasis. There are an estimated 14 million people infected by leishmaniasis worldwide with an annual incidence of 2 million and about 50.000 estimated deaths due to its visceral form caused mainly by *Leishmania donovani*, considering that the number of cases is certainly under-evaluated as leishmaniases are reportable disease in only 40 countries [1].

The current first line drugs against leishmaniases are pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), amphotericin B, pentamidine, paromomycin and miltefosine. Currently, these drugs used in the treatment of leishmaniasis present several problems including non-oral routes of administration (except for miltefosine), expensive cost of liposomal amphotericin B, high toxicity of antimonials, pentamidine, paromomycin and amphotericin B and adverse events of most clinically used molecules, leading to patients withdrawing from treatment and emergence of resistant strains [2,3]. During the last decade, antimonial resistance reached epidemic dimension in Bihar, India, where about 60% of newly diagnosed visceral leishmaniasis do not respond to these molecules nowadays [4]. Evaluation of the *in vitro* susceptibility of Indian *L. donovani* patient isolates to antimonials, amphotericin B and miltefosine indicates that cross-resistance may be emerging among these three drugs. Thus, efficacious and cheap oral antileishmanial agents to use in combination therapy are needed to safeguard against the expanding resistance problem and to overcome miltefosine's shortcomings [5].

During the last decade, only a few clinical studies about new antileishmanial drugs were carried out, even if several novel potential drug targets were identified in biochemical and molecular biology studies. Among these new targets, trypanothione reductase, cysteine peptidases, ornithine decarboxylase and cyclin-depend kinases appeared as particularly relevant [6]. Concerning the new chemical entities with antileishmanial potential, recent reviews showed that small synthetic heterocycles were good early candidates with a view to design new efficient drugs [5,7]. Among these heterocyclic molecules, studies focussing on quinoline derivatives have led to the identification of various interesting molecular scaffolds, bearing a large diversity of substituents at several positions of the quinoline ring. Bis-quinolines [8], some amodiaquine analogues [9], 2-substituted quinolines [10–12], 3-substituted quinolines [13], 4-substituted quinolines [14,15] and 8-substituted quinolines [16,17] are to mention. Sitamaquine, a 4,6,8-trisubstituted quinoline derivative (Fig. 1) targeting the parasitic succinate dehydrogenase even reached phase IIb of clinical trials as an oral antileishmanial drug for the treatment of visceral leishmaniasis [18].

Our research team, has been working on the synthesis of new heterocycles with pharmaceutical potential for years [19–25], and has developed a specific competency in the preparation of original antiprotozoal molecules targeting *Trichomonas* [26], *Plasmodium* [27], or *Leishmania* [28]. In direct continuation of preliminary studies [29–31] which were conducted in our lab on the synthesis and structural analysis of quinoline derivatives bearing antiparasitic potential, we prepared a series of nitrated quinoline derivatives and started to evaluate both their *in vitro* activity toward *L. donovani* promastigotes and their cytotoxicity on two different cell lines: J774 murine macrophage and HepG2 human hepatic cell lines, aiming at identifying original and selective antileishmanial quinolines.

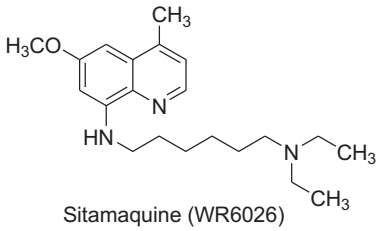
2. Results and discussion

2.1. Organic synthesis

In addition to the bibliographical data which we previously presented about antileishmanial 2-substituted-quinolines, a few nitroaromatic compounds have been reported in the literature as interesting antileishmanial compounds [5]. In the present study, 38 quinolines were evaluated among which 32 were prepared in our lab (6 were purchased). Apart molecules **2**, **35** and **36**, all synthesized molecules were quinolines bearing both a substituent at position 2 and a nitro group, mainly at position 8. Thus, a structural homogeneous quinoline series was obtained, aiming at analysing the antileishmanial structure–activity relationships in optimal conditions.

Molecules **2**, **3** and **4** were obtained in good yields, successively by trichlorination [32] and classical nitration reactions. Molecules **5**, **6** and **7** were easily prepared by a nitration reaction followed by a radical chlorination reaction using *N*-chlorosuccinimide (NCS) in carbon tetrachloride, under nitrogen atmosphere and light irradiation. Original vinylic derivatives **8**–**11** were synthesized from **6** by a $S_{RN}1$-E_2 reaction sequence [19,20,33] in variable yields, depending on the nitroalkane used. Compounds **12** and **13** were obtained by a bromination reaction in pure acetic acid, respectively by saturating the reaction medium with non-anhydrous or anhydrous sodium acetate, while molecule **14** was prepared from **13** by an acidic hydrolysis (Scheme 1).

Molecules **15**–**19**, belonging to the 6-nitroquinoline series, were synthesized from commercial quinoline **15** *via* a radical



Sitamaquine (WR6026)

Fig. 1. Sitamaquine, an antileishmanial quinoline undergoing phase IIb clinical trial.