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Research paper

Synthesis, antileishmanial activity and cytotoxicity of 2,3-diaryl- and 2,3,8-trisubstituted imidazo[1,2-*a*]pyrazines

Pascal Marchand [a, *], Marc-Antoine Bazin [a], Fabrice Pagniez [b], Guillaume Rivière [a], Lizeth Bodero [a], Sophie Marhadour [a], Marie-Renée Nourrisson [a], Carine Picot [a, b], Sandrine Ruchaud [c], Stéphane Bach [c], Blandine Baratte [c], Michel Sauvain [d, e], Denis Castillo Pareja [f], Abraham J. Vaisberg [f], Patrice Le Pape [b]

[a] Université de Nantes, Nantes Atlantique Universités, Laboratoire de Chimie Thérapeutique, Cibles et Médicaments des Infections et du Cancer, IICiMed UPRES EA 1155, UFR de Sciences Pharmaceutiques et Biologiques, 1 rue Gaston Veil, 44035 Nantes, France
[b] Université de Nantes, Nantes Atlantique Universités, Laboratoire de Parasitologie et Mycologie Médicale, Cibles et Médicaments des Infections et du Cancer, IICiMed UPRES EA 1155, UFR de Sciences Pharmaceutiques et Biologiques, 1 rue Gaston Veil, 44035 Nantes, France
[c] Sorbonne Universités, UPMC Univ Paris 06, CNRS USR3151, "Protein Phosphorylation & Human Diseases" Group, Station Biologique, Place Georges Teissier, 29688 Roscoff, France
[d] Institut de Recherche pour le Développement (IRD), UMR152, Mission IRD Casilla, 18-1209 Lima, Peru
[e] Université de Toulouse, UPS, UMR152 (Laboratoire de Pharmacochimie et Pharmacologie pour le Développement, Pharma-DEV), F-31062 Toulouse Cedex 9, France
[f] Departamento de Microbiología y Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

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ABSTRACT

A series of original 2-phenyl-3-(pyridin-4-yl)imidazo[1,2-*a*]pyrazines and the 3-iodo precursors, bearing a polar moiety at the C-8 position, was synthesized and evaluated for their antileishmanial activities. Two derivatives exhibited very good activity against the promastigote and the amastigote forms of *Leishmania major* in the micromolar to submicromolar ranges, coupled with a low cytotoxicity against macrophages and 3T3 mouse fibroblast cells. Through *Lm*CK1 inhibition assay, investigations of the putative molecular target of these promising antileishmanial compounds will be discussed.

1. Introduction

According to a recent report from the World Health Organization (WHO) [1], leishmaniases - visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL) - collectively affect 12 million people in 98 countries, and 350 million more are at risk of infection. Moreover, there are 1.3 million new cases and 40,000 deaths attributed to leishmaniases each year and leishmaniasis is included in the neglected tropical diseases (NTDs) [2]. Clinical forms differ in immunopathologies and degree of morbidity and mortality. VL caused by *Leishmania donovani* and *Leishmania infantum* is the most severe form of leishmaniasis and is usually fatal in the absence of treatment whereas CL caused by *Leishmania major*, *Leishmania amazonensis, Leishmania mexicana*, *Leishmania braziliensis*, and *Leishmania panamensis* is significantly associated with morbidity. Cutaneous leishmaniasis is more widely distributed, with about one-third of cases occurring in each of three epidemiological regions, the Americas, the Mediterranean basin, and western Asia from the Middle East to Central Asia. The ten countries with the highest estimated case counts are: Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, Peru, Sudan and Syria, and together account for 70–75% of global estimated CL incidence. More than 90% of global VL cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan [1–9].

These parasitic infections are caused by a protozoan of the *Leishmania* genus transmitted to its mammal hosts (humans, dogs,

* Corresponding author.
E-mail address: pascal.marchand@univ-nantes.fr (P. Marchand).

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monkeys, rodents,…) by the bite of an infected sandfly (*Phlebotominae*). *Leishmania* parasites present two 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 [1]. Currently, there are no effective vaccines and a number of drugs are used in the treatment of leishmaniasis. These drugs include pentavalent antimonials, amphotericin B, miltefosine, pentamidine and paromomycin. Unfortunately, many of these drugs cause side effects and high toxicities, and display a high rate of treatment failure in HIV co-infected patients. In addition, an inevitable resistance has developed in recent times in *Leishmania* parasites towards some of these drugs. Otherwise, they are costly and require long-term treatment [2–9]. Consequently, there is an urgent need to speed up the development of a new generation of more effective and safe antileishmanials.

Various classes of natural products have shown promising antileishmanial activity [10] and compounds of synthetic origin comprising a diverse group of chemical structures have been reported as antileishmanial agents [3,5,11]. These include mostly the nitrogen heterocycles for instance as quinolines [12], quinolinones [13], quinazolines [14], quinoxalines [15], acridines [16], pyrimidines [17], thienopyrimidines [18] or azoles [9].

During the last few years, our strategy to fight against leishmaniasis was the design of small heterocyclic compounds in azolylindole series [19,20] and, more recently, 2,3-diarylimidazo[1,2-*a*]pyridine series [21].

Interestingly, 2,3-diarylimidazo[1,2-*a*]pyridine derivatives exhibited good activity against the promastigote form and/or the amastigote form of *L. major*, coupled with a low cytotoxic activity against the HeLa human cell line. We studied *Lm*CK1 as a potential molecular target responsible of antiparasitic properties but, in a general approach, antileishmanial activities of the imidazo[1,2-*a*]pyridines were not clearly correlated with *Lm*CK1 inhibition. Nevertheless, the 4-pyridyl derivative **I** (Fig. 1) displayed *Lm*CK1 kinase inhibition in the submicromolar range (IC_{50} value of 0.30 μM) and IC_{50} value of 6.5 μM on *L. major* promastigote stage, constituting a good starting point for the development of new antileishmanial agents [21].

Indeed, among the different targets such as sterol biosynthetic pathway, trypanothione or methylglyoxal metabolism, parasite glycolysis, purine salvage pathway, folate biosynthesis or topoisomerases [4–6,10,22], the involvement of several parasitic protein kinases was found essential for parasite proliferation and viability [23,24]. These enzymes were validated as potential molecular targets for drug development, namely cyclin-dependent kinases (CDKs) [25–27], mitogen-activated protein kinases (MAPKs) [28–30], Aurora kinase [31], Protein kinase C (PKC) [32] or, in our field of interest, casein kinase 1 (CK1) [33–35].

In the course of our ongoing synthetic and screening programs for new biologically active imidazo[1,2-*a*]azines [21,36,37], we decided to develop bioisostere analogues of the previously described 2,3-diarylimidazo[1,2-*a*]pyridines [21]. Imidazo[1,2-*a*]pyrazines have been gaining attention in drug discovery but no biological application related to *Leishmania* diseases was found in the literature [38]. Consequently, we planned to explore such scaffold for the design of new antileishmanial agents associated with *Lm*CK1 inhibition (Fig. 1, Series A). In addition, to try to enhance *Lm*CK1 inhibition, our second strategy was to introduce a variety of polar groups, namely amines [33], at the imidazo[1,2-*a*]pyrazine C8 position, by holding constant the 4-pyridyl at the C3 position along with a phenyl or a 4-fluorophenyl substituent at the C2 position (Fig. 1, Series B).

2. Results and discussion

2.1. Chemistry

For the design of imidazo[1,2-*a*]pyrazines substituted at 2,3-ring positions, we were interested in preparing 2-phenylimidazo[1,2-*a*]pyrazine **2** as intermediate (Scheme 1).

To this end, a generally established method was the condensation of α-bromoacetophenone with 2-aminopyrazine **1** [39]. The low yield observed for the cyclization step (30%) prompted us to prepare imidazo[1,2-*a*]pyrazin-2-yl triflate **3**, as recently described by our team in imidazo[1,2-*a*]pyridine series [21,36], followed by Suzuki coupling to give the desired product **2** (Scheme 1). Thus, 2-aminopyrazine **1** reacted with ethyl bromoacetate and a subsequent treatment with *N*-phenylbis(trifluoromethanesulfonimide) led to **3** in only 33% yield. This could be due to the competitive reactivity of the N-4 nitrogen of starting 2-aminopyrazine, involved in the nucleophilic substitution mechanism, thereby preventing following cyclisation. Suzuki coupling was then performed in the presence of phenylboronic acid to produce compound **2** in satisfactory yield (67%). Considering the overall yield of the two steps of this sequence (22% *vs* 30%), this second approach was less effective. Nevertheless, the original introduction of a leaving group (OTf) at the position 2 of the imidazo[1,2-*a*]pyrazine core will allow metal-catalyzed chemistry for a broad pharmacomodulation since the corresponding halogenated derivatives are almost not described in the literature. In the aim of the obtention of 2-arylimidazo[1,2-*a*]pyrazines, a wide variety of boronic acids are commercially available for Suzuki coupling in the contrary of α-bromoacetophenones for heterocyclization reaction.

The direct arylation reaction at the 3-position was then examined using 2-phenylimidazo[1,2-*a*]pyrazine **2** and aryl halides, applying Fagnou's procedure [40,41]. In the presence of $Pd(OAc)_2$ (2 mol %), $PCy_3 \cdot HBF_4$ (4 mol %), PivOH (0.3 equiv), K_2CO_3 (1.5 equiv), the reaction proceeded smoothly (18–40 h) in DMA at 100 °C to afford 3-aryl-2-phenylimidazo[1,2-*a*]pyrazines **4a–g** in low yields due to degradation of the reaction mixture (Scheme 1, Table 1).

In the second series of compounds, the purpose of the synthetic scheme was to introduce a chlorine atom at the position 8 of the imidazo[1,2-*a*]pyrazine scaffold for further pharmacomodulation. Thus, 2,3-dichloropyrazine **5** was the starting material subjected to amination and cyclization (Scheme 2, Table 2) [42,43].

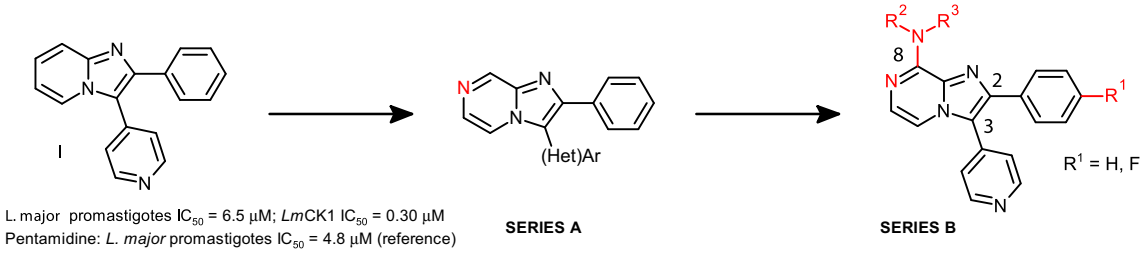

Fig. 1. Structures of target compounds.