European Journal of Medicinal Chemistry 90 (2015) 107–123

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and biological evaluation of novel 2,3-disubstituted quinoxaline derivatives as antileishmanial and antitrypanosomal agents

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ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form
29 August 2014
Accepted 8 November 2014
Available online 11 November 2014

Keywords:
Quinoxaline derivatives
Leishmania amazonensis
Trypanosoma cruzi
Anti-trypanosomatid agents

ABSTRACT

Quinoxalines belong to the *N*-containing heterocyclic compounds that stand out as having promising biological activity due to their privileged scaffold. In this work, we report the synthesis, antileishmanial, and antitrypanosomal properties of 46 new 2,3-disubstituted quinoxaline and 40 previously reported derivatives. Among all of the compounds screened for *in vitro* activity against epimastigotes and trypomastigotes of *Trypanosoma cruzi* and promastigotes of *Leishmania amazonensis* as well as mammalian toxicity on $LLCMK_2$ cells and J774 macrophages, analogues from series **5**, **6**, **7**, **9**, **12**, and **13** displayed high activity at micromolar IC_{50} and EC_{50} concentrations. Sixteen quinoxaline derivatives were selected and evaluated on *T. cruzi* and/or *L. amazonensis* amastigotes. The most active compounds were **6a-b** and **7d-e**, on all evolutive forms of *L. amazonensis* and *T. cruzi* evaluated with IC_{50} values 0.1–0.8 μM on promastigotes and epimastigotes 1.4–8.6 on amastigotes. Compounds **5k**, **12b** and **13a** were the most selective (SI = 19.5–38.4) on amastigotes of *T. cruzi*. In general their activity was directly related to the methylsulfoxyl, methylsulfonyl, and amine groups as well as the presence of chorine or bromine in the molecules. The current results indicate that these quinoxaline derivatives are novel and promising agents for further development towards a treatment for Chagas' disease and leishmaniasis.

1. Introduction

Neglected tropical diseases are significant public health problems and have been attracting increasing worldwide attention [1]. Chagas' disease is caused by the protozoan *Trypanosoma cruzi*, which is found in 21 countries and affects approximately 8 million people, with approximately 50,000 new cases per year [2]. Latin America has most of the cases of Chagas' disease, which has become a global health problem as a result of migration to non-endemic regions, such as Australia, Europe, the United States, and Canada, resulting in annual treatment costs of approximately USD$ 600 million [3,4]. Its pathogenesis is subdivided into an acute phase characterized by nonspecific inflammation, an asymptomatic indeterminate phase, and a chronic phase, during which approximately 30–40% of the cases develop irreversible cardiovascular, gastrointestinal, and neurological lesions [5,6]. The transmission of Chagas' disease generally occurs through the bite and infection with contaminated feces of insects of the subfamily Triatominae (Hemiptera, Reduviidae). It may also be transmitted through blood transfusion or congenitally and orally, such as through contaminated food [7,8].

Leishmaniasis is endemic in 98 countries worldwide, with approximately 350 million people at risk of infection and 12 million currently infected. The disease may be caused by more than 20 different species of *Leishamania* sp, which are responsible for clinical manifestations that can be classified as cutaneous,

Abbreviations: T. cruzi, *Trypanosoma cruzi*; *L. amazonensis*, *Leishmania amazonensis*; IC, inhibitory concentration; EC, effetive concentration; SI, selective index; CC, cytotoxic concentration; $LLCMK_2$, epithelial cells from the kidney from *Macaca mulatta*.

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http://dx.doi.org/10.1016/j.ejmech.2014.11.018
0223-5234/

mucocutaneous, and visceral. Cutaneous leishmaniasis is the most common clinical form of the disease, with 1.5 million new cases per year. The species that stand out in the New World are *Leishmania amazonensis*, *Leishmania braziliensis*, and *Leishmania guyanensis* [2,9,10]. Among the cutaneous leishmaniasis cases worldwide, 70–75% occur in just 10 countries, including Brazil, which experienced approximately 30,000 new cases in 2010 [11,12]. Transmission occurs through the bite of the female phlebotomine sandfly. The vector in the New World is *Lutzomyia*, and the vector in the Old World is *Phlebotomus* [13]. Clinical manifestations are characterized by single or multiple lesions that are usually located on the legs, arms, and head. They initially appear as papules and progress into nodules and finally ulcerative lesions [14,15].

Nitroderivative compounds, such as benznidazole and nifurtimox, are currently the primary treatment options for Chagas' disease, despite their reduced efficacy in the chronic phase and adverse reactions in approximately 40% of patients [16–18]. For antileishmania chemotherapy, pentavalent antimonials, such as meglumine antimoniate and sodium stibogluconate, are the first-line treatment, whereas amphotericin B, pentamidine, paromomycin, and miltefosine are the second-line treatment [19–21]. However, the available drugs for both diseases have severe side effects, long-term treatment, and variable efficacy, factors that encourage the search for new therapeutic alternatives [22].

Quinoxalines belong to the *N*-containing heterocyclic compounds that stand out as having promising biological activity because of their privileged scaffold [23,24]. They have numerous reported biological activities, including anticancer [25–27], beneficial effects for sleep disorders [28], antimycobacterial [29,30], antibacterial [31], antifungal [32], antiviral [33], anti-inflammatory, and antioxidant activities [34,35]. The antiprotozoal activity of quinoxalines is relevant, especially their antitrypanosomatid activity, which has been reported for quinoxaline 1,4-di-*N*-oxide [36,37], 3-trifluoromethylquinoxaline *N*,*N*′-dioxide [38], and 3-aminoquinoxaline-2-carbonitrile 1,4-dioxide derivatives [39]. Quinoxalines also exhibit antileishmanial activity, which has been reported for 4-substituted pyrrolo[1,2-a]quinoxalines [40], 3-phenyl-1-(1,4-di-*N*-oxide quinoxalin-2-yl)-2-propen-1-one [41,42], and 1,4-di-*N*-oxide quinoxaline derivatives [43].

Recently, we have reported the activity of 3-chloro-7-methoxy-2-(methylsulfonyl)quinoxaline, against *T. cruzi* [44]. A synergistic effect between this quinoxaline and benznidazole was observed against epimastigotes and trypomastigotes, accompanied by an antagonistic interaction against $LLCMK_2$ cells. Based on the above considerations, novel 2,3-disubstituted quinoxaline derivatives were synthesized to evaluate their *in vitro* antitrypanosomal and antileishmanial activity.

2. Results and discussion

The discovery and development of new drugs for the treatment of neglected diseases, such as leishmaniasis and Chagas' disease, is necessary and urgent. Their current treatments have several limitations, including limited effectiveness, parenteral administration, long courses of treatment, severe side effects, toxicity, and high cost, making them unaffordable for most patients [22,45]. Many studies have reported that quinoxaline derivatives are promising chemotherapeutic agents against *Leishmania* sp and *T. cruzi* [36–43].

Several methods have been reported for the synthesis of quinoxalines [47]. In the present study, we have focused on straight forward synthetic routes, especially those based on green chemistry principles, Thus, we synthesized 46 new 2,3-disubstituted quinoxaline derivatives (**3d**; **5e**, **5fa-fb**, **5g**, **5ka**; **6a-b**; **7d**; **8a**; **10a**; **11a-d**, **11f-p**; **12a-p**; **13a-b**; **14a-c**) and 40 previously reported compounds (**1**; **2a-p**; **3a-c**, **3e**; **4a-b**; **5a-d**, **5f**, **5h-k**; **7a-c**, **7e**; **9a-c**; **10b-c**; **11e**) with the goal of discovering new drugs for the treatment of Chagas' disease and leishmaniasis.

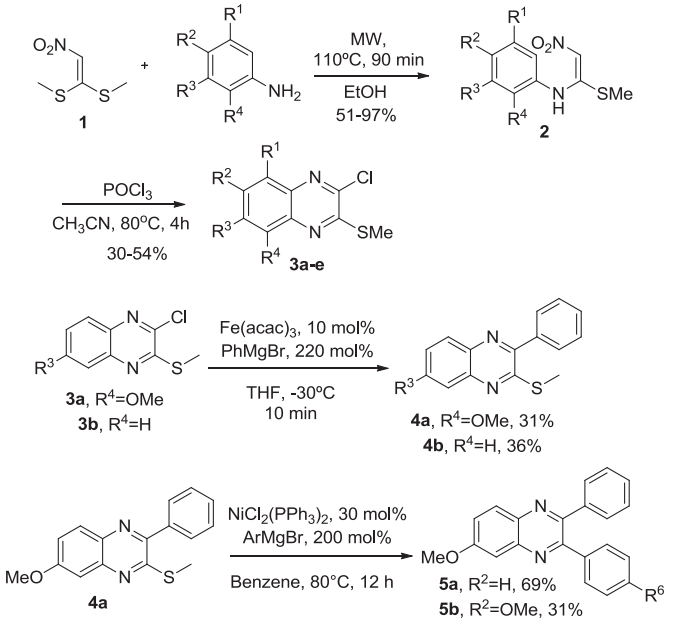
Initially, quinoxaline derivatives were prepared using a procedure described by Venkatesh et al. [48]. The first step was improved by using microwave (MW) irradiation [49] and the nitroketene *N*,*S*-acetal derivatives **2**, obtained by vinylic substitution, were cyclized to produce quinoxaline **3**. By using quinoxaline **3** as starting material, we also synthesized quinoxalines **4a-b** and **5a-b** through cross-coupling reactions (Scheme 1).

Employing a series of sulfur oxidations with *m*-chloroperbenzoic acid, and solvent-free nucleophilic substitutions, we synthesized quinoxalines **6**, **7**, **9**, **10**, **11**, **12** and **13**, using quinoxalines **3** as starting material (Scheme 2) [48].

Quinoxalines **8a** and **14a-c** were synthesized using **4a** as starting material through oxidation followed by nucleophylic substitution (Scheme 3). We have tried to synthesize aryilaminoquinoxalines **14** at room temperature without success, thus microwave irradiation was applied under the same conditions used to obtain aminosulfonylquinoxalines **11** reaching good results.

Quinoxalines **5c-k** were synthesized through the condensation of 1,2-diarylethanediones with *O*-phenylenediamine by using ultrasound irradiation as energy source (Scheme 4) [50]. Compounds **5fa**, **5fb**, and **5ka** were prepared from **5f** or **5k** by deprotection of the methoxyl group followed by *O*-alkylation.

The screening for antichagasic and antileishmanial activity was performed on the epimastigote and trypomastigote forms of *T. cruzi* and promastigote form of *L. amazonensis*. Epimastigotes and promastigotes are the extracellular replicative forms inside the insect vectors of *T. cruzi* and *L. amazonensis*, respectively. The easily cultivable and drug-sensitive epimastigote and promastigote forms make these models an excellent choice for preliminary *in vitro* screening. Trypomastigotes are an extracellular non-replicative stage of *T.* cruzi found in the bloodstream of infected vertebrate hosts. Selective toxicity is an important principle of antiparasitic therapy, therefore the cell viability was also carried out to verify their cytotoxic effects on mammalian cells ($LLCMK_2$ and J774-A1 macrophages).



Scheme 1. Synthesis of quinoxalines **3**, **4** and **5a-b.**