Bioorganic & Medicinal Chemistry Letters 20 (2010) 4831–4835

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Anti-*T. cruzi* activities and QSAR studies of 3-arylquinoxaline-2-carbonitrile di-*N*-oxides

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ARTICLE INFO

Article history:
Received 1 May 2010
Revised 17 June 2010
Accepted 19 June 2010
Available online 25 June 2010

Keywords:
Anti-trypanosomal
Chagas disease
Multivariable linear regression
Quinoxaline *N*-oxide
QSAR
Replacement method

ABSTRACT

In a continuing effort to identify new active compounds for combating Chagas disease and other neglected diseases, our research group synthesized and evaluated 23 3-arylquinoxaline-2-carbonitrile di-*N*-oxides against *Trypanosoma cruzi*. Five of them presented IC_{50} values of the same magnitude as the standard drug Nifurtimox, making them valid as new lead compounds. The optimized molecular structures of 23 derivatives represented by 1497 types of DRAGON descriptors were subjected to linear regression analysis, and the derived QSAR was shown to be predictive. In this way, we achieved a rational guide for the proposal of new candidate structures whose activities still remain unknown.

Chagas disease, or American trypanosomiasis, is one of the most important parasitic diseases in Latin America, mainly, in rural areas where poverty is widespread. It is caused by the haemoflagellate protozoan *Trypanosoma cruzi*, which is transmitted to humans by blood-sucking reduviid bugs (*Triatoma infestans* and *Triatoma rubrovaria*) that deposit their infective feces on the skin at the time of biting. Cases have also been reported in the USA and Canada, as a result of transfusion-related infection. Currently, there are an estimated 16–18 million persons infected by *T. cruzi*. Approximately 2–3 million individuals develop the typical symptoms of Chagas disease and some 100 million persons (25% of the Latin American population) are at risk of acquiring this infection.[1] It is estimated that 14,000 people die from the disease every year; this number of deaths in Latin America is greater than that of any other parasite-born disease, including malaria.[2]

This disease represents a serious public health problem in the countries and areas where it is endemic (21 countries in Central and South America) because, in addition to the fact that no effective methods of immunoprophylaxis currently exist, chemotherapy treatment for controlling this parasitic infection remains undeveloped.[1] Nifurtimox and Benznidazol are drugs that are commonly used to treat this disease; however, these compounds produce several adverse reactions and are not effective in the chronic phase of the disease. Therefore, the design, synthesis, and biological evaluation of new compounds with potential activity against *T. cruzi* is considered to be of great importance.[3]

Chagas disease is included in the collective 'neglected diseases' because it principally affects poor people in developing countries for which health interventions (as well as drug research and development) are inadequate to the existing needs.[4–6] In order to be useful worldwide, antichagasic drugs must be inexpensive so that they are routinely available to populations in need in developing countries. Therefore, our research group carried out a search for inexpensive, available reagents so as to be able to prepare new and inexpensive anti-*T. cruzi* drug candidates.

Quinoxalines and their mono- and di-*N*-oxide derivatives display a broad range of biological activities,[7] and quinoxaline di-*N*-oxides are known to undergo bioreductions under hypoxia, causing DNA damage.[8–11] Given the known activity of other classes of bioreductive agents, such as Nifurtimox,[12] it seemed logical to us to evaluate our group of structures against *T. cruzi*. As a result of the anti-Chagas research project, our group published several articles in which the synthesis and biological evaluation of a large amount of quinoxaline and quinoxaline di-*N*-oxides have been described. From these studies, several derivatives, with different patterns of substituents at the quinoxaline nucleus, were prepared with outstanding in vitro anti-trypanosomal activity (i.e., lead

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doi:10.1016/j.bmcl.2010.06.101

compounds **I–IV**, Fig. 1).[13–18] Screening of the in vitro anti-trypanosomal activity of quinoxaline-2-carbonitrile di-*N*-oxide derivatives[14,18] indicated that their potency mainly depended on the substituents in R^6/R^7 positions, with 6/7-halogenated derivatives being the most active compounds (**I–IV**, Table 1), and on the compounds lipophilicity, with the most active derivatives being the less hydrophilic (Table 1). Recently, we have theoretically demonstrated that quinoxaline di-*N*-oxide could be bioreduced into the parasite, thereby producing reactive species which cause death of the parasite.[19]

In a continuing effort to identify new active compounds to combat Chagas disease, and other neglected diseases,[20–22] our research group prepared 23 3-arylquinoxaline di-*N*-oxide derivatives, **1–23**. Several structural modifications were introduced at the lead compounds **I–IV**, by applying the isosteric and homologous strategies (Fig. 1) attempting to increase the compounds lipophilic properties. Based on the above, we proposed, as a first approximation, the elimination of nonaromatic moiety linked to C-3 of the quinoxaline scaffold (piperazinyl ring in **I–II** or carbonylamino group in **III–IV**), keeping the carbonitrile group linked to C-2, in order to obtain a series of 3-arylquinoxaline-2-carbonitrile di-*N*-oxides, **1–23**. Variations on the electronic profile of the W *para*-substituent of the phenyl moiety were carried out (**1–12**). On the other hand, the halogen atoms present in the prototypes **I–IV** were isosterically replaced by other monovalent groups. These modifications were conducted in order to establish the contributions of electronic and steric parameters for the optimization of the previous lead compounds, **I–IV**.

The 3-arylquinoxaline-2-carbonitrile di-*N*-oxides were obtained by the classical Beirut reaction.[23] The aforementioned compounds (**1–23**) were prepared, in good to excellent yields, according to the synthetic process previously reported.[20,21] Structures of the compounds were confirmed by infrared spectroscopy, nuclear magnetic resonance and mass spectrometry; purity was established by elemental analysis (Scheme 1).

Anti-*Trypanosoma cruzi* activities were determined according to the previously described method.[14,18,24] The existence of the epimastigote form of *T. cruzi* as an obligate mammalian intracellular stage has been revisited and confirmed.[25,26] Moreover, good correlation was observed between the anti-proliferative epimastigote activity and the in vivo anti-*T. cruzi* activity.[27–30] Table 2 shows the results expressed as percentage of epimastigotes *T. cruzi*, Tulahuen 2 strain, growth inhibition (PGI) at 25 μM doses and the corresponding IC_{50} values. As shown in Table 2, almost all of the compounds were active, with PGI >50%, in the preliminary assay at 25 μM. Five of these derivatives (**8**, **11**, **12**, **21**, and **23**) displayed IC_{50} values of the same order than the standard drug Nifurtimox (Nfx), when tested in vitro against epimastigote forms of *T. cruzi*, making them new, valid lead compounds.

Table 1
In vitro activity of previous lead compounds, **I–IV**[a,b] against epimastigote Tulahuen 2 strain of *T. cruzi*

Compound	PGI[c,d] (%)	IC_{50}[e] (μM)	*MLOGP*[f]
I	100	6.5	1.912
II	94	6.7	2.987
III	53.1	19.2	0.868
IV	92.4	10.8	1.616

[a] For structure see Figure. 1.
[b] From Refs. 8,12.
[c] Percentage of growth inhibition respect to untreated parasite.
[d] Inhibition of epimastigotes growth of Tulahuen 2 strain, doses = 25 μM.
[e] Fifty percentage inhibitory concentrations (μM).
[f] Moriguchi-octanol/water partition coefficient.

Some interesting structure–activity relationships can be observed from these results. The most lipophilic compound, **8** (Table 2), according to Moriguchi-octanol/water partition coefficient,[31] was one of the best anti-*T. cruzi* agents. However, in general, the elimination of piperazinyl ring linked to C-3 of the quinoxaline subunit, present in the prototypes **I** and **II**, leads to compounds which do not improve the anti-trypanosomal activity. On the other hand, the elimination of carbonylamino group linked to C-3 of the quinoxaline scaffold, present in the prototypes **III** and **IV**, leads to the most active derivative of both of these series of compounds (**21**, Table 2).

With the aim of completing the analysis of these biological results, we resorted to the quantitative structure–activity relation-

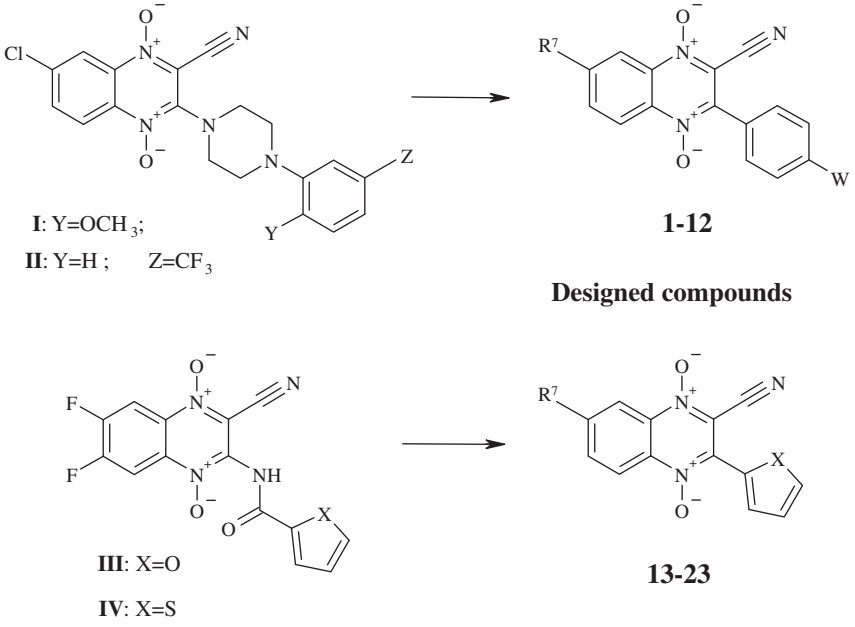

Figure 1. Design of 3-arylquinoxaline-2-carbonitrile di-*N*-oxides, **1–23**, as anti-trypanosomal drugs, obtained from previous lead compounds with structural modifications.[14,18]