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Research paper

Synthesis and trypanocidal activity of a library of 4-substituted 2-(1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)propan-2-ols

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ABSTRACT

A library of 16 4-substituted 2-(1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)propan-2-ols **17**–**32** has been synthesized for use in biological testing against *Trypanosoma cruzi*, the protozoan parasite that causes Chagas disease. The 4-substituted 2-(1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)propan-2-ols **17**–**32** were subjected to biological testing to evaluate their efficacy against intracellular *Trypanosoma cruzi* (Y strain) amastigotes infecting U2OS human cells, with benznidazole as a reference compound. The assay was performed in duplicate (two independent experiments) and submitted to High Content Analysis (HCA) for determination of trypanocidal activity. Three of the tested compounds presented relatively high trypanocidal activity (**19**, **22** and **29**), however severe host cell toxicity was observed concomitantly. Chemical optimization of the highly active compounds and the synthesis of more compounds for biological testing against *Trypanosoma cruzi* will be required to improve selectivity and so that a structure-activity relationship can be generated to provide a more insightful analysis of both chemical and biological aspects.

1. Introduction

Chagas disease, also known as American trypanosomiasis, is a chronic infectious disease endemic in Latin America. The disease is caused by the protozoan parasite *Trypanosoma cruzi*, which is transmitted to humans by blood-sucking triatomine insect vectors. Other forms of contagion are organ transplant or blood transfusion contaminated with *Trypanosoma cruzi*, transmission from mother to fetus during pregnancy (vertical transmission), or by ingestion of food or drink contaminated with live parasites [1]. The World Health Organization estimates that there are approximately 7 million people who are infected with *Trypanosoma cruzi* in the world, and although Chagas disease is endemic in Latin America, global migratory phenomena has scattered infected individuals to several non-endemic countries, including USA, Canada, Spain Australia and Japan, where parasite transmission can occur, especially for organ transplantation and transfusion of contaminated blood, since the practice of screening for *Trypanosoma cruzi* in blood and organ banks is not common in these countries [2]. It is estimated that in the United States alone there are about 300 thousand infected individuals, and a recent study suggests that a cycle of transmission of *Trypanosoma cruzi* is occurring locally [3]. Chagas disease begins when an individual becomes infected with *Trypanosoma cruzi*. Transmission by vector occurs when triatomines deposit infected faeces near the site of the bite, the bite of which compromises the physical integrity of the skin. The bite induces an inflammatory reaction that causes itching so that when the individual scratches the site of the bite the contaminated faeces are spread to the bite, allowing the parasite to penetrate the bite lesion (or the parasites can be spread to the mucous membranes, such as the eyes, where the parasites can easily enter).

The transmission form of the parasite is called the trypomastigote, and is capable of infecting various cells in the host organism. Once inside the cells the trypomastigotes differentiate into amastigotes, which are intracellular forms that are able to multiply. After a few days, the amastigotes differentiate into trypomastigotes and disrupt the host cell by moving in a tumbling fashion, they then go on to infect new cells, thus establishing the infection in the host.

The initial period of infection (acute phase) is characterized by non-specific symptoms such as fever, and diagnosis often fails due

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to the generality of the symptoms. If Chagas disease is not treated during the acute phase, the disease progresses to a chronic phase, which is also known as the indeterminate phase.

This may be asymptomatic and is the most common clinical presentation. Decades after infection, about 30% of patients develop the cardiac form of the disease, which is characterized by progressive injury to the heart muscle tissue, causing arrhythmias and cardiac failure. Some patients develop the digestive form of Chagas disease, which is characterized by loss of muscle motility in the digestive tract [4]. For many years, medical scientists thought that there was an autoimmune component involved in Chagas disease. It is now known that pathological lesions occur due to the persistence of the parasite in infected tissues and therefore it is recommended that patients receive antiparasitic treatment at any stage of disease [5].

Because it is a disease that affects mainly economically disadvantaged populations in developing countries, there has been little interest in the pharmaceutical industry to develop new therapies for the treatment of Chagas disease and therefore Chagas disease is considered a neglected disease. Currently there are only two drugs available for clinical use: the nitroimidazole compound benznidazole and the nitrofuran compound nifurtimox. Both were developed about 40 years ago and belong to the class of compounds called nitroheterocyclics, which usually deliver broad-spectrum antimicrobial activity, but with high toxicity [6].

Both drugs require prolonged administration (about 60 days) and have high efficacy and curative rate when administered during the acute phase of infection. The use of benznidazole or nifurtimox for the treatment of symptomatic chronic Chagas patients is still considered controversial. Although both exhibit antiparasitic efficacy, both are associated with substantial side effects, which often lead to the abandonment of the treatment by the patient, and are contraindicated in some circumstances, such as during pregnancy [7]. Therefore, benznidazole and nifurtimox are not satisfactory drugs and new approaches are needed for the treatment of Chagas disease.

Thus a continuous search for new drugs is required to ensure the discovery and development of alternatives to current drugs. Thankfully research in the development and testing of antitrypanosomal/trypanocidal compounds has never abated, recent examples include the 2-((4,5-disubsituted-thiazol-2-yl)amino)isoindoline-1,3-diones, 2-((E)-2-((Z)-(3,4-disubsituted-thiazol-2(3H)-ylidene)hydrazono)ethyl)isoindoline-1,3-diones, (E)-2-(2-(1-(3-bromophenyl)propylidene)hydrazinyl)-4,5-disubstituted-thiazoles and (Z)-2-((E)-(1-(3-bromophenyl)propylidene)hydrazono)-3,4,5-trisubstituted-2,3-dihydrothiazoles developed and tested by Moraes Gomes et al. [8], and the nitrotriazole-based acetamides and propanamides developed and tested by Papadopoulou et al. [9].

Trypanosoma species express several kinases, an observation that has driven efforts to identify classes of kinase inhibitors that can be useful for discovery of new parasite growth inhibitors. To identify new kinase-targeting chemotypes for target and pathway analysis and drug discovery for *Trypanosoma brucei* (the causal pathogen of human African trypanosomiasis (HAT) and related to *Trypanosoma cruzi*) Diaz et al. performed a high-throughput screen of 42,444 focused inhibitors, from the GlaxoSmithKline screening collection, against parasite cell cultures and counter-screened against human hepatocarcinoma (HepG2) cells. They identified hundreds of compounds that ranged in performance from being (1) sub-micromolar (EC_{50}) inhibitors of *T. brucei* growth with at least 100-fold selectivity over HepG2 cells, (2) hit compounds that rapidly inhibited cellular growth, and (3) compounds that showed rapid cidality. Compounds **1**–**9** (Fig. 1) were among the best performing and showed pEC_{50}s of 6.16–9.17 and pTC_{50}s of <4–4.84. Compounds **1**–**9** were also fast acting ($pEC_{50} \geq 6$ at 18 h) and cidal. The best compound tested, NEU-0001053 **7**, demonstrated parasitological cure of a murine bloodstream infection of *T. brucei rhodesiense* [10].

Woodland et al. recently performed a screen of a focused kinase inhibitor library against *Trypanosoma brucei rhodesiense* leading to the identification of several compounds (seven series, 121 in total) which showed >50% inhibition at 5 μM. Screening of these compounds in a *T. b. brucei* proliferation assay identified three compounds with the 1*H*-imidazo[4,5-*b*]pyrazin-2(3*H*)-one ring system or scaffold that showed sub-micromolar activity and excellent selectivity against the MRC5 cell line. Subsequent rounds of optimization with synthetic chemistry and biological testing led to the identification of compounds that exhibited good in vitro drug metabolism and pharmacokinetics (DMPK) properties, albeit with poor solubility. A scaffold-hopping exercise, involving more synthetic chemistry and biological testing, led to the identification of a 1*H*-pyrazolo[3,4-*b*]pyridine scaffold, which retained potency. A number of these compounds were tested in a *T. b. brucei* growth assay, which could differentiate static (growth slowing/inhibiting) and cidal action. Compounds from the 1*H*-imidazo[4,5-*b*]pyrazin-2(3*H*)-one series were found to be either static or growth-slowing and not cidal, whereas compounds with the 1*H*-pyrazolo[3,4-*b*]pyridine scaffold, such as compounds **10** and **11** (Fig. 2), were found to be cidal and showed an unusual biphasic nature in the assay, suggesting that they act by at least two mechanisms [11]

The potent and selective antitrypanosomal/trypanocidal compounds shown in Figs. 1–2 all contain a variety of different fused bicyclic heteroarenes, namely 1*H*-pyrrolo[2,3-*b*]pyridine, 1*H*-benzo[*d*]imidazole, indole, furo[2,3-*d*]pyrimidine and 1*H*-pyrazolo[3,4-*b*]pyridine, with monocyclic substituents such as aryl, heteroaryl and cycloalkyl attached. Drawing inspiration from the antitrypanosomal/trypanocidal compounds shown in Figs. 1–2, we wondered whether 1*H*-pyrrolo[3,2-*c*]pyridines (5-azaindoles), which are not represented in these literature examples, would also show antitrypanosomal/trypanocidal activity, perhaps by inhibition of trypanosomal kinases. Therefore, for this work we synthesized a library of differently substituted 1*H*-pyrrolo[3,2-*c*]pyridines (5-azaindoles) and subjected them to biological testing against *Trypanosoma cruzi*, the causal pathogen of Chagas disease.

2. Results and discussion

2.1. Synthetic chemistry

The precursor compound for the synthesis of the library, 2-(4-chloro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)propan-2-ol **16**, was synthesized in four steps from commercially available 2-chloropyridin-4-amine **12**. 2-Chloropyridin-4-amine **12** was iodinated with iodine monochloride in glacial acetic acid, in the presence of sodium acetate trihydrate, with heating at 70 °C. This produced the desired 2-chloro-3-iodopyridin-4-amine **13** in 45% yield [12]. The 2-chloro-3-iodopyridin-4-amine **13** was then bis-mesylated with methanesulfonyl chloride in anhydrous pyridine at room temperature, according to the procedure of Sakamoto et al. [13]. This produced *N*-(2-chloro-3-iodopyridin-4-yl)–*N*-(methylsulfonyl)methanesulfonamide **14** in 89% yield. Then **14** was monodemesylated with 2.75 M aqueous sodium hydroxide in tetrahydrofuran at room temperature, which produced the desired monomesylate *N*-(2-chloro-3-iodopyridin-4-yl)methanesulfonamide **15** in 87% yield [12b], [12c]. This set the stage for the key cyclization which would form the desired 1*H*-pyrrolo[3,2-*c*]pyridine (5-azaindole) ring system. Very few examples of the synthesis of substituted 1*H*-pyrrolo[3,2-*c*]pyridines have been reported over the years. Miscellaneous synthetic methods have been reported for the preparation of substituted indoles [13], [14], but relatively few have