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Original article

Synthesis, physicochemical properties of allopurinol derivatives and their biological activity against *Trypanosoma cruzi*

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ABSTRACT

Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*) leading to a huge number of infections and deaths per year, because in addition to many sufferers only having limited access to health services only an inefficient chemotherapy is available using drugs such as benznidazole and nifurtimox. Here, C6-alkyl (**2a**–**c**) and N1-acyl (**3a**–**c**) derivatives of Allopurinol (Allop, compound with activity against *T. cruzi*) were synthesized in good yields and their structures were unambiguously characterized. Only **2a**, **2b** and **3c** showed inhibitory activity against the proliferative stages of the parasite when tested at 1 μg mL^{-1} with the **3c** derivative exhibiting an IC50 value similar to that of Allop and not being toxic for mammalian cells. Relevant pharmaceutical physicochemical properties (pKa, stability, solubility, lipophilicity) were also determined as well by using Lipinski's rule, polar surface area and molecular rigidity. Taken together, the results demonstrated that the studied derivatives had optimal properties for bioavailability and oral absorption. For the stability studies, Micellar Liquid Chromatography was used as the analytical method which was fully validated according to the FDA guidelines and shown to be a suitable, sensitive and simple method for routine analysis of these Allop derivatives.

1. Introduction

Chagas disease (Chagas) or American Trypanosomiasis is caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), and remains a major health problem in Central and South America that affects nearly 8–10 million people throughout Latin America [1].

In endemic countries, *T. cruzi* is transmitted via haematophagous triatomine insect vectors that release infective forms within the feces and urine after every mammalian blood ingestion. Once in the host, parasites invade cells and undergo differentiations into intracellular amastigotes (AMAS), which after several rounds of duplication transforms them into trypomastigotes (TRYP), the form that disseminates the infection. Transmission may also occur through laboratory accidents, organ transplantation, ingestion of contaminated food as well as by blood transfusion and congenital passage [1]. The global increase in human migrations is responsible for most of the cases reported in non-endemic countries [2].

Paradoxically, 100 years after the first report describing the morphology and the life cycle of the pathogen, neither vaccines nor effective treatments for chronic cases are available. One reason for this may be that Chagas disease belongs to a group of tropical infections that are endemic mainly among low-income populations of the developing regions of Africa, Asia, and America, which are named *neglected diseases* [3]. The lack of interest of pharmaceutical companies and the absence of effective social policies from the affected states are responsible for the limited evolution toward an improved pharmacotherapy.

Chagas disease initiates as an acute phase that is usually asymptomatic. Then, after decades of chronic infection, 30–40% of the infected people develop symptoms that can include cardiac and/or digestive (megaesophagus or megacolon) forms. Despite significant progress having been made in understanding the biochemistry and physiology of the causative agent, the current etiological treatment of Chagas disease is based on rather

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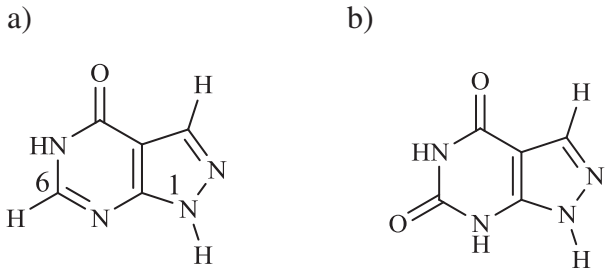


Fig. 1. Allopurinol (a) and its metabolite oxypurinol (b).

unspecific drugs developed more than four decades ago, such as nifurtimox and benznidazole (Bz). Moreover, although the use of these drugs to treat the acute phase of the disease is widely accepted, etiological treatment at the chronic phase remains controversial [4], with the undesirable side effects of both drugs being major drawbacks and frequently forcing treatment to be discontinued [5]. Research on anti-*T. cruzi* compounds has been to date based on different strategies aimed at targeting specific parasite enzymes or parasite DNA or at inducing oxidative stress damage [6,7].

Nevertheless, the development of safer and more efficient trypanocidal drugs remains a major goal in Chagas disease chemotherapy, with one possibility being to exploit differences in the purine metabolism between *T. cruzi* and host cells. Although *T. cruzi* does not synthesize purines *de novo* as in the case of as mammals, the parasite is able to concentrate the pyrazolopyrimidines within the cell and metabolize them as purines through a salvage pathway, ultimately incorporating them into nucleic acids [8,9].

Allopurinol (**1**, Allop 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one, Fig. 1a) employed for the treatment of hyperuricemia, is an hypoxantine analog used by the *T. cruzi*'s hypoxanthine–guanine phosphoribosyltransferase (HGPRT) as an alternative substrate. This enzyme can incorporate Allop into the parasite's ribonucleic acid as a non-physiological nucleotide; thus blocking the synthesis of new purine nucleotides [10,11]. The activity of Allop in chemotherapy for Chagas disease has been extensively investigated, but the results are somewhat contradictory [12–14]. In a recent pilot study, Perez-Mazliah et al. [15] showed that the combination of Allop and Bz induced significant modifications of the T and B cell responses, indicative of a reduction of the parasite burden, and thereby sustained the feasibility of administration of two antiparasitic drugs in the chronic phase of Chagas disease. In addition, these two drugs when administered together were reported to be safe and effective in the treatment of Chagas disease reactivation after heart transplantation [16].

The variable efficacy of Allop depends on the infective parasite population, which varies among geographical areas [17]. In addition, in mammals, Allop is converted into oxypurinol by xanthine oxidase (Fig. 1b) with a $t_{1/2} = 1–2$ h, which is not a substrate for HGPRT and has no anti-*T. cruzi* activity [10].

Although Allop presents trypanocidal activity, its failure in avoiding Chagas disease progression may be due in part to inadequate blood levels caused by unfavorable physicochemical properties, thus leading to versatile responses in humans.

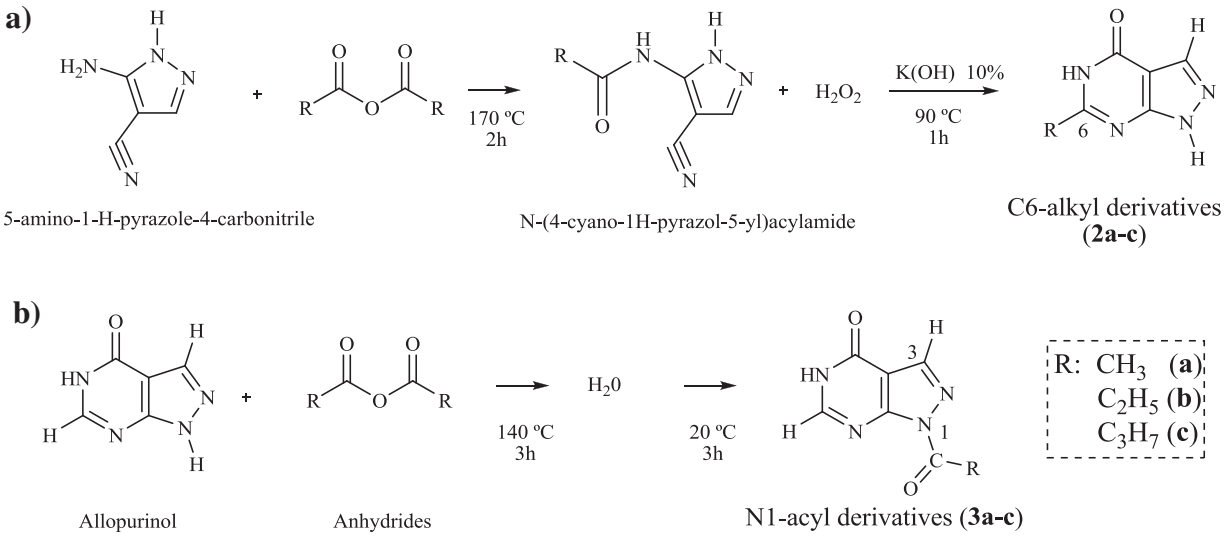
In an attempt to improve its performance, we have developed a series of derivatives of Allop by chemical modification of their specific functional groups, resulting in active anti-*T. cruzi* agents *per se* or prodrug compounds. Then, their activities against *T. cruzi* as well as their different relevant physicochemical properties, such as their integrity in buffers, simulated fluids and human plasma were determined, in addition to carrying out hydrolytic and oxidative studies, and determining acid dissociation constants, lipophilicity and solubility.

2. Results and discussion

2.1. Chemistry

Taking into account that C6 of Allop is a target of metabolism for the xanthine oxidase enzyme, a series of derivatives with alkyl groups on this position (**2a**–**c**) were synthesized in two steps, obtaining 6-methyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**2a**), 6-ethyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**2b**) and 6-propyl-1,5-di hydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**2c**) by employing the method of Cheng and Robins [18] (Scheme 1a). It is important to point out that Biagi et al. have found that these compounds to be ineffective inhibitors of this enzyme, and that neither physicochemical properties nor activity against *T. cruzi* were previously studied [19].

N1-acyl prodrugs of Allop were also synthesized (**3a**–**c**) to improve their physicochemical properties; whose design was based



Scheme 1. Synthesis procedures used for the development of derivatives. a) C6-alkyl derivatives of Allop (**2a**–**c**), b) N1-acyl derivatives of Allop (**3a**–**c**).