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Original article

New perspectives on the synthesis and antichagasic activity of 3-alkoxy-1-alkyl-5-nitroindazoles

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ABSTRACT

The synthesis and antiprotozoal activity of some 3-alkoxy-1-alkyl- (**1**, **4**) and 3-alkoxy-1-(ω-aminoalkyl)-5-nitroindazoles (**2**, **3**, **5**–**8**) against different morphological forms of *Trypanosoma cruzi* are reported. These compounds were prepared using simple alkylation reactions and, usually, taking advantage of the reactivity of some indazole-derived betaines previously studied by us. Most indazole derivatives showed *in vitro* activities similar or higher than those of the reference drug benznidazole; this fact, along with low unspecific cytotoxicities against Vero cells shown by some of them, led to very good selectivity indexes (SI). The high efficiency of 5-nitroindazoles **1** and **2** against *T. cruzi* was confirmed by further *in vitro* studies on infection rates and by an additional *in vivo* study in a murine model of acute and chronic Chagas disease. Complementary analyses of the changes in the metabolites excreted by the parasite and on the ultrastructural alterations induced after treatment with indazole derivatives **1** and **2** were also conducted.

1. Introduction

Chagas disease or American trypanosomiasis is a major parasitosis originally endemic to poor rural areas of Latin America, mainly transmitted by hematophagous triatomine insects and caused by the trypanosomatid (Kinetoplastid) protozoan parasite *Trypanosoma cruzi*. At present because of intense international migrations it can also be found in other areas such as Western Europe, USA, Australia, etc, countries in which the infection is transmitted by other routes. Only two drugs, nifurtimox and benznidazole are currently available for the treatment of Chagas disease. This chemotherapy, however, is unsatisfactory because these drugs exhibit limited efficacy in the chronic phase of the disease and also severe toxic side effects. Thus, it is clear that the development of new drugs for the treatment of this parasitosis is urgently needed. Several excellent articles covering different aspects of Chagas disease chemotherapy [1] have been published recently.

In this context, we have reported in the last years the synthesis and antichagasic properties of some 5-nitroindazole derivatives, mainly 1-substituted 3-alkoxy-1*H*-indazoles [2–4], 2-substituted 3-alkoxy-2*H*-indazoles [4] and 1,2-disubstituted indazolin-3-ones [4], as well as those of some 4-substituted and 1,4-disubstituted 7-nitroquinoxalin-2-ones [5,6]. It has been proposed [2–4,7] that the mentioned nitro-group bearing heterocycles act upon intracellular nitro reduction followed by redox cycling leading to reactive oxygen species (ROS) (like nifurtimox) [8] or producing electrophilic metabolites (like benznidazole) [8] able to damage essential biomolecules of parasites. Nevertheless, the classical mechanisms of action proposed for nifurtimox and benznidazole are currently under revision [9] and consequently, that of our nitroheterocycles need to be investigated more thoroughly. Inhibition of trypanothione reductase has also been suggested according to molecular modelling studies carried out with 7-nitroquinoxalin-2-one derivatives [6].

Considering the interest of 3-alkoxy-1-alkyl-5-nitroindazole scaffold [2–4] in the field of antichagasic agents, and that ω-(dialkylamino)alkyl chains are found in some of the most active reported indazoles [2,3] and quinoxalines [6], in this article we describe the synthesis and antichagasic properties of a new family of 5-nitroindazole-derived primary, secondary and tertiary amines

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(**2**, **3**, **5**–**8**); the reported low solubility of some indazole derivatives containing lipophilic substituents [4] led us to introduce in the present compounds an oxaalkyl chain directed to improve their solubility in aqueous media. Compounds **1** and **4** were also prepared and tested as simple models containing the 3-alkoxy-1-alkyl-5-nitroindazole scaffold (Fig. 1).

Antichagasic properties were initially evaluated *in vitro* against epimastigote, amastigote and trypomastigote forms of *T. cruzi*; Vero cells were used in order to determine unspecific cytotoxicity of our compounds. This study was complemented by infectivity assays on Vero cells carried out with products showing highest activity and selectivity index (SI) values, i.e., compounds **1** and **2**. *In vivo* trypanocidal activities of these compounds in a murine model of acute and chronic phases of Chagas disease were also determined.

Furthermore, a ^{1}H NMR study has been conducted in order to observe changes in the nature and percentage of metabolites excretion directed to obtain information about the effect of our compounds on the glycolytic pathway of *T. cruzi* epimastigotes; finally, we have also studied the ultrastructural alterations of parasite epimastigotes treated with our compounds using transmission electron microscopy (TEM).

2. Results and discussion

2.1. Chemistry

Compounds **1** and **4** were prepared by alkylation of 1-methyl-5-nitroindazol-3-ol **9** (Scheme 1). As expected [10,11], mixtures of N_1,*O*- and N_1,N_2-dialkyl derivatives were obtained, but chromatographical separation of 3-alkoxy-1-alkylindazoles (**1**, **4**) from the isomeric 1,2-dialkylindazolinones (**10**, **11**) was very simple.

Compounds **2**, **3**, **5**–**8** were prepared according to the pathway shown in the Scheme 1. The key intermediate is indazolium-3-olate **14**, belonging to a class of betaines which synthesis [12] and reactivity [13] have previously been studied by some of us. These betaines are easily available by cyclization of 2-halogenobenzohydrazides such as **13** which, in turn, was obtained by acylation of 4-aminomorpholine with the corresponding acid chloride **12** [12].

Treatment of betaine **14** with hydrobromic acid [13] yielded ω-(3-hydroxyindazolyl)alkyl bromide **15**. Alkylation of this compound with benzyl bromide or methyl iodide afforded, as expected for 1-substituted indazol-3-ols [10,11], mixtures of *O*- (**16**, **17**) and N_2-alkyl (**18**, **19**) derivatives; partial halogen exchange (Finkelstein reaction), completed by treatment with sodium iodide in acetone, takes also place during the methylation reaction (NMR). ω-(3-Alkoxyindazolyl)alkyl halides **16** and **17**, chromatographically separated from the corresponding 1,2-disubstituted indazolinones **18** and **19**, were then treated with an excess of methylamine in ethanol to afford secondary amines **2** and **5**, respectively. The latter, treated with an additional equivalent of the required halides **16** or **17**, yielded tertiary amines **3** and **6**.

Finally, treatment of iodide **17** with an excess of ammonia in ethanol gave primary amine **7** as the main reaction product, along with a small amount of secondary amine **8**; both compounds were readily separated by chromatography.

Since the control of the reaction of alkyl halides with ammonia or primary amines is difficult, this method is not usually recommended [14] for the preparation of primary or secondary amines, respectively; in our case, however, these processes were very clean, affording primary amine **7** and secondary amines **2** and **5** with excellent yields.

The structure of all compounds has been established on the basis of analytical and spectral data. Hydrazides such as **13** appear in solution (NMR) as mixtures of *Z* and *E* rotamers owing to restricted rotation around the N–CO bond [12]. In this case, the *Z* rotamer/*E* rotamer ratios, determined by ^{1}H NMR as previously reported for related compounds [12], are *ca.* 58:42 and 65:35 in $CDCl_3$ and $(CD_3)_2SO$, respectively.

As previously observed for related betaines [12], the presence on the quaternary nitrogen atom of compound **14** hinders the conformational equilibrium of morpholine ring. Thus the anisochronic NCH_2 protons can be easily distinguished in the ^{1}H NMR spectrum as H_{ax} and H_{eq} by comparison with the spectra of previously described piperidine analogues [12]. A similar effect has been observed for OCH_2 protons but in this case the assignment of the signals is not easy, and they have been distinguished in the spectral description as H_A and H_B.

On the other hand, 3-alkoxy-1-alkylindazoles (**1**, **4**, **16**, **17**) can be easily distinguished by NMR from the isomeric 1,2-disubstituted indazolinones (**10**, **11**, **18**, **19**, respectively) also arising from the alkylation of 1-substituted indazol-3-ols [11].

2.2. In vitro anti-T. cruzi evaluation

In vitro activity of compounds **1**–**8** on epimastigotes, amastigotes and trypomastigotes [15,16] of *T. cruzi*, the unspecific cytotoxicity against Vero cells and the corresponding selectivity indexes (SI) are gathered in Table 1. First we prepared an epimastigotes (extracellular insect vector stage) culture and in a further step we infected Vero cells with metacyclic forms of parasite, which were

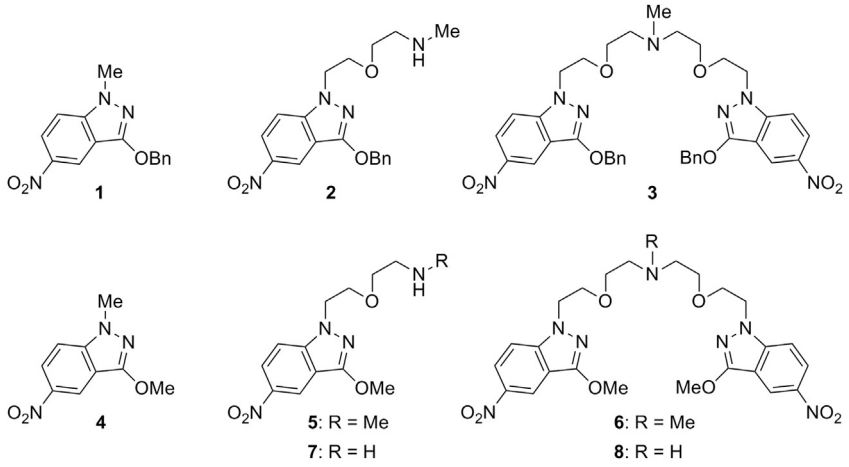

Fig. 1. Chemical structure of indazole derivatives **1**–**8** studied in the present work.