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Biological and chemical study of fused tri- and tetracyclic indazoles and analogues with important antiparasitic activity

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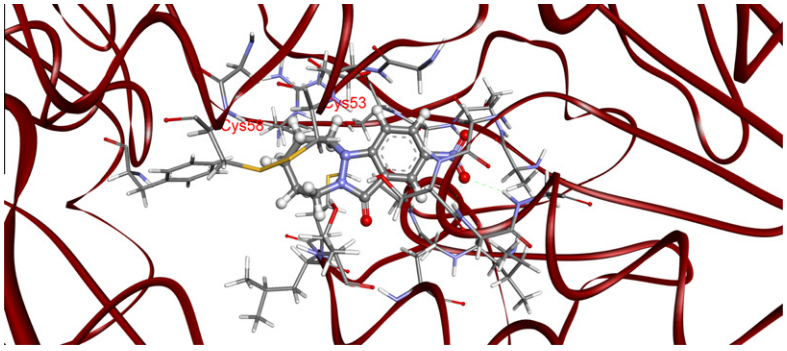
HIGHLIGHTS

- In this study we characterized the electrochemistry mechanism of tetracylic indazoles. The radicals species generated were register using electron spin resonance spectroscopy. This family was also evaluated as potential tripanocide.

GRAPHICAL ABSTRACT

Conformational mode adopted by compound **1** into the *Trypanosoma cruzi* trypanothione reductase active site.



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ABSTRACT

A series of fused tri- and tetracyclic indazoles and analogues compounds (NID) with potential antiparasitic effects were studied using voltamperometric and spectroscopic techniques. Nitroanion radicals generated by cyclic voltammetry were characterized by electron spin resonance spectroscopy (ESR) and their spectral lines were explained and analyzed using simulated spectra. In addition, we examined the interaction between radical species generated from nitroindazole derivatives and glutathione (GSH). Biological assays such as activity against *Trypanosoma cruzi* and cytotoxicity against macrophages were carried out. Finally, spin trapping and molecular modeling studies were also done in order to elucidate the potentials action mechanisms involved in the trypanocidal activity.

Introduction

Chagas disease, a bug-borne disease caused by the flagellate protozoa *Trypanosoma cruzi*, is the second most important parasitic disease in Latin America [1]. Although there have been important

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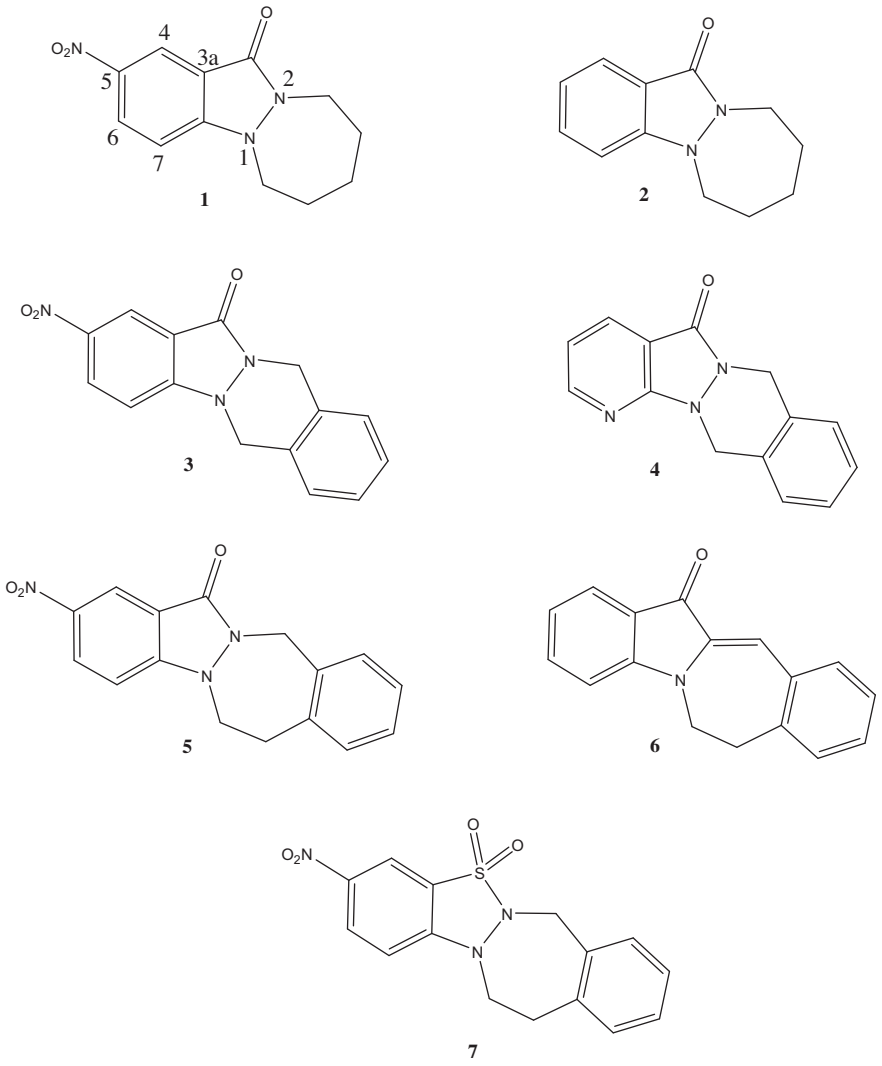

Fig. 1. Fused tri- and tetracyclic indazole derivatives and related compounds. (For reasons of nomenclature, we have used the numbering of non-condensing indazole ring, which does not correspond to the numbering of the condensed ring, see **1**.)

advances in the control of vector transmission and transfusion of this disease [2], the problem of infected people still remains unsolved [3]. The parasite life cycle involves an extracellular, proliferative stage (epimastigote) that resides in the insect vectors. Two forms occur in the mammalian hosts, a nonproliferating infective form (trypomastigote) and an intracellular proliferative form (amastigote) [4]. Without immediate prospect of a vaccine, the search for parasite's specific targets exploitable in terms of new chemotherapies is an urgent priority.

Current therapies based on two nitroheterocycles namely nifurtimox (Nfx) and benznidazole (Bzn), are still insufficient due to several limitations including severe adverse events and the fact that these are more effective in the early stages of the disease but not in the chronic stage altogether with the resistance of several strains against treatment [1,3,5,6]. These drugs act nonspecifically through the generation of highly reactive free radicals, that can react with oxygen leading to reactive oxygen species (ROS), or by covalent modification of organic macromolecules [7], which might explain some of the toxic effects observed on mammals [3,7]; however, some of their proposed modes of action are recently being challenged [8].

On the other hand, previous experimental results showed that families of nitroindazole derivatives with a flexible chain such as 3-alkoxy- or 3-hydroxy-1-[ω-(dialkylamino)alkyl]-5-nitroindazoles are electrochemically reduced with the formation of a nitroanion radical. Their reduction mechanism depends on the acidic moieties in 3-hydroxy structures [9]. A self-protonation process involving the reduction of the nitro group was also observed. Additionally, 1,2-disubstituted 5-nitroindazolin-3-ones and 2-substituted 3-alkoxy-5-nitro-2*H*-indazoles have shown the generation of the nitroanion radical species [10]. This mechanism seems to be independent of the substituent groups in the different positions on the molecule. In this sense, it is interesting to carry out studies involving a new family of fused tri- and tetracyclic indazoles (NID) compounds that lack acid group that would lead to this self-protonation process.

In the present work fused tri- and tetracyclic nitro derivatives **1** (2-nitro-7,8,9,10-tetrahydro-6*H*,12*H*-[1,2]diazepino[1,2-*a*]indazol-12-one) [11], **3** (2-nitro-6,11-dihydro-13*H*-indazolo[1,2-*b*]phthalazin-13-one) [11], **5** (2-nitro-6,17-dihydro-12*H*-indazolo[2,1-*b*][2,3]benzodiazepin-14-one) [11] and, **7** (3-nitro-12,13-dihydro-7*H*-[1-3]benzothiadiazolo[2,3-*b*][2,3]benzodiazepine-5,5-dioxide) [12] (Fig. 1) have been electrochemically studied in aprotic solvent using cyclic voltammetry (CV) technique. The nitro-anion radical species were characterized using electron spin resonance spectroscopy (ESR) and their spectra were explained using theoretical sim-