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Research paper

# Antichagasic and trichomonacidal activity of 1-substituted 2-benzyl-5-nitroindazolin-3-ones and 3-alkoxy-2-benzyl-5-nitro-2*H*-indazoles☆

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ABSTRACT

Two series of new 5-nitroindazole derivatives, 1-substituted 2-benzylindazolin-3-ones (**6**–**29**, series **A**) and 3-alkoxy-2-benzyl-2*H*-indazoles (**30**–**37**, series **B**), containing differently functionalized chains at position 1 and 3, respectively, have been synthesized starting from 2-benzyl-5-nitroindazolin-3-one **5**, and evaluated against the protozoan parasites *Trypanosoma cruzi* and *Trichomonas vaginalis*, etiological agents of Chagas disease and trichomonosis, respectively. Many indazolinones of series **A** were efficient against different morphological forms of *T. cruzi* CL Brener strain (compounds **6**, **7**, **9**, **10** and **19**–**21**: $IC_{50}$ = 1.58–4.19 μM for epimastigotes; compounds **6**, **19**–**21** and **24**: $IC_{50}$ = 0.22–0.54 μM for amastigotes) being as potent as the reference drug benznidazole. SAR analysis suggests that electron-donating groups at position 1 of indazolinone ring are associated with an improved antichagasic activity. Moreover, compounds of series **A** displayed low unspecific toxicities against an *in vitro* model of mammalian cells (fibroblasts), which were reflected in high values of the selectivity indexes (SI). Compound **20** was also very efficient against amastigotes from Tulahuen and Y strains of *T. cruzi* ($IC_{50}$ = 0.81 and 0.60 μM, respectively), showing low toxicity towards cardiac cells ($LC_{50}$ > 100 μM). In what concerns compounds of series **B**, some of them displayed moderate activity against trophozoites of a metronidazole-sensitive isolate of *T. vaginalis* (**35** and **36**: $IC_{50}$ = 9.82 and 7.25 μM, respectively), with low unspecific toxicity towards Vero cells. Compound **36** was also active against a metronidazole-resistant isolate ($IC_{50}$ = 9.11 μM) and can thus be considered a good prototype for the development of drugs directed to *T. vaginalis* resistant to 5-nitroimidazoles.



## 1. Introduction

Our research group has been pursuing during the last decade the identification of alternative therapeutic options for parasitic protozoan infections through the design, synthesis and biological activity analysis of novel nitroheterocycles. In these previous studies we reported the activity against *Trypanosoma cruzi* [1–8], *Trypanosoma brucei* [9], *Leishmania* spp. [2,10] and *Trichomonas vaginalis* [1,11–13] of 5-nitroindazole derivatives, mainly 1-substituted indazol-3-ols [11,12], 2-substituted indazolin-3-ones [11], 1-substituted 3-alkoxyindazoles [1–5,7,9], 1,2-disubstituted

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indazolin-3-ones [5,6,8,13] and 2-substituted 3-alkoxyindazoles [5,6,13].

In the present study our efforts have been centered on new indazole derivatives focusing on two parasitic infections, Chagas disease and trichomonosis. The reference drugs for both diseases, i.e., nifurtimox and benznidazole for the former, and metronidazole for the latter, are nitroheterocyclic compounds [14], and furthermore our 5-nitroindazole derivatives have previously shown, as mentioned above, activity against both protozoan parasites.

Chagas disease is a silent chronic infection caused by the trypanosomatid (Kinetoplastid) protozoan parasite *T. cruzi*. This parasitosis, originally endemic to poor rural areas of Latin America, from Mexico to Argentina, is an anthropozoonosis, mainly transmitted in this area by hematophagous triatomine insects [15,16]. The disease is currently endemic to 21 countries, where causes more than 7000 deaths per year and maintains over 25 million people at risk for the infection [17]. Now, owing to intense international migrations and secondary routes of transmission (i.e., transfusion of infected blood, contaminated organ transplantation or congenitally), it can also be found in non-endemic areas such as Western Europe, USA, or Australia [18], currently affecting about 7 million people worldwide [17].

The initial acute phase of Chagas disease, which appears after 1 week of *T. cruzi* infection, has low (<10%) mortality usually related to heart failure and/or meningoencephalitis, but it is often asymptomatic [15,19]. After 1–2 months, immune response partially controls the infection but cannot eradicate it completely. Although the infection persists for life, about 60–70% of untreated infected people never develop clinical manifestations, entering in an indeterminate phase of the disease lacking organ affectation. However, the remaining 30–40%, 10–30 years after initial infection, evolve the symptomatic chronic phase of the disease, characterized by a life-threatening cardiomyopathy and/or severe digestive problems (e.g. megacolon and megaesophagus). In fact, Chagas infection significantly contributes to the global burden of cardiovascular disease [15,16]. The differences noticed in clinical presentations occur as a consequence of six discrete typing units (DTU) of *T. cruzi* (TcI–TcVI), which along the endemic area show differences in their geographical distribution, ecotopes and mammalian host [20].

The drugs currently used to treat Chagas disease, nifurtimox and benznidazole, are decades old and have many limitations, including severe side-effects, low efficacy specially at the later chronic stage of the disease [21], and other important inconveniences such as the different response to the treatment registered among divergent *T. cruzi* strains [20]. Although benznidazole is the first-line treatment in most countries [17], the drawbacks of the current therapy make the search for new drugs urgently needed; it is especially necessary to find effective and safe compounds for the treatment of prevalent late chronic stage of the disease, where the effectiveness of benznidazole, despite some controversial reports [19,21], seems to be very low, especially to prevent heart damage progression [22].

In recent years many compounds exhibiting anti-*T. cruzi* activity, as well as different potential molecular targets of parasite, have been reported [21,23–26].

Antifungal azoles acting as sterols biosynthesis inhibitors such as posaconazole or ravuconazole seemed to be very promising candidates but recent clinical studies have been rather disappointing, showing that azoles are less effective than benznidazole, at least when used as monotherapy [15,27]. In fact, it has recently been pointed out that nitroheterocyclic derivatives still represent the only real alternative in the antichagasic fight [28].

Regarding trichomonosis, *T. vaginalis* (fam. Trichomonadidae) is the causative agent of this sexually transmitted infection (STI) accounting for over 50% of all curable STIs worldwide [29]. According to recent data from the World Health Organization, the number of new cases in adults in 2008 was estimated to be 276.4 million [30].

This protozoan is transmitted only through sexual contact. It is characterized by a wide range of clinical manifestations that can cause severe inflammation of genitourinary passages accompanied by a characteristic vaginal discharge, erythema, pruritus, dysuria, infertility or the formation of small lesions in the cervix. In men, the infection can result in non-gonococcal urethritis (NGU) and impaired sperm viability and motility [31,32]. Human trichomonosis has been associated with various complications such as problems during pregnancy, “low birth weight” (LBW), preterm births, etc. [33]. This infection also increases the risk of development of cervical [34] and prostate [35] neoplasia, and it is also related to a greater predisposition to co-infection with other STIs of bacterial or viral origin [36–38]. However, epidemiological studies have shown that at least half of the infected women and 80% of men do not show symptoms, becoming asymptomatic carriers and potential transmitters of this infection [39].

Trichomonosis is preferably treated with metronidazole, introduced to the market by 1960. Currently, metronidazole and tinidazole, both from the same family of 5-nitroimidazoles [40], are the only two drugs approved by the Food and Drug Administration (FDA) to treat this STI [41]. However, there are no effective alternatives for patients who develop side effects or hypersensitivity, or when its use is contraindicated. Moreover, it is estimated that approximately 5–10% of diagnosed cases of trichomonosis are caused by nitroimidazoles-resistant isolates [42,43].

Therefore, the search for alternative drugs for the treatment of both parasitic pathologies is urgently needed.

In the present study we have synthesized and phenotypically evaluated *in vitro* against *T. cruzi* and *T. vaginalis* two main series of new indazole derivatives: 1-substituted 2-benzyl-5-nitroindazolin-3-ones **6**–**29** (Scheme 1, series **A**) and 3-alkoxy-2-benzyl-2*H*-indazoles **30**–**37** (Scheme 1, series **B**), as well as some other products, i.e., bisindazoles **38** and **39**, and quinazolinones **40**–**43**, arising from the synthetic procedures.

The preparation and study of both series of compounds were planned considering the previously reported activity against *T. cruzi* [5,6,8] and *T. vaginalis* [13] of related compounds containing different moieties at position 2 (Me, Ph, Bn, phenethyl and 2-naphthylmethyl) and alkyl or aryl substituents (Me, Pr, iPr, Bu, Pe, Bn and Ph) at position 1 of indazolin-3-one ring (related to series **A**) or at 3-*O* of 2*H*-indazol-3-ol system (related to series **B**). The most active compounds against *T. cruzi* epimastigotes (**1**–**3**; $IC_{50}$ = 0.93–2.39 μM) [5] and *T. vaginalis* trophozoites (**4**; $IC_{50}$ = 18.51 μM) [13] from previous studies are gathered in Fig. 1.

The current new compounds **6**–**37** have been designed to explore the antiprotozoal activity of indazoles containing at the mentioned 1 or 3-*O* positions unsaturated moieties, ω-substituted alkyl chains supporting different functional groups or acyl and sulfonyl residues. Taking in mind that in previous reports [5,6] 2-benzyl group led to the best antichagasic activities, this substituent was maintained in the current series **A** and **B**. In relation to physicochemical properties, some of these chemical modifications have also been planned in order to improve the low water solubility of the previously studied indazole derivatives [5].

## 2. Results and discussion

### 2.1. Chemistry

Many compounds gathered in Scheme 1 were obtained by alkylation of the previously reported [44] 2-benzyl-5-nitroindazolin-3-one **5** with the required functionalized halides. These alkylation reactions afforded, as previously described [5],