Studies of genotoxicity and mutagenicity of nitroimidazoles: demystifying this critical relationship with the nitro group

Núbia Boechat1/+, Alcione S Carvalho1, Kelly Salomão2, Solange L de Castro2, Carlos F Araujo-Lima1, Francisco VC Mello3, Israel Felzenszwalb4, Claudia AF Aiub4, Taline Ramos Conde5, Helena PS Zamith5, Rolf Skupin6, Günter Haufe6

1Departamento de Síntese de Fármacos, Farmanguinhos 2Laboratório de Biologia Celular, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil 3Departamento de Farmacologia e Toxicologia, Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil 4Organisch-Chemisches Institut, Westfälischen Wilhelms-Universität Münster, Münster, Germany

Nitroimidazoles exhibit high microbicidal activity, but mutagenic, genotoxic and cytotoxic properties have been attributed to the presence of the nitro group. However, we synthesised nitroimidazoles with activity against the trypanosom- tigerotes of Trypanosoma cruzi, but that were not genotoxic. Herein, nitroimidazoles (11-19) bearing different substituent groups were investigated for their potential induction of genotoxicity (comet assay) and mutagenicity (Salmonella/Microsome assay) and the correlations of these effects with their trypanocidal effect and with megazol were investigated. The compounds were designed to analyse the role played by the position of the nitro group in the imidazole nucleus (C-4 or C-5) and the presence of oxidisable groups at N-1 as an anion receptor group and the role of a methyl group at C-2. Nitroimidazoles bearing NO2 at C-4 and CH3 at C-2 were not genotoxic compared to those bearing NO at C-4. However, when there was a CH3 at C-2, the position of the NO2 group had no influence on the genotoxic activity. Fluorinated compounds exhibited higher genotoxicity regardless of the presence of CH3 at C-2 or NO2 at C-4 or C-5. However, in compounds 11 (2-CH3; 4-NO2; N-CH2OHCH2Cl) and 12 (2-CH3; 4-NO2; N-CH2OHCH2F), the fluorine atom had no influence on genotoxicity. This study contributes to the future search for new and safer prototypes and provide.

Key words: nitroimidazoles - genotoxicity - mutagenicity - trypanocidal activity

The class of nitroimidazoles includes compounds that are important antiparasitic agents, which have a broad spectrum of action and high biological activity. For instance, metronidazole (1), secnidazole (2), tinidazole (3), ornidazole (4), dimetridazole (5), carnidazole (6) and panidazole (7) (Fig. 1) are some examples of drugs currently used to treat infections of anaerobic Bacteroides sp. and protozoans, such as Trichomonas sp., Entamoeba sp., Giardia sp. and Histomonas sp. (Yakugaku 1971, William et al. 1975, Buschini et al. 2007, Mital 2009, Valdez et al. 2009). In addition, nitroimidazoles have other interesting properties, including antibacterial and antifungal activities, in the control of fertility (Bone et al. 1997, Cooper et al. 1997), as radiosensitisers (Paul & Abdel-Nabi 2007, Khabnadideh et al. 2009, Lee et al. 2011) and against the recombinant reverse transcriptase of human immunodeficiency virus (HIV)-1 (Silvestri et al. 2002, Al-Soud et al. 2007).

The 2-nitroimidazole benznidazole (BZ) (8) (Fig. 2) and nifurtimox (NFX) are the only available drugs for the treatment of Chagas disease. However, these nitro derivatives exhibit poor activity in the late chronic phase, with severe collateral effects and limited efficacy against different parasitic isolates, justifying the urgent need to identify alternatives to treat chagasic patients (Soeiro & de Castro 2011, Urbina 2014). This disease is caused by Trypanosoma cruzi and affects approximately eight million individuals in Latin America. Furthermore, it is emerging in nonendemic areas, associated with the immigration of infected individuals (Gascon et al. 2010, Schmunis & Yadon 2010, França et al. 2014). Megazol (9) (Fig. 2) is a nitroimidazole-thiadiazole with high in vitro and in vivo activity against Trypanosoma cruzi, including against strains resistant to 8 (Filaridi & Brener 1982, de Castro & de Meirelles 1986, Lages-Silva et al. 1990, Salomão et al. 2010) and Trypanosoma brucei, the causative agent of human African trypanosomiasis (HAT) (Enanga et al. 1998, 2000, Boda et al. 2004). The mode of action of 9 is associated with the interference with the parasite’s oxygen metabolism, as well as acting as a trypanothione scavenger (Viód et al. 1999, Maya et al. 2003). Despite its notable trypanocidal activity, 9 was not approved for clinical use due to reports of in vitro mutagenic and genotoxic effects associated with the reduction of the nitro group (Ferreira & Ferreira 1986, Poli et al. 2002, Nesslany et al. 2004), but the nature of the mutagenic metabolite was not yet characterised.
Several nitroimidazoles possess good oral therapeutic activity against protozoal parasites; however, concerns over toxicity, mutagenicity and genotoxicity have made drug development problematic. These adverse properties appear to be related to DNA damage by the products of the bio-reduction of the nitro group. In fact, positive Ames tests were observed for 1 and 8 using Salmonella typhimurium (Rosenkranz Jr et al. 1976). Despite these results, nitroimidazoles are employed for the clinical treatment of bacterial and protozoal infections and 1 was included the World Health Organization list of Essential Medicines, a list of the arsenal of key antimicrobial drugs (WHO 2011).

Studies of the mutagenicity have shown that there are differences in the ability of mammalian cells, bacteria and protozoa to reduce nitroimidazoles (Moreth et al. 2010). However, to date, there are no conclusive results from analogous studies of mutagenicity performed in vitro in animals or in humans (Paula et al. 2009). Voogd et al. (1979) described the influences of different substituent groups in the nitroimidazole ring on the redox system, while Walsh et al. (1987) group demonstrated that the mutagenic action of 1 is affected by different substituents at position 1 of the imidazole nucleus. For example, compound 10 (Fig. 2), which is a position structural analogue of 1, maintained its biological activity without toxic effects. Although some hypotheses exist, active research in this area has yet to produce a comprehensive mechanism to explain the toxic and therapeutic activities of these compounds. Thus, further studies to elucidate the relationship between the biological activity, genotoxicity and mutagenicity of nitroimidazoles are needed.

Our group has investigated new nitroimidazoles as trypanocidal agents and their mutagenic/genotoxic activities. We have observed that these activities do not only depend on the nitro group (Boechat et al. 2001, Carvalho et al. 2004, 2006, 2007, 2008, 2014, Mello et al. 2013). Encouraged by this observation, in the present work we evaluated the genotoxic, mutagenic and antitypanocidal activities of nine nitroimidazoles (11-19) (Fig. 3). The following key structural aspects of the nitroimidazoles were examined: the importance of the position of the nitro group in the imidazole nucleus (C-4 or C-5), the incorporation of an oxidisable group at N-1 of the nitroimidazole ring, as an anion receptor group and the importance of the methyl group at C-2. This study may serve as a guide to the search for new lead compounds for the chemotherapy of human trypanosomiasis and may also identify safer compounds that may serve as the basis for investigation into the mutagenic activity of nitroimidazoles.

Although the focus of this work is studying the genotoxic and mutagenic effects of different nitroimidazole moieties, the synthesised compounds (11-19) were also assayed against T. cruzi, even though they do not possess the structural requirements required for trypanocidal activity, i.e., two aromatic and/or heteroaromatic rings.

**MATERIALS AND METHODS**

**Chemistry** - 4 or 5-nitroimidazoles with different substituents at the 2 position (11-19) were synthesised following the method described by Skupin et al. (1997). Stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO) and in all subsequent assays control sets with the highest required solvent concentrations (5%) were included.

**In vitro trypanocidal assay** - Bloodstream trypomastigotes of T. cruzi were obtained from mice intraperitoneally inoculated with the Y strain of T. cruzi and were resuspended in Dulbecco’s modified Eagle’s medium plus 10% foetal calf serum at a concentration of 10^7 parasites/mL. This suspension (100 μL) was added to an equal volume of 11 to 19 and incubated for 24 h at 37 ºC. The cell viability was determined using the fluorescein diacetate (FDA)/ethidium bromide (EtBr)-assay, in which viable cells are labelled in green, while dead ones display orange-stained nuclei (Hartmann & Speit 1997).

**In vitro treatment and cytotoxicity assay in whole blood** - Heparinised human blood was obtained by venipuncture immediately before the assays. Whole blood was treated for 2 h at 37 ºC with different concentrations of 9 (380–4,000 μM) and of 11 to 19 (148–6,400 μM). The cell viability was determined using the fluorescein diacetate (FDA)/ethidium bromide (EtBr)-assay, in which viable cells are labelled in green, while dead ones display orange-stained nuclei (Hartmann & Speit 1997).

Whole blood (50 μL) was mixed with an equal volume of a freshly prepared staining solution consisting of 30 μg/mL FDA plus 8 μg/mL EtBr in phosphate-buffered saline (PBS). Samples (50 μL) were spread on a micro-
The codes M0, M1, M2 and M3 correspond to the number of cells within damage classes 0, 1, 2 and 3, respectively. Thus, the total DNA damage score [total arbitrary units (TAU)] for 200 comets range from zero (undamaged) to 600 (maximally damaged). The experiments were performed in triplicate and the statistical significance (p < 0.05) between the treated and control groups was evaluated using Student’s one-tailed t test (Kobayashi et al. 1995, OECD iLibrary 1997).

Salmonella/Microsome mutagenicity test - Four samples with nitro group at positions 4 (16 and 17) and 5 (13 and 18) and different substituent groups at position N-1 were selected for this analysis (Fig. 3). The Salmonella/Microsome mutagenicity test evaluates mutations in the DNA of genetically modified Salmonella enterica serovar Typhimurium strains that lack the ability to synthesise histidine (His). The strains TA97, TA98, TA100, TA102 and TA1535 were utilised following the guidelines for the testing of chemicals (Maron & Ames 1983). At least five strains of bacteria were used, including four strains of S. enterica serovar typhimurium (TA1535; TA1537 or TA97a or TA97; TA98 and TA100).

A volume of 100 µL of the nitroimidazole (25-2,500 µM), 500 µL of PBS and 100 µL of the bacterial suspension (2 x 10⁷ cells/mL) were combined in a test tube. Next, 2 mL of top agar (7 g/L agar; 5 g/L NaCl; 0.0105 g/L L-histidine; 0.0122 g/L biotin; pH 7.4, 45ºC) was added and the mixture was poured into a Petri dish containing minimal agar (15 g/L agar, Vogel-Bonner E medium 10X [10 g/L MgSO₄·7H₂O; 100 g/L C₃H₈O₄H₂O; 500 g/L K₂HPO₄; 175 g/L Na(NH₄)HPO₄·4H₂O] with 20 g/L glucose. The plates were incubated at 37ºC for 72 h and the His revertant colonies were counted. The positive controls included 1.0 µg/plate 4-nitroquinoline-N-oxide (4-NQO) for TA97 and TA98, 0.5 µg/plate sodium azide for TA100 and TA1535 and 0.5 µg/plate mitomycin C for TA102. All of the chemicals were obtained from Sigma Co (USA). DMSO was used as the negative control. The sample was considered positive for mutagenicity when the following conditions were observed: the number of revertant colonies in the assay was at least twice the number of spontaneous revertants [mutagenicity index (MI) ≥ 2], a significant response to the analysis of variance (p ≤ 0.05) was observed and a reproducible, positive dose-response curve (p ≤ 0.01) was obtained.
The MI was calculated by dividing the number of His' colonies induced in the sample by the number of His' colonies in the negative control. All the experiments were performed in triplicate and were repeated twice. Statistical significance was evaluated using analysis of variance, including a one-way ANOVA and Tukey's honestly significant difference HSD post hoc analysis ($p < 0.01$). The mutagenic potency slope was obtained using the Bernstein model (Bernstein et al. 1982).

**Survival experiments -** Quantitative evaluations were performed to determine the cytotoxic effects of the nitroimidazoles by the Ames test. Ten microlitres of each treated bacterial suspension (10⁴ cells/mL) was diluted in a saline solution (NaCl, 9 g/L-0.9%). Then, 100 µL of the solution was placed on a Petri dish containing nutrient agar [0.8% bacto nutrient broth (Difco), 0.5% NaCl and 1.5% agar] and incubated at 37°C for 24 h. The colonies were counted and the survival percentage was determined with respect to the negative control. All the experiments were performed in triplicate and were repeated twice (Aiub et al. 2004).

### RESULTS

The present study focused on the use of SCGE to evaluate DNA damage induced by the nitroimidazoles 11-19 with the objective of mapping genotoxic activity, as displayed in Table I. The treatment of blood cells with 9 over the range of 380-4,000 µM for 2 h at 37°C did not reduce the cell viability. However, a highly significant ($p < 0.01$) genotoxic effect was observed at concentrations higher than of 1,562 µM. The positive control, MMS, was highly active at 160 µM ($p < 0.0001$), causing a clear genotoxic effect with a TAU value of 454.3 ± 9.2. In the range of 148-6,400 µM, the nitroimidazoles 11, 12, 16 and 18 did not alter cellular viability and caused no DNA damage compared to the control group ($p > 0.05$). On the other hand, at the same concentrations, 13, 15 and 19 caused moderate alterations to the DNA, whereas 14, 17 and 19 caused strong alterations of the DNA structure.

The nitroimidazoles were also assayed against *T. cruzi*. Compounds 11-17 and 19 exhibited an IC₅₀ in the range of 902-1,749 µM, whereas for 18, this parameter was > 2,000 µM. Under the same experimental conditions, the values for 8 and 9 were 9.7 ± 2.4 and 9.9 ± 0.8 µM, respectively (Table I). As expected, the compounds were not active trypanocides because they do not possess the structural requirements required for trypanocidal activity, i.e., two aromatic and/or heteroaromatic rings.

Fig. 4 illustrates the mutagenicity and cytotoxicity induced by nitroimidazoles 13, 16, 17 and 18 using *S. typhimurium* strains TA97, TA98, TA100, TA102 and TA1535. At concentrations between 25-2,500 µM, these compounds did not induce frameshift mutations in TA97. However, compounds 17 and 18 were capable of inducing frameshift mutations in TA98, whereas 18 also induced transversions and transitions in TA102 and base pair substitutions in TA1535. Furthermore, all of the nitroimidazoles were capable of inducing dose-dependent base pair substitutions for at least two tested doses in TA100, the most mutagen-responsive strain (Fig. 5). In addition, cytotoxic activity was present only in the higher concentration (2,500 µM) for at least one sample and strain. Using the Bernstein correlation, the number of revertant colonies per µg (rev/µg) was deduced. In addition, higher mutagenic potency correlated with an increased risk of being a mutagen.

### DISCUSSION

Although nitroimidazoles have been clinically used for chemotherapy against several parasites, the mechanisms underlying their genotoxic and biological activities are not fully understood. On the basis of the high activity of 9 against trypanosomatids (Filardi & Briner 1982, Enanga et al. 1998, Darsaud et al. 2004) together with the success of NFX-eflornithine for the treatment of HAT (Yun et al. 2010), the Drugs for Neglected Diseases initiative triggered a systematic review of more than 700 nitroheterocycles (mostly nitroimidazoles) (Torreele et al. 2010). In this context, fexinidazole (5-nitroimidazole), first described in 1978 by Winkelmann and Raether (1978), emerged as a potential candidate. The development of this compound was previously abandoned largely due to the prejudice against nitroaromatic compounds (Patterson & Wyllie 2014). More recently, analysis of pharmacological and toxicological profiles suggested that fexinidazole is a promising candidate for both HAT (Torreele et al. 2010) and Chagas disease (Bahia et al. 2012). These facts and our previous results prompted us to re-evaluate the role of the nitro group in the toxic effects of nitroimidazoles. The presence of a nitro group in a compound can result in several toxicity issues, including genotoxicity and mutagenicity (Walsh & Miwa 2011). However, at the same time, this functional group is necessary for the desired biological activity. Consequently, nitrocompounds are not included in screening libraries due to this unwanted functionality (Brenk et al. 2008) and are not synthesised in medicinal chemistry programs [reviewed in Patterson and Wyllie (2014)].

| Compound | IC₅₀/24 h (µM) | Genotoxic |
|-----------|----------------|-----------|
| 9         | 9.9 ± 0.8      | 3         |
| 11        | 1,690.9 ± 371.8| 0         |
| 12        | 1,147.3 ± 111.6| 0         |
| 13        | 1,749.7 ± 184.6| 1         |
| 14        | 1,170.7 ± 100.5| 3         |
| 15        | 1,585.8 ± 218.3| 1         |
| 16        | 1,132.4 ± 86.3 | 0         |
| 17        | 1,049.4 ± 63.7 | 3         |
| 18        | > 2,000        | 0         |
| 19        | 902.6 ± 83.4   | 1         |

0: undamaged; 1: slightly damaged; 2: moderately damaged; 3: maximally damaged (see Materials and Methods for more details).
This work investigated the importance of different functional groups of C-4 or C-5 nitroimidazoles having oxidisable groups bonded at N-1 on the biological and mutagenic activities of the compounds.

The results of the genotoxicity assays are described in Table I. Nitroimidazoles 11 and 12, which contain a NO2 group at C-4 and a CH3 group at C-2, were not genotoxic compared to 13, 14 and 15, which possess a NO2 group at C-5 and exhibited moderate to high genotoxicity. Nitroimidazole 13 had moderate mutagenic effects. We also observed a comparable result between 11 (2-CH3 and 4-NO2), which was not genotoxic and its analogue 4 (2-CH3 and 5-NO2), which is known to be a mutagenic agent (Ferreiro et al. 2002). However, when the nitroimidazole had no CH3 group at C-2, the position of the NO2 group had no influence on genotoxic activity. This is the case for 16 (4-NO2) and 18 (5-NO2), which exhibited behaviours similar to that of 11 and 12 (no genotoxicity). When comparing pairs of similar compounds, for instance, 16 (4-NO2 and N-CH2OAcCH2F) with 17 (4-NO2 and N-CH2OAcCH2Cl) and 14 (2-CH3; 5-NO2; N-CH2OHCH2OH) with 15 (2-CH3; 5-NO2; N-CH2OHCH2OH), we observed that the presence of fluorine induced genotoxicity. The fluorinated compounds 14 and 17 showed higher genotoxicity regardless of the presence of CH3 at C-2 or NO2 at C-4 or C-5. However, when comparing compounds 11 (2-CH3; 4-NO2; N-CH2OHCH2Cl) and 12 (2-CH3; 4-NO2; N-CH2OHCH2F), the fluorine atom had no influence on genotoxicity.

Four compounds were selected for the Ames assays [nitro group at positions 4 (16 and 17) and 5 (13 and 18) and different substituent groups at position N-1] with the aim of clarifying whether the increase in mutagenicity of nitroimidazoles was dependent only on the position of the nitro group or was related to the presence of more or less reactive halogens.

The results of the cytotoxicity and mutagenicity analyses are described in Fig. 4 and Table II. The four S. typhimurium strains have GC base pairs at the primary reversion site and as a result, it is known that these...
strains may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by S. typhimurium TA102, which has an AT base pair at the primary reversion site (OECD iLibrary 1997). The data showed that 17 is a highly potent mutagen (41.7 rev/µg to TA100), whereas 13, 16 and 18 are low-potency mutagens, causing approximately 8-9 rev/µg. The data in Fig. 5 further corroborate this finding, on the basis that the number of TAU in the comet assay is high for 17, similar to the number of rev/µg.

In conclusion, we observed that the type and position of different substituents bonded to the imidazole ring have a significant influence on the toxicological activity. Whereas nitroimidazoles 11 and 12 were not genotoxic, nitroimidazoles 13, 15 and 19 were moderately genotoxic and mutagenic. Nitroimidazole 16 was neither genotoxic nor mutagenic and 18 was moderately mutagenic but not genotoxic. These results demonstrate that the nitro group is not solely responsible for the mutagenic or genotoxic activity.

Furthermore, the data suggest that the nitroimidazole may be the moiety most likely to be responsible for the genotoxic and mutagenic effects, but in the analogues tested, this moiety was unable to provide anti-T. cruzi activity.

REFERENCES

Aiub CAF, Stankevicins L, da Costa V, Ferreira F, Mazzei JL, Ribeiro AS, de Moura RS, Felzenszwalb I 2004. Genotoxic evaluation of a vinifera skin extract that present pharmacological activities. Food Chem Toxicol 42: 969-973.

Al-Soud YA, Al-Masoudi NA, Hassan HG, De Clercq E, Pannecouque C 2007. Nitroimidazoles. V. Synthesis and anti-HIV evaluation of new 5-substituted piperazinyl-4-nitroimidazole derivatives. Acta Pharm 57: 379-393.

Anderson D, Yu TW, Meggregor DB 1998. Comet assay responses as indicators of carcinogen exposure. Mutagenesis 13: 539-555.

Bahia MT, Andrade IM, Martins TA, Nascimento AF, Diniz LF, Cardosa IS, Talvani A, Trunze BB, Torrelee E, Ribeiro I 2012. Fexinidazole: a potential new drug candidate for Chagas disease. PLoS Negl Trop Dis 6: e1870.

Bernstein L, Kaldor MJ, Pike MC 1982. An empirical approach to the statistical analysis of mutagenesis data from Salmonella test. Mutat Res 97: 267-281.
Boda C, Enanga B, Dumet H, Chauviere G, Labrousse F, Coquet C, Savin S, Houn G, Perie J, Dumas M, Bouteille B 2004. Plasma kinetics and efficacy of oral meglazol treatment in Trypanosoma brucei brucei-infected sheep. Vet Parasitol 121: 213-223.

Boechat N, Carvalho AS, Fernandes-Ferreira E, Soares ROA, Souza AS, Gibaldi D, Bozza M, Pinto AC 2001. Novel nitroimidazoles with trypanocidal and cell growth inhibition activities. Cytobios 105: 83-90.

Bone W, Yeung CH, Skupin R, Haufe G, Cooper TG 1997. Toxicity of ornidazole and its analogues on rat spermatozoa as reflected in motility parameters. Int J Androl 20: 347-355.

Brenk R, Schipani A, James D, Krasowski A, Gilbert IH, Frearson Buschini A, Giordani F, de Albuquerque CN, Pellacani C, Pelosi G, Boechat N, Carvalho AS, Fernandez-Ferreira E, Soares ROA, Souza AS, Ferreira RC, Ferreira LC 1986. Mutagenicity of CL 64855, a potent trypanocidal, cytotoxic and genotoxic activities and their in vitro and in vivo interactions with the 

Buschini A, Giordani F, de Albuquerque CN, Pellacani C, Pelosi G, Rossi C, Zucchi TMAD, Poli P 2007. Trypanocidal nitroimidazole derivatives: relationships among chemical structure and genotoxic activity. Biochim Pharmacol 73: 1537-1547.

Carvalho AS, Gibaldi D, Bozza M, Pinto AC, Boechat N 2006. Synthesis and trypanocidal evaluation of news 5-N-[3-(5-substituted)-1,3,4-thiadiazolyl]mamino-1-methyl-4-nitroimidazoles. Lett Drug Des Discov 3: 98-101.

Carvalho AS, Menna-Barreto RFS, Romeiro NC, de Castro S, Boechat N 2007. Design, synthesis and activity against Trypanosoma cruzi of azaheterocyclic analogs of meglazol. Med Chem Res 3: 465-465.

Carvalho AS, Salomão K, de Castro SL, Conde TR, Zamith HPS, Caffarena ER, Hall BS, Wilkinson SR, Boechat N 2014. Megazol and its bioisostere 4H-1,2,4-triazole: comparing the trypanocidal, cytotoxic and genotoxic activities and their in vitro and in silico interactions with the Trypanosoma brucei nitroreductase enzyme. Mem Inst Oswaldo Cruz 109: 315-323.

Carvalho SA, da Silva EF, Santa-Rita RM, de Castro SL, Fragal CAM 2004. Synthesis and antitrypanosomal profile of new functionalized 1,3,4-thiadiazole-2-aroylhydrazo derivatives, designed as non-mutagenic meglazol analogs. Bioorg Med Chem Lett 14: 5967-5970.

Carvalho SA, Lopes FAS, Salomão K, Romeiro NC, Wardell SMVS, de Castro SL, Silva EF, Fragal CAM 2008. Studies toward the structural optimization of new brazilzone-related trypanocidal 1,3,4-thiadiazole-2-aroylhydrazo derivatives. Bioorg Med Chem 16: 413-421.

Cooper TG, Yeung CH, Skupin R, Haufe G 1997. The antifertility potential of nitroimidazoles analogs in rats. J Androl 18: 431-438.

Darsaud A, Chevrier C, Bourdon L, Dumas M, Buguet A, Bouteille B 2004. Megazol combined with suramin improves a new diagnosis index of the early meningo-encephalitic phase of experimental African trypanosomiasis. Trop Med Int Health 9: 83-91.

de Castro SL, de Meirelles MNL 1986. Effect of drugs on Trypanosoma cruzi and on its interaction with heart muscle cell in vitro. Mem Inst Oswaldo Cruz 82: 209-218.

Enanga B, Keita M, Chauviere G, Dumas M, Bouteille B 1998. Megazol combined with suramin: a chemotherapy regimen which reverses the CNS pathology in a model of human African trypanosomiasis in mice. Trop Med Int Health 3: 736-741.

Enanga B, Ndong IMM, Boudia H, Debrueull L, Dubreuil G, Bouteille B, Chauviere G, Labat C, Dumas M, Perie J, Houin G 2000. Pharmacokinetics, metabolism and excretion of meglazol in a Trypanosoma brucei gambiens e primate model of human African trypanosomiasis: preliminary study. Arzneimittelforshung 50: 158-162.

Ferreira RC, Ferreira LC 1986. Mutagenicity of CL 64855, a potent anti-Trypanosoma cruzi drug. Mutat Res 171: 11-15.

Ferreiro GR, Badals LC, Lopez-Nigro M, Palermo A, Mudry M, Elio PG, Carballo MA 2002. DNA single strand breaks in peripheral blood lymphocytes induced by three nitroimidazole derivatives. Toxicol Lett 132: 109-115.

Filardi LS, Brener ZA 1982. Nitroimidazole-thiadiazole derivative with curative action in experimental Trypanosoma cruzi infections. Ann Trop Med Parasitol 76: 293-297.

França RRF, Carvalho AS, Branco FSC, Pinto AC, Boechat N 2014. Inhibidores potentes da enzima esterol 14α-desmetilase contra Trypanosoma cruzi. Rev Virtual Quim 6: 1483-1516.

Gascon J, Bern C, Pinazo MJ 2010. Chagas disease in Spain, the United States and other non-endemic countries. Acta Trop 115: 22-27.

Hartmann A, Speit G 1997. The contribution of cytotoxicity to DNA - effects in the single cell gel test (comet assay). Toxicol Lett 90: 183-188.

Henderson L, Wolfeys A, Fedyk J, Bourner C, Windebank S 1998. The ability of the comet assay to discriminate between genotoxins and cytotoxins. Mutagenesis 13: 89-94.

Khabnadideh S, Rezai Z, Khahi-Nezhad A, Pakshir K, Heiran MJ, Shobeiri H 2009. Design and synthesis of 2-methyl and 2-methyl-4-nitro imidazole derivatives as antifungal agents. Iranian J Pharm Sci 5: 31-36.

Kobayashi H, Sugiyama C, Morikawa Y, Hayashi M, Sofuni T 1995. A comparison between manual microscopic analysis and computerized image analysis in the single cell gel electrophoresis assay. MMS Commun 3: 103-115.

Lages-Silva E, Filardi LS, Brener Z 1990. Effect of the host specific treatment in the phagocytosis of Trypanosoma cruzi blood forms by mouse peritoneal macrophages. Mem Inst Oswaldo Cruz 85: 401-405.

Lee S, Kim S, Yun M, Lee YS, Cho S, Oh T, Kim P 2011. Synthesis and antituberucular activity of monocylic nitroimidazoles: in-sights from econazol. Bioorg Med Chem Lett 21: 1515-1518.

Maron DM, Ames BN 1983. Revised methods for the Salmonella mutagenicity test. Mutat Res 113: 173-215.

Maya JD, Bollo S, Nuñez-Vergara LJ, Squealla JA, Repetto Y, Morrello A, Perie J, Chauviere G 2003. Trypanosoma cruzi: effect and mode of action of nitroimidazoles and nitrofuram derivatives. Biochem Pharmacol 65: 999-1006.

Mello FVC, Carvalho AS, Bastos MM, Boechat N, Aiub CAF, Felzen-szwalb I 2013. Evaluation of genotoxic effects of new molecules with possible trypanocidal activity for Chagas disease treatment. Scientific World Journal 2013: 287319.

Mital A 2009. Synthetic nitroimidazoles: biological activities and mutagenicity relationships. Sci Pharm 77: 497-520.

Moreth M, Ornelas D, Gomes CRB, de Souza MVN 2010. Nitroimidazóis - uma promissora classe de substâncias para o tratamento da tuberculose. Rev Virtual Quim 2: 105-117.

Nesslany F, Brugier S, Mouries M, Le Curieux F, Marzin D 2004. In vitro and in vivo chromosomal aberrations induced by meglazol. Mutat Res 560: 147-158.

OECD iLibrary - Organisation for Economic Cooperation and Development 1997. OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 471: Bacterial Reverse Mutation Test. Available from: oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en.

Patterson S, Wyllic S 2014. Nitro drugs for the treatment of trypanosomatid diseases: past, present and future prospects. Trends Parasitol 30: 289-298.

Paul AK, Abdel-Nabi H 2007. Cancer imaging agents for positron emission tomography: beyond FDG. Curr Med Imaging Rev 3: 178-185.
Paula FR, Serrano SHP, Tavares LC 2009. Aspectos mecanísticos da bioatividade e toxicidade de nitrocompostos. Química Nova 32: 1013-1020.

Poli P, de Mello MA, Buschin A, Mortara RA, de Albuquerque CN, da Silva S, Rossi C, Zucchi TMAD 2002. Cytotoxic and genotoxic effects of megalazol, an anti-Chagas disease drug, assessed by different short-term tests. Biochem Pharmacol 64: 1617-1627.

Rosenkranz Jr HS, Speck WT, Stambaugh JE 1976. Mutagenicity of metronidazole: structure-activity relationships. Mutat Res 38: 203-206.

Salomão K, de Souza EM, Carvalho AS, Silva EF, Fraga CAM, Barbosa HS, de Castro EM 2010. In vitro and in vivo activity of 1,3,4-thiadiazole-2-arylhydrazone derivatives of megalazol on Trypanosoma cruzi. Antimicrob Agents Chemother 54: 2023-2031.

Schmunis GA, Yadon ZE 2010. Chagas disease: a Latin American health problem becoming a world health problem. Acta Trop 115: 14-21.

Silvestri R, Artico M, De Martino G, Ragno R, Massa S, Loddo R, Murgioni C, Loi AG, La Colla P, Pani A 2002. Synthesis, biological evaluation and binding mode of novel 1-[2-(diarylmethoxy)ethyl]-2-methyl-5-nitroimidazoles targeted at the HIV-1 reversed transcriptase. J Med Chem 45: 1567-1576.

Skupin R, Cooper TG, Frohlich R, Prigge J, Haufe G 1997. Lipase-catalyses resolution of both enantiomers of ornidazole and some analogues. Tetrahedron Asymmetry 8: 2453-2464.

Soeiro MNC, de Castro SL 2011. Screening of potential anti-Trypanosoma cruzi candidates: In vitro and in vivo studies. Open Med Chem J 5: 21-30.

Speit G, Hartmann A 2006. The comet assay: a sensitive genotoxicity test for the detection of DNA damage and repair. Methods Mol Biol 314: 275-286.

Torreele E, Trunz BB, Tweats D, Kaiser M, Brun R, Mazuz E, Bray MA, Pécoul B 2010. Fexinidazole - a new oral nitroimidazole drug candidate entering clinical development for the treatment of sleeping sickness. PLoS Negl Trop Dis 4: e2923.

Urbina JA 2014. Recent clinical trials for the etiological treatment of chronic Chagas disease: advances, challenges and perspectives. J Eukaryot Microbiol 62: 149-156.

Valdez C, Tripp JC, Miyamoto Y, Kalisiak J, Hruz P, Andersen YS, Brown SE, Kangas K, Arzu LV, Davids BJ, Gillin FD, Upcroft JA, Upcroft P, Fokin VV, Smith DK, Sharpless KB, Eckmann L 2009. Synthesis and electrochemistry of 2-ethenyl and 2-ethanamine derivatives of 5-nitroimidazole and antimicrobial activity against Giardia lamblia. J Med Chem 52: 4038-4053.

Viodé C, Betache N, Cenas N, Krauth-Siegel RL, Chauvière G, Bakalara N, Périé J 1999. Enzymatic reduction studies of nitroheterocycles. Biochem Pharmacol 57: 549-557.

Walsh JS, Miwa GT 2011. Bioactivation of drugs: risk and drug design. Annu Rev Pharmacol Toxicol 51: 145-167.

Walsh JS, Wang R, Bagan E, Wang CC, Wislock P, Miwa GT 1987. Structural alterations that differentially affect the mutagenic and antitrichomonal activities of 5-nitroimidazoles. J Med Chem 30: 150-156.

WHO 2011. 17th list of essential medicines. Available from: whqlibdoc.who.int/hq/2011/a95053_eng.pdf.

William J, Ross WJ, Jamieson WB 1975. Antiparasitic nitroimidazoles. 8. Derivatives of 2-(4-formylstyril)-5-nitro-1-vinylimidazole. J Med Chem 18: 2158-2161.

Winkelmann E, Raether W 1978. Chemotherapeutically active nitrocompounds. 4. 5-nitroimidazoles (Part II). Arzneimittelforschung 28: 739-749.

Yakugaku Z 1971. Studies on chemotherapeutic drugs. VII. Synthesis of nitroimidazole derivatives. 2. Preparation of 4(or 5)-nitro-5(or 4)-styrilimidazole derivatives and 4 (or 5)-((beta-phenyl-beta-hydroxy)ethyl)-5 (or 4)-nitro-imidazole derivatives and their antimicrobial activity. J Pharm Soc Japan 91: 231-239.

Yun O, Priotto G, Tong J, Flevaud L, Chappuis F 2010. NECT is next: implementing the new drug combination therapy for Trypanosoma brucei gambiense sleeping sickness. PLoS Negl Trop Dis 4: e2720.