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Cytotoxic, mutagenicity, and genotoxicity effects of guanylhydrazone derivatives

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ABSTRACT

Several studies have reported that guanylhydrazones display a variety of desirable biological properties, such as antihypertensive, antibacterial, and antimalarial behaviour. They furthermore promote anti-pneumocystosis and anti-trypanosomiasis, exhibit antitumor activity, and show significant cytotoxicity against cancer cell lines. In this work, we have evaluated the cytotoxicity, mutagenicity, and genotoxicity of two guanylhydrazones derivatives, (*E*)-2-[(2,3-dimethoxyphenyl) methylene] hydrazine carboxymidamide hydrochloride (2,3-DMeB) and (*E*)-2-[(3,4-dimethoxyphenyl) methylene] hydrazine carboxymidamide hydrochloride (3,4-DMeB), in different biological models. Both 2,3-DMeB and 3,4-DMeB induce weak cytotoxic and mutagenic effects in bacteria and yeast. The genotoxicity of these compounds was determined in a fibroblast cell line (V79) using alkaline comet assay, as well as a modified comet assay with bacterial enzymes formamidopyrimidine DNA-glycosylase (FPG) and endonuclease III (EndoIII). Both guanylhydrazone derivatives induced DNA damage. Treatment of V79 cells with EndoIII and FPG proteins demonstrated a significant effect of 2,3-DMeB and 3,4-DMeB with respect to oxidized bases. In addition, the derivatives induced a significant increase in the frequency of micronucleated cells at high doses. The antifungal and anti-trypanosomal properties of these guanylhydrazone derivatives were also evaluated, and the obtained results suggest that 2,3-DMeB is more effective than 3,4-DMeB. The biological activity of 2,3-DMeB and 3,4-DMeB may thus be related, at least in part, to their oxidative potential, as well as to their ability to interact with DNA. Considering the previously reported *in vitro* antitumor activity of guanylhydrazone derivatives in combination with the lack of acute toxicity and the fact that DNA damage is only observed at high doses should render both compounds good candidates for *in vivo* studies on antitumor activity.

1. Introduction

As guanylhydrazones promiseinteresting chemical and biological potential they have been widely studied. A common feature of guanylhydrazones, as well as biguanidines, is the presence of amidine groups attached to the aliphatic or aromatic structures [1]. Guanylhydrazones moreover contain an amidine (guanyl) group attached to the hydrazone moiety [1,2].

Given that the synthesis of guanylhydrazones is very easy, and the production costs corresponingly low [3], the research and development of guanylhydrazone-based therapeutic agents is highly attractive. Additionally, these substances play an important role as intermediates in the synthesis of heterocyclic polyfunctional nitrogen-containing compounds [4]. In recent decades, hundreds

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of guanylhydrazone derivatives have been synthesized, and their structure-activity relationship has been examined [2,5–8].

Many studies have reported a variety of desirable biological properties for guanylhydrazones, e.g. antihypertensive, antibacterial, and antimalarial behavior, as well as the promotion of anti-pneumocystis and anti-trypanosomiasis [5–8]. A guanylhydrazone with especially interesting pharmacological activity is guanabenz (Wytensin®), an active central α2-adrenoceptor agonist used as antihypertensive agent [9].

Drugs containing the guanidine group have moreover been the object of intense clinical evaluation for antitumor therapy [7,8,10–12]. These guanidines have attracted interest due to their antiproliferative activity. An example is methylglyoxal-bis(guanylhydrazone) (MGBG), a potent inhibitor of the biosynthesis of polyamines spermidine and spermine [10]. Polyamines are small cations that are crucial for many biological processes, such as the regulation of cell proliferation and macromolecular synthesis through the stabilization of DNA, as well as the methylation of tRNA [13]. Cytotoxic effects have been suggested for MGBG, arising from inhibited mitochondrial respiration, which consequently reduces ATP production [14]. Cell death is also induced by *m*-iodobenzylguanidine (MIBG) and *N*-(6-(4-chlorophenoxy)hexyl)-N9-cyano-N0-4-pyridylguanidine (CHS 828). This can be, in part, attributed to the early and rapid decrease of ATP levels and to inhibited mitochondrial respiration, which leads to an inhibition of the energy-dependent activation of caspase-mediated DNA fragmentation [10]. In addition, other guanylhydrazones exhibit antiproliferative effects, which are associated with blocking the cell cycle progression, cell accumulation occurring during the G2/M phase, a marked reduction in mitochondrial transmembrane potential, or a decrease in intracellular ATP content [8,11,15].

In the context of antitumor effects, it should also be noted that the steroid FG, a funtumine derivative substituted with a guanylhydrazone moiety, is able to induce senescence and telomere shortening in tumor cells [12,16]. More recently, the novel bis-guanylhydrazone [4,4′-diacetyldiphenylureabis(guanylhydrazone) guanylhydrazone] has been described as a potent and selective inhibitor of Chk2p, a protein kinase involved in the ATM-dependent checkpoint pathway [12].

On account of the wide-ranging biological activity of guanylhydrazone derivatives and their low cost of production, [3] we synthesized a series of guanylhydrazones. Among these, hydrochloride (*E*)-2-[(2,3-dimethoxyphenyl) methylene] hydrazine carboxymidamide hydrochloride (2,3-DMeB) and (*E*)-2-[(3,4-dimethoxyphenyl) methylene] hydrazine carboxymidamide hydrochloride (3,4-DMeB) showed antibacterial effects. The difference between these derivatives is the presence of a methoxy group at the 2 or 4 positions of the aromatic ring (Fig. 1).

Considering these previous results pertaining to 2,3-DMeB and 3,4-DMeB, and the lack of data regarding their potential biological and genotoxic effects, this study aimed to investigate the genotoxic potential of 2,3-DMeB and 3,4-DMeB by employing three experimental models: *Salmonella typhimurium*, *Saccharomyces cerevisiae*, and the permanent cell line derived from Chinese hamster lung fibroblasts (V79 cell line). Furthermore, we performed experiments to determine the antifungal and anti-trypanosome activities of 2,3-DMeB and 3,4-DMeB.

2. Materials and methods

2.1. Chemicals

Guanylhydrazone derivatives 2,3-DMeB and 3,4-DMeB were synthesized as previously described by Martins et al. [3]. The structures of the products were confirmed by infrared (IR) and multinuclear NMR (^{1}H and ^{13}C) spectroscopy, in combination with melting point tests and a comparison with literature values. The chemical purity of 2,3-DMeB and 3,4-DMeB was ≥98%. Amino acids, nitrogen bases, methyl methane sulfonate (MMS), hydrogen peroxide (H_2O_2), 4-nitroquinoline-*N*-oxide (4-NQO), morpholinepropanesulfonic acid (MOPS), cyclophosphamide (CP), aflatoxin B_1, and sodium azide were purchased from Sigma (St. Louis, MO, USA). Formamidopyrimidine DNA-glycosylase (Fpg, also known as MutM), and endonuclease III (Endo III, also known as Nth) were obtained from New England BioLabs (Ipswich, MA, USA). Yeast extract, yeast nitrogen base (YNB), bacto-peptone, bacto-agar, and Sabouraud dextrose agar were obtained from Difco Laboratories (Detroit, MI, USA). Dulbecco's modified Eagle medium (DMEM), RPMI 1640 medium, fetal bovine serum (FBS), trypsin–EDTA, L-glutamine, and antibiotics were purchased from Gibco BRL (Grand Island, NY, USA). Itraconazole was purchased from Janssen Pharmaceutical N. V. (Beerse, Belgium). The S9 fraction prepared from the livers of Sprague–Dawley rats, which were pre-treated with the polychlorinated biphenyl mixture Araclor 1254, was purchased from Moltox (Boone, NC, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

For all treatments, 2,3-DMeB and 3,4-DMeB derivatives were dissolved in 10% dimethylsulfoxide (DMSO) in distilled water. The appropriate concentrations in each assay were obtained by dilution of stock solutions immediately prior to use. The final DMSO concentration in the media never exceeded 0.2%, and the negative control was exposed to an equivalent concentration of solvent (negative control).

2.2. Strains and antifungal test

For the determination of *in vitro* antifungal activity, 39 *Candida* strains were used: *Candida albicans* (*American Type Culture Collection* – ATCC 10231, ATCC 18804, ATCC 28367, 0050-L, 0051-L, MG), *C. glabrata* (0030-L, 0013-L, 993, ATCC 2001, MG), *C. guilhermondii* (0031-L, 168, 992), *C. krusei* (ATCC 6258, ATCC 20298, 0037-L, 219, 990, MG), *C. parapsilosis* (ATCC 22019, 4063, 0052-L, 0032-L, II, MG), *C. kefyr* Y-329, *C. tropicalis* (0033-L, 0056-L, ATCC 750, 0055-L) and *C. dubliensis* (22, 23, 25, 27, 28, 29, 0029-L, ATCC 7987). The minimum inhibitory concentration (MIC) of itraconazole and guanylhydrazones was determined by using broth microdilution techniques as described by the *Clinical and Laboratory Standards Institute* (CLSI) for yeast M27-A2. The strains were subcultured on Sabouraud dextrose agar for 24 h at 35 °C. The inoculum was suspended in saline solution and adjusted to a final concentration of 0.5–2.5 × 10^3 in RPMI 1640 medium buffered to pH 7.0 with MOPS (165 mM). Cells were treated for 24 h at 35 °C, and after incubation, the MIC was deter-

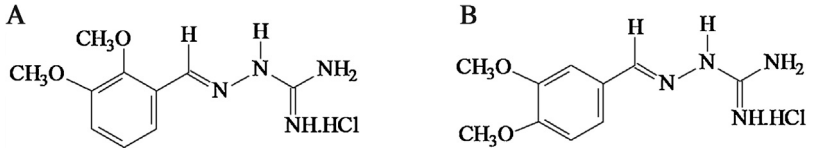

Fig. 1. Chemical structure of 2,3-DMeB (A) and 3,4-DMeB (B).