Antimicrobial and anticancer activities of bisacodyl and its deacetylated metabolite DDPM

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Abstract: The antibacterial activity of bisacodyl and its deacetylated metabolite, DDPM was investigated against Gram-positive pathogens including Staphylococcus aureus (ATCC 9144), Micrococcus luteus (LB14110), Salmonella enterica (NCTC 6017) with minimal inhibitory concentration (MIC) ranging from 12.5 to 25 µg/mL, 6.25 to 12.5 µg/mL and 25 to 50 µg/mL respectively. The results of antibacterial activity against Gram-negative foodborne Pseudomonas aeruginosa (ATCC 9027), Escherichia coli (ATCC 8739), show MIC values between 12.5-25 µg/mL and 25-50 µg/mL. The antifungal activity was also evaluated against the opportunistic pathogenic yeast Candida albicans (ATCC 2091) with MIC ranging from 12.5 to 25 µg/mL. Furthermore, the anticancer activity was evaluated against epithelial cervical cancer cell line (ATCC, Manassas, VA, USA). The results obtained indicated that bisacodyl and its deacetylated metabolite, DDPM have a cytotoxic activity at 63.69 µg/mL and 16.7 µg/mL respectively.

Keywords: Bisacodyl, DDPM, antibacterial activity, antifungal, anticancer

1. Introduction

Bisacodyl, a pyridylphenylmethane derivative, is a stimulant laxative, frequently prescribed to treat functional constipation and/or evacuate the bowels before colonoscopy. After oral administration bisacodyl is metabolized into the active metabolite bis (p-hydroxyphenyl)pyridyl-2-methane (DDPM) by intestinal brush border and/or bacteria present in the large intestine. DDPM inhibits the reabsorption of water and electrolytes and increases their secretion into the intestinal
lumen, by stimulating the epithelial cells into the large intestine [1-2]. (Scheme 1) Despite the numerous publications in the literature concerning the mechanism of laxative action of bisacodyl, there is still a lack of adequate clinical data.

Previous studies have revealed that oral administration of bisacodyl induces Na/K-ATPase pump inhibition and increased production of prostaglandin E₂ (PGE₂). These effects lead to an increased osmotic pressure in the intestinal tract, resulting in increased secretion of electrolytes, such as Na⁺ and K⁺ as well as water, contributing to the laxative effect [3-4]. Other studies have shown that the laxative effect of bisacodyl is related to a decrease in the expression level AQP3 of aquaporin-3 in mucosal epithelial cells of the colon, resulting in the inhibition of water transfer from the tract, intestinal to the vascular side leading to the development of diarrhea [5].

Bisacodyl has been also described for its antibacterial, [6] anti-inflammatory, [7] and anticancer activities [8-9]. In 2015 Sylla and co-workers described for the first time the antibacterial activity of bisacodyl against Gram-positive and Gram-negative pathogenic strains [6]. Bisacodyl showed an excellent antimicrobial activity (MIC values of 6.25-12.5 µg/mL; 3.125-12.5 µg/mL and 6.25-12.5 µg/mL) against Gram-positive strains Micrococcus luteus, Staphylococcus aureus, and Gram-negative strains Salmonella enterica were used for inhibitory tests, using levofloxacin and the fusidic acid, a broad spectrum antibiotics, as a control. The second method concerns a Friedel-Crafts hydroxyl-alkylation between of 2-pyridinecarboxaldehyde with phenol under acid catalysis. The low yield obtained of 40% could be explained by the formation of α,β regioisomer.

![Scheme 1: Preparation of the bis (p-hydroxyphenyl)pyridyl-2-methane (DDPM)](image)

Biological studies

**Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)**

Bisacodyl and its metabolite DDPM were screened for antibacterial activity against Gram-positive and Gram-negative pathogens. Gram-positive strains Micrococcus luteus, Staphylococcus aureus, Gram-negative strains Escherichia coli, Pseudomonas aeruginosa, and Salmonella enterica were used for inhibitory tests, using levofloxacin and the fusidic acid, a broad spectrum antibiotics, as a control. The minimal inhibitory concentration and minimal bactericidal concentration values are presented in Table 1. The MIC and MBC values for levofloxacin and fusidic acid were found to be < 1.52 µg/mL and ranging from 12.5 µg to 25 µg/mL.

Bisacodyl and its metabolite DDPM showed a good antimicrobial activity. They seem to be more...
bacteriostatic than bactericidal, because MBC/MIC ratio is greater than or equal to four (≥4). Even if no significant difference in activity against Gram-positive or Gram-negative bacteria was observed, Gram-positive strains seem to be more sensitive than Gram-negative strains. Consequently, these results suggest a potential use against Gram-positive bacterial infections for these compounds. Bisacodyl and DDPM showed comparable results with values of minimal inhibitory concentration against Gram-positive pathogens Staphylococcus aureus and Microoccus luteus, ranging from 25 to 50 μg/mL and 6.25 to 12.5 μg/mL respectively. Concerning the antibacterial activity against Gram-negative foodborne Pseudomonas aeruginosa, Escherichia coli, Salmonella enterica both compounds exhibited MIC values between 25-50 μg/mL. In the case of the antifungal activity evaluated against the opportunistic pathogenic yeast Candida albicans, the DDPM showed an activity greater than that of bisacodyl with a MIC ranging between 12.5 to 25 μg/mL.

### Table 1. Antimicrobial activities of bisacodyl and its deacetylated metabolite DDPM. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in μg/mL.

| Compound | Gram (+) | Gram (-) | Yeast |
|----------|----------|----------|-------|
|          | Micrococci | Staphylococci | E. Coli | Pseudomonas | Salmonella | Candida |
| Bisacodyl | [6.25-12.5] | [25-50] | [25-50] | [25-50] | [25-50] | [25-50] |
| MBC      | >100     | >100     | >100   | >100   | >100   | >100   |
| DDPM     | [6.25-12.5] | [12.5-25] | [25-50] | [25-50] | [25-50] | [12.5-25] |
| MBC      | 100      | >100     | >100   | >100   | >100   | >100   |
| Levofloxacin | <1.52 | <1.52   | <1.52  | <1.52  | <1.52  | <1.52  |
| MBC      | <1.52   | <1.52   | <1.52  | <1.52  | <1.52  | <1.52  |
| Fusidic acid | [12.5-25] | [12.5-25] | [12.5-25] | [12.5-25] | [12.5-25] | [12.5-25] |
| MBC      | 100      | 100      | >100   | >100   | >100   | >100   |

**Anticancer activity:**
The anticancer activity was evaluated on human cell lines HeLa (epithelial cervical cancer cell line) (ATCC, Manassas, VA, USA). The obtained results are presented in table 2 and figure 1.
Table 2. Anticancer activities of bisacodyl and its deacetylated metabolite DDPM (IC50) in µg/mL

| Compound       | IC50 (µg/mL) |
|----------------|--------------|
| Bisacodyl      | 63.69 ± 6.48 |
| DDPM           | 36.17 ± 5.57 |

The DDPM showed a cytotoxic activity greater than bisacodyl with an IC50 of 36.17 ±5.57 µg/mL.

3. Materials and Methods

Experimental

Materials:
All reagents were obtained from commercial sources unless otherwise noted and used as received. All reactions were monitored by analytical thin layer chromatography (TLC). TLC was performed on aluminium sheets precoated silica gel plates (60 F254, Merck). TLC plates were visualized using irradiation with light at 254 nm or in an iodine chamber as appropriate.

Physical measurements:
The structure of the products prepared by different methods was checked by comparison of their NMR, IR and MS data and by the TLC methods. TLC was visualized using irradiation with light at 254 nm or in an iodine chamber as appropriate.

Figure 2: Convention adopted to assign signals of NMR spectra

Low-resolution mass spectra (LRMS) resulting from ionization by electronic impact. Infrared spectra were recorded over the 400-4000 cm−1 range with an Agilent Technologies Cary 630 FTIR/ATR/ZnSe spectrometer.

4,4′-(pyridin-2-ylmethylene)diphenol (DDPM)

Method 1: To a 250 mL, there-necked flask under 0-5 °C containing 23.92 g phenol (0.25 mol, 3 equiv), concentrated H2SO4 was added dropwise under agitation. The mixture was stirred during 2 h. Then, the solution was brought to a temperature between 5 and 15 °C, and pyridine-2-carboxyaldehyde (0.084 mol, 9 g, 1 equiv) was added dropwise and the reaction mixture was stirred during 1 h at room temperature. The reaction mixture was cooled with an ice bath. At 0 to 10 °C, 40 mL of water were added, and the mixture was stirred for 10 min. Then pH was adjusted to 7 with 2 M NaOH solution and stirred again for 2 h. AcOEt was added and stirring continued for 10 min. 5.8 g of the desired recrystallized compound was obtained after addition of ethanol with 35% of yield.

Method 2: To an aqueous solution of KOH (99.60 mmol, 5.4 g, 4 equiv) containing 10% of EtOH was added the 4,4′-(pyridin-2-ylmethylene) bis(4,1-phenylene) diacetate (24.90 mmol, 9 g, 1 equiv) at 0 °C. The reaction was stirred at room temperature during 72 h. After reaction completion, HCl was added to the mixture until pH = 1-2, then alkalinized with Na2CO3 saturated solution until pH = 8-9. The mixture was extracted with EtOAc and organic phase was washed with water. The combined organic extracts were dried over anhydrous MgSO4, filtered and concentrated to afford 6.40 g of 4,4′-(pyridin-2-ylmethylene) diphenol as a white solid. Yield 92%. RI = 0.4, SiO2 (CH2Cl2/EtOAc 60:40). m.p : 252 °C (melting point from literature : 248-250°C) 1H NMR (400 MHz, DMSO-d6) : δ (ppm) 9.27 (s, 1H), 8.45 (dd, J1 = 4.8 Hz, J2 = 1.04 Hz, 1H, H12), 7.69 (td, J1= 7.68 Hz, J2= 1.88 Hz 1H, H10), 7.18 (m, 2H, H9,H11), 6.91(dt, J1= 8.56 Hz, J2 = 3.28 Hz, 4H, H3, H7, H3', H7'), 6.62 (dt, J1= 8.56 Hz, J2= 2.92 Hz, 4H, H4, H6, H4', H6'), 5.41(s,1H, H8), IR (cm-1): 3302 (vO-H), 3030 (vCsp2-H), 2977, 2924 and 2890 (vCsp3-H), 1611, 1593, 1510 and 1469 (vasym C–N); 1174 (v C–O)
In vitro antibacterial activity

Bacteria and growth conditions
Microorganism growth inhibition assays were performed using LB (1% Bactotryptone, 0.5% Yeast extract, 0.5% NaCl) cultures of Gram-positive pathogens: Staphylococcus aureus (ATCC 9144), Micrococcus luteus (LB14110) and Gram-negative pathogens: Salmonella enterica (NCTC 6017), Pseudomonas aeruginosa (ATCC 9027), Escherichia coli (ATCC 8739). The antifungal activity was investigated against the opportunistic pathogenic yeast Candida albicans (ATCC 2091).

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)
The compounds, dissolved in dimethylsulfoxide (DMSO), were tested in triplicate, using microplate dilution method. Minimal inhibitory concentrations (MICs) of compounds were determined according the National Committee for Clinical Laboratory Standard (NCCLS, 2002). The test was performed in sterile 96-well microplates. The compounds were dissolved in dimethylsulfoxide (DMSO). Serial two-fold dilutions of each sample to be evaluated were made to yield volumes of 100 μL per well with final concentrations ranging from 200 to 1.152 μg/mL. 100 μL of bacteria suspension with a concentration of 10^7 CFU/mL were added to each well. Negative control wells contained bacteria only in LB broth medium. After incubation at 37 °C for 20 h, the minimal inhibitory concentrations (MICs) were recorded as the lowest concentration of compound in the medium that showed no microbial growth. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to the wells to facilitate reading of the plates. If there is microbial growth, MTT turns to blue if not the medium remains yellow. Solvent medium and positive growth controls were also run simultaneously. Then from each tube, one loopful was cultured on plate count agar and incubated for 24 h at 30 °C. The lowest concentration of the compound supporting no colony formation was defined as the MBC. The levofloxacin, a broad-spectrum antibiotic and fusidic acid, a narrow spectrum antibiotic were used as references.

Anticancer activity
The continuous human cell lines HeLa (epithelial cervical cancer cell line) (ATCC, Manassas, VA, USA) were used to investigate the investigated for cytotoxicity effect of new products. This adherent cell line was grown in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10 % (v/v) fetal calf serum (FCS) (Gibco) and 2 mM L-glutamine (Sigma-Aldrich) in tissue culture flasks (Nunc, Roskilde, Denmark). It was sub cultured twice a weekend kept at 37 °C in a humidified and controlled atmosphere of 95% air and 5% CO2.

MTT cell proliferation assay
The cell proliferation test MTT [3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich) measures the rate of cell proliferation and conversely, the reduction of cell viability when metabolic events lead to apoptosis or necrosis. The yellow compound MTT (Sigma) is reduced by mitochondrial dehydrogenases to the water-insoluble blue formazan compound, depending on the viability of the cells. The cells (3 x 10^4 cells/mL) were grown on microtiter plates (200 μL of cell suspension/well) in 96 well microplates with serial dilutions of the compounds. 48 h later, 10 μL of a MTT solution (5 mg/mL in PBS) were added in each well. The plate was incubated for 4 h at 37 °C in a CO2 incubator. Then, 180 μL of MTT solvent (methanol/DMSO; v/v) were added to each sample. The preparations were mixed vigorously on a plate shaker with the cells containing formazan crystals. When all the crystals were dissolved, absorbance was measured at 570 nm with a microplate reader (Elx 800 microplate reader).

4. Conclusions
We discussed herein the antibacterial, antifungal and anticancer activity of bisacodyl and its deacetylated metabolite DDPM. The obtained results corroborate the therapeutic potentialities of the triarylmethane scaffold in drug discovery process.

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Author Contributions
All authors contributed to the drafting and revision of the article and approved the final version.

Conflicts of Interest
The authors declare no conflict of interest.

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