Mol2Net-04, **2018**, BIOCHEMPHYS-01 (pages 1- x, *type of paper*, *doi: xxx-xxxx*
http://sciforum.net/conference/mol2net-4

SciForum
Mol2Net-04

Antimicrobial and anticancer activities of bisacodyl and its deacetylated metabolite DDPM

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Received: / Accepted: / Published:

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 pyridyldiphenylmethane 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

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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 E_2 (PGE_2). 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, results in the inhibition of water transfer from the tract. intestinal to the vascular side leading to the development of diarrhea [5].

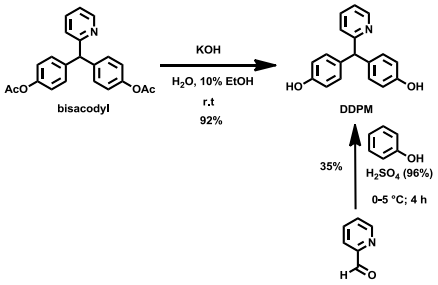
Bisacodyl has been also described for its antibacterial, [6] anti-inflammatory [7] and anticancer activities [8-9] In 2015 Sylla and co-workers described for the first time the antibacterial activity of bisacodyl against Gram-positive and Gram-negative pathogenic strains [6]. Bisacodyl showed an excellent 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*, *Staphylococcus* and *Listeria respectively*. In 2017, the same research group also evaluated the anti-inflammatory activity of bisacodyl derivatives and their corresponding *N*-oxides. The best compounds exhibited anti-inflammatory activities at 10 µM using zebrafish model.

Recently, Zeniou and co-workers described the potentialities of bisacodyl and its desacetylated metabolite DDPM to target glioblastoma cancer stem-like cells in their quiescent, more resistant state [8-9].

Taking into account the literature data concerning the new therapeutic applications of bisacodyl, we report herein the antibacterial activity of bisacodyl and its deacetylated metabolite DDPM using new pathogens strains. The antifungal activity against the opportunist yeast *Candida Albicans* and the anticancer activity against an epithelial cervical cancer cell line are also described.

2. Results and Discussion

4,4'-(Pyridin-2-ylmethylene) diphenol (DDPM) was prepared by saponification from bisacodyl or by Friedel-Crafts reaction from pyridine carbaldehyde. Saponification of bisacodyl was performed in an aqueous KOH solution containing 10% of ethanol to afford the deacetylated bisacodyl in excellent yield of 92% and clean enough to be used without supplementary purification as suggested ^{1}H NMR analysis. 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 *o,p* regioisomer.



Scheme 1: Preparation of the bis (*p*-hydroxyphenyl)pyridyl-2-methane (DDPM)

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 *Micrococcus 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**
		Micrococcus	*Staphylococcus*	*E. Coli*	*Pseudomonas*	*Salmonella*	*Candida*
Bisacodyl	MIC	[6,25-12,5]	[25-50]	[25-50]	[25-50]	[25-50]	[25-50]
	MBC	>100	>100	>100	>100	>100	>100
DDPM	MIC	[6,25-12,5]	[12,5-25]	[25-50]	[25-50]	[25-50]	[12,5-25]
	MBC	100	>100	>100	>100	>100	>100
Levofloxacin	MIC	<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	MIC	[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

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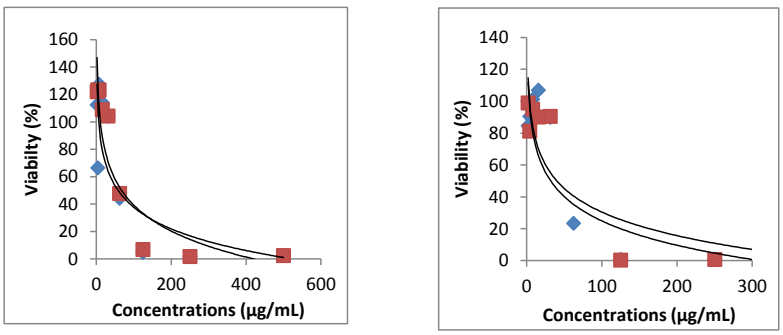

Figure 1. Anticancer effect on epithelial cervical cancer cell line of Bisacodyl (A) and DDPM (B)

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 behaviour. 1H and 13C-NMR spectra were acquired on a Bruker BioSpin GmbH spectrometer 400 MHz, at room temperature. Chemical shifts are reported in δ units, parts per million (ppm). Coupling constants (J) are measured in hertz (Hz). Splitting patterns are designed as follows: s, singlet; d, doublet; dd, doublet of doublets; dm, doublet of multiplets, ddd, doublet of doublets of doublets; m, multiplet; br, broad. Various 2D techniques and DEPT experiments were used to establish the structures and to assign the signals.

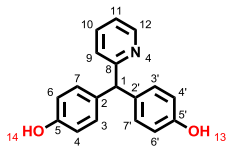

Figure 2: Convention adopted to assign signals of NMR spectra

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 at 0-5 °C containing 23.92 g phenol (0.25 moL, 3 equiv), concentrated H_2SO_4 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 alkalized with Na_2CO_3 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 $MgSO_4$, filtered and concentrated to afford 6.40 g of 4,4'-(pyridin-2-ylmethylene) diphenol as a white solid. Yield 92%. Rf = 0.4, SiO_2 (CH_2Cl_2/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 (νO-H), 3030 (νCsp2-H), 2977, 2924 and 2890 (νCsp3-H), 1611, 1593, 1510 and 1469 (νC=C); 1238 (vasym C−N); 1174 (ν 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-dimethyl thiazol-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% CO_2.

MTT cell proliferation assay

The cell proliferation test MTT [3-(4,5-dimethylthiazolyl-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 CO_2 incubator. Then, 180 µL of medium were removed from each well and 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.

5. Acknowledgments

We gratefully acknowledged Campus France (PHC UTIQUE 2017 37094WH) and the Tunisian Ministry of Higher Education and Scientific Research and Technology for financial support.
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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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