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Conception, Synthesis, Characterization and Antimicrobial Evaluation of New Ferrocene-Based Derivatives Inspired by the Bisacodyl Lead Structure

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Abstract: The antibacterial activity of bisacodyl, a drug used in therapeutic as laxative, and its ferrocenyl analogues was investigated against Gram-positive and Gram-negative foodborne pathogens including *Listeria monocytogenes*, *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enterica*, *Micrococcus luteus* and *Staphylococcus aureus*. The results showed that most of these compounds exhibit an excellent antimicrobial activity, and the bisacodyl analogues seemed to be more bactericides than bacteriostatic.

Keywords: bisacodyl; ferrocene; antibacterial activity

1. Introduction

The leaving behind of the antibiotic discovery area by many pharmaceutical companies is one of the major reasons of the discovery decline. Since the year 2000, only eight antibacterial molecules have obtained a marketing authorization. Moreover, despite the discovery over the last twenty years of compounds with an interesting antibiotic activity, few of them belong to new chemical classes or have the required properties to become drugs or to circumvent resistance problems. One of the approaches to overcome drug resistance is the search for new multi-target inhibitors.[1-3]

At present, in order to accelerate the development of drugs with relatively low costs and reduced risks, pharmaceutical companies develop new approaches from existing drugs. This methodology known as drug repurposing

allows the development of new indications for existing drugs with well-known pharmacokinetic profiles, known safety profile, already solved manufacturing issues. Concerned by the high interest in infectious disease research and considering the forgoing argues, we decided to evaluate the antimicrobial activity of bisacodyl, drug used in therapeutics as laxative. [4-5]

To our delight, the 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). These results encouraged us to develop a series of new analogues. We developed the strategy of incorporating an organometallic ferrocenyl moiety. The use of a ferrocene group to enhance the activity of antibiotics was proposed by Edwards et al. in 1976 [6]. The advantages of the introduction of ferrocenyl moiety to increase the antimicrobial activity have been widely described in the literature [7-12]. The use of ferrocene is especially attractive because it is neutral, chemically stable, a nontoxic molecule and can be easily derivatized or functionalized. Several ferrocenyl compounds have been described for their antitumor, antimalarial or antifungal properties [13-15].

All the ferrocenyl compounds that we have synthesized were characterized and evaluated on pathogen bacteria Gram positive and Gram negative. Finally the antimicrobial effect of bisacodyl and one of its analogues was also estimated [16].

2. Results and Discussion

2.1 Synthesis

The ferrocenyl arylethylpyridines and some corresponding *N*-oxide derivatives were prepared via a McMurry coupling reaction. General synthetic methods to obtain the target compounds are outlined in Scheme 1. The detailed synthesis has been described by us [16]. The key step to obtain the desired olefin intermediates involved a McMurry cross-coupling reaction between the ketone **3** and ferrocenecarboxaldehyde to afford the 2-(1-(4-methoxyphenyl)-2-ferrocenylvinyl)pyridine **4**. The olefins are obtained in two separable *E* and *Z* isomers with 30% and 49% yields respectively. These modest yields results of the possible competition between the formation of the desired cross-coupled product and the two homo-coupled compounds [17].

The demethoxylated compounds **5** and **8** were synthesized by reaction with boron tribromide in dichloromethane. The 2-(1-(4-hydroxyphenyl)-2-ferrocenylethyl)pyridine **8** was obtained in 50% yield (non-optimized yield). The 2-(1-(4-hydroxyphenyl)-2-ferrocenylvinyl) pyridine **5**, was obtained with low yields. The isomerization of the double bond can explain this result. It is known that organometallic complexes adjacent to a double bond advantage the stabilization of α carbenium ions by protonation of the double bond in acidic medium. A similar isomerization of analogous organometallic complexes has been described in the literature [18]. Acetates **6** and **9** were obtained with yields of 96% and 76% respectively. *N*-oxide ferrocenyl derivatives **10** and **11** were prepared from the corresponding ferrocenyl pyridines by oxidation with *m*-chloroperbenzoic acid in dichloromethane at room temperature. Compounds **10** and **11** were isolated after purification by flash chromatography on silica gel with non-optimized

yields of 7% and 16% respectively. All synthesized compounds were biologically evaluated.

2.2 Biological studies

Bisacodyl and its analogues were screened for antibacterial activity against Gram-positive and Gram-negative pathogens using doxycycline, a broad spectrum antibiotic, as a control. The minimum inhibitory concentration and minimum bactericidal concentration were measured for all compounds (Table 1). The MIC and MBC values for doxycycline were found to be <12.5 µg/mL and 12.5 µg/mL on *Staphylococcus aureus*.

Bisacodyl and its ferrocenyl analogues showed an excellent antimicrobial activity. All tested compounds seemed to be more bactericidal than bacteriostatic, because MBC/MIC ratio is less than or equal to four (≤ 4) [19]. Even if no significant difference in activity against Gram-positive or Gram-negative bacteria was observed, Gram-positive strains, *Micrococcus* and *Staphylococcus aureus* seemed to be more sensitive than Gram-negative strains. Consequently, there is a potential use against Gram-positive bacterial infections for these compounds.

In the ferrocenyl arylvinylpyridine series (compounds **4**, **5**, **6** and **10**) compounds **5** and **6** exhibited the greatest antimicrobial activity with MIC values between [12.5-25] µg/mL against *Micrococcus*, *Staphylococcus* and *Listeria*. No difference for antimicrobial activity was observed between isomers **5**, against both gram-positive and gram-negative bacteria. However for compound **4**, the *Z* isomer showed a higher activity compared with its *E* analogue. Compound **4b** exhibited MIC values between [12.5-25] µg/mL against *Micrococcus* and *Staphylococcus* respectively versus [25-50] µg/mL for compound **4a**. Furthermore, when *N*-oxide moiety was introduced (see compound **10**) the antimicrobial activity was comparable with that of compounds **5** and **6**. These results suggested that the functionalization with a hydroxyl group, an acetoxy group or *N*-oxide moiety can have a positive influence for the antimicrobial activity. It has been described in the literature that the introduction of a pyridine *N*- oxide may play an important role in biological activity because it may increase the metabolic stability and bioavailability. [20-22]

In the ferrocenyl arylethylpyridine series (compounds **7-11**), compound **11** exhibited the greatest antimicrobial activity with MIC values between [12.5-25] µg/mL against Gram-positive strains *Micrococcus*, *Staphylococcus* and *Listeria*. Once again these results suggested that *N*-oxide moiety plays an important role in the antimicrobial activity. Compounds **7**, **8** and **9** were less active against *Listeria*, with MIC values between [25-50] µg/mL. Compound **8** showed less anti-*Micrococcus* activity compared to others ferrocenyl analogues (MIC values between [25-50] µg/mL).

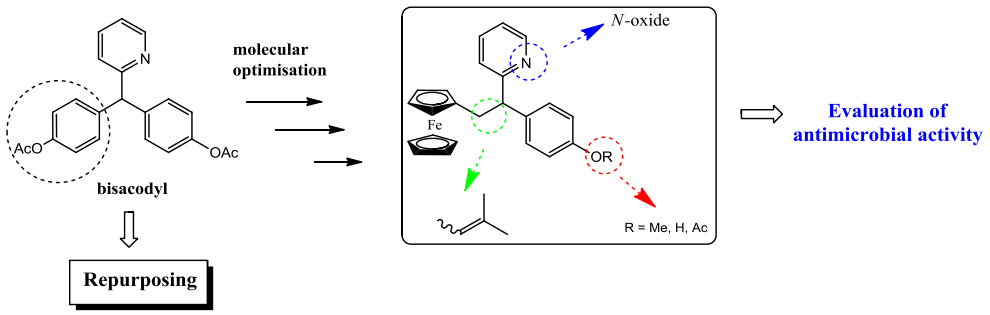
Bisacodyl exhibited the best antimicrobial activity with MIC values between [6.25-12.5] µg/mL; [3.125-12.5] µg/mL, [6.25-12.5] µg/mL for against Gram-positive strains *Micrococcus*, *Staphylococcus* and *Listeria*. Gram-negative strains *Escherichia coli*, *Enterococcus* and *Salmonella* were also sensitive to bisacodyl with MIC values between [3.125-12.5] µg/mL; [3.125-12.5] µg/mL, [6.25-12.5] µg/mL respectively. These results could suggest that an acetoxy group may be necessary to achieve excellent antimicrobial activity.

Finally the antimicrobial effect of bisacodyl and its ferrocenyl acetyl analogue 2-(1-(4-acetoxyphenyl)-2-ferrocenylethyl) pyridine **9** was estimated on *Listeria monocytogenes* and *Salmonella enterica* strains using levofloxacine

and fusidic acid as controls. The details of this estimation were described by us [16]. Antimicrobial effect was estimated (Table 2)

It may be noted that the ferrocenyl derivative **9**, is more active against *Listeria monocytogenes* (gram-positive bacteria) with EC_{50} and EC_{90} of 37 and 71 µM respectively than bisacodyl with EC_{50} and EC_{90} of 46 and 89 µM respectively. However, bisacodyl has a better activity against *Salmonella enterica* (gram-negative bacteria) with EC_{50} and EC_{90} of 50 and 69 µM respectively than compound **9** with EC_{50} and EC_{90} values of 51.5 and 85 µM respectively. According to the obtained results bisacodyl and compound **9** are more active against *Listeria monocytogenes* than *Salmonella enterica*. Therefore, these compounds are promising antimicrobials, more effective against gram-positive than against gram-negative bacteria.

Figure 1. Repurposing and pharmacomodulation of bisacodyl



Scheme 1. Synthesis of ferrocenyl derivatives (a): *n*-BuLi, *p*-anisaldehyde, THF, -78°C / r.t., 17 h; (b): NaOH, O_2, toluene, reflux, 18 h; (c): $TiCl_4$, Zn, THF, reflux, 2h then ferrocenecarboxaldehyde, 8 min; (d): H_2, Pd/C, AcOEt, r.t., 36 h; (e): BBr_3, dichloromethane, r.t., 22 h; (f): Ac_2O, NaOH, 20°C, 2 h for compound **6**, 48 h for compound **9** ; (g): *m*-CPBA, dichloromethane, r.t., 5-12 h.

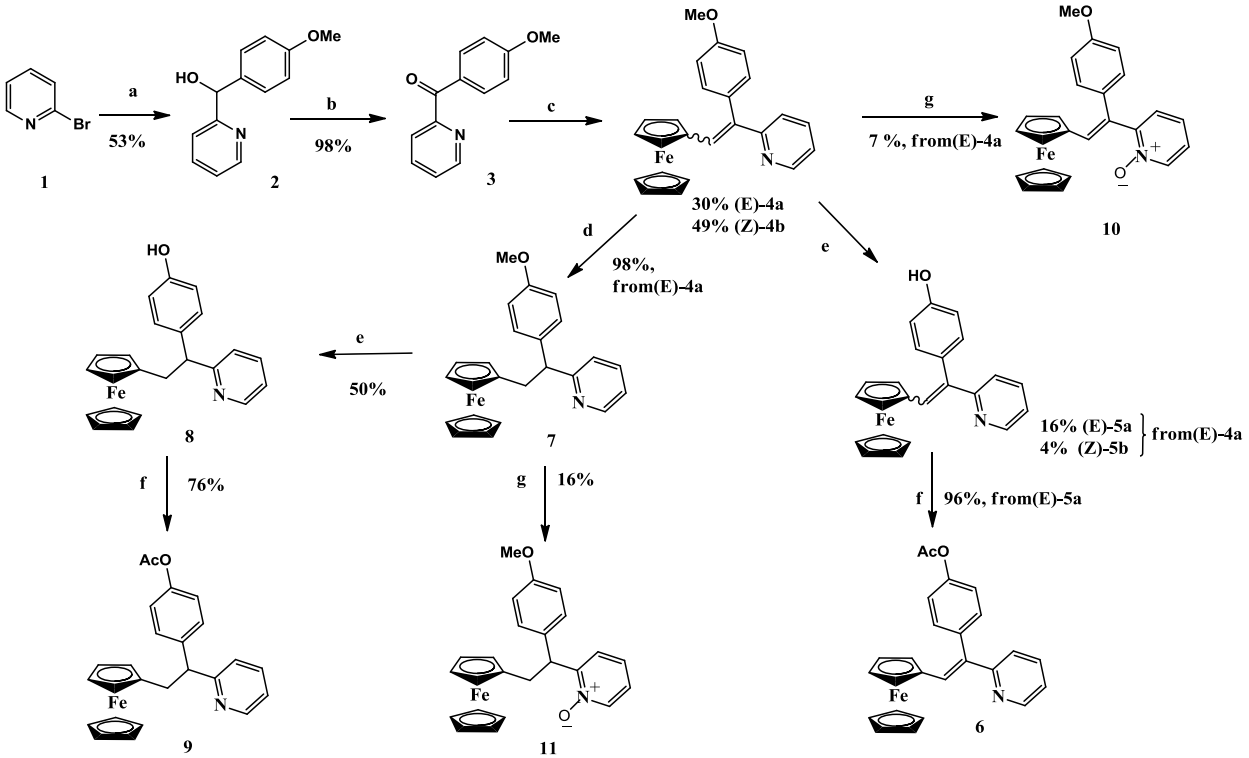


Table 1. Antimicrobial activities of bisacodyl and their ferrocenyl analogues. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in µg/mL.

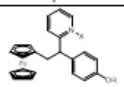
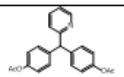
Cpd. Num			Gram (+)			Gram (-)		
			Micrococcus	*Staphylococcus*	*Listeria*	*E. Coli*	*Entero bacterium*	*Salmonella*
4a	*E isomer*	MIC	[25-50]	[25-50]	[25-50]	[25-50]	[25-50]	[12.5-25]
	R=Me	MBC	>100	100	>100	>100	>100	>100
4b	*Z isomer* R=Me	MIC	[12.5-25]	[12.5-25]	[25-50]	[12.5-25]	[25-50]	[25-50]
		MBC	100	>100	50	>100	>100	50
5a	*E isomer* R=H	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
5b	*Z isomer* R=H	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
6	*E isomer* R=Ac	MIC	[12.5-25]	[12.5-25]	[12.5-25]	[25-50]	[12.5-25]	[25-50]
		MBC	>100	>100	>100	>100	>100	>100
10	*E isomer*	MIC	[12.5-25]	[12.5-25]	[12.5-25]	[25-50]	[12.5-25]	[25-50]
	R=Me, X=O⁻	MBC	>100	>100	50	>100	>100	>100
								
7	R=Me	MIC	[12.5-25]	[12.5-25]	[25-50]	[25-50]	[25-50]	[25-50]
		MBC	100	>100	50	>100	>100	50
8	R=H	MIC	[25-50]	[12.5-25]	[25-50]	[25-50]	[12.5-25]	[25-50]
		MBC	>100	25	>100	>100	>100	100
9	R=Ac	MIC	[12.5-25]	[12.5-25]	[25-50]	[25-50]	[25-50]	[25-50]
		MBC	50	>100	50	>100	>100	50
11	R=Me, X=O⁻	MIC	[12.5-25]	[12.5-25]	[12.5-25]	[12.5-25]	[25-50]	[12.5-25]
		MBC	>100	>100	100	>100	>100	>100
bisacodyl		MIC	[6.25-12.5]	[3.125-6.25]	[6.25-12.5]	[3.125-6.25]	[3.125-6.25]	[6.25-12.5]
		MBC	50	12.5	50	>50	50	25
doxycycline		MIC	-	<12.5	-	-	-	-
		MBC	-	12.5	-	-	-	-

Table 2. EC_{50} and EC_{90} values of bisacodyl and its ferrocenyl analogue **9** in µM.

Compounds	**strains**	**EC_{50}**	**EC_{90}**
bisacodyl	*Listeria*	46	89
	Salmonella	50	69
compound **9**	*Listeria*	37	71
	Salmonella	51.5	85
fusidic acid	*Listeria*	38	75
	Salmonella	47	68
levoflaxacine	*Listeria*	3	10
	Salmonella	1	3

3. Materials and Methods

All reagents were obtained from commercial sources unless otherwise noted, and used as received. Heated experiments were conducted using thermostatically controlled oil baths and were performed under an atmosphere oxygen-free in oven-dried glassware. All reactions were monitored by analytical thin layer chromatography (TLC) or by Gas chromatography-Mass spectrometry (GC-MS). TLC was performed on aluminium sheets precoated silica gel plates (60 F_{254}, Merck). TLC plates were visualized using irradiation with light at 254 nm or in an iodine chamber as appropriate. Flash column chromatography was

carried out when necessary using silica gel 60 (particle size 0.040-0.063 mm, Merck).
All synthesized compounds were characterized by NMR, IR, MS data and by the TLC behavior.
The experimental procedures and the characterization have been previously described [16].
In vitro antibacterial activity
Microorganism growth inhibition assays were performed using LB (1% Bactotryptone, 0.5% Yeast extract, 0.5% NaCl) cultures *of Listeria monocytogenes* (ATCC 7644), *Escherichia coli* (ATCC 10536), *Enterococcus faecalis* (ATCC 19434), *Salmonella enterica* (ATCC 13314), *Micrococcus luteus* (ATCC 9341) and *Staphylococcus aureus* (ATCC 6538).

All synthesized compounds 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 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 100 to 12.5 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 30°C for 16 h, the minimal inhibitory concentrations (MICs) were recorded as the lowest concentration of compound in the medium that showed no microbial growth. 3-(4,5-dimethyl thiazol-2-yl)-2,5- diphenyltetrazo lium 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 estimation of the antimicrobial effect against microbial strains was performed by the method of micro dilution in ELISA plates. A stock solutions of the tested products were prepared in DMSO or water, depending on their solubility. In Elisa plates and for each product a series of nine wells containing 100 µL of culture medium with decreasing concentration of the product was prepared by the successive ½ dilution. A 100 µL of overnight shaking microbial culture, incubated at adequate temperature, depending on bacterial strains, was used to inoculate the plate wells containing different concentrations of compounds. The final concentration of each product, for a series of eight wells was 300 µM, 150 µM, 75 µM, 37.5 µM, 18.75 µM, 9.37 µM, 4.68 µM, 2.34 µM and 1.17 µM
The plates were incubated with shaking overnight at the same temperature and their OD was measured at 600 nm. A negative control (uninoculated wells) and a positive control (seeded and without antimicrobial compound wells) were prepared under the same experimental conditions.
The inhibitory activity of the tested compounds was calculated according to the formula:
IA (%) = 100−100 (OD 600 (x) / OD 600 (i))
where (x) is the microbial culture containing the inhibitor and (i) is the microbial culture without inhibitor.

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4. Conclusions

We described the antimicrobial activity of bisacodyl and its ferrocenyl analogues. The compounds were tested on Gram-positive pathogens: *Micrococcus*, *Staphylococcus*, *Listeria* and Gram-negative pathogens, *Escherichia coli*, *Enterococcus* and *Salmonella*. The results obtained revealed that these compounds are potentially more effective against gram-positive than against gram-negative bacteria. The importance of anti-infective research field motivates us to continue the study of based bisacodyl skeleton compounds in order to find new potential antimicrobial molecules. The influence of ferrocene and other chemical substitutions on the antimicrobial activity will be the subject of further investigations.

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Author Contributions
The French team, MG, MS-IV and CF, is responsible for the synthesis and characterization of compounds and the Tunisian team, FT and ME is responsible for microbiological testing. All authors contributed to the drafting and revision of the article and approved the final version.

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Conflicts of Interest
The authors declare no conflict of interest.

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