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Design and synthesis of multifunctional ferrocene derivatives as potent antimicrobial agents

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Abstract: In recent year, the chemistry of ferrocene and the design of new compounds containing the ferrocene unit, has received a surge in interest, owing to their utility in many fields such as organic synthesis, catalysis and biotechnology material. In the framework, of attending a new multifunctional ferrocenyl derivatives, two precursors namely 1,2-dimethyl amine thiophosphine ferrocene (1) and a ferrocene functionalized in 1,2 position by thiophosphine function (2), were fully characterized by HRMS, IR, NMR ^{1}H, ^{13}C, ^{31}P and UV spectroscopy, then were *in vitro* tested for antibacterial activity potential against Gram-negative: *Escherichia coli:* ATCC 25922 (for 1,2), *Klebsiella pneumonia:* ATCC 700603 (for 1,2), *Pseudomonas aeruginosa* : ATTC27853 (for 2), *Proteus Sp* (for 2), *Morganella morganii* (for 1,2), *Enterrobacter sp* (for1) ; Gram-positive: *Staphylococcus aureus:* ATCC 25923 (for 2), *Staphylococcus aureus 2S:* ATCC 43300 (for 1,2), *Bacillus Sp* (for 1,2), fungus: *Aspergillus Niger* (for 2), *Fusarium oxysprium* (for 2), *Alternaria Sp* (for 2) and *Penicillium sp* (for 1,2); using disc diffusion method on solid medium MH and Sabouraud respectively. Obtained results were compared to antibiogram and reveled an interesting antimicrobial potent varying from 7 to 10mm of inhibition for compound 1 and 7 to 15mm of inhibition for compound 2, which indicates mostly an improvement of antimicrobial activity with chiral substitution of ferrocene moiety with amine and thiophosphine functions, this encourage future investigations on chirality and substitution role in the improvement of antimicrobial activity.

Keywords: ferrocene; thiophosphine; planar chirality; antimicrobial activity

1. Introduction

Molecular hybridization of different bioactive agents is a promising approach for developing hybrid compounds with improved therapeutic effects **[1,2].**

The introduction of a ferrocenyl group into drug or drug-like molecules has certainly effect on molecular properties, for example, promising drug candidates have emerged from the field of bioorganometallic chemistry such as ferrocifens and Ru(ii) arene complexes, which are under pre-clinical protocole **[3].**

In fact, Bioorganometallic chemistry investigates the link between classical organometallicchemistry to biology and medicine [4–7], and one of the best studied organometallic compounds is ferrocene [4,8–13]. Thus, some ferrocene conjugates show anticancer, antifungal, antiparasitic and antibacterial properties [14]. However, there is a lack of investigations on the mode of action of ferrocene conjugated to natural products, peptides or other bioactive compounds regarding their
biological activities ,which is of key importance for the design of new drugs to combat infectious

disease [15].

More over, the excessive use of antibiotics nowadays, presents a real potential danger as bacteria are very adaptable and develop rapidly resistances[16] and according to the World Health Organization 558,000 people developed antimicrobial resistant in 2017 [17–19].

In view of this of global warming [20,21] a continuous development of new drugs to fight existing infectious diseases and new pathogens as well as resistance is a great challenge of the 21st century. Especially the search for active substances made by Bioorganisms, so called secondary metabolites [22–24], but also approved drugs or peptides have been derivatized with organometallic moieties resulting in new molecules with interesting biological effects [4–7].

In this perspective, the present work investigated in addition to an efficient regioselective synthesis and structural analysis of ferrocene derivatives, an *in vitro* antimicrobial screening and assessing of corresponding minimal inhibitory concentrations (MIC) of tested compounds on several referential strains, then the comparison of obtained results in order to evaluate the impact of substitution and planar cherality on therapeutic response.

2. Material and methods

All chemicals and reagents used in this work were of analytical grade and purchased from Sigma-Aldrich Company Ltd. (Germany). NMR ADVANCE-DPX 250 and Bruker 300 in CDCl3, HRMS API-365 (Perkin Elmer Sciex) ESI ,Infrared (IR) spectra were recorded with a Shimadzu FTIR-8201 PC spectrometer between 400 and 4000 cm-1 (KBr discs), UV spectra were recorded with a **UV-Vis** Biowave spectrophotometer.

2.1. Chemistry

General procedure: studied compounds (1-2) were synthesized according to published procedures [25, 26].

Briefly, in a Schlenk tube, under argon, were added 13,5g (55,6mmol) de N,N diméthylaminométhyl ferrocéne in 80ml of distilled water, the solution is cooled to -85°C, then 42ml(1,6M) of n-buthyllinium in hexane solution is slowly added, the mixture is stirred for 3h at RT, afterward, it is cooled again at – 85°C , then 27ml (97,2mmol) of distilled diphenylphosphine chloride are added, organic phase is extracted, dried then filtered. 1,7gr (53mmol) of sulfur in 100ml of dichloromethane are added to the obtained solid and the solution is refluxed under Argon for 2h, the crud material is purified by flash chromatography on silica gel to yield 95% and 05% of purred compounds 1 and 2 respectively. The reactions were monitored by NMR.

2.2.6. Antimicrobial activity

The antimicrobial susceptibility and resistance tests of compounds 1, 2 were carried out according to the Agar disc-diffusion testing developed in 1940 [27] and screened against referential strains Gram-negative: *Escherichia coli:* ATCC 25922 (for 1,2), *Klebsiella pneumonia:* ATCC 700603 (for 1,2), *Pseudomonas aeruginosa* : ATTC27853 (for 2), *Proteus Sp* (for 2), *Morganella morganii* (for 1,2), *Enterrobacter sp* (for1) *;* Gram-positive: *Staphylococcus aureus:* ATCC 25923 (for 2), *Staphylococcus aureus 2S:* ATCC 43300 (for 1,2), *Bacillus Sp* (for 1,2), fungus *Aspergillus Niger* (for 2), *Fusarium oxysprium* (for 2), *Alternaria Sp* (for 2) and *Penicillium sp* (for 1,2).

*F*or comparison IPM, SR, CTX and AMX were used as standards.Discs (Whatman No. 1, 6 mm diameter) are impregnated with each extract and then applied to the surface of the specific agar plates which have been seeded by spreading the microbial suspension. The seeding is carried out in such a way to ensure a homogeneous distribution of the bacteria. The petri dishes are incubated during 24 hours at the appropriate temperature 37C° in the laboratory oven, and the resulting inhibition zone diameter was measured in millimeters .

Antimicrobial activity is determined in terms of the diameter of the inhibition zone produced around the discs.

2.3. Statistical analysis of data

Statistical analyses were performed using Excel 2018 software, measurements were realized in triplicate. $p < 0.05$ is regarded as statistically significant.

3. Results and discussion

3.1. Synthesis

Compound 1, was synthesized in a large scale according to a published procedure [25]; and compound 2 have been isolated as a by-product in presence of an excess of sulfur and PPh_2PCl for a long time, leading to target compounds in one pot reactions with good yields. This efficient regioselective reaction introduce easily new functions of interest, in addition to planar chirality to ferrocene scaffold.

3.1.1. (R/S)- 2,2-dimethyl, 1-((2-diphenylthiophosphino) ferrocene) methyl amine

(R/S)-1 $C_{25}H_{26}FeNPS$

Yield: 91%, yellow solid, NMR 1H (300MHz, $CDCl_3$):(ppm):7.77-7.28 (m,10H, PPh_2),4.57- 4.2 (m, 8H, Cp et subst. Cp), 3.52 (d, J=12,5 ,2H,CH_2),1.24 (s, 6H, CH_3). NMR 31P (300MHz, $CDCl_3$):(ppm):41.89 (PPh_2), IR cm^{-1} : 1095 (C=N) , 482 (Fc-), 1392, 1431 (CH_3 deformation), 2781-2954 (CH_3 valence), 3047 *(CH=),* UV λ, nm: 237 (Cp).

3.1.2. (R/S)-1, 2-diphenylthiophosphino – 2(2-diphenyldithiophosphinatomethyl)

ferrocene

(R/S)-2 $C_{35}H_{30}FeP_2S_3$

Yield: 91%, brown solid, NMR 1H (500 MHz,$CDCl_3$): (ppm):7.8-7.7 (m, 8H, Ph), 7.5-7.4 (m, 12H, Ph), 4.87 (dd, JHH = 13.2Hz, JHP=14.0Hz, CH_2), 4.52 (m, 1H, subst. Cp), 4.290(s, 5H, Cp), 4.289 (d, JHH=13.2Hz, CH_2), 4.16 (m, 1H, subst.Cp), 3.78 (m, 1H, subst. Cp). NMR 13C (500 MHz,$CDCl_3$):(ppm): 134.7(d, JCP=87.0Hz, quat. PPh_2), 134.5 (d, JCP =85.1Hz, quat. PPh_2), 134.4(d,JCP=84.0Hz, quat. PPh_2), 133.4(d, JCP =82.7Hz, quat. PPh_2), 132.3(d, JCP =10.6Hz, PPh_2),132.0(d, JCP=10.8Hz, PPh_2), 131.7(d, JCP =3Hz, PPh_2), 131.65(d, JCP =11Hz, PPh_2), 131.6(d, JCP =3Hz, PPh_2), 131.39(d, JCP =3Hz, PPh_2), 131.37(d, JCP =11Hz, PPh_2), 131.34(d, JCP =3Hz, PPh_2), 128.6(d, JCP =13.3Hz, PPh_2), 128.21(d, JCP =13.4Hz, PPh_2), 128.19(d, JCP =12Hz, PPh_2), 128.1 (d, JCP =12.4Hz, PPh_2), 88.6 (dd, JCP =12.0 and 5.4 Hz, quat. Cp), 74.6(d, JCP =11.7 Hz, 2 C subst. Cp), 73.9(d, JCP =97Hz, quat. Cp), 71.0(s, Cp), 69.4 (d, JCP =10.3Hz, subst. Cp), 30.6 (s, CH_2). NMR 31P (500 MHz,$CDCl_3$): (ppm):63.9 (SP(S)Ph_2), 40.8 (FcP(S)Ph_2), HR MS (ESI+): 664.1286 (100%, 664.033 for $C_{35}H_{30}P_2S_3Fe$: M), IR cm^{-1} :*451(*R1R2P(S)S) , 644 (R1R2R3P(S)), 1427 (P-Ph), 706 (S-C), *1574 (aromatics),* 1639 (P-C=C), *818 (H benzen),* 490 (Fc), UV λ, nm: 263.6 (Cp), 250 (Ph).

3.1.3. Bis(3-acetyl-6-methyl-2-oxo-2H-pyran-4-olato)bis(dimethyl formamide) zinc(II)

[Zn(DHA)2.2DMF] **(3).** Yield: 91%, mp=178°C, white solid, IR spectrum, ν, cm–1:1670 (C=O,lactones), 1575 (C=O, acetyl), 3377 (C-O, hydroxyl), 620 (O-M), 1000 (C-O-C). UV λ, nm: 251, 267, 285.

2.5.6. Antimicrobial activity

Antimicrobial potential of ferrocene derivatives 1 and 2 was screened against several referential strains, from which the following results will be discussed:

Gram-negative:

Escherichia coli: ATCC 25922 tested for both compounds 1,2 exhibits a resistance even at high doses.

Klebsiella pneumonia: ATCC 700603 , compound 1 exhibits a better MIC= 3,13µ/ml than compound 1 (MIC= 50µ/ml), however inhibition diameter are substantially the same.

Pseudomonas aeruginosa : ATTC27853 and *Proteus Sp* tested only for compound 2, exhibits the same MIC= 1,56 µ/ml.

Morganella morganii (for 1,2), exhibits a MIC= 0,78µ/ml for compound 1, and was resistant to compound 2.

Enterrobacter sp (for1) ; tested only for 1, exhibits a MIC= 3,13µ/ml.

Gram-positive:

Staphylococcus aureus: ATCC 25923 (for 2), was resistant to tested compound 2.

Staphylococcus aureus 2S: ATCC 43300 tested for both compounds 1,2 exhibit a MIC= 3,13µ/ml for compound 1, and was resistant to compound 2.

Bacillus Sp (for 1,2), was resistant to compound 2, and gave a MIC=1,56µ/ml for compound 1.

Fungus:

Aspergillus Niger (for 2), compound 2gave a MIC=1,56µ/ml.

Fusarium oxysprium (for 2), tested compound 2 exhibits a MIC=3,13µ/ml.

Alternaria Sp (for 2) was resistant to tested compound 2.

Penicillium sp (for 1,2) was resistant to compound 2, on the other hand compound 1 exhibits an interesting MIC=0,78µ/ml .

Therefore, investigations on antimicrobial potential of compounds 1 and 2, exhibit ferrocene moiety functionalized in position 1 by an amine group and position 2 by thiophosphine group mostly as better antimicrobial agent in term of sensitivity and MIC values than when functionalized in position 1 by diphosphine thioyl group and thiophosphine group in position 2, this is probably due to hydrogene binding present in both structures. [15]

3. Figures, Tables and Schemes

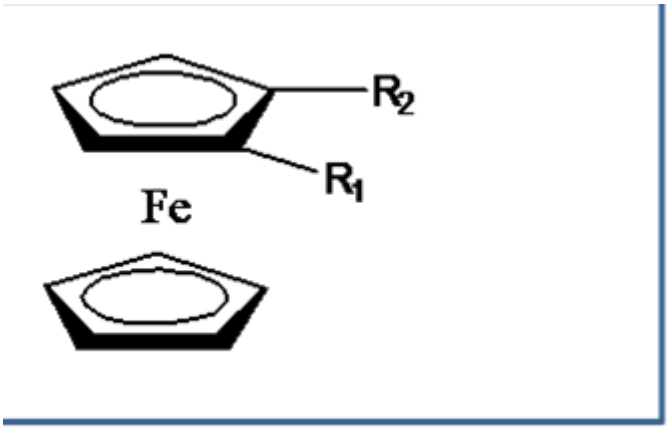

Figure 1: Planar chirality induced by 1,2 functionalization of Ferrocene moiety.

4. Conclusion

Ferrocene planar chiral derivatives 1 and 2 were efficiently synthesized, characterized and fully screened for its antimicrobial potential against several gram+, gram- and fungus referential strains. The obtained bidendate ligands, exhibit mostly better results in case of functionalization with amine and thiophosphine groups. Investigations on molecular structures and comparison with observed effect could help to explain the structure activity relationship that may or not improve observed therapeutically effect of functionalized ferrocene.

This encouraging results lead to invest more ferrocene derivatives as potent antimicrobial agents and face microbial resistant phenomenon in clinical and alimental medium.

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