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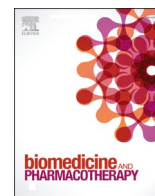
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Review

An overview on the antibacterial properties of juglone, naphthazarin, plumbagin and lawsone derivatives and their metal complexes

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ABSTRACT

Bacterial resistance development represents a serious threat to human health across the globe and has become a very serious clinical problem for many classes of antibiotics. Hence, there is a constant and urgent need for the discovery and development of new effective antibacterial agents to stem the emergence of resistant bacteria. 1,4-naphthoquinones are an important class of natural products and have been known for decades as a privileged scaffold in medicinal chemistry regarding their many biological properties. The significant biological properties of specific 1,4-naphthoquinones hydroxyderivatives have drawn the attention of researchers in order to find new derivatives with an optimized activity, mainly as antibacterial agents. Based on juglone, naphthazarin, plumbagin and lawsone moieties, structural optimization was realized with the purpose of improving the antibacterial activity. Thereupon, relevant antibacterial activities have been observed on different panels of bacterial strains including resistant ones. In this review, we highlight the interest of developing new 1,4-naphthoquinones hydroxyderivatives and some metal complexes as promising antibacterial agents alternatives. Here, we thoroughly report for the first time both the antibacterial activity and the chemical synthesis of four different 1,4-naphthoquinones (juglone, naphthazarin, plumbagin and lawsone) from 2002 to 2022 with an emphasis on the structure-activity relationship, when applicable.

1. Introduction

The discovery of antibiotics (ABs) in the 1940 s is considered the major therapeutic advance of the 20th century. AB has revolutionized the treatment of patients with bacterial infections worldwide. To survive and adapt to environmental changes, bacteria develop different resistance mechanisms in response to the use of antibiotics, which is called antibiotic resistance (ABR) [1]. This phenomenon allows the appearance of new diseases or the reappearance of diseases that were previously controlled by ABs [2]. Control of bacterial infections has become

increasingly tenuous [3], where AB use and misuse have played a key role and are still a major cause of the emerging public health crisis of ABR [4–7]. Moreover, the declining interest in AB research by pharmaceutical industries contributed to an increased occurrence of hard to treat bacterial infections as well [8,9]. For example, daptomycin is one of the last representative of the novel antibiotic class of lipopeptide antibiotics that was introduced to the clinic with a new mechanism of action and marketed in 2003 [10]. The World Health Organization (WHO) has warned that none of the AB currently being developed against ABR bacteria would be enough to tackle this plague that is

Abbreviations: AB, Antibiotic; ABR, Antibiotic resistance; AcOH, Acetic acid; WHO, World health organization; Ac₂O, Acetic anhydride; ADMET, Absorption, distribution, metabolism, excretion and toxicity; B. cereus, Bacillus cereus; B. mycooides, Bacillus mycooides; B. subtilis, Bacillus subtilis; BSA, Bovine serum albumine; BBB, Blood brain barrier; C. albidus, Cryptococcus albidus; CAN, Cerium ammonium nitrate; CHS, Conventional heating synthesis; DBU, 1,8-Diazabicyclo[5.4.0]undec-7-ene; DCC, Dicyclohexylcarbodiimide; DHNA, 1,4-dihydroxy-2-naphthoate; DMAP, 4-dimethylaminopyridine; DMSO, Dimethyl sulfoxide; E. cloacae, Enterobacter cloacae; E. faecalis, Enterococcus faecalis; E. coli, Escherichia coli; K. pneumoniae, Klebsiella pneumoniae; K. oxytoca, Klebsiella oxytoca; MSSA, Methicillin sensitive S. aureus; MRSA, Methicillin resistant S. aureus; M. smegmatis, Mycobacterium smegmatis; M. tuberculosis, Mycobacterium tuberculosis; MIC, Minimal inhibitory concentration; Na₂CO₃, Sodium carbonate; NET₃, Triethylamine; p-TsOH, para-Toluene sulfonic acid; P. mirabilis, Proteus mirabilis; P. aeruginosa, Pseudomonas aeruginosa; RBC, Red blood cells; r.t., Room temperature; S. marcescens, Serratia marcescens; S. aureus, Staphylococcus aureus; S. simulans, Staphylococcus simulans; S. epidermidis, Staphylococcus epidermidis; SAR, Structure-activity relationship; UAS, Ultrasound assisted synthesis; VISA, Vancomycin-intermediate S. aureus.

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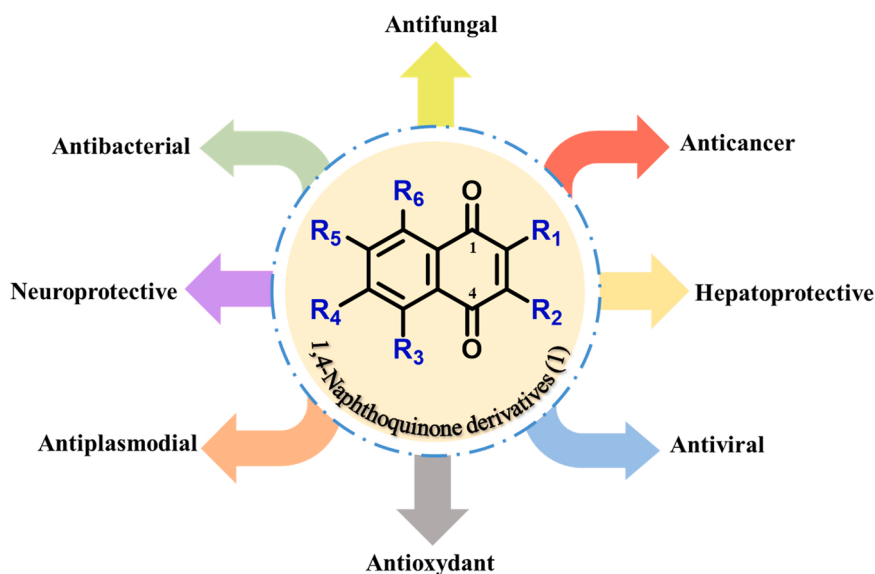


Fig. 1. Some biological properties of 1,4-naphthoquinone.

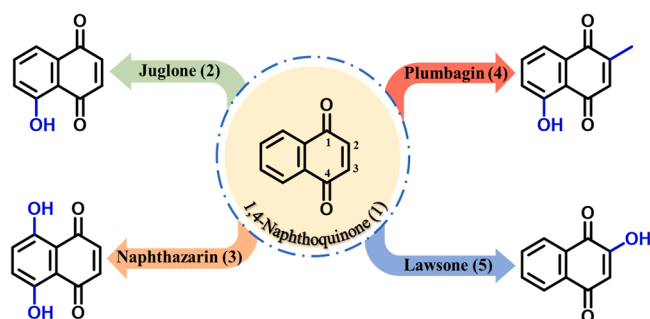


Fig. 2. 1,4-naphthoquinone hydroxyderivatives (1), juglone (5-hydroxy-1,4-naphthoquinone (2), naphthazarin (5,8-dihydroxy-1,4-naphthoquinone (3), plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone (4), lawsone (2-hydroxy-1,4-naphthoquinone (5).

expected to kill millions of people in 2050 [11]. Recent research works suggest that the spread of drug-tolerant bacteria kills around 700,000 people a year, and could reach 10 million by 2050, about the same number of deaths due to cancer each year [12]. Therefore, the development of new classes of molecules that possess antibacterial activities is still considered as an emergency [13].

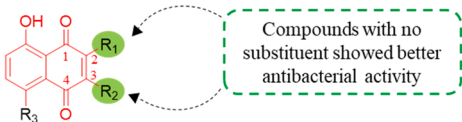
Historically plants have provided a source of inspiration for novel drug compounds and have made a large contribution to human health and well-being [14,15]. Among these compounds, the 1,4-naphthoquinone structure [16–20] (1, Fig. 1,2) has been extensively studied and modified especially in medicinal chemistry due to its various biological properties (antibacterial, antiviral, antifungal, anticancer, antioxidant, hepatoprotective, neuroprotective and antiplasmodial activities) [21–29] (Fig. 1). These structural modifications have led to the development of 1,4-naphthoquinones with broad biological properties or multi-target compounds.

For example, researchers have developed some derivatives showing dual activity as anticancer and antifungal [30] or even a triple activity as antiviral, anticancer and antifungal [31]. Besides the interesting and promising biological activities of 1,4-naphthoquinone, it is important to note that this moiety is small enough to cross the blood brain barrier (BBB). Therefore, it is also considered as an attractive scaffold used to find new drugs for neurodegenerative disorders and has been reported as an acetyl-cholinesterase activity and amyloid β_{42} aggregation inhibitor to treat Alzheimer's disease [27]. The well-known

hydroxyderivatives of 1,4-naphthoquinone belong to one of the largest and diverse groups of plant metabolites [32], occur naturally in leaves, roots, husks and bark of plants especially in Juglandaceae [33], Boraginaceae [34], Plumbaginaceae [35] and Lythraceae [36] families. Over many years these plants have been used as a cosmetic agent for dyeing human keratinous materials and human keratinous fibers such as hair, eyelashes and eyebrows and skin [37–40]. In folk medicine, thanks to their rich composition in 1,4-naphthoquinone and its hydroxyderivatives families [41], plants like Juglandaceae and Plumbaginaceae were used as a topical remedy for dermal inflammation and excessive perspiration of the hands and feet [42]. It is also a common home remedy for the treatment of chronic eczema, dyspepsia, piles, leprosy, anasarca, worms, cough, phlegm, flatulence and biliousness [43–47]. The range of biological effects of naturally occurring and synthetic 1,4-naphthoquinones hydroxyderivatives is diverse and includes anti-proliferative activities [48–52], antibacterial and fungal properties [53–56], inhibition of platelet aggregation [57], anti-inflammatory and antiallergic activity [58,59] as well as antimalarial and antileishmanial activities [60–62]. Hence, all of these examples support that 1,4-naphthoquinone family may be an excellent model for the development of active compounds.

Among this family of compounds, four hydroxynaphthoquinones derivatives were selected for their biological relevance and will be further described in this review: juglone 2 (5-hydroxy-1,4-naphthoquinone), naphthazarin 3 (5,8-dihydroxy-1,4-naphthoquinone), plumbagin 4 (5-hydroxy-2-methyl-1,4-naphthoquinone) and lawsone 5 (2-hydroxy-1,4-naphthoquinone) (Fig. 2). The high reactivity of these naturally occurring hydroxyderivatives (2–5), and the well-established chemical modifications on 1,4-naphthoquinone scaffold raised a huge interest for further development of new types of substances with wide biological applications [63–65].

This is the first time that a review has been conducted on both the chemical synthesis of natural based 1,4-naphthoquinone hydroxyderivatives (2–5) and their antibacterial activities with a thorough interest in structure-activity relationship from 2002 to 2022. The review is divided in two parts. The first part is dedicated to the synthesis of 1,4-naphthoquinone hydroxyderivatives, the description of their antibacterial activities. In line with the expanding interest in metal complexes as drugs, the second part deals with the metal complexes of 1,4-naphthoquinone hydroxyderivatives and their associated antibacterial activity. In both cases, a structure-activity relationship has been carried out when applicable.



Compounds	MIC ($\mu\text{g/mL}$) on <i>S. aureus</i>	
	without BSA	With BSA
3; R ₁ =H, R ₂ =H, R ₃ =OH	60	125
8; R ₁ =H, R ₂ =H, R ₃ =NH ₂	30	60
9; R ₁ =OH, R ₂ =H, R ₃ =NH ₂	30	80

Fig. 3. SAR of juglone derivatives realized by A. Riffel et al. [66].

2. Synthesis, antibacterial activity, structure-activity relationship of juglone, naphthazarin, plumbagin and lawsone derivatives

2.1. Synthesis, antibacterial activity and structure-activity relationship of juglone derivatives

In 2002, A. Riffel et al. developed a new series of compounds derived from juglone [66]. A total of twelve compounds were synthesized according to Farina procedure [68]. The antimicrobial effect of some naphthoquinones has been reported including their structure-activity relationship (SAR) (Fig. 3). This study indicated that compounds with a free keto group at position 1 would be more likely to be active. Moreover, active compounds must possess either no substituent or a mesomeric electron-donating group at R₁ or R₂ positions. It would enhance the hydrogen bonding capacity and allow the compound to bind more strongly to its site of action as compound 8 [66,68].

The best compounds presented MIC values in the 30–125 $\mu\text{g/mL}$ range on *Staphylococcus aureus* (*S. aureus*) isolated from the hospital of Pronto Socorro de Porto Alegre, Brazil, whereas some others showed MIC values higher than 500 $\mu\text{g/mL}$ on the same strain. The most effective compound in this series is the 5-amino-8-hydroxy-1,4-naphthoquinone 8 (Table 1) with a MIC of 30 $\mu\text{g/mL}$ without bovine serum albumin (BSA) and 60 $\mu\text{g/mL}$ with BSA (1%) on clinical isolates strain of *S. aureus* strain [66]. Based on its activity, compound 8 was selected in order to determine its pharmacological properties *in vivo* and its potential for antimicrobial therapy.

The synthesis of 8 is described in Scheme 1. The first step was the treatment of 1,5-dinitronaphthalene 6 with a concentrated fuming sulfuric acid in the presence of elemental sulfur as a reducing agent ($\text{H}_2\text{SO}_4/\text{S}_8$) and H_2O to lead to the formation of 4,8-diaminonaphthalene-1,5-dione 7. Compound 3 was obtained either with the hydrolysis of compound 7 or from 8 using acidic condition and heating. No yield was indicated for the formation of compounds 7 and 3. Compound 8 was obtained by hydrolysis of compound 7 under mild acidic conditions at room temperature (r.t.) in 65% yield after purification on column chromatography. Unfortunately, the synthesis of compound 9 is not described [68].

In 2007, A. Mahapatra et al. also developed a series of plant-derived hydroxynaphthoquinone. 7-methyljuglone scaffold was investigated due to its increased efficiency of redox cycling generating O_2 and H_2O_2 and may thereby cause oxidative stress [69]. During the investigation of South African medicinal plants traditionally used to treat pulmonary diseases, significant antibacterial activity of the crude extract of *Euclea natalensis* was observed and that of diospyrin 10, isolated from this plant, against drug-resistant strains of *Mycobacterium tuberculosis* (*M. tuberculosis*) [70,71] (Fig. 4). A. Mahapatra et al. were inspired by diospyrin 10 and decided to synthesize a series of nineteen 7-methyljuglone derivatives as depicted in Scheme 2, which were then evaluated

for their antibacterial activity. Finally, a study of SAR studies was carried out.

According to SAR studies, compounds 13–15 and 18 exhibited the greatest activity among all the screened derivatives (Fig. 4). Compound 15 was the most potent with a MIC value of 0.5 $\mu\text{g/mL}$ on *M. tuberculosis* H37Rv versus 1 $\mu\text{g/mL}$ for juglone 2 and 8 $\mu\text{g/mL}$ for diospyrin 10 (Fig. 4). The screening results showed that the presence of methyl group at R₂ position, conferred the best MIC value. In the halide series, the activity increased when the halide was bulky and decreased according to the halide electronegativity. Interestingly, the 7-methyl substituted compound 15 showed more potency than its 6-methyl isomer 18, but an opposite behavior has been observed for the 8-chloro derivatives 14 and 17 [69]. However, when it comes to the substrate efficiency, compound 15 was not the most potent substrate for the NADPH-dependent enzyme mycothiol disulfide reductase, which is one of the several potential biological targets for the antitubercular activity of naphthoquinones.

The synthesis of 13–18 is described in Scheme 2. Compounds 13–17 were prepared by the reaction of a phenol derivative 11 with maleic anhydride 12 under Friedel-Crafts conditions using aluminum AlCl_3 and NaCl followed by an acidification with 12 M HCl in moderate yields (25–65%) after crystallization in CH_2Cl_2 . The reductive cleavage of the chlorine substituent of 17 was achieved by treatment with SnCl_2 in 4 M HCl and THF after filtration in FeCl_3 to give compound 18 (65% yield) [72].

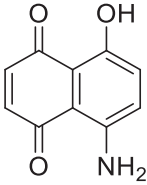
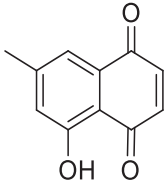
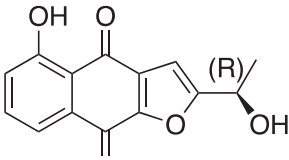
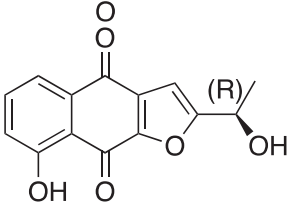
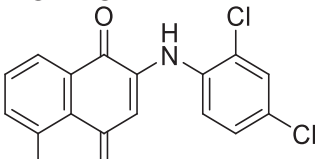
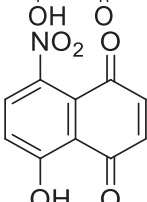
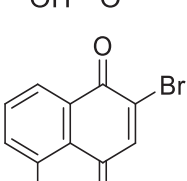
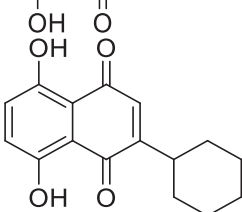
In 2009, M. Yamashita and coworkers developed a series of naphthoquinones based on the naphtho[2,3-*b*]furan-4,9-dione skeleton. The synthesized compounds are secondary metabolites found in the inner bark of *Tabebuia avellanae* and were stereoselectively synthesized and evaluated for their antibacterial activity [73]. All compounds showed a potent to modest antibacterial activity against several Gram-positive bacteria (0.78–6.25 $\mu\text{g/mL}$) in comparison with penicillin G and amphotericin B, two reference antibiotics (0.39–1.56 $\mu\text{g/mL}$) (Fig. 5).

Compounds (R)-24a and (R)-24b showed the best antibacterial activity against different Gram-positive bacterial strains. They presented MICs of 0.78 $\mu\text{g/mL}$ and 1.56 $\mu\text{g/mL}$ respectively against *Bacillus subtilis* (*B. subtilis*) NBRC3134, MICs of 3.13 $\mu\text{g/mL}$ and 6.25 $\mu\text{g/mL}$ respectively against *S. aureus* NBRC13276, MICs of 0.78 $\mu\text{g/mL}$ and 1.56 $\mu\text{g/mL}$ against *Bacillus mycoides* (*B. mycoides*) ATCC 11778 [73] (Table 1). None of the compounds were active on Gram-negative bacteria at concentrations up to 100 $\mu\text{g/mL}$. From these results, it has been deduced that the presence of the hydroxyl group at C₅ position in 1,4-naphthoquinone scaffold slightly contributed toward increasing antimicrobial activity (Fig. 5).

As described in Scheme 3, the synthesis of 24a and 24b started with the commercially available 1,5-hydroxynaphthalene 19, which was oxidized by the air in the presence of CuCl in the dark to afford 2 in 47% yield. Oxidative amination of 2 with dimethylamine (2.0 M solution in THF) in toluene at 40 °C gave 2-dimethylaminojuglone 20a and 3-dimethylaminojuglone 20b in 42% and 11% yields respectively. The deamination of 20a and 20b with 10% aqueous HCl solution quantitatively yielded 2,5-dihydroxy-1,4-naphthoquinone 21a and 21b. Compounds 21a and 21b were reacted with 3,4-dibromobutan-2-one in the presence of DBU in THF to access to compounds 22a and 22b in 70% and 72% yields respectively. Naphthodihydrofurans 22a and 22b were treated with MnO_2 in chloroform to provide the desired naphthofurans 23a and 23b in 71% and 84% yields respectively. The asymmetrical Noyori reduction of compounds 23a and 23b was catalyzed by the chiral Ru(II) complex $\text{Ru}[(R,R)\text{-Tsdpen}]$ (p-cymene), in a formic acid–triethylamine mixture and CH_2Cl_2 , to give the corresponding secondary alcohols (R)-24a and (R)-24b in excellent yields (89% for 24a and 97% for 24b) and good enantiomeric excess (ee) (96% for 24a and 97% for 24b). The corresponding enantiomers (S)-24a and (S)-24b were similarly prepared using $\text{Ru}[(S,S)\text{-Tsdpen}]$ (p-cymene) as a chiral catalyst.

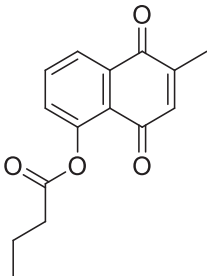
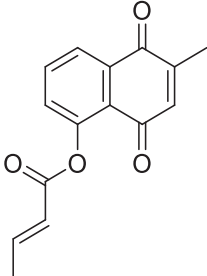
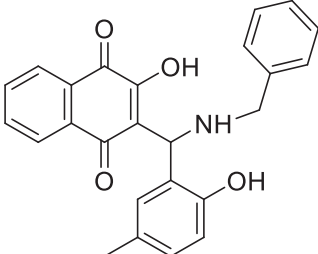
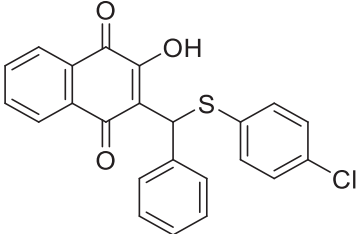
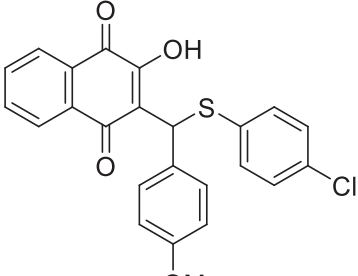
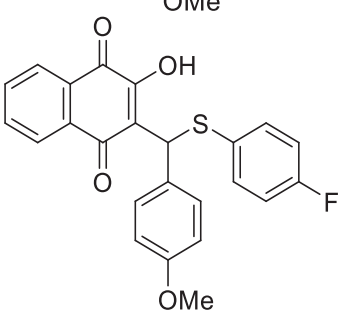
In 2014, L.I. Lopez-Lopez et al. focused their studies on the derivatives of 1,4-naphthoquinone and considered that juglone 2

Table 1
Summary of the most active compounds from the different works reported above.

Structure	Compound	MIC (bacterial strains) ($\mu\text{g/mL}$)	Supposed mechanism of inhibition	Reference
	8	30 <i>S. aureus</i>	Increased efficiency of redox cycling	[66]
	15	0.5 <i>M. tuberculosis</i> H37Rv	Subversive substrates with similar disulfide reductases found in <i>M. tuberculosis</i> .	[69]
	24a	0.78 <i>B. subtilis</i> NBRC3134 3.13 <i>S. aureus</i> NBRC13276 0.78 <i>B. mycooides</i> ATCC 11778	nd	[73]
	24b	1.56 <i>B. subtilis</i> NBRC3134 6.25 <i>S. aureus</i> NBRC13276 1.56 <i>B. mycooides</i> ATCC 11778	nd	[73]
	26	0.49 <i>S. aureus</i> 31.25 <i>E. faecalis</i> ATCC29212 62.50 <i>E. coli</i> ATCC 23922	nd	[74]
	29	63.5 <i>B. cereus</i> ATCC 10876 30.6 <i>S. aureus</i> ATCC 25923 63.5 <i>E. coli</i> ATCC 25922 63.5 <i>K. pneumoniae</i> ATCC 13883	nd	[75]
	45	16 <i>S. aureus</i>	Highly reactive radical anion intermediates, which are responsible for the oxidative stress	[76]
	53	0.5 MSSA 0.5 MRSA	nd	[78]

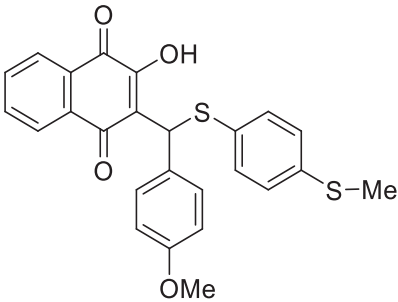
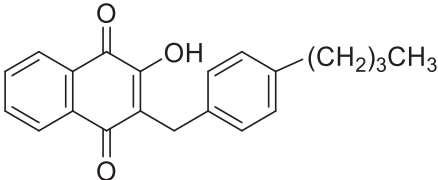
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Table 1 (continued)

Structure	Compound	MIC (bacterial strains) ($\mu\text{g/mL}$)	Supposed mechanism of inhibition	Reference
	59	4.0 <i>M. smegmatis</i> mc ² 155 19.9 <i>M. tuberculosis</i> H37Rv	Inhibition of menaquinone biosynthesis pathway	[79]
	60	4.9 <i>M. smegmatis</i> mc ² 155 3.9 <i>M. tuberculosis</i> H37Rv	Inhibition of menaquinone biosynthesis pathway	[79]
	66	7.7 <i>E. coli</i> ATCC 25922 15.4 <i>S. aureus</i> ATCC 25923 34.6 <i>B. subtilis</i> ATCC 6633	Generation of reactive oxygen species by two successive reduction processes to form radical anion and dianion species that are toxic to bacteria	[80]
	72	32 <i>S. epidermidis</i> ATCC 12228, 32 <i>S. aureus</i> ATCC 25923	Inhibition of biofilm formation of MRSA	[81]
	84	4 <i>E. coli</i> ATCC 25922 8 <i>P. aeruginosa</i> ATCC 27853 16 <i>P. aeruginosa</i> ATCC 15442	Inhibited biofilm formation at the initial and later process of biofilm formation	[83]
	85	8 <i>P. aeruginosa</i> ATCC 27853 16 <i>P. aeruginosa</i> ATCC 15442	Inhibited biofilm formation at the initial and later process of biofilm formation	[83]

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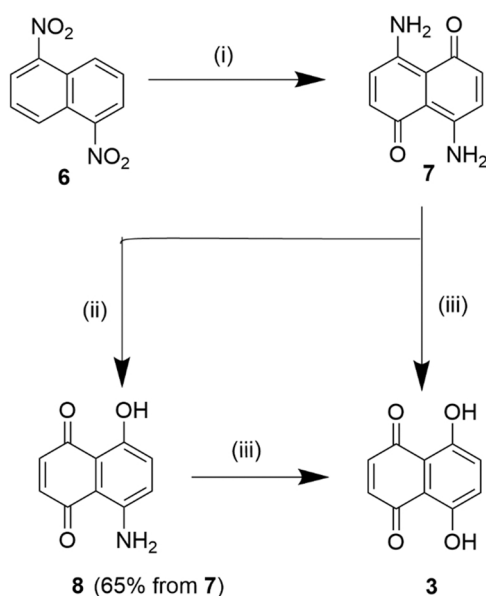
Table 1 (continued)

Structure	Compound	MIC (bacterial strains) ($\mu\text{g/mL}$)	Supposed mechanism of inhibition	Reference
	90	16 <i>E. coli</i> ATCC 25922 64 <i>P. aeruginosa</i> ATCC 27853 64 <i>P. aeruginosa</i> ATCC 15442	Inhibited biofilm formation at the initial and later process of biofilm formation	[83]
	104	1.25–1.9 MSSA ATCC 29213 0.6 MSSA ATCC 6538 1.25–2.5 VISA ATCC 700699 1.25–2.5 MRSA USA300, ATCC BAA1717 1.25–2.5 MRSA ATCC BAA44	Ability to dissolve biofilm	[84]

*nd: not determined.

derivatives were very important intermediates in organic synthesis to produce analogs with an interesting antibacterial activity, thanks to their tendency to accept one or two electrons [74]. Considering that the nature of the substituents in the naphthoquinone structure modify the electron-accepting capacity, they developed a series of 2-(anilino)-5-hydroxy-1,4-naphthoquinone derived from juglone **2**. This series showed very interesting activities ranged from 0.49 to 125 $\mu\text{g/mL}$ against both Gram-positive or Gram-negative strains [74] (Fig. 6).

Compounds **26–28** showed a great antibacterial activity against clinical isolated *S. aureus* strain with MIC ranged from 0.49 to 0.98 $\mu\text{g/mL}$. The best MIC value was obtained for compound **26** on clinical *S. aureus* (MIC = 0.49 $\mu\text{g/mL}$) (Table 1). Compound **26** showed a MIC of 31.25 $\mu\text{g/mL}$ against *Enterococcus faecalis* (*E. faecalis*) ATCC29212 strain



Scheme 1. Reagents and conditions: (i) fuming $\text{H}_2\text{SO}_4/\text{S}_8$, 0 °C, 1 h at r.t., 10 min at 50 °C; (ii) H_3O^+ ; (iii) H_3O^+ at 50 °C; (iv) $\text{Ac}_2\text{O}/\text{NaOAc}$ in Ac_2O , 48 h at r.t.

and MIC of 62.50 $\mu\text{g/mL}$ against *Escherichia coli* (*E. coli*) ATCC 23922 strain (Fig. 6).

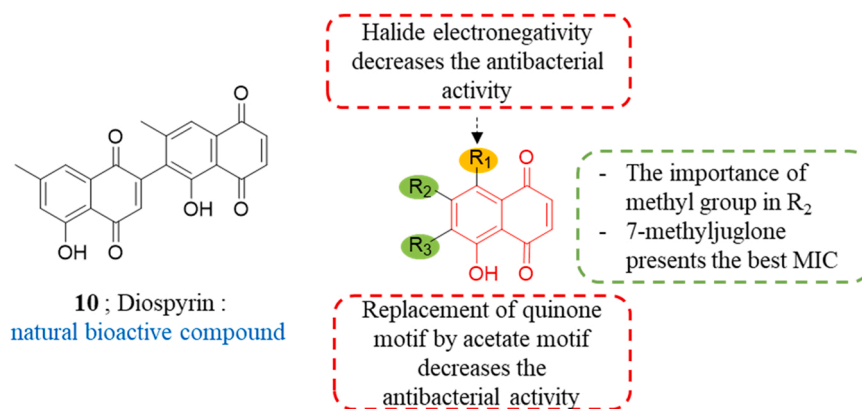
The synthesis of compound **26–28** is described in Scheme 4. The synthetic strategy for compound **26–28** consists in the reaction between juglone **2** with aniline derivatives **25** following a regioselective Michael addition using methanol as solvent and $\text{CeCl}_3 \cdot 7 \text{H}_2\text{O}$ as a catalyst. To optimize the reaction yield, different conditions were used such as conventional heating synthesis (CHS) versus ultrasound assisted synthesis (UAS). Except for compound **26** (81% yield by UAS and 60% by CHS), CHS and UAS gave comparable results for compound **27** (74% by UAS and 72% by CHS) and **28** (79% by UAS and 73% by CHS) [74].

In 2012 T.D.C. Tan et al. synthesized and evaluated a new series of eight nitro and halogen compounds substituted derivatives of juglone **2** [75]. The new series were subjected to antibacterial assay against four bacterial strains: *Bacillus cereus* (*B. cereus*) ATCC 10876, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 13883.

Compound **29** displayed the most notable activity against *S. aureus* ATCC 25923 with a MIC of 30.6 $\mu\text{g/mL}$ versus 125.3 $\mu\text{g/mL}$ for juglone **2** (Table 1). Juglone substituted with a nitro group showed a better activity than ones substituted with halogen atoms. This indicated a possible relationship between the nitro substituent of compound **29** and its notable activity against *S. aureus* compared to juglone **2** [75] (Fig. 7).

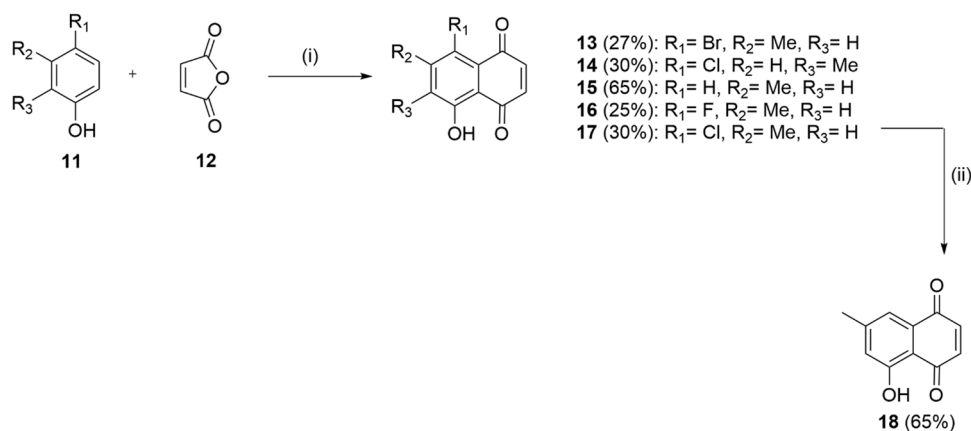
The synthesis of **29** is described in Scheme 5. Compound **29** was obtained from the direct nitration of juglone **2** with $\text{Ni}(\text{NO}_3)_2$ with catalytic *p*-toluenesulfonic acid in acetone. The reaction was refluxed at 50 °C for 24 h and gave compound **29** in 11% yield after purification by column chromatography and recrystallization from chloroform. Substituted juglone derivatives **31–34**, were prepared from Friedel-Crafts acylation reaction from the respective 3-bromo-4-methylphenol, 4-bromophenol, 4-chloro-3-methylphenol and 4-chlorophenol with maleic anhydride in the presence of molten $\text{AlCl}_3\text{-NaCl}$. At the end of the reaction, addition of a mixture of hydrochloric acid and ice afforded compounds **31–34** with respective 10%, 24%, 8% and 10% yields (Scheme 5).

Different structural modifications were carried out on the juglone ring so far (Fig. 8). The authors showed that the introduction of halide in R_4 positions decreases the antibacterial activity (compounds **13**, **14**, **16** and **17**) [69] and the best antibacterial activity was obtained for **15**.



Compounds	MIC (µg/mL) on <i>M. tuberculosis</i> H37Rv
13; R ₁ = Br, R ₂ = Me; R ₃ = H	5.0
14; R ₁ = Cl, R ₂ = H; R ₃ = Me	5.0
15; R ₁ = H, R ₂ = Me; R ₃ = H	0.5
16; R ₁ = F, R ₂ = Me; R ₃ = H	10
17; R ₁ = Cl, R ₂ = Me; R ₃ = H	10
18; R ₁ = H, R ₂ = H; R ₃ = Me	5.0
2; Juglone	1
10; Diospyrin	8

Fig. 4. SAR of juglone derivatives realized by A. Mahapatra et al. [69].



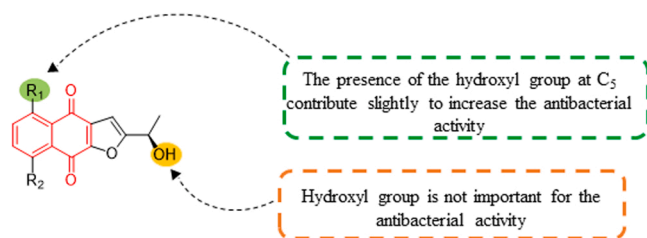
Scheme 2. Reagents and conditions: (i) AlCl₃, NaCl at 180 °C then 12 M HCl, 2 min; (ii) SnCl₂ in 4 M HCl/THF at 60 °C, 2–4 h.

However, other authors showed that the introduction of OH or NO₂ functions at R₄ position increases the antibacterial activity (Compound 29) [75]. Incorporations of substituted or unsubstituted aromatic rings were also achieved at R₁ and R₂ positions (compounds 24a, 24b, 26, and 45) [73]. All of these optimization works presents a good start to improve the antibacterial activity of juglone, still further studies are required to lead us to conclusions about a lead compound derived from juglone. For example, trying to introduce a variety of substituted aromatic groups or aliphatic chains of different length as well as

heterocycles known to confer interesting biological activities in medicinal chemistry would be valuable.

2.2. Synthesis, antibacterial activity, and structure-activity relationship of naphthazarin derivatives

Based on the potential oxidizing or dehydrogenation mechanism of action of naphthoquinone, in 2016 J.M. Sánchez-Calvo et al. carried out a SAR on fifty naphthazarin derivatives [76]. The compounds were



Compounds	MIC ($\mu\text{g/mL}$) on Gram-positive		
	<i>B. subtilis</i> NBRC3134	<i>S. aureus</i> NBRC13276	<i>B. mycooides</i> ATCC 11778
23a ; R ₁ = OH, R ₂ = H	1.56	1.56	0.78
24a ; R ₁ = OH, R ₂ = H	0.78	3.13	0.78
24b ; R ₁ = H, R ₂ = OH	1.56	6.25	1.56
Penicillin G	0.39	0.39	1.56
Amphotericin B	nd	nd	nd

Fig. 5. SAR of juglone derivatives realized by M. Yamashita et al. [73].

screened for their antibacterial activity against two Gram-negative bacteria *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*) and two Gram-positive bacteria *E. faecalis* ATCC 29212, and *S. aureus*. Forty-five compounds were generated by useful synthesis and five compounds were purchased for comparison purposes.

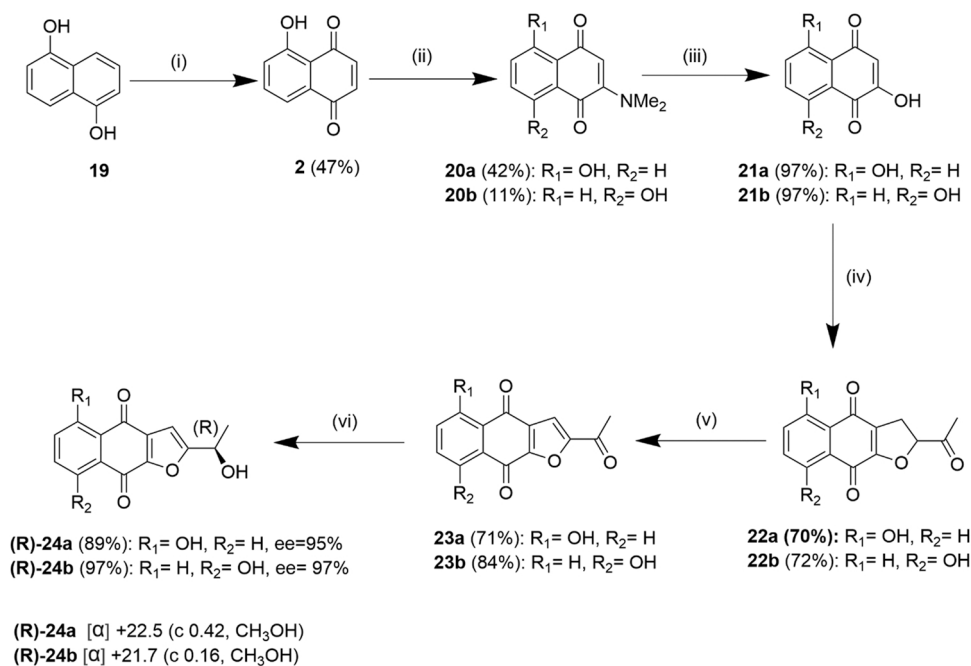
In this study, naphthazarin **3** showed a modest antimicrobial activity against *E. coli*, *E. faecalis* and *S. aureus* with MIC values from 64 to 128 $\mu\text{g/mL}$. The methoxylation at C₅ and/or C₈ positions led to inactive compounds against all of the studied bacterial strains. The authors specified that the activity of these compounds could be influenced by the partition coefficient logP. The logP value is used in this quantitative structure–property relationships study as a measure of lipophilicity. LogP values lower than 1 and close or over 3 were associated with

inactive or low activity compounds which include all compounds with a methoxy group at C₅ and C₈ positions. It was shown as well, that the presence of two free keto groups at the C₁ and C₄ positions is essential for the antibacterial activity. Structures without keto groups at the C₁ and C₄ positions remained inactive even when some substituents were added or removed. J. M. Sanchez-Calvo et al. also showed that the addition of halogen groups in the structure of naphthoquinone could produce a significant improvement in the antimicrobial activity which was the case for bromo substituent at C₂ position, increasing the activity on Gram-positive bacteria. In general, halogenated compounds in this series showed the highest activities [76] (Fig. 9). Compound **45** (Table 1) showed an interesting antibacterial activity against *S. aureus* with a MIC of 16 $\mu\text{g/mL}$, but not lower than the molecule of reference Gentamicin which presented a MIC of 0.5 $\mu\text{g/mL}$ against *S. aureus*. The compounds are suspected to bypass the cellular electron transfer normally performed by quinones to form highly reactive radical anion intermediates, in turn responsible for the oxidative stress observed in the cells [77].

The synthesis of **38**, **42**, **44**, **45** and **48** is described in Scheme 6. Compound **38** was prepared from *p*-dimethoxybenzene **35** and dichloromaleic anhydride **37** in the presence of AlCl₃ and NaCl, heated for 2 min at 180 °C then treated with water and concentrated HCl. The reaction was allowed to cool at r.t. overnight to form compound **38** in 97% yield. Then, **38** reacted with SnCl₂ to give compound **40** in 80% yield. Compound **40** was then heated with NaOH (5 M) during 90 min at 60 °C. The mixture was then acidified with HCl in order to obtain a crude naphthazarin **3** with a yield of 93%. Naphthazarin **3** was brominated with Br₂ in acetic acid and heated at 80 °C under a nitrogen atmosphere to produce **42** and **44** (Yields are not given). Compound **45** was prepared following the same strategy as for the preparation of **44** (No detail is indicated concerning this synthesis).

The permethylation of **40** with dimethyl sulfate and potassium hydride gave **46** with a yield of 95%. This compound was used to afford **47** (97%) by oxidation with ceric ammonium nitrate (CAN) followed by the bromination mediated with Br₂ to produce **48** with a yield of 99% [76].

In 2021, another series derived from naphthazarin and juglone was developed by V. Duvauchelle et al. [78]. Forty-three molecules have been synthesized and evaluated for their antibacterial properties against various clinical and reference Gram-positive methicillin sensitive



Scheme 3. Reagents and conditions: (i) CuCl, CH₃CN, air, r.t.; (ii) Me₂NH, toluene, THF, 40 °C; (iii) 10% HCl, dioxane, reflux; (iv) 3,4-dibromobutan-2-one, DBU, THF, r.t.; (v) MnO₂, CHCl₃, reflux; (vi) 5 mol% Ru[(*R,R*)-Tsdpen] (p-cymene) HCO₂H–Et₃N (5:2) CH₂Cl₂, r.t., 24 h.

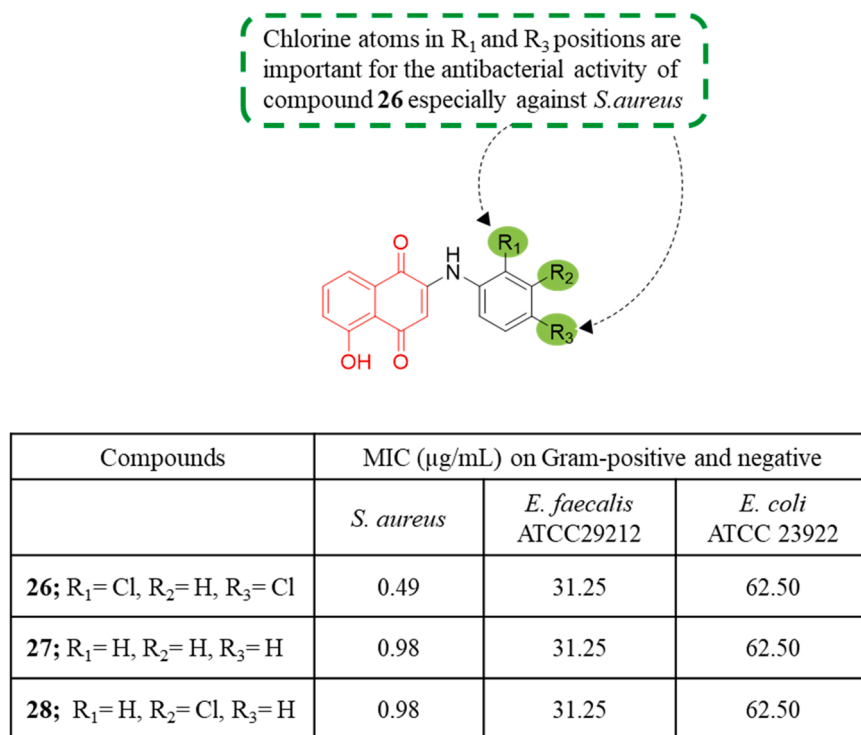
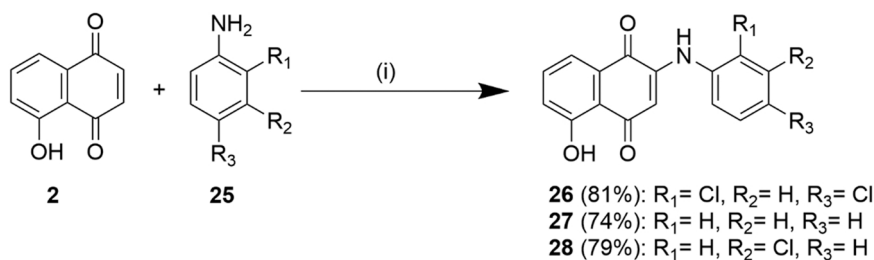


Fig. 6. Antibacterial activity of juglone derivatives developed by L.I. Lopez-Lopez et al. [74].



Scheme 4. Reagents and conditions: (i) CeCl₃•7 H₂O, MeOH, r.t., using UAS.

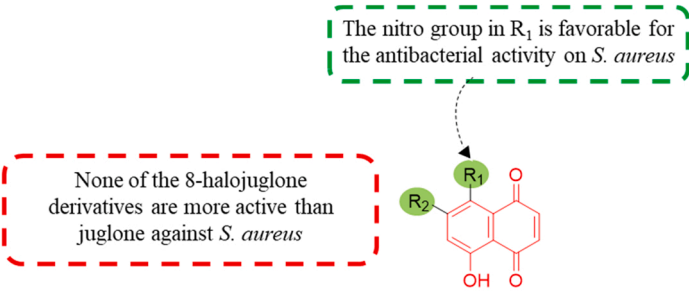
S. aureus (MSSA), clinical Gram-positive methicillin resistant *S. aureus* (MRSA) and clinical and reference Gram-negative bacteria *E. coli* and *P. aeruginosa*. As a comparative, the authors measured the MIC of cloxacillin to be of 0.25 µg/mL on MSSA and 2 µg/mL on MRSA and so vancomycin with a MIC of 0.25 µg/mL on both MSSA and MRSA strains.

In this study, naphthazarin derivatives were generally found to be less potent than juglone ones. Four compounds (**51-54**, Fig. 10) exhibited interesting activities against both sensitive and resistant *S. aureus* strains. Compounds **51** and **52** showed a MIC value of 1 µg/mL on MSSA (clinical and ATCC 29213) and a MIC value of 2 µg/mL on MRSA. Compounds **53** and **54** showed MIC of 0.5 µg/mL on MSSA, and MIC of 0.5 µg/mL for **53** and 2 µg/mL for **54** on MRSA. Only mono-alkylated compounds showed an interesting antibacterial activity. The C₂ and C₃ dialkylation significantly decreased the antibacterial activity mostly due to solubility issues. The authors demonstrated that when the alkyl chain was grafted on C₃ as for compound **53**, the antibacterial activity was higher than for C₂ alkylated derivatives (Fig. 10). Moreover, synergistic studies were carried out on the four most potent compounds and in combination with two reference antibiotics (cloxacillin and vancomycin). Compound **53** with a cyclohexyl group grafted on C₃ was the only compound showing synergistic effect with both cloxacillin and vancomycin on MRSA. In presence of **53**, MICs values of vancomycin and cloxacillin were lowered up to 1/16th of the original MIC (from 0.5 to 0.03 µg/mL for vancomycin and from 2 to 0.125 µg/mL for

cloxacillin). Cytotoxicity test was also performed on mammalian red blood cells (RBC). No hemolytic activity was observed, which indicates that the tested molecules are not hemolytic even at 7.5 MIC concentration. *In silico* prediction of ADME profile parameters was performed on these series of compounds as well and demonstrated that all of them would have a good pharmacokinetic profile (*i.e.*, high gastrointestinal absorption, cLogP > 5, BBB permeability).

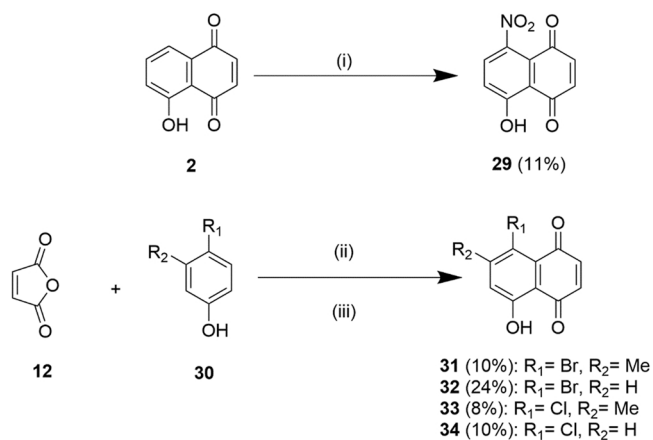
The synthesis of compounds **51-54** was described in Scheme 7. This synthesis has been achieved using a Minisci-type direct C–H alkylation. Commercially available juglone **2** reacted with propylpentanoic acid **49** or cyclohexane carboxylic acid **50** in the presence of ammonium persulfate in DMSO/H₂O (600:1 v/v) and heated under reflux for 22 h at 40 °C to afford respectively compound **51** and **54** in 17% and 8% yields. The same synthesis procedure was performed using commercial naphthazarin **3** to respectively give compounds **52** and **53** in 23% and 33% yields. (Fig. 11).

Despite the interesting structure of naphthazarin with two hydroxyl groups at C₅ and C₈ positions, few SAR studies have been performed on this structure overall. The authors synthesized mono and dibrominated naphthazarin (compounds **42**, **44**) and methoxylated naphthazarin in the two hydroxyl groups (compound **48**) [76]. The antibacterial activity slightly increased for the brominated derivatives, but a decreased when the two hydroxyl groups were methoxylated. The antibacterial activity remains low with MIC values ≥ 64 µg/mL. Interesting results were



Compounds	MIC ($\mu\text{g/mL}$) on Gram-positive and negative			
	<i>B. cereus</i> ATCC 10876	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>K. pneumoniae</i> ATCC 13883
29 ; R ₁ =NO ₂ , R ₂ =H	63.5	30.6	63.5	63.5
31 ; R ₁ =Br, R ₂ =Me	125	250	125	125
32 ; R ₁ =Br, R ₂ =H	126	249.4	126	126
33 ; R ₁ =Cl, R ₂ =Me	124.3	244.2	124.3	124.3
34 ; R ₁ =Cl, R ₂ =H	124.8	124.8	124.8	124.8
2 ; Juglone	62.6	125.3	62.6	62.6
Gentamicin	-	-	12.08	12.08
Tetracycline	7.11	< 7.11	-	-

Fig. 7. Juglone derivatives realized by T.D.C. Tan et al. [75].



Scheme 5. Reagents and conditions: (i) Ni(NO₃)₂, 6 H₂O, p-TsOH, acetone, 50 °C, 24 h; (ii) AlCl₃-NaCl at 180–185 °C, 2 min; (iii) H₃O⁺.

obtained by second authors with MIC values $\leq 0.5 \mu\text{g/mL}$ (compound **53**) with linear and branched aliphatic groups in R₁ and R₂. It may also be worthwhile to study the activity of other functional groups such as aromatic or heteroaromatic groups on other positions. It is also interesting to study not only the antibacterial activity of the derivatives, but also the antibiotic adjuvant activity of these derivatives that could be another strategy to fight bacterial resistance [78].

2.3. Synthesis, antibacterial activity and structure-activity relationship of plumbagin derivatives

A few studies have been conducted to optimize the activity of

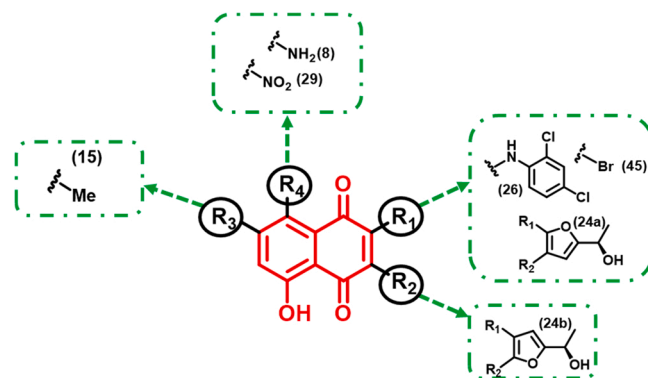
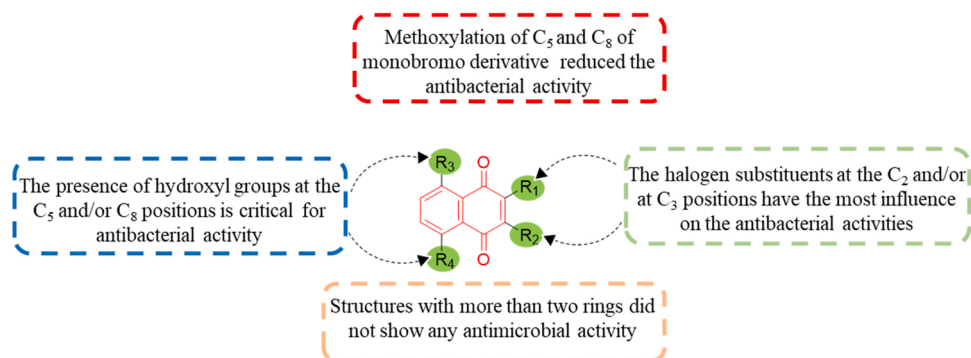


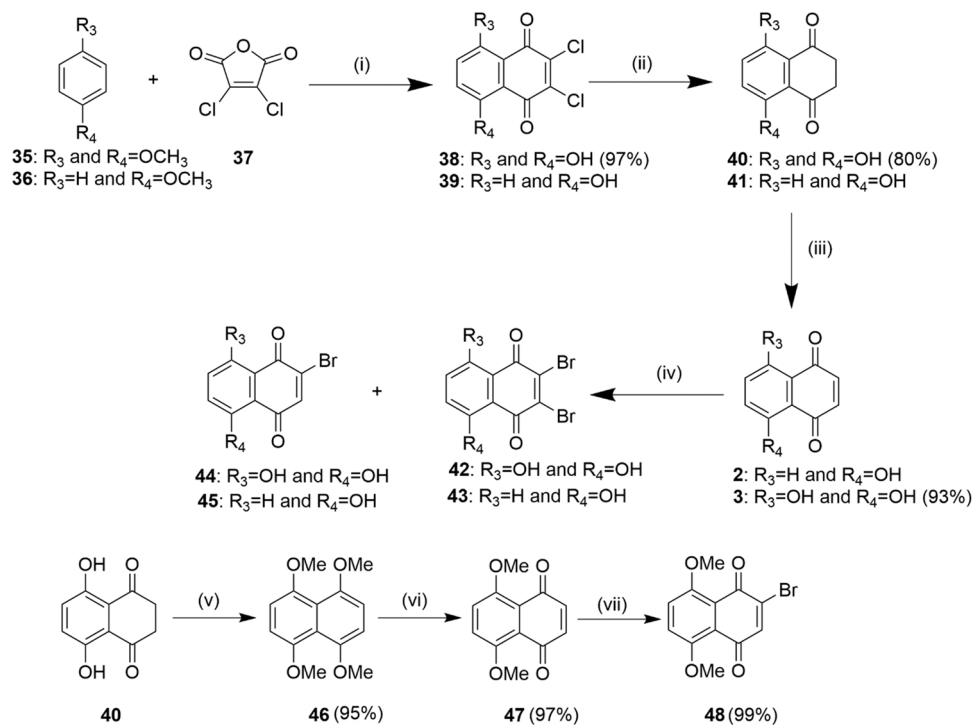
Fig. 8. SAR studies performed on juglone scaffold.

plumbagin **4**. In 2010, R. Mathew et al. have designed a series of eight plumbagin derivatives, in order to develop analogs of menaquinone (vitamin K₂) which is a naturally occurring substrate in bacteria and essential for the growth of mycobacteria [79]. Furthermore, plumbagin **4** has a basic structural scaffold similar to 1,4-dihydroxy-2-naphthoate (DHNA) which is an important intermediate product in the menaquinone biosynthetic pathway. For this reason, they started by derivatizing the phenolic group of plumbagin **4** (the SAR was not discussed). All several plumbagin derivatives have been synthesized and evaluated for their antibacterial activity [79]. Among the different eight derivatives of plumbagin, five of them had inhibitory effect on the wild-type of *Mycobacterium smegmatis* (*M. smegmatis*) mc² 155. Compound **59** showed to be the most potent with a MIC of 4.0 $\mu\text{g/mL}$ against *M. smegmatis* mc² 155 (Table 1). Moreover, on the wild-type strain of *M. tuberculosis* H37Rv, **60** showed the best activity with a MIC value of 3.9 $\mu\text{g/mL}$ [79].

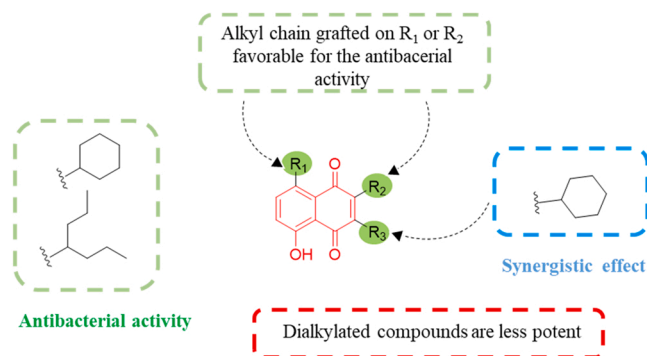


Compounds	MIC (μg/mL) on Gram-positive and negative		
	<i>E. coli</i> ATCC 25922	<i>E. faecalis</i> ATCC 29212	<i>S. aureus</i>
38 ; R ₁ =Cl, R ₂ =Cl, R ₃ =OH, R ₄ =OH	64	64	128
42 ; R ₁ =Br, R ₂ =Br, R ₃ =OH, R ₄ =OH	64	-	64
44 ; R ₁ =Br, R ₂ =H, R ₃ =OH, R ₄ =OH	64	64	64
45 ; R ₁ =Br, R ₂ =H, R ₃ =H, R ₄ =OH	128	128	16
48 ; R ₁ =Br, R ₂ =H, R ₃ =OCH ₃ , R ₄ =OCH ₃	256	256	256
3 ; Naphthazarin	64	128	128
Gentamicin	0.5	-	0.5

Fig. 9. SAR of naphthazarin realized by J.M. Sánchez-Calvo et al. [76].



Scheme 6. Reagents and conditions: (i) AlCl₃-NaCl, 180 °C, 2 min, H₂O, HCl, r.t. overnight; (ii) SnCl₂, HCl (4 M), 140 °C, 4 h; NaOH (5 M), 60 °C, 90 min, HCl (20%); (iii) NaOH (5 M), 60 °C, 90 min; (iv) Br₂, AcOH, N₂ atm, 80 °C; (v) Me₂SO₄, KH, DMF 0–40 °C; (vi) CAN/H₂O, CH₃CN, 0 °C; (vii) Br₂, CHCl₃, -5 °C, Et₃N, 0 °C.



Compounds	MIC ($\mu\text{g/mL}$) on Gram-positive	
	MSSA	MRSA
51 ; $R_1 = \text{H}$, $R_2 = \text{H}$, $R_3 = \text{n-heptan-4-yl}$	1	1
52 ; $R_1 = \text{OH}$, $R_2 = \text{n-heptan-4-yl}$, $R_3 = \text{H}$	1	2
53 ; $R_1 = \text{OH}$, $R_2 = \text{H}$, $R_3 = \text{cyclohexyl}$	0.5	0.5
54 ; $R_1 = \text{H}$, $R_2 = \text{cyclohexyl}$, $R_3 = \text{H}$	0.5	2
Cloxacillin	0.25	0.25
Vancomycin	0.25	0.25
Cefotaxime	-	-

Fig. 10. SAR studies realized by V. Duvauchelle et al. [78].

The presence of the butyrate or crotonate ester on the phenolic core at the C_5 position increased the antibacterial activity of plumbagin 4 (Fig. 12).

The most potent compounds **59-62** were synthesized using two different methods as described in Scheme 8. According to the method 1, compounds **59** and **60** were prepared by esterification using butyric acid **55** or crotonic acid **56**, in CH_2Cl_2 , pyridine and dicyclohexylcarbodiimide (DCC) at 0°C , followed by the addition of plumbagin **4** and a catalytic amount of 4-dimethylaminopyridine (DMAP) at r.t. overnight. Compounds **61** and **62** were prepared following the second method, by esterification in the presence of benzoic acid **57** and acetic acid **58** in thionyl chloride at 0°C then heated at 50°C for 3 h to give the acyl chlorides intermediates. Freshly prepared acyl chlorides were added dropwise at 0°C to a solution of plumbagin **4**, in CH_2Cl_2 and pyridine. The reaction was stirred for 3 h at r.t. to give compound **61** and **62**. All final compounds were purified by column chromatography. The yield for each compound is not indicated, but it is reported by the authors that in general the yield is 75% when using method 1 and 80% when using method 2. (Fig. 13).

Derivatives of plumbagin were synthesized to extend the structure by esterification of the hydroxyl function at C_5 position. The observed antibacterial activities of these derivatives are encouraging (compounds **59, 60, 61** and **62**) [79], but remain lower than the activity of naturally

occurring plumbagin **4** (Compound **59** for *M. smegmatis* and compound **60** for *M. tuberculosis*). Plumbagin is also an interesting scaffold in terms of antibacterial activity but also from a chemical synthetic point of view. The existence of the methyl group in C_2 facilitates the synthesis of new derivatives especially on C_3 . In our point of view, it is interesting to synthesize further derivatives of plumbagin by substituting C_3 with different aliphatic, aromatic or heteroaromatic groups. Vitamin K is known to have a similar structure to plumbagin with a methyl group at C_2 and different substituents at C_3 (K_1 , K_2 and K_3) but with a lack of the hydroxyl function at position C_5 compared to plumbagin, which makes this structure interesting for the synthesis of vitamin K derivatives in order to see the importance of the hydroxyl group of plumbagin in the

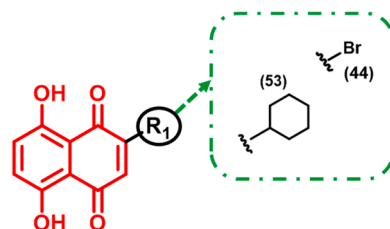
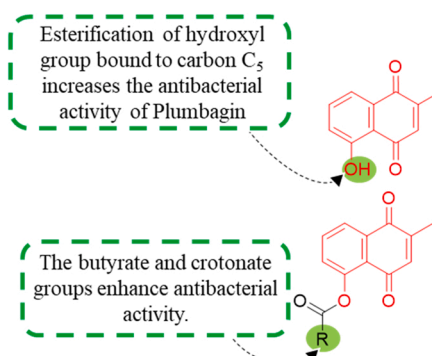
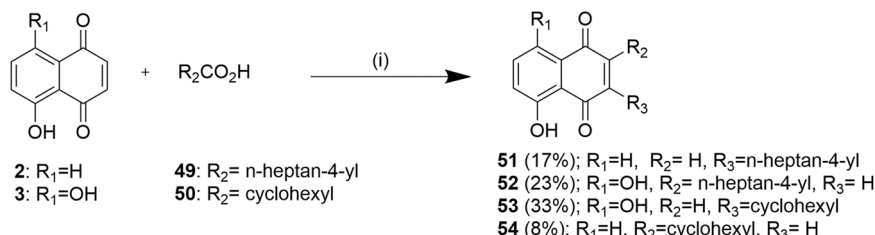


Fig. 11. SAR studies performed on naphthazarin scaffold.

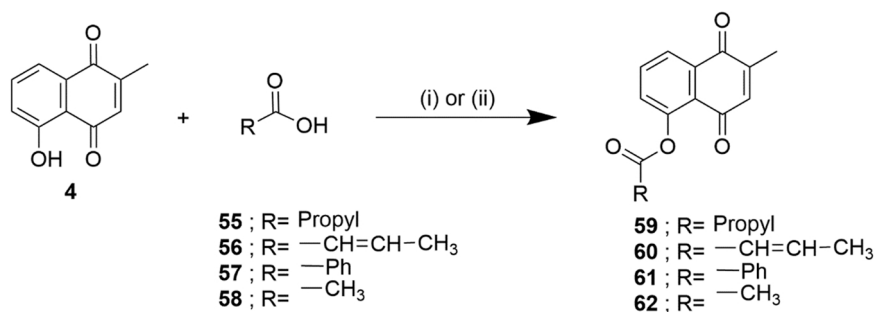


Compounds	MIC ($\mu\text{g/mL}$) on Gram-positive and negative	
	<i>M. smegmatis</i> mc2 155	<i>M. tuberculosis</i> H37Rv
59 ; $R = \text{-propyl}$	4.0	19.9
60 ; $R = \text{-CH=CH-CH}_3$	4.9	3.9
61 ; $R = \text{-Ph}$	6.0	8.0
62 ; $R = \text{-Me}$	6.4	8.0
4 ; Plumbagin	2.5	2.3

Fig. 12. Plumbagin derivatives realized by R. Mathew et al. [79].



Scheme 7. Reagents and conditions: (i) $(\text{NH}_4)_2\text{S}_2\text{O}_8$, DMSO/ H_2O (600:1 v/v), reflux for 22 h at 40°C .



Scheme 8. Reagents and conditions: Method 1: (i) CH_2Cl_2 , 0°C , DCC, pyridine, DMAP_{cat} , overnight, r.t.; Method 2: (ii) CH_2Cl_2 , 0°C , SOCl_2 , 50°C , 3 h then CH_2Cl_2 , pyridine, 0°C , 5 min, r.t., 3 h.

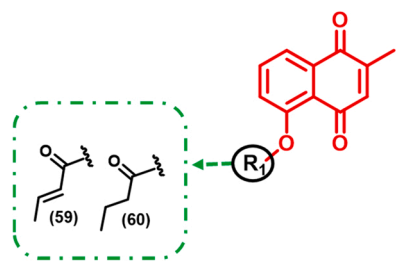
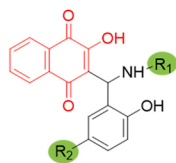


Fig. 13. SAR of studies performed on plumbagin scaffold.

The substitution of the secondary amine in R_1 by a benzyl group improves the antibacterial activity by comparing with aliphatic chain

The existence of the methyl group in R_2 increased interestingly the antibacterial activity



Compounds	MIC ($\mu\text{g}/\text{mL}$) on Gram-positive and negative		
	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923
65 ; $R_1 = \text{Bn}$, $R_2 = \text{H}$	66.8	66.8	66.8
66 ; $R_1 = \text{Bn}$, $R_2 = \text{Me}$	34.6	7.7	15.4
67 ; $R_1 = \text{C}_4\text{H}_9$, $R_2 = \text{Me}$	> 73.03	> 73.03	> 73.03
68 ; $R_1 = \text{C}_4\text{H}_9$, $R_2 = \text{H}$	> 70.2	63.2	> 70.2
Chloramphenicol	6.5 - 29.1	29.1	12.9 - 29.1

Fig. 14. Lawsonone derivatives realized by A.P. Neves et al. [80].

antibacterial activity. It is always interesting to study all possible positions of the 1,4-naphthoquinone ring if it is chemically synthetically possible.

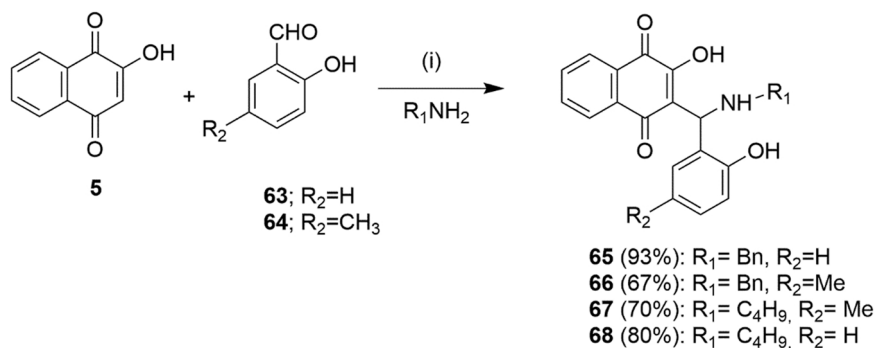
2.4. Synthesis, antibacterial activity and structure-activity relationship of lawsonone derivatives

Regarding lawsonone **5** derivatives, in 2009 A.P. Neves et al. indicated that the most admitted mechanism for the antimicrobial activity of naphthoquinones was based on the generation of reactive oxygen species (ROS) by two successive reduction processes to form radical anion

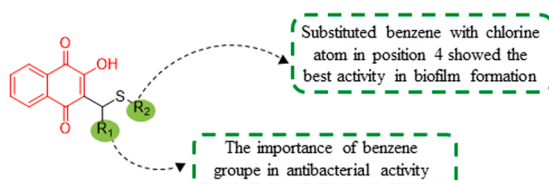
and dianion species that are toxic to bacteria [80]. For this reason, they have designed and synthesized a series of novel Mannich bases derived from lawsonone **5**, substituted benzaldehydes and various primary amines as well as their Cu^{2+} complexes. The antimicrobial activity of all compounds has been evaluated on seven different strains of bacteria: *B. cereus* ATCC 33019, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. Most of the Mannich bases were more active than their metal complexes, probably due to their lower solubility. Compounds substituted at the C_3 position, either with $R_2 = \text{Me}$ or benzyl, and with $R_1 = \text{benzyl}$ or butyl inhibited strongly the bacterial growth especially for *E. coli* and *S. aureus* (Fig. 14). The nature of the R_1 and R_2 substituents appeared to be important for the antibacterial activity. Based on their results, compound **66** with a benzyl substituent on R_1 and a methyl group on R_2 showed a higher antibacterial activity than compound **67** with a butyl substituent on R_1 and methyl substituent on R_2 . Compound **66** strongly inhibited the growth of *E. coli* with MIC value of $7.7 \mu\text{g}/\text{mL}$, *S. aureus* at $15.4 \mu\text{g}/\text{mL}$, and *B. subtilis* at $34.6 \mu\text{g}/\text{mL}$ (Table 1). Compared to chloramphenicol, used as a positive control here, compound **66** exhibited a better antibacterial activity against these three bacteria strains (Fig. 14).

The synthesis of **65–68** is described in Scheme 9. Compounds **65–68** were prepared following a Mannich reaction between lawsonone **5** in the presence of the corresponding amines (benzylamine or butylamine) and aldehydes (2-hydroxybenzaldehyde (**63**) and 2-hydroxy-5-methylbenzaldehyde (**64**)). The compounds **65–68** were obtained with 67–93% yields.

In 2016, a series of lawsonone derivatives were developed by C.S. Moreira et al. [81]. Thirty-six analogs based on lawsonone with different substituents were synthesized and evaluated as antibacterial agents, against some ESKAPE pathogens bacteria in particular *E. faecalis* ATCC29212, *Staphylococcus epidermidis* (*S. epidermidis*) ATCC 12228, *Staphylococcus Simulans* (*S. simulans*) ATCC 27851, *E. coli* ATCC 25922 (the SAR was not discussed in this study) (Fig. 15, Scheme 10). The antibacterial evaluations were performed in three different ways. The first one was realized using disc diffusion assays with $30 \mu\text{g}/\text{disc}$ in the presence of a panel of bacteria. Among the thirty-six compounds tested, nineteen were only active on Gram-positive bacteria ($7 \leq \text{halo (mm)} \leq 16$), similar to vancomycin activity which is the reference antibiotic ($13 \leq \text{halo (mm)} \leq 16$). Compound **74** was the only one to show antibacterial activity on *E. coli* ATCC 25922. The second antibacterial activity screening was the determination of the MIC following the microdilution method. According to the observed results the thirty-six compounds showed a MIC ranged from 32 to $256 \mu\text{g}/\text{mL}$ except compound **78** which showed the lowest observed MIC ($16 \mu\text{g}/\text{mL}$) on *S. epidermidis* particularly. The third performed test was the evaluation of the biofilm formation against planktonic cells of MRSA (BMB9393), where compound **72** ($R_1 = \text{C}_6\text{H}_5$ and $R_2 = 4\text{-ClC}_6\text{H}_4$, MIC = $64 \mu\text{g}/\text{mL}$) interestingly showed the best results, even better than those observed for vancomycin (MIC = $2 \mu\text{g}/\text{mL}$ (data not shown)). Compound **72** showed a significant reduction of the biofilm mass ranging from 39% to 49% at 1

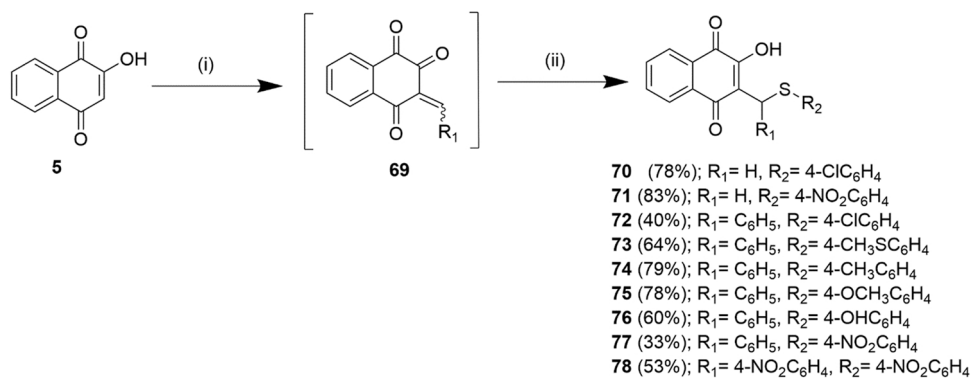


Scheme 9. Reagents and conditions: (i) EtOH, r.t., 12 h in the dark.



Compounds	MIC (µg/mL) on Gram-positive and Gram-negative				
	<i>E. faecalis</i> ATCC29212	<i>S. aureus</i> ATCC 25923	<i>S. epidermidis</i> ATCC 12228	<i>S. simulans</i> ATCC 27851	<i>E. coli</i> ATCC25922
70 ; R ₁ = H, R ₂ = 4-ClC ₆ H ₄	128	64	64	64	ND
71 ; R ₁ = H, R ₂ = 4-NO ₂ C ₆ H ₄	256	256	256	256	ND
72 ; R ₁ = C ₆ H ₅ , R ₂ = 4-ClC ₆ H ₄	128	32	32	ND	ND
73 ; R ₁ = C ₆ H ₅ , R ₂ = 4-CH ₃ SC ₆ H ₄	ND	ND	256	ND	ND
74 ; R ₁ = C ₆ H ₅ , R ₂ = 4-CH ₃ C ₆ H ₄	ND	ND	ND	ND	ND
75 ; R ₁ = C ₆ H ₅ , R ₂ = 4-OCH ₃ C ₆ H ₄	ND	ND	128	ND	ND
76 ; R ₁ = C ₆ H ₅ , R ₂ = 4-OHC ₆ H ₄	256	64	128	64	ND
77 ; R ₁ = C ₆ H ₅ , R ₂ = 4-NO ₂ C ₆ H ₄	256	256	256	256	ND
78 ; R ₁ = 4-NO ₂ C ₆ H ₄ , R ₂ = 4-NO ₂ C ₆ H ₄	256	128	16	256	ND
Ciprofloxacin	ND	ND	ND	ND	27
Vancomycin	13	16	14	16	ND

Fig. 15. Lawsone derivatives realized by C.S. Moreira et al. [81].

Scheme 10. Reagents and conditions: (i) R₁CHO, EtOH, MW (150 °C, 20 min); (ii) R₂-SH.

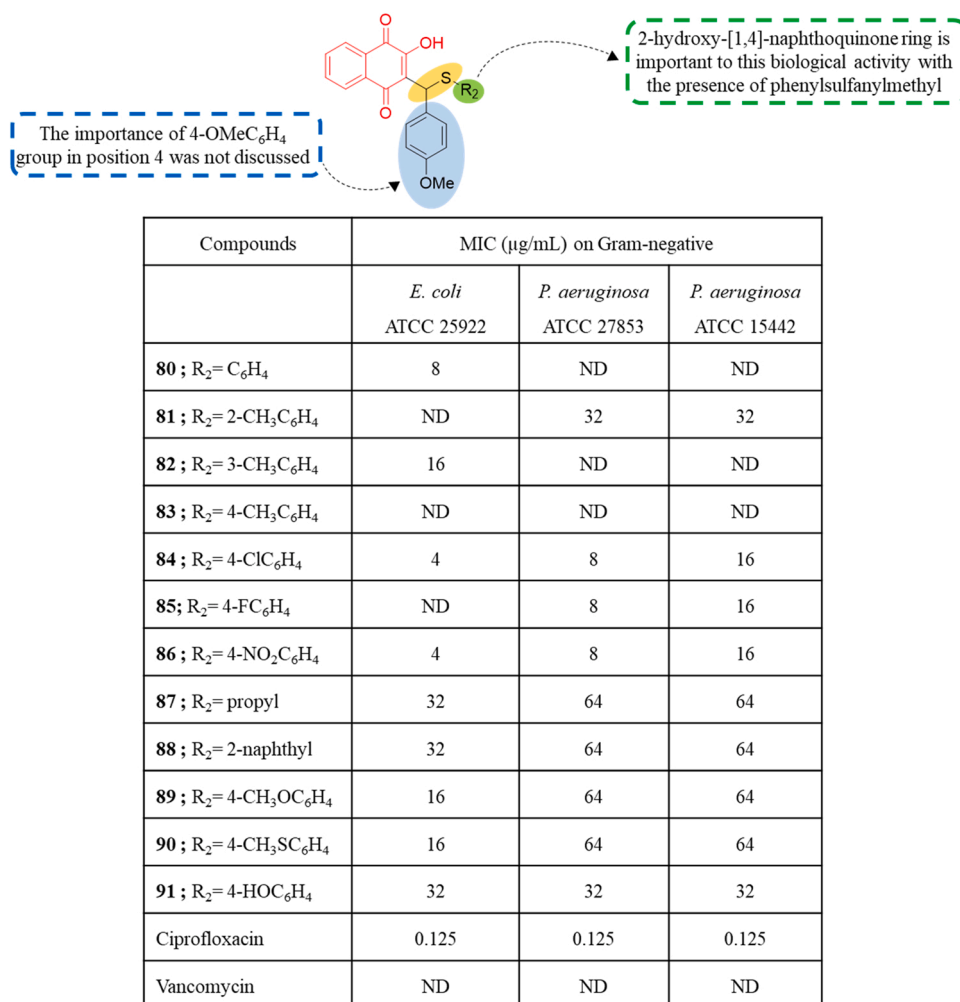


Fig. 16. Lawsone derivatives realized by J. S. Novais et al. [83].

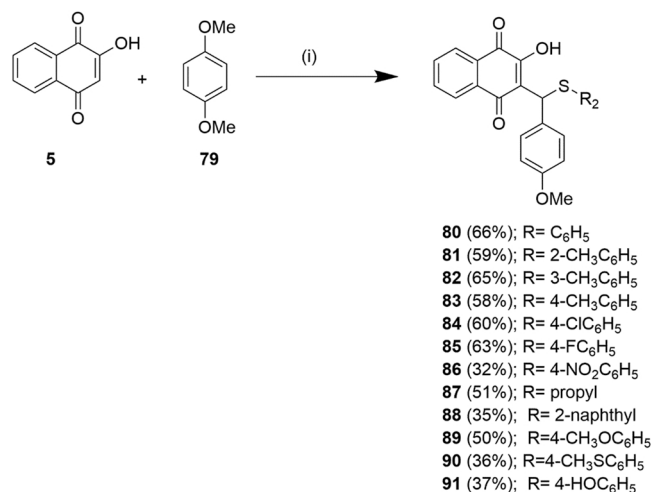
time and 2 times MIC compared with vancomycin which only showed a reduction of 14% of the biofilm formation. An *in silico* evaluation of the ADMET profile was performed, compounds showed promising ADMET parameters made making of these molecules good candidates for future antibacterial treatments.

The synthesis of compound **72** was described by A. Sharma et al. in 2013, starting with a Knoevenagel condensation reaction between the lawsone **5** with the appropriate aldehyde in EtOH under a microwave irradiation (150 °C, 20 min) to generate the intermediate o-quinone methide **69** *in situ*, followed by a nucleophilic addition of 4-chlorobenzenethiol to give compound **72** in 40% yield (Scheme 10) [82].

In 2018, the same research group published another article about twelve new lawsone derivatives searching for potential lead compounds against bacterial biofilm formation [83] (Fig. 16, Schema 11). All the compounds were tested against Gram-positive bacterial strains (*E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. simulans* ATCC 27851, *S. epidermidis* ATCC 12228 and an hospital MRSA) and on Gram-negative bacterial strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 15442, *Proteus mirabilis* (*P. mirabilis*) ATCC 15290, *Serratia marcescens* (*S. marcescens*) ATCC 14756, *K. pneumoniae* ATCC 4352 and *Enterobacter cloacae* (*E. cloacae*) ATCC 23355). In order to evaluate antibacterial activity of these synthesized compounds, a first qualitative evaluation was performed using the disk diffusion method in the presence of synthesized compounds at 30 μg/disc. Ten compounds showed an antibacterial activity (**80**, **82**, **84–91**), with inhibitory zones in the range of 9–30 mm against *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 and ATCC 15442. Among these, four compounds (**84**, **86**,

87 and **91**) were also active against *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, MRSA, *S. epidermidis* ATCC 12228 and *S. simulans* ATCC 27851 with inhibitory zones in the range of 10–13 mm. Afterwards, the MIC values of the synthesized compounds were determined. Mostly, the antibacterial activity was observed against Gram-negative bacteria *E. coli* ATCC 25922, *P. aeruginosa* (ATCC 27853 and ATCC 15442) with a MIC ranged from 4 to 64 μg/mL suggesting a highly selective profile for the latter strains. A similar activity was observed on the two studied *P. aeruginosa* strains, ATCC 27853 and ATCC 15442 supporting that the antibacterial profile was not limited to one specie strain. Finally, all active compounds were tested as inhibitors of the *P. aeruginosa* ATCC 15442 biofilm formation. The inhibitory effect on biofilm formation in the presence of synthesized compounds at 1/8 time to 1/2 time the MIC was compared with the inhibition of ciprofloxacin. At 1/2 time to 1/4 time the MIC, compound **89** prevented almost 65% of the biofilm formation compared with ciprofloxacin, unable to show a reduction in the biofilm formation in the same conditions. In addition, compounds **84** and **90** were able to reduce mature *P. aeruginosa* ATCC 15442 biofilm. The obtained results showed that these compounds were able to reduce the formation of *P. aeruginosa* ATCC 15442 biofilm in two different stages which is very promising. Hemocompatibility and theoretical pharmacokinetic profiles, in addition to toxicological analysis supported the potential for further investigations to determine the mechanisms involved in this selective and promising profile inhibition towards Gram-negative bacteria [83].

The synthesis of compounds **84**, **89** and **90** is the same as described for compound **72** in Scheme 10. The first step consists in a Knoevenagel



Scheme 11. Reagents and conditions: (i) R₂-SH, EtOH, MW (150 °C, 20 min).

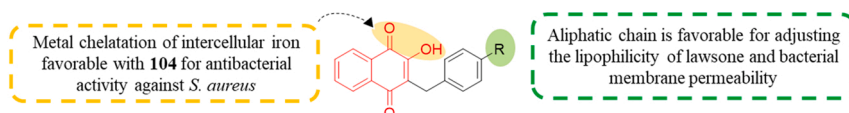
condensation between lawsone and *p*-anisaldehyde followed by the nucleophilic addition of different thioethers derivatives under microwave irradiation (150 °C, 20 min), to give compounds **84**, **89** and **90** in 60%, 50% and 36% yields respectively (Scheme 11).

In 2020, R. Song et al., have chosen lawsone as a pharmacophore and suggested that the substitution of the H atom at the C₃ with an R group can prevent lawsone from unspecific binding to nucleophiles such as azote atoms in DNA bases and thiol groups in proteins [84]. In addition, cellular toxicity of lawsone is related to its ability to produce ROS and it has been hypothesized that lawsone derivatives could be less cytotoxic than lawsone itself. A series of five lawsone derivatives were designed and synthesized. The synthesized compounds were evaluated as potential antibacterial agents against different sensitive and resistant strains of *S. aureus*: MSSA (ATCC 29213, ATCC 6538), vancomycin-intermediate *S. aureus* (VISA) (ATCC 700699), MRSA (USA300, ATCC BAA 1717, ATCC BAA44) to identify a lead exhibiting bactericidal activity. In most cases, the synthesized compounds showed antibacterial activity with MIC valued in the range of 0.6–128 µg/mL.

Among the five synthesized compounds, **104** was proven to have the strongest antimicrobial activity against MRSA strains (ATCC BAA-44 and ATCC BAA-1717, 1.25 ≤ MIC ≤ 2.5 µg/mL) with values similar to those observed for vancomycin and daptomycin (MIC = 1 µg/mL) (Table 1, Fig. 17). It has also been demonstrated that **104** exhibited a bacteriostatic effect at a 4 times MIC dose and a bactericidal effect at 8 times MIC using time-kill assay on MRSA (ATCC BAA-44). Moreover, **104** significantly inhibit the formation of biofilm formed by MRSA at a dose of 1 time MIC (5 µM or 1.5 µg/mL). The research group examined whether the antibacterial effect of **104** was associated with a bacterial membrane disruption by in MRSA (ATCC BAA-44) the uptake of propidium iodide (PI) which is a cell impermeable nucleic acid intercalating dye. In order to do so, they treated MRSA with **104** at a dose of 16 µM (4 × MIC) and observed a significant increase in the absorption of the PI dye indicating a membrane-lytic ability of the compound. However, the treatment of MRSA with lawsone or vancomycin at the same concentration had little effect on the uptake of PI. To further investigate the activity, selectivity studies of **104** for *S. aureus* against mammalian cells were realized, respectively on human dermal fibroblasts (HDFs) and mouse red blood cells (mRBCs). The obtained results confirmed that the treatment of **104** (at a dose of 4 times MRSA MIC) did not alter the morphology of mRBCs. Considering the critical role of metal metabolism in bacterial survival and the ability of lawsone to chelate metal ions, particularly Fe ions, the research group performed several evaluations with **104** and iron. The results include that the presence of **104** reduced the level of intracellular iron in MRSA concomitantly with exposure to the increasing concentrations of **104**, suggesting the iron-chelating effect of the compound.

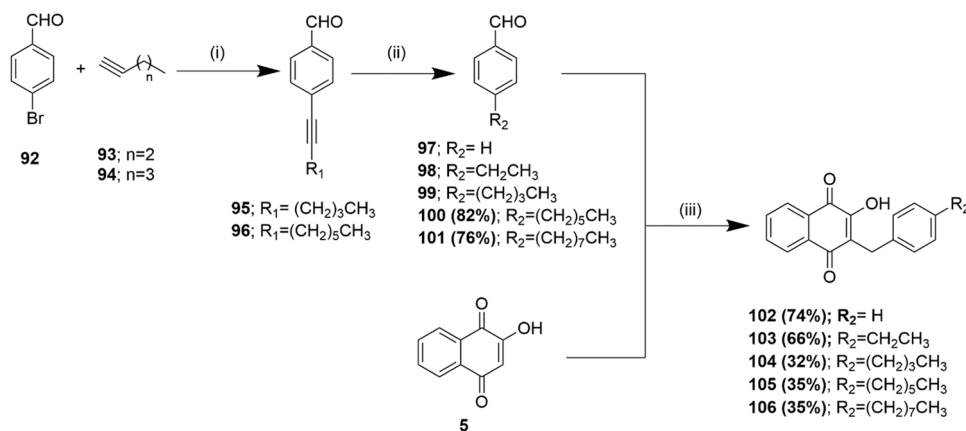
Compound **104** was synthesized in three steps. The first step has been carried out from 4-bromobenzaldehyde **92** with 1-hexyne **94** in the presence of bis(triphenylphosphine)palladium (II) dichloride PdCl₂(PPh₃)₂ and Copper(I) iodide (CuI) in Et₃N to give compound **95** used in the next step without further purification. Then compound **95** was reduced by catalytic hydrogenation with activated palladium/carbon (Pd 10%) in ethyl acetate to give compound **99** with no determined yield. Compound **104** was obtained in 32% yield, via an organocatalytic three-component reductive alkylation (TCRA) reaction between **99**, **5** and Hantzsch ester under proline catalysis in CH₂Cl₂ [85] (Scheme 12).

Various studies have been carried out on lawsone derivatives



Compound	MIC (µg/mL) Gram-positive				
	MSSA (ATCC 29213)	MSSA (ATCC 6538)	VISA (ATCC 700699)	MRSA (USA300, ATCC BAA1717)	MRSA (ATCC BAA44)
102 ; R= H	128	>128	128	>128	>128
103 ; R= CH ₂ CH ₃	32	32	128	64	32
104 ; R= (CH ₂) ₃ CH ₃	1.25–1.9	0.6	1.25–2.5	1.25–2.5	1.25–2.5
105 ; R= (CH ₂) ₂ CH ₃	>128	>128	>128	>128	>128
106 ; R= (CH ₂) ₇ CH ₃	>128	>128	>128	>128	>128
5 ; Lawsone	16	32	64	128	32
Vancomycin	0.5	1	4	1	1
Daptomycin	0.5	1	4	1	1
Ofloxacin	0.25	0.25	16	0.25	8
Ciprofloxacin	0.25	0.25	32	0.25	16

Fig. 17. Lawsone derivatives realized by R. Song et al. [84].



Scheme 12. Reagents and conditions: (i) PdCl₂(PPh₃)₂, CuI, Et₃N, 50 °C, overnight, N₂; (ii) H₂, Pd/C (10%), EtOAc, 6 h, yields of **97**, **98** and **99** were not mentioned; (iii) L-Proline, Hantzsch ester, CH₂Cl₂, 24 h at r.t.

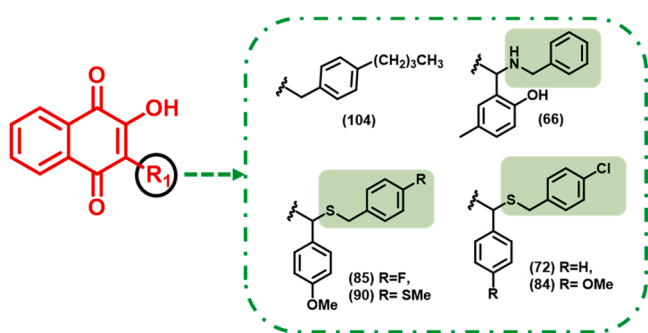


Fig. 18. SAR studies performed on lawsone scaffold.

Table 2

Inhibitory zones (mm) of ligand **108** and its metal complexes.

	108	109: Fe (II)	110: Co (II)	111: Ni (II)	Ciprofloxacin
<i>B. cereus</i>	13.5	15.5	24.0	0.0	33.0
<i>E. coli</i>	9.0	18.5	23.0	0.0	32.0
<i>K. oxytoca</i>	15.0	14.0	21.0	0.0	36.0
<i>P. aeruginosa</i>	19.0	0.0	29.0	0.0	26.5
<i>S. aureus</i>	0.0	0.0	22.5	9.5	29.0
<i>P. mirabilis</i>	12.5	25.0	26.0	0.0	23.0

(Fig. 18), extending its structure and especially on C₃ substituted with benzyl (**104**), amino (**66**), sulfanyl (**72**, **84**, **85**, **90**) groups. In general, an improvement in antibacterial activity was observed, especially for compound **104** [84] which drew our attention, showing a better antibacterial activity than lawsone **5** and some reference antibiotics on MSSA (ATCC 6538) and VISA (ATCC 700699). A compound like **104** is very interesting and could be taken as a hit for future optimization work especially for its antibiofilm activity on planktonic bacteria. Indeed, it would provide an interesting alternative to fight against biofilms, involved in antibiotic resistance.

3. Synthesis and antibacterial activity of metal complexes of 1,4-naphthoquinones hydroxyderivatives

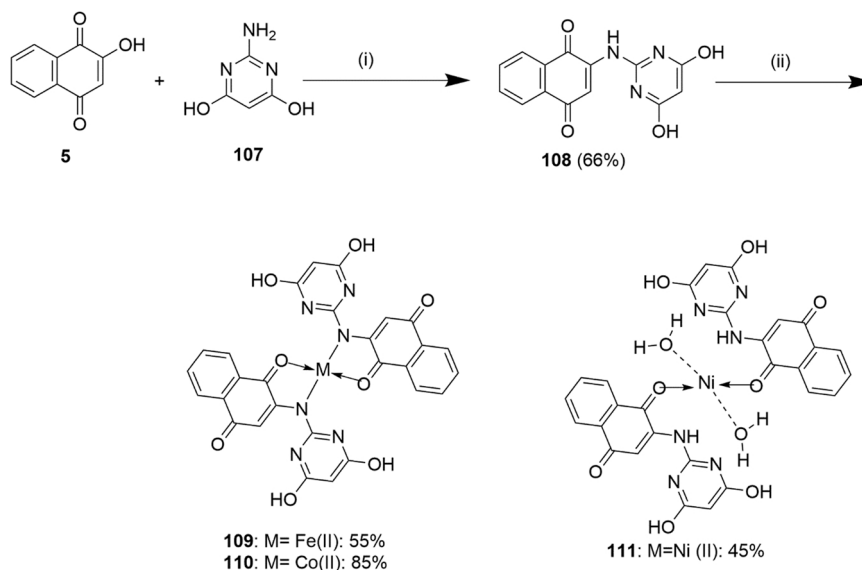
The amount of metal complexes outlined for their antibacterial activity remains overshadowed by those describing their antiproliferative properties [86]. However, the number of works highlighting the use of metal complexes as antibacterial agents has grown for a few years in balance with the renewed interest in antibiotics development [87–89]. The association between metal complexes and toxicity is yet a deeply held belief far from the truth [88]. The best counter-example is

Salvarsan, known as the first arsenic complex used for its antibacterial properties against syphilis [91]. While the 1,4-naphthoquinone moiety can be functionalized with various biologically active groups as discussed above, other studies have focused on their metal complexes. In fact, naphthoquinone moiety is a very relevant and suitable scaffold for metal complexes driven by its particular redox behavior [92] improving its activity at such a point that some 1,4-naphthoquinones hydroxyderivatives complexes can sometimes exhibit a better antibacterial profile than the free ligand.

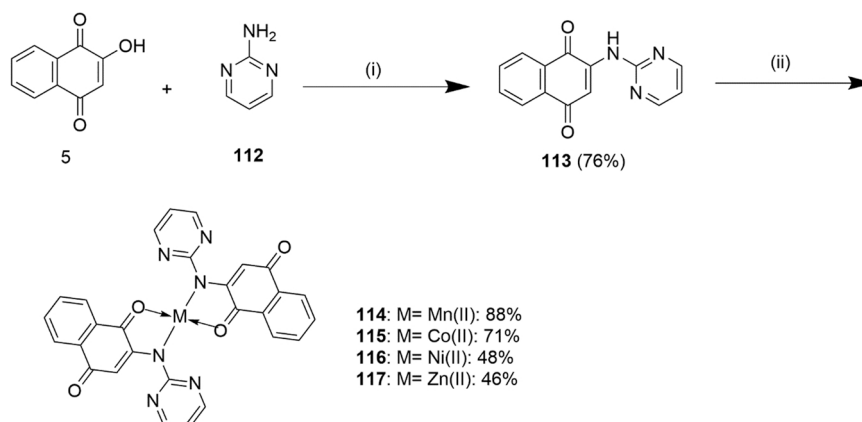
Most of the naphthoquinone metal complexes further described are bidentate and coordinate with the ketone at the position 1 and the nitrogen at the position 2. To our knowledge, a total of 7 different metals have been used to form such complexes: iron, nickel, cobalt, manganese, zinc, copper and palladium, all exhibiting an oxidation state of +II.

This approach has recorded a huge success over the past decades and most of these studies have been focused on lawsone's derivatives for the simple reason that it is the easiest way to afford the 2-amino derivatives. Consistently, several metal-based lawsone **5** derivatives have been synthesized and screened against multiple bacterial strains, for example MRSA, *E. faecium* and penicillin resistant *S. pneumoniae* strains. The emphasis on the development of effective antibacterial drugs is to synthesize compounds with a broad antimicrobial spectrum potential, a low toxicity, a good permeability across the microbial membrane, an improved selectivity and perhaps novel mechanisms of action. In order to enhance bacterial outer membrane permeability, and adjust hydrophobicity problems of antibacterial agents, in 2018, C. Festus et al. [93] have synthesized a new naphthoquinone/pyrimidine base ligand **108** and their metal complexes **109**, **110** and **111**. Literature reports indicate that pyrimidine is a key structural moiety in vitamins, coenzymes and uric acid drugs while also being responsible for the properties of many natural and synthetic biomolecules [94,95].

The ligand 2-(4,6 dihydroxypyrimidin-2-ylamino)1,4-naphthoquinone **108** and its Fe(II) **109**, Co(II) **110**, and Ni(II) **111** complexes were evaluated against Gram-positive bacteria species (*B. subtilis* and *S. aureus*) and Gram-negative bacteria species (*Klebsiella oxytoca* (*K. oxytoca*), *E. coli*, *P. mirabilis*, and *P. aeruginosa*) using the agar well diffusion technique. The antibacterial screening results showed that compound **108** showed an interesting activity against all the tested bacteria, with inhibitory zones in the range of 9.0–19.0 mm, yet completely tolerated by *S. aureus*. The metal (II) complexes Fe(II) **109**, Co(II) **110** and Ni(II) **111** have shown a better antibacterial activity against the tested bacteria compared to compound **108**. The authors suggest that the observed enhanced activity of the complexes can be attributed to the chelation effect, which increases the antibacterial activity primarily due to partial sharing of the positive charge on the metal ion with the donor groups of the ligand and possible electron delocalization on the aromatic rings. Fe(II) **109** was active against *B. subtilis*,



Scheme 13. Reagents and conditions: (i) MeOH/ Na_2CO_3 , reflux, 6 h, 66%; (ii) EtOH, corresponding metal salt, reflux, 6 h, Et_3N .



Scheme 14. Reagents and conditions: (i) MeOH, 45 °C, 6 h, 76%; (ii) EtOH, corresponding metal acetate, 50 °C, 6 h, Et_3N .

Table 3

Inhibitory zones (mm) of ligand **119** and its metal complexes.

	<i>E. coli</i>	<i>K. oxytoca</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. cereus</i>
119	19	23	24	22	19
120: Zn (II)	24	24.5	7	7	8
121: Ni (II)	26	25.5	26	25.5	24.5
122: Cu (II)	28	24	23	24	31
123: Co (II)	23	22	26	26	19
124: Mn (II)	0.0	0.0	0.0	0.0	13.5
125: Pd (II)	0.0	0.0	0.0	0.0	0.0
Streptomycin	23	31	29	23.5	28.5

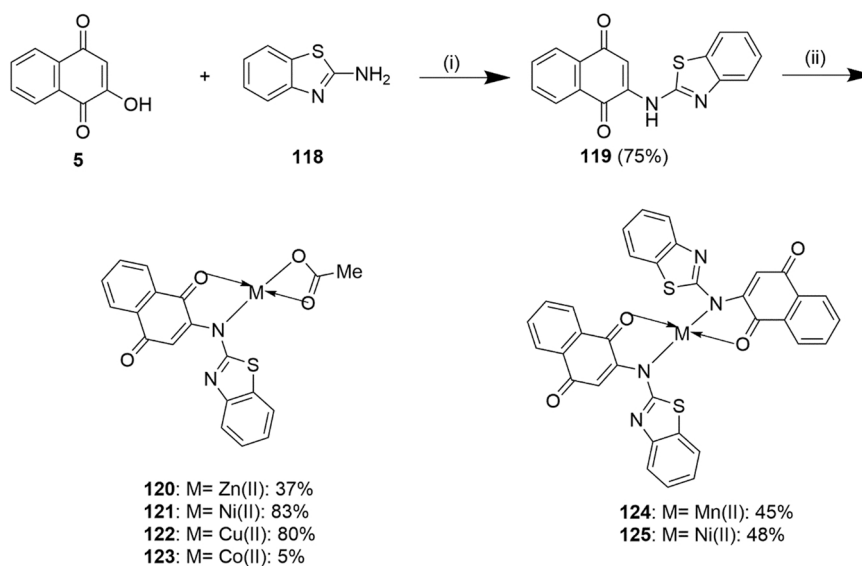
E. coli, *K. oxytoca* and *P. mirabilis* with inhibitory zones of 14.0–25.0 mm, but was inactive against *P. aeruginosa* and *S. aureus*. Ni (II) **111** did not show any activity against the tested bacteria except on *K. oxytoca*, with an inhibitory zone of 9.5 mm. Co(II) **110** was the most active on all tested bacteria with inhibitory zone of 21.0–29.0 mm (Table 2). To sum up, the Fe(II) and Co(II) (**109** and **110**) complexes exhibited a better antibacterial activity compared with reference drug ciprofloxacin against *P. mirabilis* (25.0 mm for **109** and 26.0 mm for **110**) and *P. aeruginosa* (29.0 mm for **110**) (Table 2).

2-(4,6-dihydropyrimidin-2-ylamino) naphthalene-1,4-dione **108** was synthesized by direct C-N bond formation via a coupling reaction

between lawsone **5** and 2-amino-4,6-dihydropyrimidine **107** in the presence of a sodium carbonate solution. After 6 h under reflux, the products precipitated by cooling the solution on ice. The precipitates were collected by filtration and recrystallized from ethanol to give compound **108** in 66% yield. The metal complexes were prepared by dissolving compound **108** in ethanol and by adding an equimolar concentration of each respective metal salt (Fe(II) sulfate hexahydrate, Co (II) acetate tetrahydrate, Ni(II) acetate tetrahydrate). The solution was buffered with triethylamine and refluxed for 6 h. Solid precipitates were collected, washed with ethanol, recrystallized in methanol and dried to afford the different metal complexes of Fe (II) **109**, Co (II) **110** and Ni (II) **111** with yields of 55%, 85% and 45% respectively (Scheme 13).

In 2018, C. Festus et al. published another research article about naphthoquinone pyrimidine base ligand, using this time 2-(pyrimidin-2-ylamino) 1,4-naphthoquinone **113** [96].

The ligand **113** and its metal complexes (Mn(II) **114**, Co(II) **115**, Ni (II) **116**, Zn(II) **117**) were screened *in vitro* for their antimicrobial activity against some clinical Gram-positive isolates (*B. subtilis* and *S. aureus*) and Gram-negative bacteria species (*K. oxytoca*, *E. coli*, *P. mirabilis* and *P. aeruginosa*) using the well diffusion technique. The compounds were generally active against both Gram-positive and Gram-negative bacteria with varying inhibitory zones values, excepted for compound Ni(II) **116** which was inactive on *E. coli*, *K. oxytoca*, *S. aureus* and *P. mirabilis*. In most cases, metal complexes Mn(II) **114**, Co(II) **115**,



Scheme 15. Reagents and conditions: (i) EtOH, 6 h, 76%; (ii) MeOH, corresponding metal salts, 50 °C, 3 h, Et₃N.

Table 4
Inhibitory zones (mm) of ligand **128** and **129** and their metal complexes.

Compounds	<i>P. aeruginosa</i>
128	13.5 – 27
129	11 – 15
130: [Ni 128 (H ₂ O) ₂ (CH ₃ COO ⁻)]	23 – 27.5
131: [Ni 129 (H ₂ O) ₂ (CH ₃ COO ⁻)]•H ₂ O	13 – 20.5
132: [Co 129 (H ₂ O) ₂ (CH ₃ COO ⁻)]	NS
133: [Co (128) ₂ (H ₂ O) ₂]•2 H ₂ O	23 – 27.5
134: [Pd 128(H ₂ O)Cl]	13.5 – 18.5
135: [Pd 129(H ₂ O)Cl]	NS

*NS: Not screened

Ni(II) **116** and Zn(II) **117** showed a better activity than the free ligand **113**. Compound Zn(II) **117** displayed a good activity on *B. cereus* (20.0 mm), *K. oxytoca* (23.0 mm), *P. aeruginosa* (19.5 mm), and *P. mirabilis* (20.3 mm). Compound Mn(II) **114** exhibited a good activity as well on *B. cereus* (15.0 mm), *E. coli* (10.0 mm), *K. oxytoca* (15.5 mm), *P. aeruginosa* (21.0 mm), *S. aureus* (9.5 mm) and *P. mirabilis* (13.5 mm). Compound Co(II) **115** was active on all tested bacterial organisms, and even more active on *P. mirabilis* than the standard drug ciprofloxacin (in the reference document, the exact values of the inhibition zones obtained were not provided for all synthesized compounds on the different studied bacterial strains).

2-(pyrimidin-2-ylamino)-1,4-naphthoquinone **113** was synthesized in the same way as previously reported for compound **108** by direct C-N bond formation via a coupling reaction between 2-amino-pyrimidine **112** and lawsone **5**. 2-aminopyrimidine **112** was added to a methanolic solution of lawsone **5** and heated for 6 h at 45 °C. The formed

precipitates were cooled to r.t., recovered by filtration and recrystallized from methanol to give compound **113** in 76% yield. The metal complexes were prepared by dissolving compound **113** in ethanol, and to this solution the respective metal (Mn(II), Co(II), Ni(II) and Zn(II)) acetate was added in dry ethanol at 50 °C. The obtained solution was buffered with triethylamine to maintain a pH of 9 and refluxed for 6 h. The formed precipitate was collected by filtration, washed with ethanol and dried to afford the different metal complexes **114–117** with yields comprised between 46% and 88% (Scheme 14).

In 2017, A.A. Osowole et al. have introduced a benzothiazole core on lawsone **5** structure to improve the antibacterial activity of this scaffold [97]. According to the literature, benzothiazole is an important heterocyclic compound in drug design and development [98–100]. Starting from the free ligand **119** 2-(1,3-benzothiazol-2-ylamino)-1,4-naphthoquinone, the authors synthesized the corresponding metal complexes Zn(II) **120**, Ni(II) **121**, Cu(II) **122**, Co(II) **123**, Mn(II) **124**, Pd(II) **125**. The ligand and their metal complexes were screened *in vitro* for their antimicrobial activity against Gram-positive bacteria species *B. cereus* and *S. aureus* and Gram-negative bacteria species *K. oxytoca*, *E. coli* and *P. aeruginosa* by measuring the inhibitory zone of microbial growth around the well. The antibacterial screening results showed that the bacterial strains were susceptible to the ligand **119** and all of its metal complexes (Table 3).

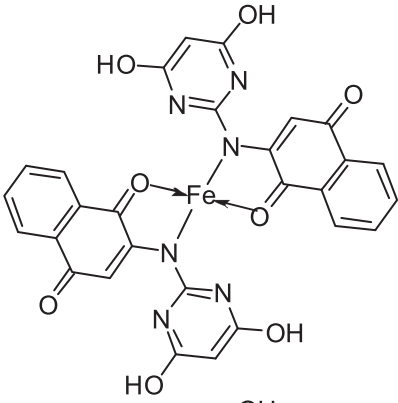
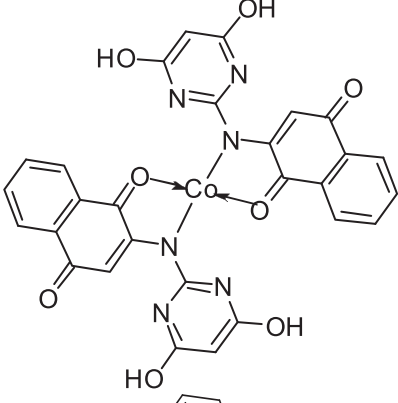
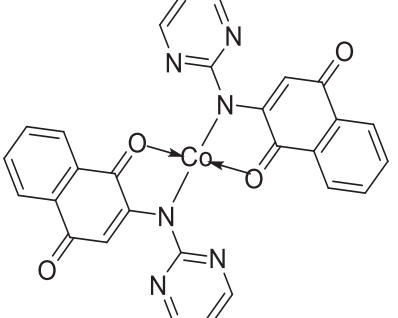
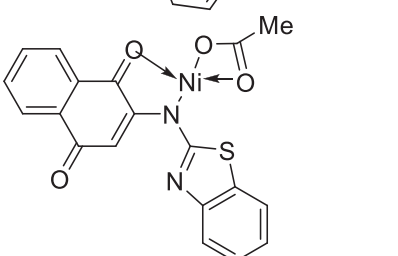
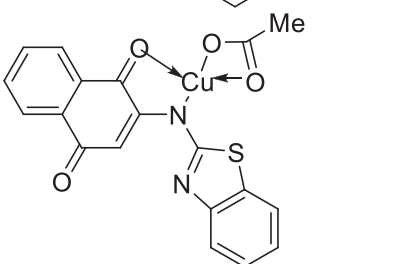
Compound **119** showed a lower antibacterial activity than its metal complexes except Mn(II) **124** and Pd(II) **125**. Regarding the others complexes Zn(II) **120**, Ni(II) **121**, Cu(II) **122**, Co(II) **123**, they exhibited a wide spectrum antibacterial activity against *E. coli* (19–28 mm), *B. cereus* (8.0–31 mm), *K. oxytoca* (23–25.5 mm), *P. aeruginosa* (7.0–26 mm), and *S. aureus* (7.0–26 mm) (Table 3). Pd(II) **125** did not

Table 5
MIC (mg/mL) results of ligand **128** and **129** and their metal complexes.

Compounds	<i>E. coli</i>	<i>K. oxytoca</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. cereus</i>
128	0.1	0.25	0.1	0.1	0.1
129	NS	NS	0.5	NS	NS
130: [Ni 128(H ₂ O) ₂ (CH ₃ COO ⁻)]	0.05	0.1	0.1	0.05	0.1
131: [Ni 129(H ₂ O) ₂ (CH ₃ COO ⁻)]•H ₂ O	0.5	0.25	0.2	0.1	0.1
132: [Co 129(H ₂ O) ₂ (CH ₃ COO ⁻)]	NS	0.5	0.5	0.1	NS
133: [Co (128) ₂ (H ₂ O) ₂]•2 H ₂ O	0.05	0.25	0.1	0.05	0.1
134: [Pd128(H ₂ O)Cl]	0.25	NS	0.2	NS	NS
135: [Pd129(H ₂ O)Cl]	NS	0.5	0.1	NS	0.1
Streptomycin	0.05	0.05	0.05	0.05	0.05

*NS: Not screened.

Table 6
Summary of the most active metal complexes from the different works reported above.

Structure	Compounds	Inhibitory zone (mm) or MIC (mg/mL) (bacterial strains)	Supposed Mechanism of inhibition	Reference
	109	18.5 mm <i>E. coli</i>	nd	[93]
	110	29 mm <i>P. aeruginosa</i>	nd	[93]
	115	20 mm <i>E. coli</i> 21.5 mm <i>K. oxytoca</i>	nd	[96]
	121	24.5–26 mm <i>E. coli</i> , <i>B. cereus</i> , <i>K. oxytoca</i> , <i>P. aeruginosa</i> , <i>S. aureus</i>	nd	[97]
	122	23–31 mm <i>E. coli</i> , <i>B. cereus</i> , <i>K. oxytoca</i> , <i>P. aeruginosa</i> , <i>S. aureus</i>	nd	[97]

(continued on next page)

Table 6 (continued)

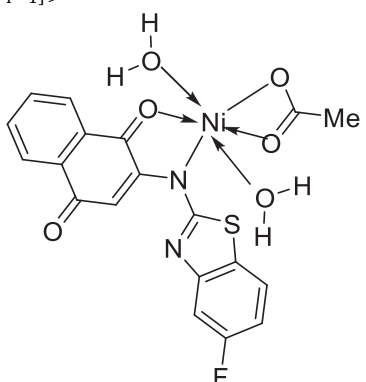
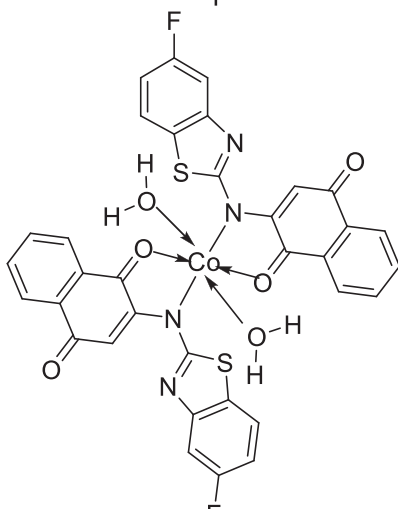
Structure	Compounds	Inhibitory zone (mm) or MIC (mg/mL) (bacterial strains)	Supposed Mechanism of inhibition	Reference
	130	0.05 mg/mL <i>E. coli</i> , <i>S. aureus</i>	nd	[64]
	133	0.05 mg/mL <i>E. coli</i>	nd	[64]

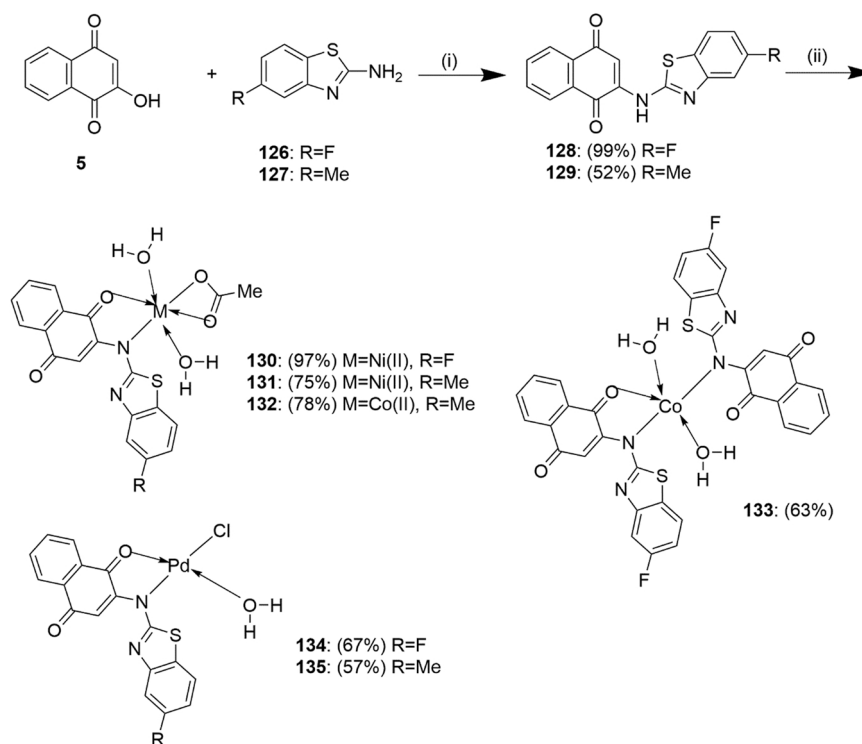
exhibit activity, and the Mn(II) **124** was only active on *B. cereus* with inhibitory zones of 13.5 mm. The authors explained this unexpected behavior by the fact that chelation could reduce the polarity of metal ions and increase the delocalization of p-electrons over the whole chelated ring, leading to a global increasing of the complex's lipophilicity. This consequently would improve the penetration of the complexes into the lipid membrane and cause death of the bacteria.

Compound **119** was obtained as described in Scheme 15, by Michael addition of 2-aminobenzothiazole **118** to a solution of lawsone **5** in ethanol. The solution was refluxed for 6 h after which a precipitate was formed. The resultant precipitate was filtered, dried to recover compound **119** in 75% yield. The corresponding metal complexes were obtained dissolving compound **119** in methanol and adding an equimolar concentration of the desired metal (II) salts (chloride, nitrate and acetate), dissolved in methanol while stirring and heating at 50 °C. The resulting homogeneous solution was then buffered with Et₃N to a pH of 9 and refluxed for 3 h. The formed precipitates were filtered under vacuum, washed with methanol, and dried to give **120** [Zn-**119**(OAc)]•4 H₂O, **121** [Ni-**119**(OAc)]•3 H₂O, **122** [Cu-**119**(OAc)]•H₂O, **123** [Co-**119**(OAc)]•H₂O, **124** [Mn-(**119**)₂]•2 H₂O, **125** [Pd-(**119**)₂]•H₂O in 5–83% yields (Scheme 15).

A.C. Ekennia et al. have synthesized two analogs of compound **119**, 2-[(5-fluoro-1,3-benzothiazol-2-yl)amino]-1,4-naphthoquinone **128** and 2-[(5-methyl-1,3-benzothiazol-2-yl)amino]-1,4-naphthoquinone **129** and their Pd(II), Ni(II) and Co(II) metal complexes [64]. The antibacterial activities of the free ligands **128** and **129** and their metal complexes were then evaluated. The antibacterial activities of **128** and

its metal complexes showed that the bacterial organisms were susceptible to the fluoro-substituted compounds with an inhibitory zone range of 13.5–27 mm. The organisms were more susceptible to **130** and **133** compared to **128** and **134**. Metal complex **130** showed the largest inhibitory zone range of 23.0–27.5 mm compared to the other compounds, while **134** gave the lowest inhibitory zone range of 13.5–18.5 mm (Table 4). In addition, *E. coli* was more susceptible to **130** compared to streptomycin at the screening concentration of 10 mg/mL. *S. aureus* was also more susceptible to **130** and **133** than streptomycin at the same concentration. **130** had the best MIC among the screened compounds on *E. coli* which is equal to the MIC of the reference antibiotic (streptomycin) (Table 5). To conclude, the fluoro-substituted ligand **128** and its metal complexes exhibited a higher antibacterial activity compared to the methyl-substituted ligand **129** and its metal complexes, probably due to the fluoro group responsible for the increase of the lipophilicity properties through cell membranes of bacterial strains. (Table 6).

Compounds **128** and **129** and their corresponding metal complexes were obtained in the same manner than compound **119** using 2-amino-6-fluorobenzothiazole **126** for compound **128** and 2-amino-6-methylbenzothiazole **127** for compound **129**, with a yield of 99% for compound **128** and 97%, 63%, 67% for its complexes [Ni **128** (H₂O)₂(CH₃COO⁻)]**130**, [Co(**128**)₂(H₂O)₂]•2 H₂O **133** and [Pd(**128**)(H₂O)Cl] **134** respectively. A yield of 52% was obtained for compound **129** and 75%, 78%, 57% for its metal complexes [Ni **129** (H₂O)₂(CH₃COO⁻)]•H₂O **131**, [Co **129** (H₂O)₂(CH₃COO⁻)] **132**, and [Pd **129** (H₂O)Cl] **135** respectively (Scheme 16).



Scheme 16. Reagents and conditions: (i) EtOH, CH₃CO₂H, 3 h; (ii) EtOH, corresponding metal salts, 3 h, Et₃N.

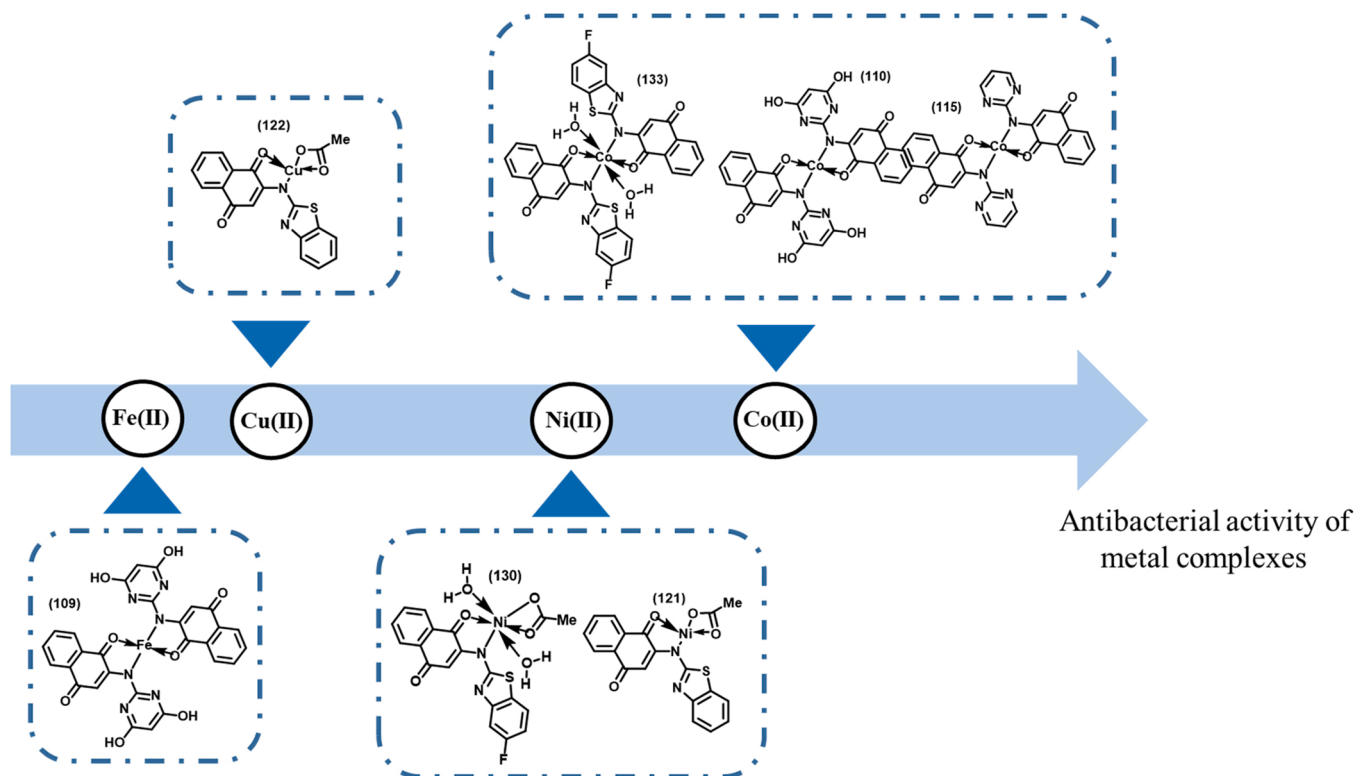


Fig. 19. Schematic representation of the metal contribution to the antibacterial activity of the synthesized complexes.

For instance, research remains focused on complexes from lawsone **5** but we can expect a chemical diversification with the growing interest around naphthoquinones complexes. Most of the time, the same ligand has been used to coordinate two naphthoquinones (**109**, **115**, **133**, Fig. 5), but complexes with one naphthoquinone and acetate have also

been synthesized (**121**, **122**). Over the past 20 years, a total of 7 different metals were chosen with the main goal to improve the antibacterial activity, among those, iron(II), copper(II), nickel(II) and cobalt(II) complexes stand out from the crowd with respective improvements in the antibacterial activity compared to the free ligands. Notably, we

can highlight the iron complex **109** with a pyrimidone moiety has only been described once with promising results on *E. coli* with an inhibitory zone of 18.5 mm and *P. mirabilis* with an inhibitory zone of 25.0 mm compared to the ligand alone with an inhibitory zone of 9 and 12.5 mm, respectively [93]. Copper complexes have been less studied than the former ones but still gave interesting results with an inhibitory zone range of 23.0–31.0 mm on both Gram-positive and Gram-negative panel of bacteria (122, Fig. 19) [97].

Nickel has been often used and showed a global very promising activity with an inhibitory zone range of 13.0–27.5 mm and a MIC range of 0.05–0.5 mg/mL (121, 130, Fig. 19) (Festus et al.; Ekennia et al.). Cobalt has been widely used to form complexes and showed the best and the most consistent broad-spectrum inhibition on both Gram-positive and Gram-negative bacteria with an inhibitory zone range of 19.0–29.0 mm and a MIC range of 0.05–0.5 mg/mL (110, 115, 133, Fig. 19) [64,97].

4. Conclusions

The 1,4-naphthoquinone derivatives have been recognized as a promising new class of compounds that can be used to treat bacterial infections. The worrying threat posed by bacteria combined with its ability to develop resistance have emulated some researchers to work on antibiotic therapy and describe numerous studies included in this review. These research studies identified several compounds derived from the 1,4-naphthoquinone core with interesting antibacterial activities. Only a few were selected for *in vivo* studies. However, for most of them, the mechanisms of action are still unclear or not elucidated yet. Toxicity studies on mammalian cell lines have been carried out but not in all studies reported above, especially on metal complexes which are known to present secondary effects in clinical phase and lead to therapeutic dose reduction or discontinuation. In general, encouraging progress has been realized in the reported articles in order to fight against antibiotic resistance. The use of natural bioactive compounds and their optimization is relevant, especially regarding the broad spectrum of biological activity potential and the very weak cytotoxic effect of molecules like 1,4-naphthoquinone hydroxyderivatives. However, the extraction of these active compounds from plants is not straightforward as it is often associated with impurities. Chemical synthesis finds its place because it allows to obtain compounds with a variety of substituent positions on the 1,4-naphthoquinone moiety alongside molecular docking studies that could help scientists developing more guided derivatives. It is although worth noting that the urgency to develop molecules which can go to the advanced stages of clinical studies to get them from the lab to the bedside is as important today as ever.

CRedit authorship contribution statement

ZB was responsible for the management of the work, overall supervision. CM wrote the manuscript with ZB. VD and PM contributed to proofreading and critical revision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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