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In situ palladium/N-heterocyclic carbene complex catalyzed carbonylative cross-coupling reactions of arylboronic acids with 2-bromopyridine under CO pressure: efficient synthesis of unsymmetrical arylpyridine ketones and their antimicrobial activities

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Abstract
The carbonylative Suzuki cross-coupling of 2-bromopyridine with various boronic acids to prepare unsymmetrical arylpyridine ketones has been carried out using palladium/N-heterocyclic carbene complexes as catalysts prepared in situ. The selectivity and the rate of these reactions are highly dependent on the conditions, i.e., nature of the palladium catalyst precursor, solvent, temperature and CO pressure. The main side-products arise from direct, non-carbonylative cross-coupling. Under the optimum conditions, arylpyridine ketones are recovered in high yields (60–88%). The antibacterial activities of the corresponding benzimidazole salts **2** were tested against Gram positive and negative bacteria using the agar dilution procedure, and their IC50 values have been determined.

Introduction

Following Arduengo's isolation of free N-heterocyclic carbenes (NHC) [1], NHCs have gained an important place as ancillary ligands for transition metals, partially as alternatives to phosphine ligands [2–7]. Although NHCs and phosphines are considered to be similar as ligands, their steric and electronic behaviors are rather different. The NHCs have some advantages compared to phosphines, such as high thermal stability, lower toxicity, resistance to oxidation [8, 9], strong σ-donor [10] and weak π-acceptor capability, as well as easily tuneable electronic and steric properties and straightforward synthesis. Complexes of NHC ligands have been synthesized for almost every transition metal [11–13]. Among NHC complexes, the palladium complexes have come to the fore due to their success as catalysts for cross-coupling reactions [14–17]. Although excellent progress has been made with mononuclear complexes [18–28], different types of NHC-Pd complexes and their catalytic properties have recently started to attract attention [29–35]. At present, palladium-catalyzed cross-coupling reactions represent over 60% of the carbon–carbon bond formation reactions used in medicinal chemistry [36]. In particular, the discovery of palladium-catalyzed C–C coupling reactions, such as Heck–Mizoroki coupling [37, 38] in the early 1970s and Suzuki–Miyaura coupling [39] in the early 1980s, has been exploited in a wide range of fields, from natural product synthesis to materials science, as well as biologically important molecules including drugs and agrochemicals [40]. A great number of in situ generated NHC-Pd catalysts have been reported for Suzuki cross-coupling reactions [47–49]. Herrmann reported the first Pd-NHC catalyst for Suzuki cross-coupling in 2002 [50, 51]. Subsequently, Nolan [52–62], Glorious [63–65], Organ [66–71] and others [40, 72–74] reported different Pd-NHC catalysts for Suzuki–Miyaura cross-couplings. α-Pyridyl ketones are useful intermediates in

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the synthesis of various natural products and drugs [41, 42]. Different methodologies have been proposed to access this important class of products. Many of them are based on the stoichiometric reaction of a pyridyl organometallic derivative with an electrophile; i.e., selective ortho-metallation of pyridine with lithiated reagents followed by reaction with amides [43, 44], anhydrides or aroyl chlorides to pyridyl Grignard reagents [45, 46], pyridyl-tellurides [47], pyridyl-zinc [48, 49] or pyridyltrialkylstannyl derivatives [50, 51]. Other routes involve oxidation of hydroxy-, imino- and benzyl-substituents on pyridyl rings [52–57], or use multistep procedures [58, 59]. However, these aforementioned methodologies generally require expensive and/or toxic reagents (oxidizers), can give poor yields, and/or are only effective with specific substrates. In this regard, to our knowledge, only three examples of Suzuki carbonylative cross-coupling of 2-pyridine halides with 3-bromoquinoline have been reported, giving the expected benzoyl products in up to 66% yield [60–62]. Among the methodologies described in the literature, the Suzuki reaction is one of the most practical for the formation of nitrogen-containing heterobiaryls through alternative cross-coupling paths (Scheme 1) [63–65]. The first path involves cross-coupling of a 2-pyridyl boronic acid/ester or borate with an aryl halide (Scheme 1, path I). In recent years, a variety of activated boronates have been reported for the construction of biaryl systems containing a 2-pyridyl moiety [66]. However, path I is limited by the accessibility and/or stability of the boron-containing reagents. Additionally, a phosphine ligand is necessary for path I [67–71]. Path II uses a 2-halogenated pyridine as one of the coupling partners. The groups of Buchwald [72, 73], Fu [74], Plenio [75], and others [76, 77] have developed ligand-promoted approaches for the formation of 2-aryl-substituted pyridine derivatives from 2-halopyridines. Recently, ligand-free protocols for the synthesis of heterobiaryls containing a 2-pyridyl moiety via path II have attracted attention because they are simpler and cheaper than their ligand counterparts [78–80]. Unfortunately, the ligand-free Suzuki reaction usually proceeds with low reactivity with respect to 2-halopyridines.

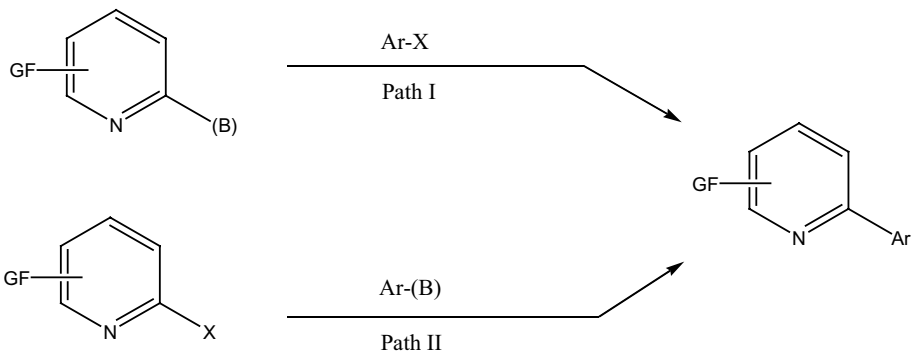
We have previously obtained results [81] that suggest that an in situ four-component system consisting of $Pd(OAc)_2$, 1,3-dialkylbenzimidazolium halide and K_2CO_3 under a CO atmosphere catalyzes carbonylative cross-coupling of 2-bromopyridine with various boronic acids to give unsymmetrical arylpyridine ketones. We were therefore interested to see whether analogous in situ palladium N-heterocyclic carbene complexes would show good activity in carbonylative cross-coupling reactions of arylboronic acids with 2-bromopyridine under CO. We now report that the proper choice of reaction conditions enables the easy and selective transformation of a variety of arylboronic acids to unsymmetrical arylpyridine ketones. In addition, all of the obtained compounds were tested for in vitro antibacterial activities against different strains.

Results and discussion

Synthesis and characterization of benzimidazolium salts 2a–e

The N,N-disubstituted benzimidazolium salts **2a–e** (Scheme 2) were synthesized in two steps. The first involves the introduction of an N-alkyl/group aryl to the benzimidazole, enhancing the reactivity of the other the nitrogen atom. Addition of the other alkylating agent to the remaining nitrogen atom gives the desired N,N-disubstituted benzimidazolium salt[45]. Benzimidazolium salts **2a–e** were prepared in good yields of 89–96% by treatment of the N,N-substituted benzimidazoles (A), with appropriate substituted benzyl halides in DMF at 70 °C for 48 h, as previously described [82].

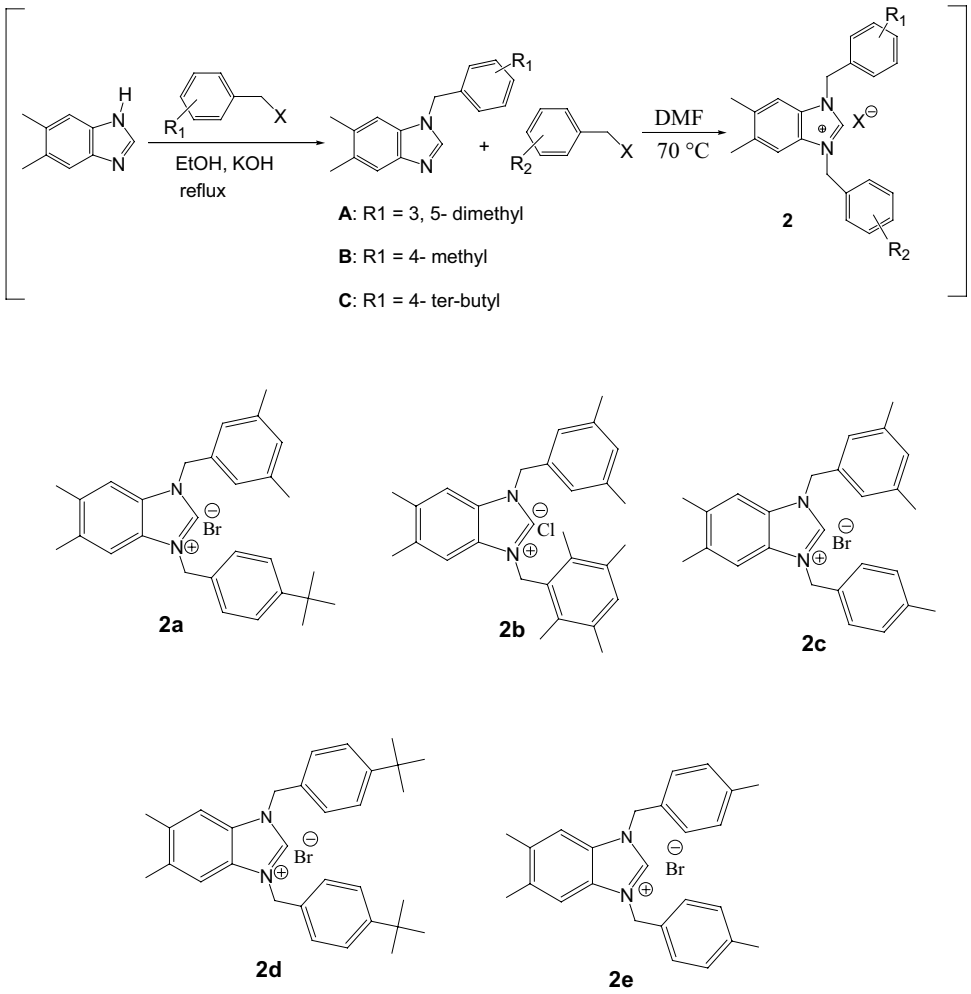
The benzimidazolium salts **2a–e** are stable to air and moisture, both in the solid state and in solution. They were



(B)= boronic acid, boronate ester, borate

Scheme 1 Alternative synthetic paths for 2-aryl-substituted pyridine derivatives

Scheme 2 General preparation and structure formulae of benzimidazolium salts **2a–e**



characterized by ^{1}H-NMR, ^{13}C{1H} NMR, IR and elemental analysis techniques.

The ^{1}H-NMR spectra of salts **2a–e** showed the NCHN protons as singlets at 11.58, 11.17, 11.54, 11.63 and 11.57 ppm, respectively. In the 13C{^{1}H} NMR spectra, the N*C*HN carbon of benzimidazolium salts is usually observed at around 142 ± 4 [82]. For the salts **2a–e,** it was observed at 152.1, 142.9, 141.8, 163.0 and 141.6 ppm, respectively. In the IR spectra, the ν(C=N) bands for salts **2a–e** were observed at 1566, 1547, 1566, 1670 and 1599 cm^{-1}, respectively. These values are in good agreement with previously reported values for benzimidazolium salts [83]. The benzylic –CH_2– proton signals $H_{1',1''}$ for the benzimidazolium salt **2e** as representative were observed at 5.67 and 5.76 ppm, respectively, while the aromatic protons appeared at δ between 6.97 and 7.37 ppm. The carbene carbon signals of the salt **2e** were observed at δ 141.6 ppm in the ^{13}C NMR spectrum, while the $C_{1',1''}$ carbon signals were at δ 51.1 ppm. The mass spectrum of salt **2e** gave the most prominent peak at m/z = 253; this can be explained by the fragmentation pattern shown in Scheme 3.

Scheme 3 Mechanism of fragmentation of **2e** leading to the m/z = 253 peak

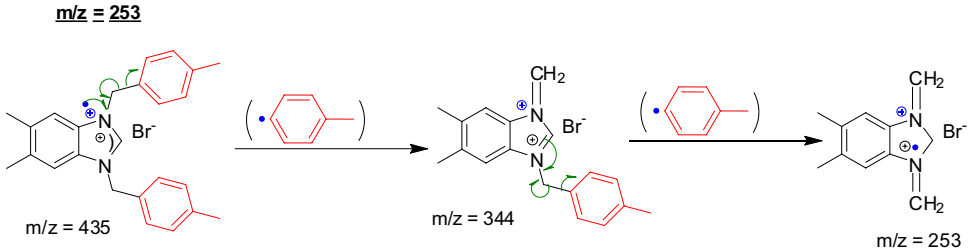
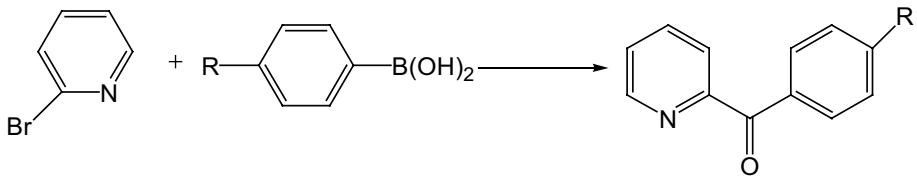


Table 1 Optimization of reaction conditions for the carbonylative cross-coupling reaction of 2-bromopyridine with phenyl boronic acid

Entry	Ligand (mol%)	Base	Temperature (°C)	Pressure of CO (bar)	Solvent	Yield
1	3	K_2CO_3	100	20	DMF	–
2	3	K_2CO_3	100	20	1,4-dioxane	55%
3	3	K_2CO_3	100	20	THF	65%
4	3	*t*-BuOK	100	20	THF	50
5	3	Cs_2CO_3	100	20	THF	70%
6	3	K_2CO_3	100	10	THF	55%
7	3	K_2CO_3	100	40	THF	60%
8	3	K_2CO_3	110	20	THF	70%
9	3	K_2CO_3	120	20	THF	65%
10	5	K_2CO_3	110	20	THF	85%
11	5	K_2CO_3	120	20	THF	70%
12	6	K_2CO_3	110	20	THF	85%

Conditions: 2-Br-Py (1 mmol), $Pd(OAc)_2$, base (2 mmol), benzimidazole **2e**, $PhB(OH)_2$ (1.1 mmol), CO, solvent (10 mL), T°, overnight

Scheme 4 Carbonylative cross-coupling reaction of 2-bromopyridine with phenylboronic acid



Palladium catalysts based on N-heterocyclic carbene ligands have previously been exploited for the effective carbonylative coupling of different chloropyridines with phenylboronic acid [84]. However, the results to date suggest that commercial Pd-based catalysts are not effective for this transformation, probably because of poisoning by CO [85]. Thus, we have studied the behavior of benzimidazole salt **2e** as a ligand in the presence of $Pd(OAc)_2$ for the carbonylative cross-coupling of 2-bromo pyridine with different boronic acids.

The reaction of 2-bromopyridine with phenyl boronic acid was chosen as a model system in order to optimize the reaction conditions (Scheme 3, Table 1) under different CO pressures at 110 °C, using various solvents and bases with $Pd(OAc)_2$ and two equivalents of **2e**. The results are summarized in Table 1. Direct non-carbonylative cross-coupling is the major side reaction, and 2-bromopyridine was recovered with a good final selectivity (entry 12), Scheme 4.

Selected results are shown in Table 1. Conditions; benzimidazolium salt **2e**, CO, base, $Pd(OAc)_2$ for Table 1, R=H.

First, DMF, 1.4-dioxane and THF were used as solvents, (Table 1, entries 1–3). No reaction was observed in DMF (Table 1, entry 1). Use of 1,4-dioxane also gave unsatisfactory results in this case in terms of the selectivity for the product (Table 1, entry 2). However, when the reaction was carried out in tetrahydrofuran, the selectivity was markedly increased and the desired product was obtained with a yield of 65% (entry 3). Use of alkali carbonates such as K_2CO_3 and $CsCO_3$ as base gave good yields (entries 3, 5). On varying the reaction temperature, the best yield was obtained at 110 °C (entry 8). Increasing the temperature to 120 °C resulted in a decrease in the yield to 65% (entry 9). We therefore selected 110 °C as the optimum temperature.

Based on these results, the optimized reaction conditions were determined as: 2-bromopyridine (1.0 mmol), arylboronic acid (1.1 mmol), benzimidazole **2e** (5 mol %), $Pd(OAc)_2$ (0.025 mmol) and K_2CO_3 (2.0 mmol) in THF at 110 °C under a CO atmosphere (20 bar).

To further investigate the scope and limitations of this methodology, we carried out cross-couplings of 2-bromopyridine with various aryl boronic acids under the optimized conditions. As shown in Table 2, the coupling of phenylboronic acid with 2-bromopyridine gave exclusively phenyl(pyridin-2-yl)methanone in 75% yield, showing high efficiency and good selectivity (Table 2, entry 1). Using 4-methylphenyl boronic acid instead of phenylboronic acid, the cross-coupling was completed in 67% yield (Table 2, entry 2). The cross-coupling of 2-bromo pyridine with (4-formylphenyl) boronic acid proceeded well to give the expected product in excellent yield (entry 4), while 4-boronobenzoic acid also afforded a satisfactory 88% (entry 5). Scheme 5.

The chemical structures of the arylpyridine ketones were established by FTIR, ^{1}HNMR and ^{13}C-NMR.

Table 2 Results of carbonylative cross-coupling reaction of 2-bromopyridine with arylboronic acids

Entry	Boronic acid	Yield (%)
1	R=H	75
2	R=CH_3	67
3	R=OCH_3	65
4	R=CHO	90
5	R=COOH	88

Conditions: 2-Br-Py (1 mmol), $Pd(OAc)_2$ (0.025 mmol), K_2CO_3 (2 mmol), benzimidazolium salt 2**e** (5 mol%), arylboronic acid (1.1 mmol), CO (20 bars), 110 °C, THF (10 mL), one night

Antibacterial activities

The benzimidazolium salts **2a–e** were evaluated for antimicrobial activity against three Gram-positive bacteria, namely *Micrococcus luteus LB 14110, Staphylococcus aureus* ATCC 6538 *and Listeria monocytogenes* ATCC 19117, plus two Gram-negative bacteria (*Salmonella Typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 49189). The results of these experiments are presented in Table 3. All of these benzimidazole salts exhibit antimicrobial activity against one or more strain of bacteria. Although salt **2e** showed high antimicrobial activity, salt **2b** showed high activity against Gram-positive and Gram-negative bacteria in high and low concentrations.

In parallel, minimal inhibitory concentration (MIC) values of the benzimidazole salts were determined against *M. luteus* and *L. monocytogenes* and the Gram-negative bacterium *S. typhimurium*. Ampicillin was used as a positive control. As shown in Table 4, the MIC values range from 1.5 to 1.8 mg/mL against *M. luteus*; 1.8–2.4 mg/mL for *L. monocytogenes* and 2.1–2.6 mg/mL for *S. typhimurium*.

Conclusion

In this study, we have shown that the carbonylative Suzuki cross-coupling of 2-bromopyridine and arylboronic acids catalyzed by our palladium complexes provides an efficient and general route to a variety of unsymmetrical arylpyridine ketones. Further development of this chemistry using 2-chloropyridine substrates will be reported in due course. In addition, the benzimidazolium salts **2a–e** showed significant antimicrobial activity compared with a standard antibiotic.

Table 3 Antibacterial activity of the benzimidazolium salts **2a–e**

Microorganism indicator	Compound	Inhibition zone (mm)
Micrococcus luteus LB 14110	**2a**	15 ± 0.4
	2b	16 ± 0.3
	2c	14 ± 0.2
	2d	17 ± 0.4
	2e	13 ± 0.4
Staphylococcus aureus ATCC 6538	**2a**	13 ± 0.4
	2b	14 ± 0.3
	2c	14 ± 0.2
	2d	13 ± 0.2
	2e	12 ± 0.3
Listeria monocytogenes ATCC 19117	**2a**	15 ± 0.4
	2b	16 ± 0.3
	2c	14 ± 0.2
	2d	13 ± 0.4
	2e	14 ± 0.5
Salmonella typhimurium ATCC 14028	**2a**	18 ± 0.5
	2b	20 ± 0.2
	2c	18 ± 0.1
	2d	19 ± 0.3
	2e	18 ± 0.5
Pseudomonas aeruginosa ATCC 49189	**2a**	17 ± 0.5
	2b	18 ± 0.2
	2c	17 ± 0.1
	2d	19 ± 0.3
	2e	18 ± 0.5

Experimental section

Materials and methods

All manipulations were performed using standard Schlenk techniques under an argon atmosphere. Chemicals were purchased from Sigma–Aldrich and used without further purification. All solvents were purified and dried with an MBraun SPS 800 solvent purification system. Column chromatography was performed using silica gel 60 (70–230 mesh). ^1H and ^{13}C NMR spectra were recorded on an AC-300

Scheme 5 Carbonylative cross-coupling reaction of 2-bromopyridine with arylboronic acids. The results are summarized in Table 2

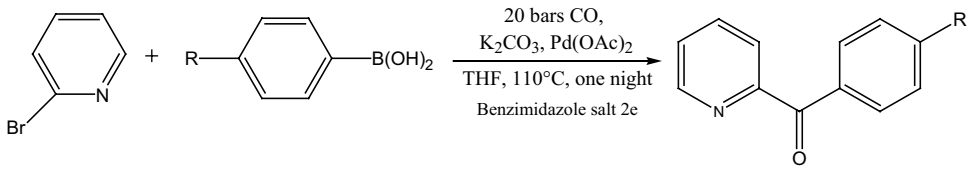


Table 4 Minimum inhibitory concentrations (MICs) for benzimidazolium salts **2a–e**

Microorganism indicator	Compounds	MIC (mg/mL)
Micrococcus luteus	**2a**	1.8
	2b	1.7
	2c	1.5
	2d	1.6
	2e	1.8
	Ampicillin	0.0195
Listeria monocytogenes	**2a**	2.2
	2b	2.3
	2c	2.4
	2d	1.8
	2e	1.9
	Ampicillin	0.039
Salmonella typhimurium	**2a**	2.6
	2b	2.3
	2c	2.2
	2d	2.1
	2e	2.4
	Ampicillin	0.625

Bruker spectrometer at 300 MHz and 75 MHz, respectively. Chemical shifts, δ, are reported in ppm relative to the internal standard TMS for both ^{1}H and ^{13}C NMR. The products were characterized by GC. Quantitative GC analyses were performed with a Shimadzu GC-2010 Plus gas chromatography. NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, m = multiplet. IR spectra were recorded on a 398 spectrophotometer (Perkin-Elmer, King Saud University, Riyadh, Saudi Arabia). Mass spectra were recorded on a ((DART-TOF–MS) instrument at King Saud University. Microanalyses were obtained on an Elementar Vario El III Carlo Erba 1108 elemental analyzer (King Saud University), and the obtained values were within ± 0.3% of the theoretical values. Melting points were determined with a Kofler bench instrument at Isste of Borj cedria (Hammam Lif, University of Carthage, Borj Cedria, Tunisia).

The N-substituted benzimidazoles (A–B) and the benzimidazolium salts **2a–e** were prepared according the reported procedures [82].

Antibacterial activities were determined according the reported procedures [82].

Gram-positive bacteria: *Micrococcus luteus* LB 14110, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 19117, and Gram-negative bacteria: *Salmonella Typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 49189. Bacterial cultures were grown overnight in Luria-Bertani (LB) agar medium composed of (g/L): peptone 10; yeast extract 5; and NaCl 5 at pH 7.2 under aerobic conditions and constant agitation (200 rpm) at 30 °C for *M. luteus*, and *L. monocytogenes* and at 37 °C for *S. aureus*, S. *typhimurium* and *P. aeruginosa* ATCC 49189, and then diluted 1:100 in LB medium and incubated for 5 h under constant agitation (200 rpm) at the appropriate temperature.

The agar well diffusion method was employed for determination of the antibacterial activities of the compounds, using the published method [86]. Briefly, the test compounds were allowed to diffuse out into LB agar medium on a plate freshly seeded with a suspension of the required microorganism (0.1 mL of 10^8 cells per mL). Each compound was dissolved in DMSO/water (1/9; v/v) to a final concentration of 20 mg/mL, and then, 100 µL of the solution was placed into the corresponding well. The plate was incubated at the appropriate temperature for each microorganism, keeping it at 4 °C for 2 h. The antibacterial activity was assayed by measuring, in millimeters, the diameter of the inhibition zone formed around the well. This determination was done in triplicate, and the reported diameter of inhibition is the average of the three tests.

The antimicrobial activities of the compounds were also assayed by their minimum inhibitory concentrations (MICs) in accordance with NCCLS guidelines M7-A_6 and M38-P [87]. Tests were performed in sterile 96-well microplates with a final volume in each well of 100 µL. The test compounds (20 mg/mL) were dissolved in DMSO/water (1/9; v/v). These solutions were transferred to the wells in order to obtain twofold serial dilutions and to produce the concentration range of 0.0048–20 mg/mL.

To each test well, 10 µL of cell suspension was added to give final inoculum concentrations of 10^6 CFU/mL for each microorganism. Positive growth control wells consisted of the microorganisms only in the medium. Ampicillin was used as a positive control. The plates were covered with sterile plate covers and incubated at the appropriate temperature for each microorganism. The MIC was defined as the lowest concentration of the test compound at which the microorganism showed no visible growth after incubation. As an aid to visualization of microorganism growth, 25 µL of thiazolyl blue tetrazolium Bromide (MTT), indicator solution (0.5 mg/mL) dissolved in sterile water was added to the wells and incubated at room temperature for 30 min.

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