

Synthesis of New 3-(2-Chloroquinolin-3-yl)-5-Phenylisoxazole Derivatives via Click-Chemistry Approach

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Aqui relatamos a síntese de novos 3-(2-cloroquinolin-3-il)-5-fenilisoxazóis (**3a-j**) substituídos por *click chemistry* com rendimentos bons a moderados. Esta abordagem baseia-se na cicloadição regioseletiva catalisada por cobre(I) entre diferentes óxidos de nitrila derivados de 2-cloroquinolina-3-carbaldeídos (**2a-j**) e fenilacetileno. Finalmente, estes derivados foram testados para a sua avaliação antibacteriana *in vitro* contra três bactérias clínicas Gram-negativas: *Escherichia coli*, *Pseudomonas aeruginosa* e *Acinetobacter baumannii* utilizando métodos convencionais.

Herein, we report the synthesis of new substituted 3-(2-chloroquinolin-3-yl)-5-phenylisoxazole (**3a-j**) by click chemistry in good to moderate yields. This approach is based on the regioselective copper(I)-catalyzed cycloaddition between different nitrile oxides derived from 2-chloroquinoline-3-carbaldehyde (**2a-j**) and phenylacetylene. Finally these derivatives were screened for their antibacterial evaluation *in vitro* against three Gram-negative clinical bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using standard methods.

Keywords: quinoline, isoxazol, 1,3-dipolar cycloaddition, click chemistry

Introduction

Quinolines and their derivatives are an important group of heterocycles that possess a variety of commercial applications, such as pharmaceuticals, fragrances and dyes.¹ These nitrogen compounds are widely found in natural products, from classic examples, such as the alkaloids isolated from the bark of the cinchona tree,² to the antitumoral agent dynemicin A.³

In recent years quinoline derivatives fused and/or substituted with heterocyclic rings were demonstrated to have significant biological activities. For example, the insertion of the benzimidazole ring at 4-position showed antimicrobial and antifungal activity (Figure 1, compound A).⁴ The substitution of a triazole ring at 3-position of the 2-chloro-quinoline derivatives enhances antimicrobial and antifungal properties (compound B).⁵ In addition, antimicrobial and antituberculosis activity was found by insertion of the oxazole⁶ (compound C) and isoxazol⁷ (compound D) ring in the quinoline core.

Isoxazole is a structure of special interest in the field of medicinal chemistry, and several biological activities for its derivatives have been reported. These include GABA_A antagonist,⁸ multi-resistant drug transport inhibition (MDR-1),⁹ antitumoral,¹⁰⁻¹² tyrosine-kinase receptor antagonist,¹³ antimicrobial,¹⁴ antiviral,¹⁵ antinociceptive,¹⁶ and anti-asthmatic activities.¹⁷

Click chemistry is a powerful reaction for making carbon-heteroatom-carbon bonds from widely available reagents in a reliable, quick and economic manner. It is also of importance in the process of drug discovery, chemical biology and proteomics.¹⁸ One of its most common applications, copper(I)-catalyzed regioselective cycloaddition of azides and terminal alkynes,¹⁹ has been examined in a large number of reports.²⁰⁻²⁴

As part of our research group's interest in quinoline synthesis²⁵ and considering the good results achieved by synthesizing quinolines connected to other heterocycles, we envisaged combining the quinoline and the isoxazole in the same unit. This would be a notable advance in the synthesis of bioactive derivatives. Here we report the synthesis and initial screening of the antibacterial activity of the title compounds arrived at by the click chemistry approach.

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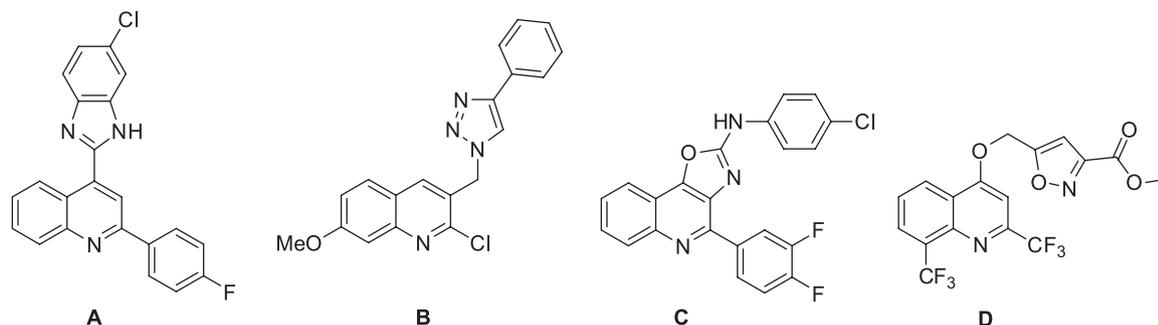


Figure 1. Examples of quinolines fused and/or substituted with heterocyclic rings.

Results and Discussion

Isoxazolic synthesis has been widely studied^{26,27} and a large number of methods are known in the literature, including cyclocondensations between hydroxylamine and 1,3-dicarbonyl compounds,²⁸ α - β -unsaturated carbonyls²⁹⁻³¹ and α - β -unsaturated nitriles.³² However, the copper-catalyzed version of acetylenes with dipoles such as nitrile oxides has been of great help in the regioselective synthesis of 3,5-disubstituted isoxazoles³³ (Figure 2). In this investigation the quinoline system containing the key aldehyde functionality was prepared with the Vilsmeier reagent and the isoxazole ring synthesis was carried out using a regioselective copper(I)-catalyzed cycloaddition reaction.

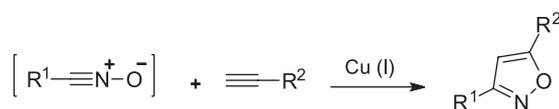


Figure 2. General synthesis of 3,5-disubstituted isoxazoles.

Chemistry

Initially different commercially-substituted anilines were quickly transformed by the acetylation reaction in acetic acid and acetic anhydride in reflux for 3-4 hours, obtaining all the corresponding N-arylacetylides in high yields (**1a-j**). 2-chloroquinolin-3-carbaldehydes (**2a-j**), were prepared with the Vilsmeier reagent (DMF+POCl₃) and confirmed by FT-IR analyses where an intense absorption band between 1680-1695 cm⁻¹ corresponding to the aldehyde group (Table 1).

3-(2-chloroquinolin-3-yl)-5-phenylisoxazoles (**3a-j**) were synthesized by click chemistry approach using a regioselective copper(I)-catalyzed cycloaddition reaction (Scheme 3). We first synthesized the aldoxime by reacting of 2-chloro-quinolin-3-carbaldehydes (**2a-j**) with hydroxylamine hydrochloride in presence of NaOH. The nitrile oxide was obtained *in situ* by treatment with Chloramine-T, and the final compounds were achieved

Table 1. Synthesis of 2-chloro-quinolin-3-carbaldehydes **2a-j**^a

entry	R ¹	R ²	R ³	R ⁴	Yield ^b / %
a	H	Cl	H	H	45
b	Me	H	H	Me	81
e	H	Me	H	H	85
d	OMe	H	H	OMe	80
e	H	F	H	H	40
f	H	H	H	H	89
g	H	Br	H	H	45
h	H	OMe	H	H	91
i	H	I	H	H	58
j	Me	H	Me	H	86

^aReaction conditions: DMF, POCl₃, 85 °C, 24 h; ^bisolated yield.

by cycloaddition reactions with the aromatic alkyne catalyzed by copper. The highly reactive nitrile oxides are chemically obtained by means of HCl elimination from hydroximinoyl chlorides commonly prepared through the use of a halogenating reagent (NaOCl, Cl₂ or NCS) in presence of a base (most frequently Et₃N). However, the double functionality of Chloramine-T permits its use as a halogenating-dehalogenating agent in this reaction in a quick, simple and clean manner.

The infrared (IR) spectrum of this compound **3a-J** shows an absorption band due to stretching (C=N) of the isoxazolic ring in the range of 1588-1598 cm⁻¹ (Table 2). All the derivatives were confirmed by ¹H nuclear magnetic resonance (NMR), ¹³C NMR and mass spectra.

The presence of the compounds was confirmed by analysis of the formation of the isoxazolic ring, where

Table 2. Synthesis of compounds **3a-j**^a

entry	Product	M.P. / °C	Yield ^b / %
1		198-200	60
2		152-154	74
3		145-147	75
4		165-167	69
5		199-201	61
6		150-152	79
7		212-214	64
8		166-168	77
9		196-198	70
10		182-184	85

^aReaction conditions: (i) NH₂OHHCl, NaOH, EtOH, r.t., 45 min. (ii) Chloramine-T trihydrate, r.t., 15 min. (iii) CuSO₄, Cu, phenylacetylene, r.t., 8 h; ^bisolated yield.

the isoxazolic proton CH-(4) is observed as a singlet with $\delta = 7.09$ - 7.12 ppm. In the ¹³C NMR spectrum, the C-(4) of the isoxazolic ring resonates at $\delta = 100.8$ - 101.0 ppm.

Antibacterial studies

The *in vitro* antibacterial activity of the new synthesized derivatives (**3a-j**) was carried out using a microdilution method at different concentrations (125-2000 $\mu\text{g mL}^{-1}$) and assayed against three different Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (clinical isolates), using G-Penicillin as a control. Compounds **3a-j** bearing electron-withdrawing and electron-donating substituents on the benzene ring of the quinoline did

not show any antibacterial activity to these three Gram-negative bacteria.

Conclusions

A new series of quinolines containing the isoxazole moiety 3-(2-chloroquinolin-3-yl)-5-phenylisoxazole (**3a-j**) has been synthesized using a click chemistry approach from good to moderate yields. These new compounds have been screened for antibacterial activity, but none exhibited specific activity. It can be concluded that the substitution of isoxazole at 3-position of the quinoline ring does not cause inhibition on the tested bacterias. Further investigation of synthetic methodology for the quinoline-isoxazole framework is in progress.

Experimental

Melting points were recorded on an Electrothermal 9100 instrument and are uncorrected. IR spectra were obtained with a Nicolet Nexus 470-FTIR spectrometer as KBr pellets and are reported in wavenumbers (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were measured on a Bruker AM-400 spectrometer (400 MHz ^1H NMR and 100 MHz ^{13}C NMR), using CDCl_3 as solvent. Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts (δ) and J values are reported in ppm and Hz, respectively. Multiplicities are shown as the abbreviations: s (singlet), d (doublet), t (triplet), m (multiplet). High-resolution electrospray ionization mass spectrometry (ESI-MS) and ESI-MS/MS analyses were conducted in a high-resolution hybrid quadrupole (Q) and orthogonal time-of-flight (TOF) mass spectrometer (Waters/Micromass QTOF micro, Manchester, UK) with a constant nebulizer temperature of 100 °C. Reaction progress and the purity of the compounds were monitored by means of TLC using Merck Kieselgel 60 (230-240 mesh). All reagents were purchased from Merck and Sigma Aldrich Co. and used without further purification. All solvents used were dried and distilled prior to use.

General procedure for the synthesis of 3-(2-chloroquinolin-3-yl)-5-phenylisoxazol (**3a-j**)

The corresponding precursor, 2-chloroquinolin-3-carbaldehyde (**2a-j**) (1.0 equiv.) was added to a hydroxylamine solution (1.1 equiv.) in ethanol. Then NaOH was added (1.1 equiv.) to the reaction mixture, which was then stirred at room temperature for 45 minutes. The oxime formation was corroborated by thin-layer chromatographic (TLC) analysis. Chloramine-T trihydrate (1.1 equiv.) was added, followed by $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.03 equiv.) and Cu (0.01 equiv.). When a change of color was observed, 10 mL of THF followed by phenylacetylene (1.1 equiv.) were added to the solution and stirred for 8 hours. Once finished, the reaction was filtered to remove copper salts, washed with water, dried with anhydrous Na_2SO_4 and recrystallized from ethanol. The derivatives which could not be purified using recrystallization techniques were purified using column chromatography in silica gel, using an 8:2 Hexane/EtOAc mixture as the mobile phase, except the derivative **2d**, which was purified using only dichloromethane as the mobile phase.

3-(2,6-Dichloroquinolin-3-yl)-5-phenylisoxazole (**3a**)

Yield: 60% as a pale yellow solid; m.p. 198-200 °C; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2920, 1589, 1569, 821; ^1H NMR (400 MHz, CDCl_3) δ 8.54 (s, 1H, quinoline), 8.01 (d, 1H, J 9.3 Hz,

quinoline), 7.89 (s, 2H, phenyl), 7.87 (s, 1H, quinoline), 7.74 (dd, 1H, J 8.8, 2.0 Hz, quinoline), 7.52 (m, 3H, phenyl), 7.1 (s, 1H, isoxazol); ^{13}C NMR (100 MHz, CDCl_3) δ 170.5 (C-isoxazol), 160.0 (C-isoxazol), 148.3 (C-quinoline), 146.1 (C-quinoline), 138.6 (CH-quinoline), 133.6 (C-quinoline), 132.5 (CH-quinoline), 130.5 (CH-quinoline), 129.9 (CH-phenyl), 129.1 (CH-phenyl), 127.3 (C-quinoline), 127.0 (C-quinoline), 126.6 (CH-quinoline), 125.9 (CH-phenyl), 123.8 (C-phenyl), 100.7 (CH-isoxazol); (QTOF-MS) m/z calculated for $\text{C}_{18}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}$: 340.02; found: 340.00.

3-(2-Chloro-5,8-dimethylquinolin-3-yl)-5-phenylisoxazole (**3b**)

Yield: 74% as a white solid; m.p. 152-154 °C; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2914, 1588, 1572, 828; ^1H NMR (400 MHz, CDCl_3) δ 8.74 (s, 1H, quinoline), 7.89 (dd, 2H, J 8.1, 1.2 Hz, phenyl), 7.55 (m, 1H, quinoline), 7.51 (m, 3H, phenyl), 7.33 (d, 1H, J 7.3 Hz, quinoline), 7.13 (s, 1H, isoxazol), 2.77 (s, 3H, quinoline- CH_3), 2.69 (s, 3H, quinoline- CH_3); ^{13}C NMR (100 MHz, CDCl_3) δ 170.2 (C-isoxazol), 160.9 (C-isoxazol), 147.5 (C-quinoline), 146.5 (C-quinoline), 136.7 (C-quinoline), 134.4 (CH-quinoline), 133.0 (CH-quinoline), 131.4 (C-quinoline), 130.4 (CH-quinoline), 129.0 (CH-phenyl), 127.8 (C-quinoline), 127.2 (C-quinoline), 126.2 (CH-phenyl), 125.9 (CH-phenyl), 121.7 (C-phenyl), 101.0 (CH-isoxazol), 18.5 (CH_3 -quinoline), 17.7 (CH_3 -quinoline); (QTOF-MS) m/z calculated for $\text{C}_{20}\text{H}_{15}\text{ClN}_2\text{O}$: 334.09; found: 334.84.

3-(2-Chloro-6-methylquinolin-3-yl)-5-phenylisoxazole (**3c**)

Yield: 75% as a white solid; m.p. 145-147 °C; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2914, 1592, 1569, 821; ^1H NMR (400 MHz, CDCl_3) δ 8.52 (s, 1H, quinoline), 7.96 (d, 1H, J 8.8 Hz, quinoline), 7.87 (d, 2H, J 7.8 Hz, phenyl), 7.64 (m, 2H, quinoline), 7.5 (m, 3H, phenyl), 7.09 (s, 1H, isoxazol), 2.55 (s, 3H, quinoline- CH_3); ^{13}C NMR (100 MHz, CDCl_3) δ 170.2 (C-isoxazol), 160.5 (C-isoxazol), 147.1 (C-quinoline), 146.4 (C-quinoline), 139.1 (C-quinoline), 137.8 (CH-quinoline), 133.9 (CH-quinoline), 130.4 (CH-quinoline), 129.0 (CH-phenyl), 128.0 (CH-quinoline), 127.1 (C-quinoline), 126.8 (CH-phenyl), 126.7 (C-quinoline), 125.8 (CH-phenyl), 122.6 (C-phenyl), 100.9 (CH-isoxazol), 21.6 (CH_3 -quinoline); (QTOF-MS) m/z calculated for $\text{C}_{19}\text{H}_{13}\text{ClN}_2\text{O}$: 320.07; found: 320.85.

3-(2-Chloro-5,8-dimethoxyquinolin-3-yl)-5-phenylisoxazole (**3d**)

Yield: 69% as a yellow solid; m.p. 165-167 °C; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2914, 1588, 1569, 825; ^1H NMR

(400 MHz, CDCl₃) δ 9.00 (m, 1H, quinoline), 7.88 (m, 2H, phenyl), 7.51 (m, 3H, phenyl), 7.10 (s, 1H, isoxazol), 7.05 (d, 1H, *J* 8.8 Hz, quinoline), 6.82 (dd, 1H, *J* 8.8, 1.5 Hz, quinoline), 4.05 (s, 3H, quinoline-OCH₃), 3.97 (s, 3H, quinoline-OCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 170.1 (C-isoxazol), 160.6 (C-isoxazol), 148.9 (C-quinoline), 148.3 (C-quinoline), 147.9 (C-quinoline), 139.7 (C-quinoline), 135.2 (CH-quinoline), 130.3 (C-quinoline), 129.0 (CH-phenyl), 127.1 (CH-phenyl), 125.8 (CH-phenyl), 122.3 (C-phenyl), 120.1 (C-quinoline), 109.6 (CH-quinoline), 104.9 (CH-quinoline), 100.9 (CH-isoxazol), 56.1 (OCH₃-quinoline), 55.8 (OCH₃-quinoline); (QTOF-MS) *m/z* calculated for C₂₀H₁₅ClN₂O₃: 366.08; found: 366.78.

3-(2-Chloro-6-fluoroquinolin-3-yl)-5-phenylisoxazole (**3e**)

Yield: 61% as a white solid; m.p. 199–201 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 2920, 1595, 1572, 825; ¹H NMR (400 MHz, CDCl₃) δ 8.59 (s, 1H, quinoline), 8.09 (dd, 1H, *J* 9.3, 4.9 Hz, quinoline), 7.89 (dd, 2H, *J* 7.8, 1.5 Hz, phenyl), 7.59 (m, 1H, quinoline), 7.55 (d, 1H, *J* 2.9 Hz, quinoline), 7.51 (m, 3H, phenyl), 7.11 (s, 1H, isoxazol); ¹³C NMR (100 MHz, CDCl₃) δ 170.6 (C-isoxazol), 160.0 (C-isoxazol), 148.5 (C-quinoline), 146.4 (C-quinoline), 138.6 (C-quinoline), 135.1 (CH-quinoline), 130.6 (CH-quinoline), 130.0 (C-quinoline), 130.0 (CH-phenyl), 129.1 (CH-phenyl), 127.8 (C-quinoline), 127.0 (C-phenyl), 125.9 (CH-phenyl), 123.9 (CH-quinoline), 121.7 (CH-quinoline), 100.8 (CH-isoxazol); (QTOF-MS) *m/z* calculated for C₁₈H₁₀ClFN₂O: 324.05; found: 324.57.

3-(2-Chloroquinolin-3-yl)-5-phenylisoxazole (**3f**)

Yield: 79% as a white solid; m.p. 150–152 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 2914, 1598, 1572, 820; ¹H NMR (400 MHz, CDCl₃) δ 8.65 (s, 1H, quinoline), 8.10 (d, 1H, *J* 8.3 Hz, quinoline), 7.94 (d, 1H, *J* 8.6 Hz, quinoline), 7.90 (dd, 2H, *J* 7.9, 1.6 Hz, phenyl), 7.84 (m, 1H, quinoline), 7.65 (m, 1H, quinoline), 7.53 (m, 3H, phenyl), 7.12 (s, 1H, isoxazol); ¹³C NMR (100 MHz, CDCl₃) δ 170.5 (C-isoxazol), 160.1 (C-isoxazol), 144.8 (C-quinoline), 139.0 (C-quinoline), 138.9 (CH-quinoline), 131.0 (CH-quinoline), 130.9 (C-quinoline), 130.5 (C-quinoline), 129.1 (CH-phenyl), 127.0 (CH-quinoline), 125.9 (CH-phenyl), 122.0 (CH-quinoline), 121.7 (CH-phenyl), 111.3 (CH-quinoline), 111.1 (C-phenyl), 100.8 (CH-isoxazol); (QTOF-MS) *m/z* calculated for C₁₈H₁₁ClN₂O: 306.06; found: 306.71.

3-(6-Bromo-2-chloroquinolin-3-yl)-5-phenylisoxazole (**3g**)

Yield: 64% as a white solid; m.p. 212–214 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 2920, 1588, 1569, 825; ¹H NMR (400 MHz, CDCl₃) δ 8.55 (s, 1H, quinoline), 8.09 (d, 1H,

J 2.0 Hz, quinoline), 7.96 (m, 1H, quinoline), 7.90 (d, 2H, *J* 2.0 Hz, phenyl), 7.88 (s, 1H, quinoline), 7.54 (m, 3H, phenyl), 7.11 (s, 1H, isoxazol); ¹³C NMR (100 MHz, CDCl₃) δ 170.3 (C-isoxazol), 160.4 (C-isoxazol), 148.0 (C-quinoline), 147.8 (C-quinoline), 139.7 (CH-quinoline), 131.6 (CH-quinoline), 130.4 (C-quinoline), 129.0 (CH-phenyl), 128.3 (CH-quinoline), 128.0 (CH-phenyl), 127.7 (CH-quinoline), 127.1 (C-quinoline), 126.7 (C-phenyl), 125.8 (CH-phenyl), 122.8 (C-quinoline), 100.8 (CH-isoxazol); (QTOF-MS) *m/z* calculated for C₁₈H₁₀BrClN₂O: 383.97; found: 384.30.

3-(2-Chloro-6-methoxyquinolin-3-yl)-5-phenylisoxazole (**3h**)

Yield: 77% as a white solid; m.p. 166–168 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 2914, 1595, 1569, 821; ¹H NMR (400 MHz, CDCl₃) δ 8.54 (s, 1H, quinoline), 7.98 (d, 1H, *J* 9.3 Hz, quinoline), 7.90 (dd, 2H, *J* 7.9, 1.3 Hz, phenyl), 7.52 (m, 3H, phenyl), 7.47 (dd, 1H, *J* 9.0, 2.7 Hz, quinoline), 7.16 (d, 1H, *J* 2.7 Hz, quinoline), 7.12 (s, 1H, isoxazol), 3.97 (s, 3H, quinoline-OCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 170.2 (C-isoxazol), 160.5 (C-isoxazol), 158.5 (C-quinoline), 145.3 (C-quinoline), 143.9 (C-quinoline), 138.3 (CH-quinoline), 130.4 (C-quinoline), 129.7 (C-quinoline), 129.0 (CH-phenyl), 127.8 (CH-quinoline), 127.1 (CH-phenyl), 125.8 (CH-phenyl), 124.4 (C-phenyl), 122.8 (CH-quinoline), 105.2 (CH-quinoline), 100.9 (CH-isoxazol), 55.6 (OCH₃-quinoline); (QTOF-MS) *m/z* calculated for C₁₉H₁₃ClN₂O₂: 336.07; found: 336.81.

3-(2-Chloro-6-iodoquinolin-3-yl)-5-phenylisoxazole (**3i**)

Yield: 70% as a white solid; m.p. 196–198 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 2914, 1586, 1569, 825; ¹H NMR (400 MHz, CDCl₃) δ 8.51 (s, 1H, quinoline), 8.30 (d, 1H, *J* 1.5 Hz, quinoline), 8.05 (dd, 1H, *J* 8.8, 2.0 Hz, quinoline), 7.88 (dd, 2H, *J* 7.6, 1.7 Hz, phenyl), 7.80 (d, 1H, *J* 9.3 Hz, quinoline), 7.53 (m, 3H, phenyl), 7.1 (s, 1H, isoxazol); ¹³C NMR (100 MHz, CDCl₃) δ 170.5 (C-isoxazol), 160.0 (C-isoxazol), 148.6 (C-quinoline), 146.7 (C-quinoline), 140.3 (CH-quinoline), 138.3 (CH-quinoline), 136.6 (CH-quinoline), 130.5 (C-quinoline), 129.9 (CH-quinoline), 129.1 (CH-phenyl), 128.2 (C-quinoline), 127.0 (CH-phenyl), 125.9 (CH-phenyl), 123.7 (C-phenyl), 100.8 (CH-isoxazol), 93.3 (C-quinoline); (QTOF-MS) *m/z* calculated for C₁₈H₁₀ClIN₂O: 431.95; found: 432.58.

3-(2-Chloro-5,7-dimethylquinolin-3-yl)-5-phenylisoxazole (**3j**)

Yield: 85% as a white solid; m.p. 182–184 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 2917, 1592, 1575, 823; ¹H NMR (400 MHz, CDCl₃) δ 8.71 (s, 1H, quinoline), 7.89 (m, 2H, phenyl), 7.7 (s, 1H, quinoline), 7.51 (m, 3H, phenyl), 7.29 (s, 1H,

quinoline), 7.11 (s, 1H, isoxazol), 2.70 (s, 3H, quinoline-CH₃), 2.55 (s, 3H, quinoline-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 170.2 (C-isoxazol), 160.8 (C-isoxazol), 148.6 (C-quinoline), 147.6 (C-quinoline), 142.2 (C-quinoline), 136.3 (C-quinoline), 135.1 (CH-quinoline), 130.5 (C-quinoline), 130.4 (CH-quinoline), 129.0 (CH-phenyl), 127.2 (CH-phenyl), 125.9 (CH-phenyl), 125.6 (C-phenyl), 124.3 (C-quinoline), 121.2 (CH-quinoline), 101.0 (CH-isoxazol), 22.0 (CH₃-quinoline), 18.6 (CH₃-quinoline); (QTOF-MS) *m/z* calculated for C₂₀H₁₅ClN₂O: 334.09; found: 334.87.

Antibacterial studies

The microorganisms used in the study were the three Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, clinically isolated strains. Antibacterial assays were carried out using the doubling dilution method in 96-well microtiter plates. Bacterial suspensions were obtained from overnight cultures in Luria broth base nutrient broth (Gibco BRL, Scotland) grown at 25 °C and diluted to approximately 10⁵ colony-forming units (CFU) *per* well in fresh medium. The compounds were dissolved in methanol as 5 mg mL⁻¹ stock solutions. These solutions were serially 2-fold diluted and added to each well, resulting in concentrations ranging from 125 to 2000 µg mL⁻¹. The final concentration of MeOH in the assays did not exceed 2%. The plates were kept at 25 °C overnight (12 h). After incubation, 20 µL of 0.5 mg mL⁻¹ aqueous 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, MO) were added to each well and re-incubated for 30 min to detect living bacteria. The absorbance was read in a universal microplate reader (Multiskan EX Thermo, Finland) at 405 nm. The results of the experimental wells were expressed as a percentage of cell viability in the controls, and the median inhibitory concentration (IC₅₀) values were graphically obtained from the dose-response curves. Penicillin G (Sigma-Aldrich, St. Louis, MO) was used as standard antibacterial.

Supplementary Information

Supplementary data are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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