Design, Synthesis and Antimicrobial Evaluation of Some Novel Quinoline Derivatives

Abstract
In an attempt to find new bio-active antimicrobial molecules, a series of quinoline-3-carbonitrile and 2-chloroquinoline derivatives were synthesized by multistep reactions. Antimicrobial screening of title compounds (3-24) was carried out against Gram-positive and Gram negative bacteria and fungi using agar diffusion technique. To understand the interaction of binding sites with bacterial protein receptor, the docking study was performed using topoisomerase II DNA gyrase enzymes. The newly synthesized compounds 14, 10 and 22 showed significant potency against different bacterial strains compared with Ciprofloxacin, Ampicillin and Gentamicin. While compounds 14, 22 and 6a had strong antifungal activity comparable to Amphotericin B. The results of in vitro antimicrobial activity and docking study revealed that the synthesized compounds have potential antimicrobial activity and can be further optimized and developed as a lead compound.

Keywords: Quinoline; Antimicrobial; Vilsmeier–Haack; Chalcone; Docking

Introduction

Background
The Global Burden of Disease Study (GBDS) estimates that infectious diseases were responsible for 22% of all deaths and 27% of disability-adjusted life years (DALYs) worldwide according to WHO [1]. Antimicrobial resistance among clinically important bacteria is widely acknowledged as a major global public health threat [2]. Most of the currently used antimicrobial drugs are associated with adverse effects, such as hepatotoxicity and hypersensitivity [3,4]. The widespread distribution of infectious diseases, the presence of antimicrobial resistance and the increased side effect of the antimicrobial drugs enhance temptation to design new more effective, safe and economic drugs to treat such infectious diseases and make the field of antimicrobial drug discovery to be of high priority.

The discovery and development of antimicrobial agents provided many classes of compounds. Among them, quinoline derivatives are still an important class of therapeutically useful antimicrobial drugs that control infectious diseases including tuberculosis [5-7]. It is well-known that the quinoline nucleus and its derivatives play a vital role in the search on wide antimicrobial activity spectrum. Structure-activity relationship (SAR) studies revealed that the antimicrobial activity in this heterocyclic class of quinoline molecules depends on the nature of the peripheral substituent’s and their spatial relationship within the quinoline skeleton [8]. Moreover, several new pyrazole [9], isoxazole [10], pyrimidine [11], guanidine [12], nicotinonitrile [13], thiazole [14], imidazole [15], hydrazine [16], piperazone [17], nitrile [18] and morpholine [19] moieties were reported to possess antimicrobial activity.

Rationale of molecular design
In fact, introducing chloroquine into treatment of malaria more than 60 years ago triggered a new era of quickly developing antimicrobial drugs through nalidixic acid and fluoroquinolones with potent activity against a wide spectrum of significant bacterial pathogens such as norfloxacin, and ciprofloxacin [19] (Figure 1). Depending on ligand based drug design particularly a molecular hybridization approach that involves the coupling of two or more groups with relevant biological properties [20], it was decided to select compounds nalidixic, norfloxacin, and ciprofloxacin as lead compounds.

The design and synthesis of the titled compounds were carried out with two objectives: the first was: the synthesis of quinoline nucleus with peripheral substituents at 2 and 3 position (comp. 3&5). The second objective was: the chemical modification of the synthesized compounds by molecular hybridization approach that involves the coupling of two or more groups with relevant biological properties (Figure 1). The first type consisted from quinoline nucleus and non-heterocyclic tail separated by open chain bridge (compounds 8-17). The second one consisted from quinoline nucleus and non-heterocyclic tail separated by heterocyclic bridge (compounds 18-24).

This approach may allow the construction of new quinoline hybrids with antimicrobial properties with different chemical isosters in order to study the SAR of these compounds and the effect of each substituent on their antimicrobial activity. In addition, we hope to get new potent, safe and effective antimicrobial agents with lower side effects.
**Materials and Methods**

**Molecular modeling**

Docking was carried out on bacterial type II A topoisomerase protein downloaded from protein data bank (PDB), (code 4BUL, resolution 2.6 Å), using discovery studio 2.5 software. The 3D crystal structure of topoisomerase receptor (code 4BUL) was downloaded from PDB, water molecules were removed. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Protein was subjected to energy minimization and applying of CHARMM (Chemistry at HARvard Macromolecular Mechanics) force fields for charge, and MMFF94 force field for partial charge, then prepared for docking by optimization the parameters. Molecular docking was performed using (CDOCKER) protocol which is an implementation of the CDOCKER algorithm. CDOCKER is a grid-based molecular docking method that employs CHARMM-based molecular dynamics (MD) scheme to dock ligands into a receptor binding site. The receptor is held rigid while the ligands are allowed to flex during the refinement.

**Chemistry**

All melting points were carried on Gallen Kamp point apparatus and are uncorrected. The infrared spectra were recorded on Brucker-Vector-22-F TIR spectrophotometer using the potassium bromide disc technique. The 1H-NMR spectra were recorded or varian-Gemini-300-MHz spectrophotometer.
using DMSO-d₆ as a solvents and TMS as internal reference. The chemical shift values were recorded in 6 ppm downfield the TMS signal. The Mass spectra were recorded on AziH-ph-AR-XO2 Mass spectrometer. Elemental analyses were performed on CHN analyzer and all compounds were within ± 0.4 of the theoretical values. All spectral measurements have been performed at the Micro analytical Center, Cairo University, Egypt. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets coated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases.

N-phenylacetamide 1 (1) [21], 2-Chloroquinoline-3-carboxylic acid (2) [22], 2-chloro quinoline-3-carbonitrile(4) [22], 2-mercapto quinoline-3-carbonitrile(5) [22] were obtained according to the reported procedures (schemes 1-3).

1-(4-aminophenyl)-3-(2-chloroquinolin-3-yl)Prop-2-en-1-one (3)

To a stirred and ice-cooled aqueous solution of sodium hydroxide (10 mmol, 50% w/w) and absolute methanol (25 ml), 2-chloroquinoline-3-carboxylic acid (2) (1.91 g, 10 mmol) was added portion wise followed by 4-aminocacetophene (1.35 g, 10 mmol). The reaction mixture was stirred vigorously for 3 h while temperature was maintained below 20°C until the reaction mixture became thick. The reaction mixture was left in the refrigerator overnight. The formed precipitate was filtered off under vacuum and washed with copious amount of water until the filtrates became neutral to litmus paper, washed with ice-cold ethanol (20 ml), and then recrystallized from ethanol to afford compound 3 as a yellow solid. Yield: 85%; m.p. 130°C. IR (KBr) δ ppm: 3070 (CH aromatic), 1650 (C=O). MS (m/z): 226 (C₇H₇NOS, 100%), 153 (C₅H₄N, 22.18%), M⁺ (C₅H₄N, 53%). Anal. Calc. for: (C₇H₇NOS) (M.W. = 228): C, 73.57; H, 8.03; N, 8.58; Found: C, 73.11; H, 7.94; N, 8.26%.

2-(Butylthio)quinoline-3-carbonitrile (6).

Yellow solid. Yield: 93%; m.p. 185 °C IR (KBr) cm⁻¹: 3075 (CH aromatic), 2954 (CH aliphatic), 2215 (CN). ¹H NMR (DMSO-d₆) δ ppm: 8.94 (s, 1H, quinoline-H₄), 8.00 (d, 1H, J = 8 Hz, quinoline-H₅), 7.91 (t, 1H, J = 9 Hz, quinoline-H₆), 7.64 (t, 1H, J = 9 Hz, quinoline-H₇), 7.60 (d, 1H, J = 8 Hz, quinoline-H₈), 3.40 (t, 2H, J = 5.7 Hz, S-CH₂), 2.20 (t, 2H, J = 7.2 Hz, CH₃), 1.48 (p, 2H, J = 7.5 Hz, CH), 0.97 (t, 3H, J = 5.5 Hz, CH₃). MS (m/z): 242 (C₁₈H₁₄N₂S, 22.18%), M⁺ (C₁₈H₁₄N₂S, 100%), 153 (C₁₃H₉N, 53%). Anal. Calc. for: (C₁₈H₁₄N₂S) (M.W. = 228): C, 69.39; H, 5.8; N, 11.57; Found: C, 69.11; H, 5.4; N, 11.36%.

2-(Decylthio)quinoline-3-carbonitrile (6).

Brownish solid. Yield: 82%; m.p. 270 °C IR (KBr) cm⁻¹: 3050 (CH aromatic), 2950 (CH aliphatic), 2200 (CN). ¹H NMR (DMSO-d₆) δ ppm: 8.94 (s, 1H, quinoline-H₄), 8.00 (d, 1H, J = 8 Hz, quinoline-H₅), 7.90 (t, 1H, J = 9 Hz, quinoline-H₆), 7.88 (t, 1H, J = 9 Hz, quinoline-H₇), 7.64 (d, 1H, J = 8 Hz, quinoline-H₈), 3.39 (t, 2H, J = 6.75 Hz, S-CH₂), 2.13 (p, 2H, J = 6.70 Hz, CH₂), 1.46 (p, 2H, J = 6.50 Hz, CH₂), 1.22 (s, 10H, J = 5.5 Hz, CH₂). MS (m/z): 326 (C₂₁H₁₄N₂S, 18.76%), M⁺ (M.W. = 326). 185 (C₁₆H₁₂N₂S, 100%). 153 (C₁₁H₇N, 25%). Anal. Calc. for: (C₂₁H₁₄N₂S) (M.W. = 326): C, 73.57; H, 8.03; N, 8.58; Found: C, 73.11; H, 7.94; N, 8.26%.

2-Allyl(quinoline-3-carbonitrile (6). 2-(Allylhydroxy)quinoline-3-carbonitrile (6a).

General method: A mixture of 2-mercaptoquinolin-3-carbonitrile (5) (1.86 g, 10 mmol) and anhydrous sodium acetate (1.25g, 15 mmol) and an appropriate alkylation halide namely, ethyl bromide, butyl bromide, n-decyl bromide and allyl bromide (10 mmol) in ethanol (30 ml) was heated to reflux for 4h. On cooling, the precipitate product was collected by filtration and recrystallized from ethanol to afford compounds 6a respectively.

2-(Ethylthio) quinoline-3-carbonitrile (6a).

White solid. Yield: 83%; m.p. 120 °C. IR (KBr) cm⁻¹: 3070 (CH aromatic), 2985 (CH aliphatic), 2210 (CN). ¹H NMR (DMSO-d₆) δ ppm: 8.96 (s, 1H, quinoline-H₄), 8.01 (d, 1H, J = 8 Hz, quinoline-H₅), 7.94 (t, 1H, J = 9 Hz, quinoline-H₆), 7.91 (t, 1H, J = 9 Hz, quinoline-H₇), 7.66 (d, 1H, J = 8 Hz, quinoline-H₈), 3.39 (q, 2H, J = 7.2 Hz, S-CH₂), 1.40 (t, 3H, J = 6.75 Hz, CH₃). MS (m/z): 214 (C₁₈H₁₄N₂S, 70.40%, M⁺), 180 (C₁₆H₁₂N₂S, 100%), 153 (C₁₃H₉N₂S, 33%). Anal. Calc. for: (C₁₈H₁₄N₂S) (M.W. = 214): C, 67.26; H, 4.70; N, 13.07; Found: C, 66.93; H, 4.61; N, 12.95%.

N-Substituted phenyl)-2-[(3-Cyaanoquinolin-2-yl)thio] acetamide (7a).

General method: A mixture of 2-mercaptoquinolin-3-carbonitrile (5) (1.86 g, 10 mmol) and anhydrous sodium acetate (1.25g, 15 mmol) and the appropriate chloroacetanilides namely, 4-chloro acetanilide, 2-chloro acetanilide, 4-methyl acetanilide and 4-methoxy acetanilide (10 mmol) in absolute ethanol (30 ml) was heated under reflux for 4 h. The reaction mixture was cooled to room temperature. The formed precipitate was collected and recrystallized from absolute ethanol to afford compounds 7a respectively.

N-(4-Chlorophenyl)-2-[(3-cyaanoquinolin-2-yl)thio] acetamide (7).

Yellowish white solid: Yield: 85%; m.p. 235 °C IR (KBr) cm⁻¹: 3295 (NH), 3080 (CH aromatic), 2900 (CH aliphatic), 2222 (CN), 1675 (C=O). ¹H NMR (DMSO-d₆) δ ppm: 10.27 (s, 1H, N-H,
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D.O-exchangeable  ), 8.8 (s, 1H, quinoline-H4), 8.01 (d, 1H, J = 8 Hz, quinoline-H5), 7.94 (t, 1H, J = 9 Hz, quinoline-H7), 7.91 (t, 1H, J = 9 Hz, quinoline-H6), 7.56 (d, 1H, J = 8 Hz, quinoline-H8), 7.49 (d, 2H, J = 6.9 Hz, phenyl-H2, H6), 7.4 (d, 2H, J = 6.9, phenyl-H3, H5), 4.25 (2s, 2H, CH2), 3.9 (s, 4H, 2CH2). MS (m/z): 275 (C17H11Cl2N3O, 1.54%, M+ +2), 273 (C17H11Cl2N3O, 0.9, M+), 257 (C16H11N3OS, 89.9%, M+), 255 (C16H11N3OS, 75% (C3H3, 8%). Anal. Calc. for: (C17H11Cl2N3O) (M.W. = 353): C, 61.10; H, 3.42; N, 11.88%; Found: C, 61.47; H, 3.25; N, 11.54%.

N-(2-Chloro-phenyl)-2-(3-cyanoquinolin-2-yl)thioacetamide (7)

Yellowish white solid. Yield: 80%; m.p. 229 °C (KBr cm−1): 3295 (NH aromatic), 2900 (CH aliphatic), 2221 (CN), 1665 (C=O). 1H NMR (DMSO-d6) δ ppm: 10.1 (s, 1H, N–H, D2O-exchangeable), 9.15 (s, 1H, quinoline-H4), 7.93 (d, 1H, J = 8 Hz, quinoline-H5), 7.90 (t, 1H, J = 9 Hz, quinoline-H7), 7.81 (t, 1H, J = 9 Hz, quinoline-H6), 7.66 (d, 1H, J = 8 Hz, quinoline-H8), 7.5-7.72 (m, 4H, aromatic protons), 4.35 (3s,2H,CH2) (MS /m/z): 355 (C17H11Cl2N3O, 0.37%, M+ +2), 353 (C17H11Cl2N3O, 0.9%, M+), 228 (C16H11N2O, 100%), 229 (C16H11N2O, 89%), 199 (C16H11N2S, 9%), 153 (C16H11N3S, 75% (C3H3, 8%). Anal. Calc. for: (C17H11Cl2N3O) (M.W. = 353): C, 61.10; H, 3.42; N, 11.88%; Found: C, 61.47; H, 3.25; N, 11.54%.

N-(2-Methyl-phenyl)-2-(3-cyanoquinolin-2-yl)thioacetamide (7)

Brown solid. Yield: 85%; m.p. 222 °C. (IR (KBr cm−1): 3300 (NH aromatic), 2900 (CH aliphatic), 2221 (CN), 1665 (C=O). 1H NMR (DMSO-d6) δ ppm: 10.27 (s, 1H, N–H, D2O-exchangeable), 9.10 (s, 1H, quinoline-H4), 8.01 (d, 1H, J = 8 Hz, quinoline-H5), 7.90 (t, 1H, J = 9 Hz, quinoline-H7), 7.81 (t, 1H, J = 9 Hz, quinoline-H6), 7.66 (d, 1H, J = 8 Hz, quinoline-H8), 7.6 (d, 2H, J = 9 Hz, phenyl-H2, H6), 7.4 (d, 2H, J = 6.9, phenyl-H3, H5), 4.2 (2s, 2H, CH2), 2.6 (2s, 3H, CH3). MS (m/z): 333 (C17H11N2O, 10.5%, M+), 257 (C16H11N2O, 100%), 229 (C16H11N2O, 89%), 199 (C16H11N2S, 9%), 153 (C16H11N3S, 75% (C3H3, 8%). Anal. Calc. for: (C17H11N2O) (M.W. = 333): C, 68.45; H, 4.53; N, 12.60%; Found: C, 68.12; H, 4.41; N, 12.07%.

N-(4-Methoxy-phenyl)-2-(3-cyanoquinolin-2-yl)thioacetamide (7)

Grey solid. Yield: 85%; m.p. 230 °C. (IR (KBr cm−1): 3261 (NH), 3071 (CH aromatic), 2900 (CH aliphatic), 2225 (CN), 1666 (C=O). 1H NMR (DMSO-d6) δ ppm: 10.28 (s, 1H, N–H, D2O-exchangeable), 9.00 (s, 1H, quinoline-H4), 8.01 (d, 1H, J = 9 Hz, quinoline-H5), 7.90 (t, 1H, J = 9 Hz, quinoline-H7), 7.81 (t, 1H, J = 9 Hz, quinoline-H6), 7.66 (d, 1H, J = 8 Hz, quinoline- H8), 7.60 (d, 2H, J = 6.9 Hz, phenyl-H2, H6), 7.4 (d, 2H, J = 6.9, phenyl-H3, H5), 4.25 (2s, 2H, CH2), 3.99 (3s, 3H, OCH3) MS (m/z): 349 (C19H15N302S, 10.5%, M+), 257 (C12H8N2O2S, 100%), 229 (C12H8N2O2S, 75.7%, 199 (C11H7N2S, 9.3%), 153 (C10H5N2S, 17.8%), 75 (C6H3, 13%). Anal. Calc. for: (C19H15N302S) (M.W. = 349): C, 65.31; H, 4.33; N, 12.03%; Found: C, 65.02; H, 4.10; N, 11.97%.

General method for synthesis of compounds 8-17

A mixture of 2-Chloroquinoline-3-carbaldehyde (2) (1.91 g, 0.01 mole) and glacial acetic acid (0.5 ml), and an appropriate primary amine derivatives namely, 2-amino-3-methylquinazolin-
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General method for synthesis of compounds 18-21

3-[2-(Chloroquinolin-3-yl)-6-(4-aminophenyl)pyrimidin-2-yl]-1,1-dimethylguani-dine (18)

light red powder. Yield: 59%; m.p.: 190 °C (CH NMR (DMSO-d6) δ ppm: 9.05 (s, 1H, quinoline-H4), 8.86 (s, 1H,CH=N), 8.53 (d, 1H, J = 8 Hz, benzothiazol-H7), 7.99 (d, 1H, J = 8 Hz, quinoline-H8), 7.95 (d, 1H, J = 8 Hz, benzothiazol-H7), 7.76 (t, 1H, J = 8 Hz, quinolin-H7), 7.70 (t, 1H, J = 8 Hz, quinolin-H6), 7.5 (d, 2H, J = 6.8 Hz, benzothiazol-H5,6H), MS (m/z): 325 ([C, H, C, N, S], 26.6%, M+ + 2), 323 ([C, H, C, N, S], 76.6%, M+), 208 ([C, H, C, N, S], 32.2%), 177 ([C, H, C, N, 100%]. Anal. Calc. for: (C, H, C, N) (M.W. = 323): C, 63.06; H, 3.11; N, 12.98%; Found: C, 63.31; H, 2.99; N, 12.73%.

1-(2-Chloroquinolin-3-yl)-N-(5-morpholinomethane) (16)

light yellow powder. Yield: 65%; m.p.: 201 °C (CH NMR (DMSO-d6) δ ppm: 10.38 (1s, 1H, quinoline-H4), 8.88 (s, 1H, CH=N), 8.14 (d, 1H, J = 8 Hz, quinoline-H5), 7.99 (d, 1H, J = 8 Hz, quinoline-H8), 7.95 (d, 1H, J = 8 Hz, benzothiazol-H7), 7.93 (d, 1H, J = 6 Hz, phenyl-H2, H6), 7.87 (t, 1H, J = 8 Hz, quinolin-H7), 7.69 (t, 1H, J = 9 Hz, quinolin-H6), 7.62 (d, 2H, J = 6 Hz, phenyl-H3, H5), 2.8 (q, 2H, J = 6.7 Hz, CH.), 1.2 (t, 3H, J = 6.5 Hz, -CH3). MS (m/z): 415 ([C, H, C, O, N] 1.24%, M+ *2), 413 ([C, H, C, O, N] 3.72%, M+), 378 ([C, H, O, N] 22.2%), 189 ([C, H, C, O, N] 100%). Anal. Calc. for: (C, H, C, O, N) (M.W. = 413): C, 72.55; H, 4.87; N, 10.15%; Found: C, 72.31; H, 4.89; N, 10.43%.

1-(2-Chloroquinolin-3-yl)-N-(thiazol-2-yl) methanimine (13)

light yellow powder. Yield: 65%; m. p.: 210 °C (CH NMR (DMSO-d6) δ ppm: 9.04 (s, 1H, quinoline-H4), 8.88 (s, 1H, CH=N), 8.14 (d, 1H, J = 8 Hz, quinoline-H5), 7.99 (d, 1H, J = 8 Hz, quinoline-H8). 7.87 (t, 1H, J = 9 Hz, quinolin-H7), 7.69 (t, 1H, J = 9 Hz, quinolin-H6), 7.62 (d, 2H, J = 6 Hz, thiazol-H2, H3). MS (m/z): 275 ([C, H, C, S, N] 1.16%, M+ *2), 273 ([C, H, C, S], 4.51%, M+), 238 ([C, H, N, S, 12.2%], 189 ([C, H, C, O, N] 100%). Anal. Calc. for: (C, H, C, O, N) (M.W. = 273): C, 57.04; H, 2.95; N, 15.35%; Found: C, 57.31; H, 2.89; N, 15.43%.
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4-(4-aminophenyl)-6-(2-chloroquinolin-3-yl) pyrimidine-2(1H)-thione (20)

Dark yellow solid. Yield: 40%; m.p. 150 °C (IR (KBr) cm\(^{-1}\)): 3290 (NH), 3350 (NH) 3050 (CH aromatic). 1\(^{\text{H}}\)NMR (DMSO-\(d_6\)) δ ppm: 12.30 (s, 1H, NH, D-O-exchangeable proton), 8.50 (s, 1H, quinoline-H4), 8.30 (d, 1H, J = 15 Hz, quinoline-H5), 8.10 (d, 1H, J = 9 quinoline-HB), 7.98 (t, 1H, J = 8 Hz, quinoline-H1), 7.88 (d, 2H, J = 7 Hz, phenyl-H2,H6), 7.4 (t, 1H, J = 9 Hz, quinoline-H6), 7.1 (d, 2H, J = 9 Hz, phenyl-H3,H5), 6.5 (s, 1H, pyrimidinone-thion), 4.1 (s, 2H, NH, D-O-exchangeable proton). MS (m/z): 366 [C\(_{14}\)H\(_{13}\)ClN\(_3\)S, 0.15%, M\(^+\)2], 364 [C\(_{15}\)H\(_{15}\)N\(_3\)S, 0.55%, M], 329 [C\(_{15}\)H\(_{15}\)N\(_3\)S, 4.4%], 77 [C\(_{7}\)H\(_{10}\)]

6-(2-Chloroquinolin-3-yl)-4-(4-aminophenyl)pyrimidin-2-amine (21)

Yellow solid. Yield: 60%; m.p. 195 °C (IR (KBr) cm\(^{-1}\)): 3300 (NH\(_3\)), 3050 (CH aromatic-H's). 1\(^{\text{H}}\)NMR (DMSO-\(d_6\)) δ ppm: 8.73 (s, 1H, quinoline-H4), 8.17 (d, 1H, J = 9 Hz, quinoline-H5), 8.1 (s, 1H, pyrimidine-H5), 7.9 (d, 1H, J = 9 Hz, quinoline-H5), 7.5 (t, 1H, J = 9 Hz, quinoline-H6), 7.4 (d, 2H, J = 9 Hz, phenyl-H2,H6), 7.1 (t, 2H, J = 9 Hz, phenyl-H3,H5), 6.5 (s, 2H, NH\(_2\) of pyrimidine, D-O-exchangeable), 5.5 (s, 2H, NH of phenyl, D-O-exchangeable). MS (m/z): 349 [C\(_{19}\)H\(_{14}\)ClN\(_3\), 1.3%, M\(^+\)], 347 [C\(_{19}\)H\(_{14}\)ClN\(_2\), 4.01%, M\(^+\)-2], 312 (C\(_{15}\)H\(_{15}\)N\(_3\), 9.15%, M\(^+\)-13), 215 (C\(_{17}\)H\(_{12}\)ClN\(_3\), 16%), 118 (C\(_{11}\)H\(_6\)ClN\(_2\), 100%). Anal. Calc. for: [C\(_{19}\)H\(_{14}\)ClN\(_3\)] (M.W. = 347): C, 65.61; H, 4.60%; N, 20.14%; Found: C, 65.95; H, 3.96; N, 20.03%.

Antimicrobial Screening

Preparation of bacterial and fungal suspensions and solution of the tested compounds

The newly synthesized compounds were dissolved in DMSO at 1 mg/ml concentration and tested against Aspergillus fumigatus, Geotrichum candidum, Syncephalastrum racemosum, Candida albicans, Streptococcus pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli obtained from the Regional Center for Mycology and Biotechnology, Faculty of Science, Al-Azhar University. Ampicillin and gentamicin were used as antibacterial standards and amphotericin B was used as antifungal standard using agar diffusion technique [24] and minimum inhibitory concentration (MIC) [25]. The standards were dissolved in DMSO at 1 mg/ml concentration. The bacterial and the fungal suspension were prepared by inoculating fresh stock cultures into separate broth tubes, each containing 7 ml of nutrient broth. The inoculated tubes were incubated at 37 °C for 24 and 48 h respectively.

Agar diffusion test for detection of antibacterial and antifungal activity:

Nutrient agar for bacterial growth and Potato-dextrose-agar for fungal growth were dissolved and distributed in 25 ml quantities in 100 ml conical flasks and was sterilized in an autoclave at 121°C for 20 minutes. The media were poured in Petri dishes and...
allowed to set for 30 minutes at room temperature. Cultures of each organism were spread with a dry sterile swab on the surface of the previously prepared plates. Cups of 6 mm at equal distances were made in each plate. In each plate one cup was used for control (DMSO) and other for standard (Ciprofloxacine, Ampicillin, Gentamicin, and Amphotericin B) where the other cups were used for the tested compounds. The plates were incubated at 37 °C for 24 h in bacterial growth and 48 in fungal growth then the plates were examined for inhibition zones.

**Determination of minimal inhibitory concentration (MIC):**

The inoculums were prepared by taking a loopful of stoke culture to about 100 ml of nutrient broth, in 250 ml clean and sterilized conical flasks. The flasks were incubated at 27 °C for 24 h before use. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly, into potato dexrose-agar medium. Then the plates were incubated at 25 °C for 48 hr. The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The experiments were performed in triplicate in order to minimize the errors.

**Results and Discussion**

**Molecular modeling**

The obtained results indicated that all studied ligands have similar position and orientation inside the putative binding site of type IIA topoisomerase protein. The selected compounds (14, 22 and 10) showed good binding energies ranging from −15.60 to -33.11 kcal/mol (Table 1). The proposed binding mode of the ligand (ciprofloxacine) binding free energy was -33.32 kcal/mol with RMSD value of 2.8. It showed the important interactions with the residues at the active site of type IIA topoisomerase protein (figure 2A). The piprazine group formed a hydrogen bond of 2.02 Å with Asp 1083. The nitrile group formed a further hydrogen bond with a distance of 2.09 Å with Asp 1083.

The proposed binding mode of compound 10 (affinity value of −33.11 kcal/mol and one H-bonds) is virtually the same as that of ciprofloxacine (Figure 2B). The hydrazine group formed a hydrogen bond with a distance of 2.06 Å with Asp 1083. The nitrile group formed a further hydrogen bond with a distance of 2.30 Å with Ser 1084.

The proposed binding mode of compound 22 (affinity value of - 27.28 kcal/mol and one H-bond) is virtually the same as that of ciprofloxacine (Figure 2C). The pyrimidione group formed a hydrogen bond with a distance of 2.06 Å with Asp 1083. The proposed binding mode of compound 10 (affinity value of −33.11 kcal/mol and one H-bonds) is virtually the same as that of ciprofloxacine (Figure 2D). The hydrazino group formed a hydrogen bond with a distance of 2.10 Å with Asp 1083. These interactions of compounds 14, 22 and 10 with type IIA topoisomerase protein explain the high binding free energies and the biological activities.

**Chemistry**

The sequence of reactions used in the synthesis of the target compounds is illustrated in Schemes 1–3. Vilsmeier formulation of N-phenylacetamide(1) afforded 2-chloroquinoline-3-carbaldehyde (2) followed by condensation with 4-amino acetophenone to afford 1-(4-aminophenyl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one (chalcone) (3). Treatment of 2 with hydroxyl amine produced 2-chloroquinoline-3-carbonitrile (4) then reaction of 4 with thiourea yielded 2-mercaptoquinoline-3-carbonitrile (5), and subsequent treatment with both alkyl halides and substituted chloroacetanilide to to afford 6a and 7a respectively (Scheme 1). Compound 2 was condensed with different primary amine or substituted hydrazide derivatives to afford the title compounds 8-17 (Scheme 2). Compound 3 underwent a 1,3-dipolar cyclo-addition reaction with seven different dipoles namely hydrazine hydrate, hydroxylamine hydrochloride, thiourea, guanidine hydrochloride, urea, metformine hydrochloride, and malononitrile, to produce the compounds 18-24 respectively (Scheme 3).

**Table 1: The docking binding free energies of compounds (2-24)**

| Comp. No | Binding free energy (kcal/mol) | Comp. No | Binding free energy (kcal/mol) |
|----------|-------------------------------|----------|-------------------------------|
| 2        | -16.30                        | 12       | -15.60                        |
| 3        | -18.10                        | 13       | -17.80                        |
| 6a       | -23.45                        | 14       | -26.60                        |
| 6b       | -18.85                        | 15       | -17.98                        |
| 6c       | -17.44                        | 16       | -18.33                        |
| 6d       | -24.22                        | 17       | -19.34                        |
| 7a       | -22.50                        | 18       | -22.80                        |
| 7a’      | -16.23                        | 19       | -22.11                        |
| 7c       | -22.70                        | 20       | -19.45                        |
| 7d       | -22.10                        | 21       | -20.56                        |
| 8        | -24.50                        | 22       | -27.28                        |
| 9        | -20.90                        | 23       | -21.10                        |
| 10       | -33.11                        | 24       | -24.01                        |
| 11       | -25.33                        | Ciprofloxacine | -33.32                |

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Figure 1: A) Binding interaction of Ciprofloxacin. B) Binding interaction of compound 14. C) Binding interaction of compound 22. D) Binding interaction of compound 10

Scheme 1: General procedure for preparation of target compounds 6a-d, 7a-d
Design, Synthesis and Antimicrobial Evaluation of Some Novel Quinoline Derivatives

Scheme 2: General procedure for preparation of target compounds 8-17

Scheme 3: General procedure for preparation of target compounds 18-24
Antimicrobial screening

Antibacterial and antifungal activities of all newly synthesized compounds were tested by measuring the inhibitory effect of such compounds against some Gram-positive, Gram-negative bacteria and some fungi using agar diffusion technique and minimum inhibitory concentration (MIC). The newly synthesized compounds were evaluated for their in vitro antibacterial activity against Gram-positive namely Staphylococcus aureus (SA) and Bacillus subtilis (BS) and Gram-negative Pseudomonas aeruginosa (PA) and Escherichia coli (EC). They were also evaluated for their in vitro antifungal activity against Aspergillus fumigatus (AF), Geotricum candidum(GC), Syncephalasterum mraceomosum (SR), Candida albicans (CA). Ampicillin (AMP) was the standard used for the evaluation of antibacterial activity against gram positive bacteria and Gentamicin (Gent) was used as a standard in assessing the activity of the tested compounds against gram negative bacteria, and Ciprofloxacin (Cf) was used as broad spectrum antibacterial positive control, while Amphotericin B (Amp-B) was taken as reference for the antifungal effect. The inhibitory effects of the synthetic compounds against these organisms are given in (Table 2&3) and (Figure 3&4).

Table 2: Antifungal activities and antibacterial activities against Gram-positive and gram- negative organisms of compounds 3- 24.

| Comp. | Gram Positive Bacteria | Gram Negative Bacteria | Fungi |
|-------|------------------------|------------------------|-------|
| | Sp | Bs | Ec | Pa | Af | Sm | Gn | Ca |
| 2 | 14±0.63 | 16±0.24 | 12±0.72 | NA | 13±0.58 | 13±0.24 | 14±0.72 | NA |
| 3 | 18±0.19 | 19±0.47 | 18±0.58 | NA | 17±0.38 | 16±0.23 | 19±0.34 | NA |
| 6a | 20±0.55 | 21±0.52 | 20±0.58 | NA | 17±0.58 | 19±0.19 | 21±0.58 | NA |
| 6b | 19±0.37 | 21±0.72 | 19±0.63 | NA | 16±0.58 | 18±0.19 | 21±0.58 | NA |
| 6c | 17±0.37 | 19±0.63 | 16±0.63 | NA | 15±0.58 | 17±0.25 | 22±0.78 | NA |
| 6d | 21±0.63 | 23±0.58 | 21±0.58 | NA | 18±0.63 | 21±0.72 | 24±0.58 | NA |
| 7a | 20±0.72 | 21±0.58 | 22±0.58 | NA | 19±0.58 | 18±0.25 | 17±0.63 | NA |
| 7b | 15±0.44 | 16±0.58 | 13±0.63 | NA | 15±0.44 | 18±0.58 | 19±0.37 | NA |
| 7c | 20±0.37 | 21±0.28 | 20±0.44 | NA | 15±0.19 | 14±0.19 | 13±0.37 | NA |
| 7d | 20±0.43 | 21±0.53 | 16±0.25 | NA | 18±0.58 | 17±0.25 | 16±0.38 | NA |
| 8 | 22±0.44 | 22±0.25 | 20±0.44 | NA | 20±0.44 | 22±0.58 | 22±0.37 | NA |
| 9 | 17±0.63 | 18±0.58 | 15±0.58 | NA | 16±0.58 | 17±0.63 | 17±0.63 | NA |
| 10 | 25±0.72 | 27±0.63 | 22±0.72 | NA | 22±0.58 | 22±0.44 | 24±0.54 | NA |
| 11 | 22±0.72 | 25±0.72 | 21±0.72 | NA | 20±0.72 | 21±0.72 | 23±0.72 | NA |
| 12 | 11±0.44 | 14±0.67 | 10±0.46 | NA | 20±0.72 | 21±0.72 | 24±0.72 | NA |
| 13 | 17±0.25 | 16±0.63 | 15±0.44 | NA | 12±0.72 | 14±0.72 | 17±0.72 | NA |
| 14 | 25±0.27 | 31±0.58 | 23±0.25 | NA | 24±0.72 | 26±0.72 | 28±0.72 | NA |
| 15 | 17±0.18 | 17±0.19 | 20±0.19 | NA | 11±0.72 | 14±0.72 | 15±0.72 | NA |
| 16 | 12±0.26 | 14±0.27 | 12±0.57 | NA | 18±0.72 | 20±0.72 | 21±0.72 | NA |
| 17 | 16±0.72 | 17±0.72 | 15±0.72 | NA | 15±0.63 | 16±0.58 | 22±0.72 | NA |
| 18 | 20±0.72 | 23±0.72 | 20±0.72 | NA | 20±0.72 | 21±0.52 | 24±0.72 | NA |
| 19 | 20±0.72 | 20±0.72 | 17±0.72 | NA | 20±0.72 | 21±0.58 | 22±0.58 | NA |
| 20 | 16±0.72 | 18±0.72 | 20±0.72 | NA | 22±0.58 | 24±0.58 | 25±0.58 | NA |
| 21 | 19±0.72 | 20±0.72 | 21±0.72 | NA | 21±0.58 | 22±0.58 | 22±0.58 | NA |
| 22 | 22±0.72 | 23±0.72 | 21±0.72 | NA | 23±0.72 | 25±0.52 | 26±0.72 | NA |
| 23 | 19±0.72 | 19±0.72 | 16±0.72 | NA | 18±0.72 | 20±0.72 | 21±0.72 | NA |
| 24 | 21±0.8 | 23±0.58 | 22±0.58 | NA | 22±0.72 | 23±0.72 | 24±0.72 | NA |
| Amp | 23±0.8 | 32±0.58 | nt | nt | nt | nt | nt | nt |
| Gent | - | - | 21±0.58 | 17±0.63 | nt | nt | nt | nt |
| CF | 23±0.6 | 22±0.51 | 23±0.33 | 22±0.54 | nt | nt | nt | nt |
| Amp.B | Nt | Nt | Nt | 23±0.63 | 19±0.72 | 28±0.58 | 25±0.63 | Nt |

NA=No activity, SA= Staphylococcus aureus, BS =Bacillus subtilis, PA= Pseudomonas aeruginosa, EC= Escherichia coli, AF= Aspergillus fumigates, GC= Geotricum candidum, SR= Syncephalasterum mraceomosum, CA= Candida albicans, AMP= Ampicillin, Gent= Gentamicin, Amp-B= Amphotericin B, CF: Ciprofloxacin, nt= not tested

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Table 3: Antimicrobial activities of the tested standards and synthesized compounds as MICs (μg/mL).

| Comp. | Sp   | Bs   | Ec   | Pa   | Af   | Sm   | Gn   | Ca   |
|-------|------|------|------|------|------|------|------|------|
| 4     | 1.95 | 0.98 | 1.95 | NA   | 3.9  | 1.95 | 0.98 | NA   |
| 14    | 0.49 | 0.24 | 0.98 | NA   | 0.49 | 0.49 | 0.24 | NA   |
| 22    | 0.98 | 0.98 | 1.95 | NA   | 0.98 | 0.98 | 0.49 | NA   |
| Amp   | 0.98 | 0.24 | nt   | nt   | nt   | nt   | nt   | nt   |
| Gent  | nt   | nt   | 1.95 | 15.63| nt   | nt   | nt   | nt   |
| CF    | 1.95 | 1.95 | 1.95 | 3.9  | nt   | nt   | nt   | nt   |
| Amp. B| nt   | nt   | nt   | nt   | 0.98 | 3.9  | 0.24 | 0.49 |

NA=No activity, SA= Staphylococcus aureus, BS = Bacillus subtilis, PA= Pseudomonas aeruginosa, EC= Escherichia coli, AF = Aspergillus fumigates, GC= Geotricum candidum, SR= Syncaphalaster mracemosum, CA= Candida albicans, AMP= Ampicillin, Gent= Gentamicin, Amp-B= Amphotericin B, CF: Ciprofloxacin, nt = not tested

Figure 3: Antibacterial activities of compounds 6b-24

Figure 4: Antibacterial activities of the synthesized compounds
SAR

The substitutions on position 3 of quinoline nucleus were more active than that on position 2. Scaffold containing heterocyclic bridge was more potent than that active open chain bridge. Hydrazones were more active then imines. Nitrile groups were more potent than heterocyclic moieties. Oxazoles were more active than pyrazoles. Thioalkyl derivatives equipotent with N-phenyl thioacetanilides ones. Thioalkenes were more potent than short thioalkyl chains, and that were more potent than long thioalkyl ones. Electron withdrawing groups and para-substitutions of N-phenyl thioacetanilide derivatives were more potent than electron donating groups and ortho-substitutions respectively.

Conclusion

In the present work, we synthesized novel series of quinoline-3-carbonitrile and 2-chloroquinoline derivatives. Screening for title compounds was carried for their potential antibacterial and antifungal activity. Most of the tested compounds revealed better activity against the Gram-positive rather than the Gram negative bacteria. All test compounds were found to be inactive against *Pseudomonas aeruginosa*. Compounds 14, 10 and 22 exhibited excellent activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* compared with the standards drugs, while compounds 14, 22 and 6, have strong antifungal activity against *Aspergillus fumigatus*, *Syncephalasterum racemosum*, *Geotrichum candidum*, comparable to Amphotericin B. Finally, none of the synthesized compounds gave any activity against *Candida albicans*. Molecular docking study revealed that the synthesized compounds have potential antimicrobial activity and can be further optimized and developed as a lead compound.

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