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RESEARCH ARTICLE

Synthesis, antimicrobial activity and molecular modeling study of substituted 5-aryl-pyrimido[5,4-c] quinoline-2,4-diones

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Abstract

A series of pyrimido[5,4-c]quinoline-2,4-dione derivatives **5a–k** were synthesized in moderate yields *via* a thermolysis reaction of equimolar ratio of 5-arylidine-1,3-dimethylbarbituric acid derivatives **3a–d** with aniline derivatives **4a–d** at 150–180 °C for 1–2 h. Eight of the synthesized compounds were chosen for a primary *in vitro* one-dose anticancer assay performed using the full NCI 60 cell panel. Only compound **5b** showed moderate GI% at the used dose (10 μM) against four of the tested cell lines corresponding to leukemia SR (GI%: 51), non small-cell lung cancer HOP-92 (GI%: 63), melanoma UACC-62 (GI%: 53) and renal cancer UO-31 (GI%: 69). On the other hand, antimicrobial screening of the whole set of the synthesized compounds was performed against three *Gram +ve* and two *Gram –ve* bacterial strains. Results of the antimicrobial screening showed that compounds **5d**, **5e**, **5f**, **5h** and **5k** have broad-spectrum antibacterial efficacy being moderately active against all the tested *Gram +ve* and two *Gram –ve* bacteria. Also, compound **5a** showed interesting results being only active against *Streptococcus faecalis* and both tested *Gram –ve* strains *viz. E. coli* and *P. aeruginosa*. In order to compare the binding mode of the most active compounds **5e** and **5f** along with the inactive compound **5c** we docked these compounds into the empty binding site of topoisomerase II DNA gyrase (PDB ID: 1KZN), and results were compared with the bound inhibitor Clorobiocin.

Keywords: Antimicrobial, anticancer, 5-arylidine-1,3-dimethylbarbituric acid, pyrimido[5,4-c]quinoline-2,4-dione, molecular modeling

Introduction

Pyrimidines and their annelated derivatives are of particular importance being widely spread in nature chiefly in the nucleobases found in nucleic acids *viz* the three pyrimidines cytosine, thymine, and uracil as well as the purines adenine and guanine[1]. Consequently, derivatives thereof have been extensively studied and many have been found useful in the field of chemotherapy. Among the pyrimidine-containing chemotherapeutics currently in use are the anticancer 5-fluorouracil, the antiviral zidovudine and the antifungal flucytosine in addition to many other antimicrobial agents[2–7].

On the other hand, quinolines play a fairly important role as chemotherapeutics, many derivatives of which being of significant importance as synthetic antimalarials, a disease that claims 1 to 3 million lives annually, for example chloroquine[8,9]. Also, many antibiotics and UT antiinfectives are based on the quinoline scaffold, like norfloxacin, levofloxacin and others[10,11].

In DNA replication, one group of enzymes has proved to be effective target for therapeutic agents, which is topoisomerase enzyme. DNA gyrase is a type II topoisomerase found in all bacteria and controls the topological state of DNA[12]. DNA gyrase consists of two subunits GyrA (875 amino acids) and GyrB (804 amino acids) with the active species being a heterotetramer A2B2[13]. Mechanistic studies have revealed the steps involved in the gyrase supercoiling reaction[14]. This process involves

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the wrapping of DNA around the A2B2 complex, cleavage of this DNA on both strands, and the passage of a segment of DNA through the double strand break. Relegation of the break results in the introduction of two negative supercoils. These processes require the binding and hydrolysis of ATP[15]. Inhibition of DNA gyrase blocks relaxation of supercoiled DNA, relaxation being a requirement for transcription and replication. DNA gyrase is a selective target for antibacterial agents, such as the most studied quinolone and coumarin antibiotics. Quinolone drugs (e.g. ciprofloxacin) affect the protein subunit GyrA and coumarins (1-benzopyran-2-ones) (e.g. novobiocin, clorobiocin) act on GyrB[16]. Computer docking technique plays an important role in the drug design and discovery, as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent fashion[17,18], and predicting the binding geometry and hydrogen bonds formed with the surrounding amino acids as well as the mode of the ligand to the receptor, which all contribute to the docking score. MOE as a flexible docking program facilitates the prediction of favorable protein–ligand complex structures with reasonable accuracy and speed (MOE 2008.10 of Chemical Computing Group. Inc.).

Given the promising properties of pyrimidines and quinolines and in continuation of our interest in preparing biologically active heterocycles, we decided to study the effect of combining the pyrimidine and quinoline scaffolds in one molecule on the biological activity of such a ring system. Therefore, this study covers the synthesis and molecular modeling study of a series of substituted 5-aryl-pyrimido[5,4-c]quinoline-2,4-diones and evaluation of their anticancer and antimicrobial activities.

Experimental section

Biology

Anticancer screening of pyrimido[5,4-c]quinoline-2,4-dione derivatives

Experimental design The chosen compounds were subjected to the *in vitro* disease-oriented NCI 60 human cells screening panel assay to be evaluated for their *in vitro* anticancer activity. In this protocol, all compounds submitted to the screen are tested initially at a single high dose (10 μM) in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. A 48 h continuous drug exposure protocol was used, and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth[19–21]. The data obtained are a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI %) caused by the tested compounds.

Experimental procedure Cells were plated in 96-multiwell microtiter plate (10^4 cells/well) for 24 h before treatment with the test compound to allow attachment of cells to the wall of the plate. Tested compounds were dissolved in DMSO and diluted with saline to the appropriate volume. The compounds under test were added to the cell monolayer. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader at a wave length of 570 nm.

Antimicrobial screening of pyrimido[5,4-c]quinoline-2,4-dione derivatives

Experimental design An extensive assessment of the antimicrobial activity of the 11 newly synthesized pyrimido[5,4-c]quinoline derivatives **5a–k** was performed. The screening was carried out against 5 different microbial strains; three organisms representing *Gram +ve* bacteria *viz. Bacillus subtilis, Staphylococcus Aureus and Streptomyces faecalis*; two *Gram –ve* bacterial strains *Escherichia coli* and *Pseudomonas aeruginosa*. The activity of the tested compounds was evaluated using disc diffusion method comparing the diameter of the inhibition zone produced by the new derivatives with those of the standard antibacterial agent Tetracycline.

Experimental procedure Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method[22–25].

Blank paper discs with a diameter of 8.0 mm were impregnated with 10 μL of the stock solutions of the tested compounds and placed on agar where the chemical diffused from the disc into the agar only around the disc. Organisms susceptible to the tested compound do not grow in the area around the disc in what is known as a "Zone of inhibition" whose diameters were measured (in mm) with slipping calipers.

Standard discs of Tetracycline (Antibacterial agent) served as positive controls for antimicrobial activity while filter discs impregnated with 10 μL of the solvent (DMSO) were used as a negative control.

Docking methodology

Docking studies were performed using MOE software Version 2008.10. The X-ray crystal structure of the antimicrobial agent Clorobiocin bound to topoisomerase II DNA gyrase was obtained from the RCSB Protein Data Bank (PDB ID: 1KZN). The enzyme was prepared for docking as follows: (a) Clorobiocin interactions were determined to reveal the different types of interaction and a validation for the docking was performed (Figure 1). (b) Clorobiocin and solvent molecules were removed from the binding site. (c) The hydrogens were added with their standard geometry. (d) The newly synthesized compounds were constructed and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/molA. (e) Docking of the energy minimized ligand molecules was carried out using the default MOE-dock

parameters. The docking aims at searching for favorable binding conformations. The quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand-DNA gyrase-B assembly dG calculated in kcal/molA (S value in MOE)[26,27].

Chemistry

Melting points were recorded using a Gallenkamp melting-point apparatus and are uncorrected. Thin-layer chromatography (TLC) analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets and detected under ultraviolet (UV) light. Infrared (IR) spectra were recorded using KBr wafer technique on a Shimadzu 5800 Fourier transform FT-IR Spectrometer at College of Science, King Faisal University. ^{1}H NMR spectra were recorded employing a Varian Unity Plus 300 spectrometer and chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a GC-MS (Schimadzu QP-1000 EX) spectrometer. Elemental analyses were performed on Perkin-Elmer 2400 analyser at the microanalytical laboratories of the Faculty of Science, Cairo University and are within ±0.4 of the theoretical values. All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific. All solvents were reagent grade. 5-Benzylidene-1,3-dimethylpyrimidine-2,4,6-trione (**3a**) was prepared according to the reported literature[28].

Preparation of 5-arylidine-1,3-dimethylbarbituric acids 3b–d

5-(4-Methoxybenzylidene)-1,3-dimethylpyrimidine-2,4,6-trione (3b): To a solution of 1,3-dimethylbarbituric acid (15.6 g, 100 mmol) and 4-methoxybenzaldehyde (13.62 g, 100 mmol) in methanol (150 mL) was added few drops of conc. HCl. The reaction mixture was heated at reflux for 3 h and the precipitate was filtered off, washed with methanol and recrystallized from ethanol/EtOAc to afford compound **3b** in 79% yield, mp 154–155 °C; Lit.[29] mp 149–151 °C.

5-(3,4-Dimethoxybenzylidene)-1,3-dimethylpyrimidine-2,4,6-trione (3c): Adopting the same methodology used for preparation of **3b**, starting with 3,4-dimethoxybenzaldehyde instead of 4-methoxybenzaldehyde. Yield 89%, mp 229–231 °C; Lit.[29] mp 226–228 °C; Lit.[30] mp 208–210 °C. IR (KBr) ν' 3122, 3004 (aromatic CH), 2947, 2906, 2839 (CH_3), 1720, 1651 (CO), 1598, 1556, 1502 (C=C) cm^{-1}. ^{1}H NMR ($CDCl_3$); δ 3.39, 3.40 (2s, 6H; 2 × CH_3), 3.97, 3.98 (2s, 6H; 2 × OCH_3), 6.94 (d, J = 8.7 Hz, 1H), 7.78 (dd, J = 8.7 Hz, 2.1 Hz, 1H), 8.38 (d, J = 2.1 Hz, 1H), 8.48 (s, 1H).

5-(2-Ethoxybenzylidene)-1,3-dimethylpyrimidine-2,4,6-trione (3d): Adopting the same methodology used for preparation of **3b**, starting with 2-ethoxybenzaldehyde instead of 4-methoxybenzaldehyde. Yield 85%, mp 165–166 °C; Lit.[31] melting point not reported. IR (KBr) ν' 3114, 3095, 2987, 2950, 2900 (CH), 1730, 1670 (CO), 1577, 1541 (C=C) cm^{-1}. ^{1}H NMR ($CDCl_3$); δ 1.37 (t, J = 7.2 Hz, 3H), 3.28 (s, 3H), 3.35 (s, 3H), 4.07 (q, J = 7.2 Hz, 2H), 6.84 (d, J = 8.4 Hz, 1H), 6.92 (t, J = 7.2 Hz, 1H), 7.39 (t, J = 7.2 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 8.85 (s, 1H). MS (EI) m/e (rel.int.); 288 (M^+, 24), 243 (100). Anal. Calc. for $C_{15}H_{16}N_2O_4$ (288.30): C, 62.49; H, 5.59; N, 9.72. Found. C, 62.21; H, 5.73; N, 9.48.

General Procedure for preparation of pyrimido[5,4-c] quinoline-2,4-diones 5a–k

A mixture of 5-arylidene-1,3-dimethylbarbituric acid derivatives **3a–d** (10 mmol) and aniline derivatives **4a–d** (10 mmol) was fused in an oil bath at 150–180 °C for 1–2 h. The reaction mixture was cooled and triturated with ethanol, the precipitated products were filtered off and purified by either recrystallization from EtOH/DMF or column chromatography on silica gel using petroleum ether (35–60 °C)/EtOAc (7:3) as an eluent.

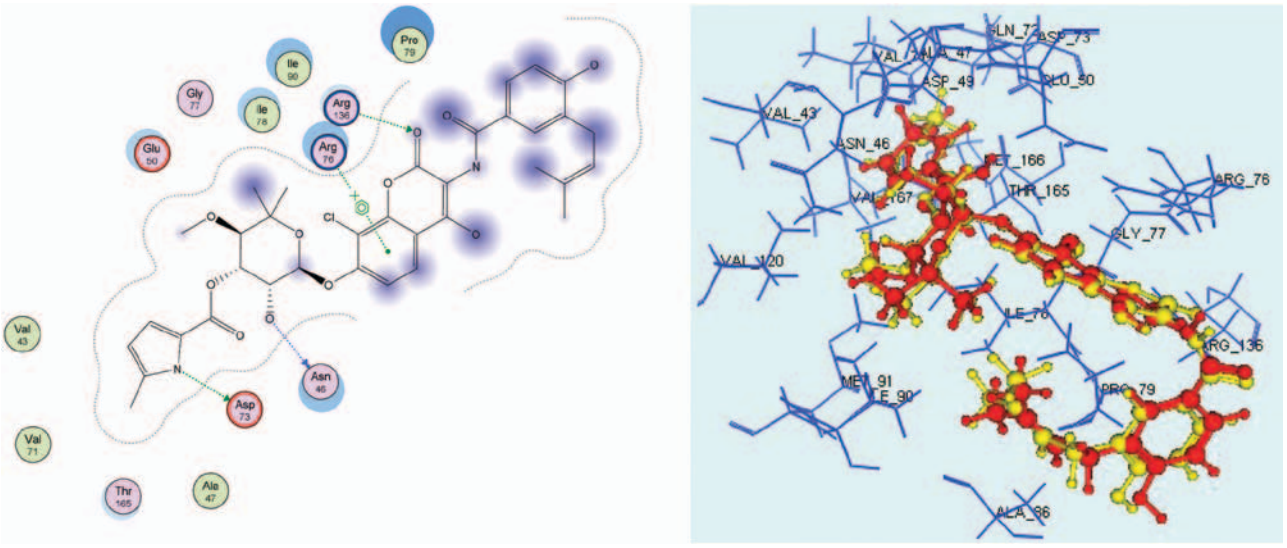


Figure 1. **Left panel:** 2D sketch of the binding mode of Clorobiocin into its binding site of DNA gyrase-*B*, showing three hydrogen bonds as green dotted lines (with residues Arg136, Asp73 and Asn46) and one Pi-cationic interaction with Arg76; **right panel:** Docking validation of Clorobiocin with DNA gyrase-B: crystal structure ligand (red) docked ligand (yellow); RMSD 0.41 Å.

1,3,9-Trimethyl-5-phenylpyrimido[5,4-c]quinoline-2,4-dione (5a): adopting the reported methodology[32], mp >300 °C.

5-(4-Methoxyphenyl)-1,3,9-trimethylpyrimido[5,4-c]quinoline-2,4-dione (5b): adopting the reported methodology[32], mp 251–252.5 °C.

5-(3,4-Dimethoxyphenyl)-1,3,9-trimethylpyrimido[5,4-c]quinoline-2,4-dione (5c): Yield 53 %, mp 276–277 °C (EtOH/DMF). IR (KBr) ν' 2991, 2935, 2837 (CH), 1708, 1668 (CO), 1635, 1606, 1569 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d_6); δ 2.35 (s, 3H), 3.18 (s, 3H), 3.69 (s, 3H), 3.70 (s, 3H), 3.87 (s, 3H), 6.72 (dd, J = 8.1 Hz, 2.1 Hz, 1H), 6.82 (d, J = 2.1 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 7.16 (d, 2.1 Hz, 1H), 7.68 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H). MS (EI) m/e (rel.int.); 391 (M^+, 100), 392 (M^+ + 1, 20), 376 (10), 363 (10). Anal. Calc. for $C_{22}H_{21}N_3O_4$ (391.42): C, 67.51; H, 5.41; N, 10.74. Found. C, 67.16; H, 5.62; N, 10.89.

9-Chloro-5-(3,4-dimethoxyphenyl)-1,3-dimethylpyrimido[5,4-c]quinoline-2,4-dione (5d): Yield 47 %, mp 276–278 °C (EtOH/DMF). IR (KBr) ν' 3080, 3008, 2958, 2927, 2837 (CH), 1712, 1672 (CO), 1602, 1568, 1517 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d_6); δ 3.17 (s, 3H), 3.67 (s, 3H), 3.70 (s, 3H), 3.87 (s, 3H), 6.75 (dd, J = 8.1 Hz, 1.8 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 2.4 Hz, 1H), 7.83 (dd, J = 9.0 Hz, 2.4 Hz, 1H), 7.95 (d, J = 9.0 Hz, 1H). MS (EI) m/e (rel.int.); 411 (M^+, 100), 412 (M^+ + 1, 12), 396 (49). Anal. Calc. for $C_{21}H_{18}ClN_3O_4$ (411.84): C, 61.24; H, 4.41; N, 10.20. Found. C, 61.19; H, 4.49; N, 10.13.

5-(3,4-Dimethoxyphenyl)-8,10-dimethoxy-1,3-dimethylpyrimido[5,4-c]quinoline-2,4-dione (5e): Yield 40 %, mp 302–303.5 °C (EtOH/DMF). IR (KBr) ν' 2997, 2943, 2837 (CH), 1708, 1668 (CO), 1618, 1585, 1552 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d_6); δ 3.11 (s, 3H), 3.38 (s, 3H), 3.63 (s, 3H), 3.68 (s, 3H), 3.81 (s, 3H), 3.91 (s, 3H), 6.41 (d, J = 2.1 Hz, 1H), 6.57 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 6.74 (d, J = 2.1 Hz, 1H), 6.88–6.91 (m, 2H). MS (EI) m/e (rel.int.); 437 (M^+, 100), 438 (M^+ + 1, 32), 422 (26), 407 (11). Anal. Calc. for $C_{23}H_{23}N_3O_6$ (437.45): C, 63.15; H, 5.30; N, 9.61. Found. C, 63.23; H, 5.46; N, 9.72.

8,10-Dimethoxy-5-(4-methoxyphenyl)-1,3-dimethylpyrimido[5,4-c]quinoline-2,4-dione (5f): Yield 38 %, mp >300 °C (EtOH/DMF). IR (KBr) ν' 2954, 2912, 1835 (CH), 1708, 1668 (CO), 1635, 1616, 1560 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d_6); δ 3.13 (s, 3H), 3.33 (s, 3H), 3.68 (s, 3H), 3.83 (s, 3H), 3.94 (s, 3H), 6.44 (s, 1H), 6.89 (d, J = 8.4 Hz, 2H), 6.94 (s, 1H), 6.99 (d, J = 8.4 Hz, 2H). MS (EI) m/e (rel.int.); 407 (M^+, 100), 408 (M^+ + 1, 20), 379 (20). Anal. Calc. for $C_{22}H_{21}N_3O_5$ (407.42): C, 64.86; H, 5.20; N, 10.31. Found. C, 64.49; H, 5.41; N, 10.32.

9-Chloro-1,3-dimethyl-5-phenylpyrimido[5,4-c]quinoline-2,4-dione (5g): Yield 45 %, mp 263-265 °C (EtOH/DMF). IR (KBr) ν' 3078, 3026, 2956, 2866 (CH), 1716, 1676 (CO), 1652, 1602, 1566 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d_6); δ 3.18 (s, 3H), 3.71 (s, 3H), 7.14 (d, J = 2.4 Hz, 1H), 7.22–7.26 (m, 2H), 7.52–7.55 (m, 3H), 7.88 (dd, J = 9.0 Hz, 2.4 Hz, 1H), 8.01 (d, J = 9.0 Hz, 1H). MS (EI) m/e (rel.int.); 351, 353 (M^+, 100, 36: chlorine isotopes), 316 (17). Anal. Calc. for $C_{19}H_{14}ClN_3O_2$ (351.79): C, 64.87; H, 4.01; N, 11.94. Found. C, 64.55; H, 4.06; N, 12.03.

8,10-Dimethoxy-1,3-dimethyl-5-phenylpyrimido[5,4-c]quinoline-2,4-dione (5h): Yield 40 %, mp >300 °C (EtOH/DMF). IR (KBr) ν' 3067, 3006, 2952 (CH), 1708, 1664 (CO), 1618, 1585, 1556 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d_6); δ 3.12 (s, 3H), 3.16 (s, 3H), 3.69 (s, 3H), 3.95 (s, 3H), 6.43 (s, 1H), 6.95–7.09 (m, 4H), 7.31 (s, 2H). MS (EI) m/e (rel.int.); 377 (M^+, 100), 378 (M^+ + 1, 71), 349 (45), 234 (40). Anal. Calc. for $C_{21}H_{19}N_3O_4$ (377.39): C, 66.83; H, 5.07; N, 11.13. Found. C, 66.59; H, 5.27; N, 11.39.

5-(3,4-Dimethoxyphenyl)-9-methoxy-1,3-dimethylpyrimido[5,4-c]quinoline-2,4-dione (5i): Yield 43 %, mp 253–255 °C(EtOH/DMF). IR (KBr) ν' 3010, 2950 (CH), 1709, 1668 (CO), 1652, 1622, 1569, 1558, 1506 (C=N, C=C) cm^{-1}. ^1H NMR ($CDCl_3$); δ 3.40 (s, 3H), 3.69 (s, 3H), 3.87 (s, 3H), 3.93 (s, 3H), 4.00 (s, 3H), 6.71–6.81 (m, 3H), 7.06 (d, J = 8.1 Hz, 1H), 7.48 (dd, J = 9.0 Hz, 2.7 Hz, 1H), 8.05 (d, J = 9.0 Hz, 1H). MS (EI) m/e (rel.int.); 407 (M^+, 100), 392 (7). Anal. Calc. for $C_{22}H_{21}N_3O_5$ (407.42): C, 64.86; H, 5.20; N, 10.31. Found. C, 64.73; H, 5.18; N, 10.25.

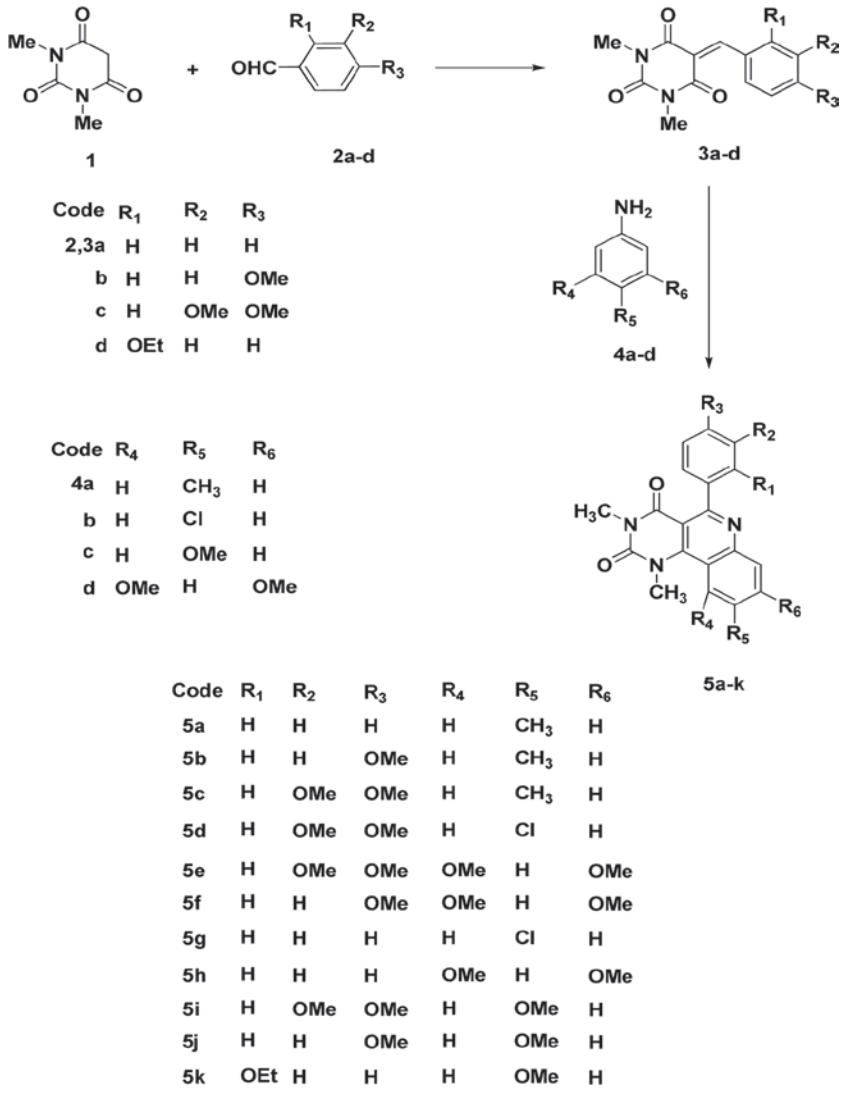
9-Methoxy-5-(4-methoxyphenyl)-1,3-dimethylpyrimido[5,4-c]quinoline-2,4-dione (5j): purified by column chromatography on silica gel using petroleum ether (35–60 °C)/EtOAc (7:3) as an eluent, R_f = 0.40. Yield 55 %, mp 268–269.5 °C. IR (KBr) ν' 3001, 2962, 2941 (CH), 1709, 1664 (CO), 1635, 1622, 1569 (C=N, C=C) cm^{-1}. ^1H NMR ($CDCl_3$); δ 3.40 (s, 3H), 3.69 (s, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 6.71 (d, J = 2.7 Hz, 1H), 7.10 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 7.47 (dd, J = 9.0 Hz, 2.7 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H). MS (EI) m/e (rel.int.); 377 (M^+, 100), 362 (6). Anal. Calc. for $C_{21}H_{19}N_3O_4$ (377.39): C, 66.83; H, 5.07; N, 11.13. Found. C, 66.95; H, 5.12; N, 10.88.

5-(2-Ethoxyphenyl)-9-methoxy-1,3-dimethylpyrimido[5,4-c]quinoline-2,4-dione (5k): purified by column chromatography on silica gel using petroleum ether (35-60 °C)/EtOAc (7:3) as an eluent, R_f = 0.46. Yield 46 %, mp 255–257 °C. IR (KBr) ν' 3060, 2979, 2954 (CH), 1710, 1660 (CO), 1622, 1568 (C=N, C=C) cm^{-1}. ^1H NMR ($CDCl_3$); δ 1.05 (t, J = 7.2 Hz, 3H), 3.40 (s, 3H), 3.68 (s, 3H), 3.88 (s, 3H), 4.00 (q, J = 7.2 Hz, 2H), 6.70 (d, J = 2.7 Hz, 1H), 7.07–7.14 (m, 3H), 7.43–7.50 (m, 2H), 7.94 (d, J = 9.0 Hz, 1H). MS (EI) m/e (rel.int.); 391 (M^+, 58), 346 (100). Anal. Calc. for $C_{22}H_{21}N_3O_4$ (391.42): C, 67.51; H, 5.41; N, 10.74. Found. C, 67.39; H, 5.54; N, 10.46.

Results and discussion

Chemistry

In view of the significant therapeutic value of quinolines, it was considered worthwhile to incorporate quinoline moieties into the C5–C6 position of the oxypyrimidine nucleus. Thus, a series of the pyrimido[5,4-c]quinoline-2,4-dione derivatives **5a–k** were synthesized in moderate yields *via* a thermolysis reaction of equimolar ratio of benzalbarbituric acid derivatives **3a–d** with the



Code	R_1	R_2	R_3
2,3a	H	H	H
b	H	H	OMe
c	H	OMe	OMe
d	OEt	H	H

Code	R_4	R_5	R_6
4a	H	CH_3	H
b	H	Cl	H
c	H	OMe	H
d	OMe	H	OMe

Code	R_1	R_2	R_3	R_4	R_5	R_6
5a	H	H	H	H	CH_3	H
5b	H	H	OMe	H	CH_3	H
5c	H	OMe	OMe	H	CH_3	H
5d	H	OMe	OMe	H	Cl	H
5e	H	OMe	OMe	OMe	H	OMe
5f	H	H	OMe	OMe	H	OMe
5g	H	H	H	H	Cl	H
5h	H	H	H	OMe	H	OMe
5i	H	OMe	OMe	H	OMe	H
5j	H	H	OMe	H	OMe	H
5k	OEt	H	H	H	OMe	H

Figure 2. Synthesis of pyrimido[5,4-c]quinoline-2,4-dione derivatives **5a–k**.

respective aniline derivatives **4a–d** at 150–180 °C for 1–2 h. The structures of pyrimido[5,4-c]quinolines **5a–k** were confirmed from their elemental analyses as well as their spectral data. Thus, ^{1}H NMR spectrum of **5c** does not show the signals corresponding to AA'-BB' system for a *p*-substituted benzene ring, however, it showed a new ABX system attributed for 1,2,4-trisubstituted benzene. Previously, we reported a suggested mechanism for the formation of **5a**[32]. In general, the suggested mechanism for the formation of pyrimido[5,4-c]quinolines **5a–k**, involves addition reaction of aniline derivative **4a–d** to the 5-arylidine derivative **3a–d**, followed by cyclization to dihydropyrimido[5,4-c]quinoline intermediate which was subsequently autooxidized (Figure 2). 5-Arylidine-1,3-dimethylbarbituric acids **3a–d** were prepared *via* a direct acid-catalyzed condensation reaction between equimolar ratios of 1,3-dimethylbarbituric acid and the respective benzaldehyde derivatives **2a–d** in methanol as a solvent. The structures of compounds **3c** and **3d** were confirmed from their spectral data. Thus, ^{1}H NMR spectrum of **3c** showed ABX system attributed for 1,2,4-trisubstituted benzene moiety indicated by the presence of three protons signals attributed to H-2; H-5 and H-6; and singlet signal at δ 8.48 attributed for a methine proton, in addition to four singlet signals attributed for protons of four methyl groups.

Biology

Anticancer screening of pyrimido[5,4-c]quinoline-2,4-dione derivatives

Eight of the synthesized compounds **5a–h** were chosen by the National Cancer Institute (NCI, Betheseda, MD, USA) for the *in vitro* disease-oriented human cells screening panel assay to be evaluated for their *in vitro* cytotoxic activity. A primary *in vitro* one-dose anticancer assay was performed using the full NCI 60 cell panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda, MD, USA. These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48 h continuous drug exposure protocol was used, and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth.

Table 1. *In vitro* antimicrobial activity of pyrimido[5,4-c]quinoline-2,4-diones **5a–k**.

	Inhibition Zone Diameter (mm)				
Compd #	*B. subtilis* (Gm +ve)	*Staph. Aureus* (Gm +ve)	*Strept. faecalis* (Gm +ve)	*E. coli* (Gm –ve)	*P. aeruginosa* (Gm –ve)
Tetracycline	32	28	31	30	31
(5a)	–	–	11	11	11
(5b)	–	–	11	–	–
(5c)	–	–	–	–	–
(5d)	13	13	14	14	14
(5e)	16	16	15	15	16
(5f)	15	15	14	15	15
(5g)	–	–	–	11	–
(5h)	15	14	16	15	16
(5i)	–	13	13	13	11
(5j)	–	11	–	11	–
(5k)	13	14	13	14	14

Data obtained from results of the anticancer screening of the eight tested quinoline derivatives can be summarized as follows, while seven of the tested compounds showed no growth inhibitory activity against all the 60 tested cancer cell lines at the used dose (10 μM), only compound **5b** possessed some cytotoxic activity. It showed moderate GI% against four of the tested cell lines corresponding to leukemia SR (GI%: 51), non small-cell lung cancer HOP-92 (GI%:63), melanoma UACC-62 (GI%: 53) and renal cancer UO-31 (GI%: 69). It also exhibited poor activity against seven cell lines comprising three breast cancer cell lines (MCF, MDA-MB-231/ATCC and HS 578T) with GI% 31, 38 and 33, respectively; two non-small cell lung cancer cell lines HOP-62 (GI%: 30) and NCI-H522 (GI%: 37); one renal cancer cell line (A498, GI%: 32) and one leukemia cell line (RPMI-8226, GI%: 30).

Antimicrobial screening of pyrimido[5,4-c]quinoline-2,4-dione derivatives

The newly synthesized compounds **5a-k** were tested against a panel of susceptible and resistant *Gram +ve* bacteria *(Bacillus subtilis, Staphylococcus Aureus and Streptomyces faecalis*) and *Gram* –ve *(Escherichia coli* and *Pseudomonas aeruginosa)* bacteria. Results of the antimicrobial screening (Table 1) showed that compounds **5d**, **5e**, **5f**, **5h** and **5k** have broad-spectrum antibacterial efficacy being moderately active against all the tested *Gram +ve* and *Gram –ve* bacteria. Compound **5i** was active against four of the tested bacterial strains. Interestingly, compound **5b** showed selectivity only towards *Streptococcus faecalis* which is known to be a relatively resistant strain of *Gram +ve* bacteria. Also, compound **5a** showed interesting results being only active against *Streptococcus faecalis* and both tested *Gram –ve* strains *viz. E. coli* and *P. aeruginosa*. Finally, Compound **5j** was only slightly active against *Staph. aureus* and *E.coli*.

In conclusion, results of the antibacterial screening showed that pyrimido[5,4-c]quinoline-2,4-diones **5d**, **5e**, **5f**, **5h** and **5k** have broad-spectrum antibacterial efficacy being moderately active against all the tested *Gram +ve* and two *Gram –ve* bacteria as compared to the standard drug Tetracycline. The following few lines display the antibacterial activity of some pyrimidine and quinoline derivatives either in the market or a few of those recently reported for the sake of comparison. Whereas, pyrimidine is pharmacologically inactive, its synthetic derivatives possess an important role in modern medicine[33]. Amongst the 2,4-diaminopyrimidine drugs, trimethoprim, an antibacterial drug is also a selective inhibitor and selectively inhibits bacterial enzyme dihydrofolate reductase (DHFR)[34]. Brodimoprim, is also found to be an effective antibacterial compound[35]. Recently, some novel reported pyrazolopyrmidine-diones showed highest antimicrobial activity against *Pseudomonas aeruginosa, Enterococcus faecalis and Bacillus pumilus* as compared to the standard drug Streptomycin[36].

On the other hand, quinolones represent a well-known class of synthetic broad-spectrum antibacterial agents including the market antibiotics levofloxacin and ciprofloxacin[37,38]. These drugs are active against a diverse assembly of *Gram –ve* and *Gram +ve* bacteria. It is well stated that the pharmacophore of this group of drugs is the quinolone ring system which is responsible for the biological activity of these drugs[39]. Furthermore, Holla *et al.*[40] described that their newly synthesized quinoline substituted pyrazolo[3,4-d]pyrimidin-4-ones showed maximum antibacterial activity against *staphylococcus aureus* in comparison to the standard drug in that study Streptomycin. Recently, a series of novel tetrahydroquinoline annulated heterocycles exhibited good antibacterial activity against microorganisms of which one of them was found to be as active as the antibiotic ciprofloxacin[41].

Finally, while our newly synthesized compounds are less potent than the pyrimidines or quinolines in the market they compare well with some reported ones.

Docking studies

In an attempt to explain the observed antimicrobial data on a structural basis, automated docking studies were carried out using MOE 10.2008 program installed on 2.0G Core 2 Duo. The protein–ligand complex based on the X-ray crystal structure of the topoisomerase II DNA gyrase-B enzyme with its bound inhibitor

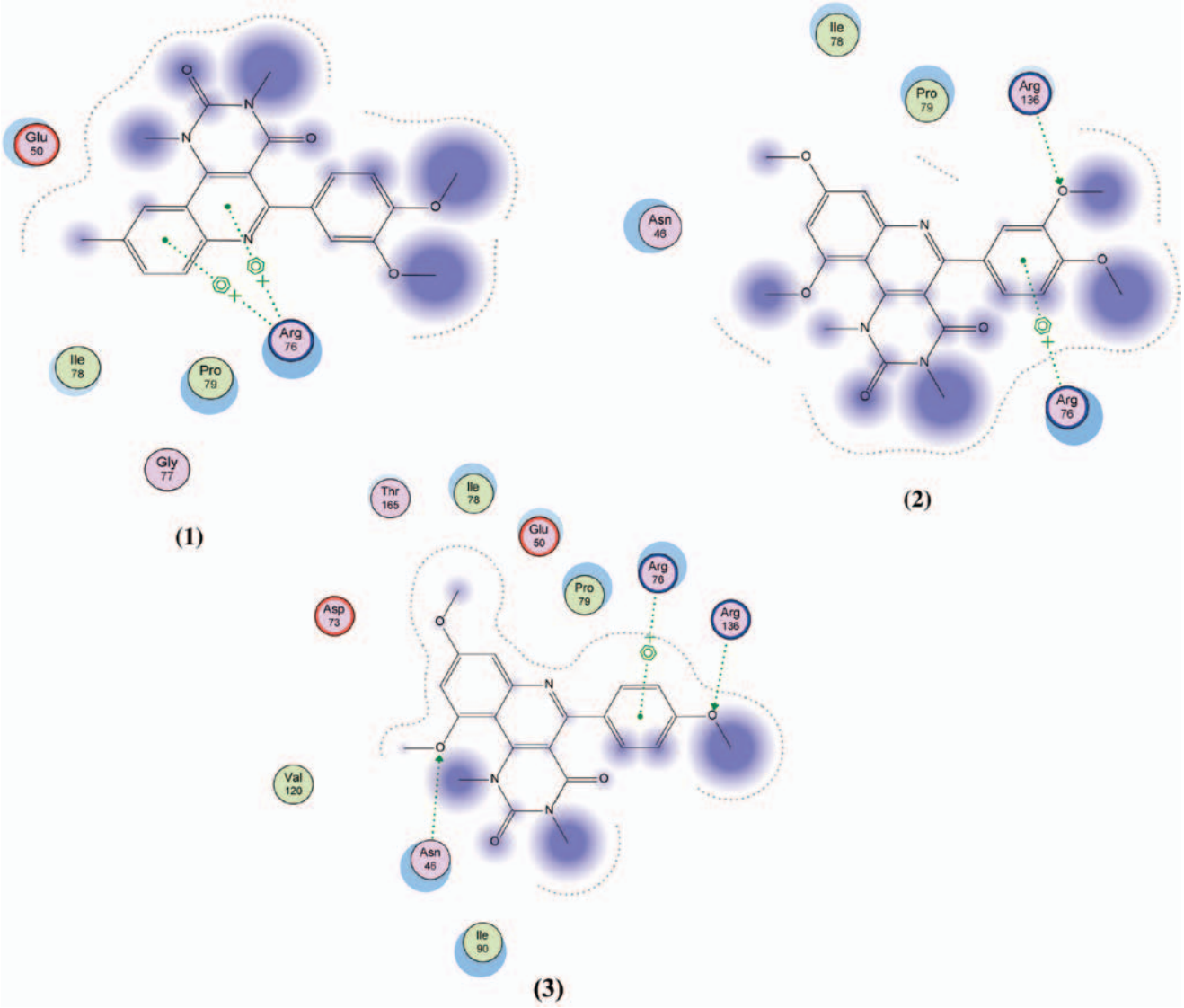


Figure 3. 2D Ligand interaction of the 5-aryl-pyrimido[5,4-c]quinoline-2,4-diones with DNA gyrase-*B*; **panel (1)** compound **5c** showing only pi-cationic interaction with Arg76; **panel (2)** compound **5e** showing pi-cationic interaction with Arg76 and H-bond with Arg136; **panel (3)** compound **5f** showing pi-cationic interaction with Arg76 and two H-bonds with Arg136 and Asn46.

Clorobiocin available through the RCSB Protein Data Bank (PDB entry 1KZN) was employed for the docking study of the new compounds[42]. The active site of the enzyme was defined to include residues within a 10.0Å radius to any of the inhibitor atoms. The binding poses as well as the binding interaction modes of the docked compounds with the surrounding amino acids in the active pocket of DNA gyrase-B were used to try to understand and rationalize the observed antimicrobial activity of the most active compounds **5e** and **5f** along with the inactive compound **5c**. The binding mode of topoisomerase II DNA gyrase-B (1KZN) with its bound inhibitor Clorobiocin (Figure 1) showed that the binding complex involves 3 hydrogen bonds with residues Arg136, Asp73 and Asn46 and one Pi-cationic interaction with Arg76. Docking validation was performed with an obtained RMSD value of 0.41Å ensuring precision and reproducibility of the docking process (Figure 1). The energy minimized structures of the most active compounds **5e** and **5f** as well as of the inactive compound **5c** with were docked in the active site of topoisomerase II DNA gyrase-*B*. The 2D ligand interactions of these 3 derivatives with DNA gyrase-B displayed in Figure 3 shows that the inactive compound **5c** possesses only a pi–cationic interaction with Arg76 which is also conserved with the other two compounds **5e** and **5f**. Moreover, the active compounds **5e** and **5f** demonstrated the presence of extra H-bond interactions (one with Arg136 for **5e;** two with Arg136 and Asn46 for **5f**) which can be regarded as the reason behind their improved activity (Figure 3). For further understanding, 3D overlay of Clorobiocin, **5c**, **5e** and **5f** in DNA-gyrase active pocket was performed which revealed that the orientations of **5e** and **5f** are similar with a slight displacement however **5c** possessed a totally

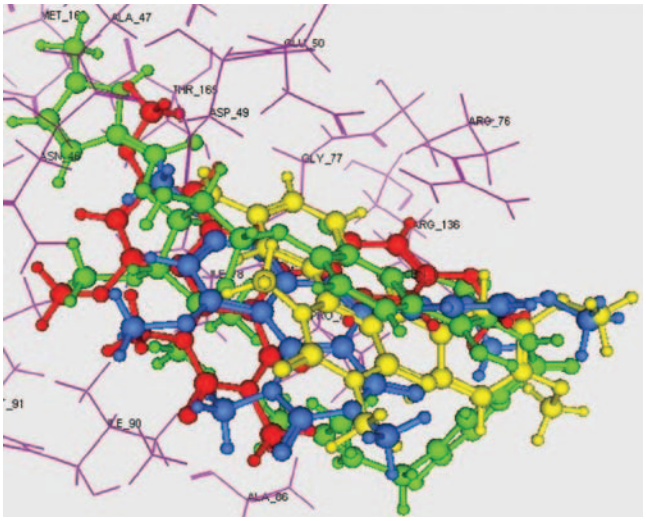


Figure 4. 3D overlay of Clorobiocin (green), **5c** (yellow), **5e** (blue) and **5f** (red) in DNA-gyrase active pocket.

different orientation with the pyrimido[5,4-c]quinolone fused ring system slightly protruding out of the active pocket thus preventing the formation of essential binding interactions (Figure 4).

After studying the docking poses and binding modes of the docked compounds, the necessity of hydrogen bond formation for enhancing the activity of this class of compounds can be highly advocated.

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Declaration of interest

The authors declare no conflict of interest.

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