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Green Synthesis, Characterization and Biological Evaluation of New Pyrazino Pyrido Quinolone Derivatives under Catalyst free Conditions

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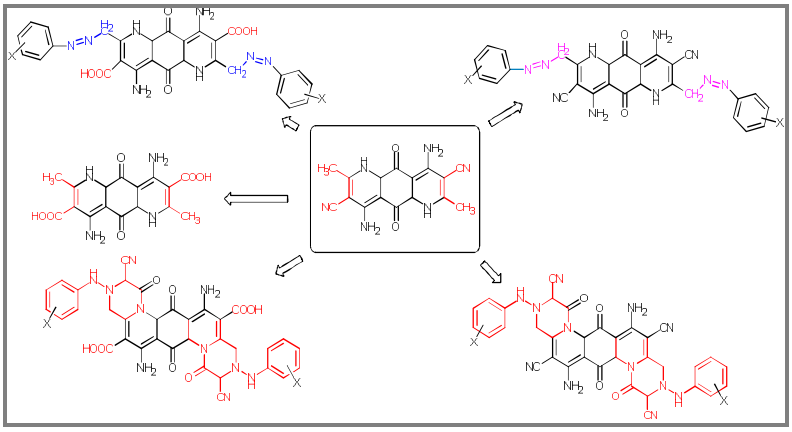
ABSTRACT

*A series of six pyrazino pyrido quinolone derivatives (**3a-c**,**6a-c**) was synthesized by treating azodyes **(2a-c)** (4,9-diamino-5,10-dioxo-2,7-bis((phenyl,4-hydroxy phenyl,4-chlorophenyl)diazenyl)methyl)-1,5,5a,6,10,10a-hexahydropyrido[2,3-g]quinoline-3,8-dicarbonitrile) and **(5a-c)** (4,9-diamino-5,10-dioxo-2,7-bis-(phenyl,4-hydroxyphenyl,4-chlorophenyl)diazenyl)methyl)-1,5,5a,6,10,10a-hexahydro-pyrido [2,3-g]quinoline-3,8-dicarboxylic acid) with ethylcyanoacetate and ethanol in microwave oven. The structural identification of these products was described on the basis of spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS) and elemental analyses. The results revealed that the proposed simple and green procedure gave the best yields (80-90%) over a very short time (25-38 s). The anti-bacterial activities of the newly synthesized compounds were evaluated in vitro against species of bacteria, including gram-positive and gram-negative. Furthermore, their antifungal activities were also tested against Aspergillus flavus and Candida albicans. The results showed that .among the synthesized compounds; compound **(3a)** exhibited the highest antibacterial and antifungal activities.*

Graphical Abstract



Keywords: Green Chemistry, Microwave, Quinolones, Catalyst-free conditions, Biological activity.

INTRODUCTION

In the past few decades, the synthesis of new heterocyclic compounds with promising biological activity has aroused significant attention due to their extensive applicability in medicinal and pharmaceutical chemistry. The presence of quinone nucleus in the chemical structure of several natural products and pharmacologically active compounds has encouraged to the development of new procedures for their synthesis [**1**]. Cyclization reactions that make possible direct transformation of quinones into different derivatives has undergone renaissance in recent decades since the change of the reactivity of these compounds rely on the substituents present in the rings of heterocyclic quinines [**2**]. The synthesis of quinolones derivatives has been of sizable interest due to the intriguing potential of the oxygen heterocycles that contribute to potential antimicrobial [**3, 4**] antiplatelet [**5**], antioxidant [**6**], antimalarial [**7-9**], anticancer [**10, 11**], antibacterial [**12**], anti-inflammatory [**13**] and antiasthmatic properties [**14**].

Aryl substituted quinolones were assumed to act as ligands for receptors like leukotriene [**15**], tyrosine kinase [**16**], 5-lipoxygenase [**17**]. Moreover, these compounds are considered valuable reagents for the synthesis of nano and nanostructures with better photonic and electronic properties [**18, 19**].

Literature surveys revealed several protocols for the synthesis of substituted quinolones, most of them involve cyclization reactions starting from benzene (or cyclohexane) derivatives substituted with nitrogen function [**20, 21**]. The classic and conventional synthetic routes include the Skraup, Friedlander, Doebner-von Millet, Pfitzinger and Combes quinolone syntheses. However, most of these methods are inadequate for the synthesis of di- and trisubstitued quinolone derivatives and require highly acidic and/or oxidizing media, high temperatures and long reaction times [**22**]. Furthermore, these synthetic methods usually involve the use of excess of reagents and produce an important amount of toxic wastes, which leads to environmental pollution. The discovery of new green approaches towards the synthesis of chemical entities has attracted tremendous research interest from scientists engaged in the ecological footprint of the chemical industry. These methods have the advantages to reduce simultaneously the use of large amount of toxic organic solvents, costs related to the prolonged heating and treatment of produced waste [**23**].Considering the negative impact of chemical methods, the development of new alternative green procedures for the synthesis of quinolones derivatives is highly desirable. Ethanol is a highly recommended green solvent for organic synthesis as alternative to toxic organic solvents because of its solvation with all type of media and low toxicity [**24**].On the other hand, microwave irradiation has several advantages over the conventional heating such as: rapidity and uniform heating of the reaction medium, higher efficiency in reduction of heat generated from unwanted side reactions and reduction of heat generated from unwanted side reactions [**25**].

As an extension of our general scope in the synthesis of novel antimicrobial agents[**26-28**],this study describes the development of novel, simple and green methodology for the synthesis of 4, 9-diamino-5,10-dioxo-2,7 bis(p-substituted phenyldiazenyl) methyl)-1,5,5a,6,10,10a-hexahydro- pyrido [2,3-g]quinoline-3,8-dicarbonitrile(**2a-c**) and 4,9-diamino-5,10-dioxo-2,7-bis-p-substituted phenyl diazenyl)methyl)-1,5,5a,6,10,10a-hexahydro pyrido [2,3-g]quinoline-3,8-dicarboxylic acid(**5a-c**) through reaction of compound (**1**) or (**4**) with diazonium salts of aromatic amine (aniline, 4-Aminophenol, 4-Chloroaniline) which reacts with two mole of ethyl cyano acetate in ethanol at microwave oven to obtain new bis((4-Substituted phenyl)amino)-pyrazino[1,2- pyridoquinoline tetra carbonitrile (**3a-c**) and bis(4-Substituted phenylamino)-1,2,3,4,7,7a ,9,10,11, 12, 15,15a-dodecahydro pyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-5,13-dicarboxylic acid (**6a-c).**

All of the newly synthesized derivatives were subjected to antimicrobial activity tests against four species of bacteria, including gram-positive, *Bacillus subtilis*, *Staphylococcus aureus* and gram-

negative, *Escherichia coli*, *Pseudomonas aeruginosa* bacteria. The antifungal activities of the new compounds were also evaluated against *Aspergillus flavus* and *Candida albicans*.

MATERIALS AND METHODS

All the melting points were measured using an electro thermal IA 9100 system open capillaries and are uncorrected. Micro analytical data were performed using a Vario El-Mentar apparatus, Organic Microanalysis Section, Micro analytical Center, Cairo University, Giza, Egypt. The results of the microanalysis were found to be in agreement with the calculated values (± 0.3). The IR spectra were recorded on KBr disks on a Perkin-Elmer 1650 spectrophotometer, Micro Analytical Center, Cairo University, Giza, Egypt. ^{1}H and ^{13}C NMR spectra were determined on a JEOL 300 MHz in DMSO-d_6, Micro analytical Center, Cairo University, Giza, Egypt. The chemical shifts were expressed in ppm relative to TMS as an internal reference. Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan)**,** Microanalytical Center, Cairo University, Giza, Egypt. Compound (**1)** was prepared according to the reported procedures [27].

General Synthesis of Azo dye(4,9-diamino-5,10-dioxo-2,7-bis((phenyl,4-hydroxy phenyl,4-chloro phenyl)diazenyl)methyl)-1,5,5a,6,10,10a-hexahydropyrido[2,3-g]quinoline-3,8-dicarbonitrile) (2a-c) from compound (1): To a solution of compound (**1)** [27] (0.01 mol) in ethanol, diazonium salt of different aromatic amine (0.02 mol) were added drop wise. The reaction mixture was magnetically stirred for1h in ice bath (0°C). The reactions were carried out through TLC using chloroform/benzene (3:1) as eluent. When the reaction was found to be complete, the solvent was evaporated under reduced pressure and the reaction mixture was cooled at -5°C in an ice bath. The obtained precipitate hence formed was filtered off and recrystallized from methanol to give the corresponding compounds (**2a-c**).

4,9-diamino-5,10-dioxo-2,7-bis(phenyldiazenyl)methyl)-1,5,5a,6,10,10a-hexahydro-pyrido[2,3-g]quinoline-3,8-dicarbonitrile(2a): Yield 54 %; mp. 280-282°C; IR (KBr): ν_{max}/cm^{-1} =1500-1521(2N=N), 1669 (2CO), 2217 (2CN), 3100 -3400 (2NH, 2NH$_2$); ^{1}H NMR (DMSO-d_6, ppm): δ_H = 2.14 (s, 4H, J = 7.04 Hz, 2CH$_2$), , 4.26 (s, 2H, J = 4.10 Hz, 2C*H*NH), 8.45 (brs, 4H, 2NH$_2$),7.02-8.1(m,12H,aromatic protons). ^{13}C NMR (DMSO-d_6, ppm): δ_C=49.68,61.59, 84,113.6, 115.91, 47,154.27,160.9,196.58, 122.68, 125.77, 129.26, 150.98; MS, *m*/*z* (%): 530 (M+, 48.2). Anal. Calcd. for $C_{28}H_{22}N_{10}O_2$ (530.54); required C, 63.39; H, 4.18; N, 26.40; found: C, 63.41; H, 4.21; N, 26.43.

4,9-diamino-2,7-bis-(4-hydroxyphenyl)diazenyl)methyl)-5,10-dioxo-1,5,5a,6,10,10a-hexahydro-pyrido[2,3-g]quinoline-3,8-dicarbonitrile(2b): Yield 59 %; mp. 265-267 °C; IR (KBr): ν_{max}/cm^{-1} =1500-1521(2N=N), 1665 (2CO), 2215 (2CN), 3100-3400 (NH$_2$, 2NH$_2$), 3435 (2OH); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.14 (s, 4H, J = 7.04 Hz, 2CH$_2$), 4.26 (s, 2H, J = 4.10 Hz, 2CHNH), 5.41 (brs, 2H,2OH), 8.45 (brs, 4H, 2NH$_2$),9.02(s,2H,2NH),7.1-8.2(m,8H,aromatic protons). ^{13}C NMR (DMSO-d6, ppm): δC =49.68, 61.59,84,113.6,115.91, 47, 154.27, 160.9, 196.58, 122.68, 125.77, 129.26, 150.98;MS, *m*/*z* (%): 562 (M+, 55). Anal. Calcd. for $C_{28}H_{22}N_{10}O_4$ (562.54); required: C, 59.78; H, 3.94; N, 24.90; found: C, 59.80; H, 3.97; N, 24.94.

4,9-diamino-2,7-bis-(4-chlorophenyl)diazenyl)methyl)-5,10-dioxo-1,5,5a,6,10,10a-hexahydro-pyrido [2,3-g]quinoline-3,8-dicarbonitrile(2c): Yield 80 %; mp. 250-253°C; IR (KBr): ν_{max}/cm^{-1} =1500-1526(2N=N), 1670 (2CO), 2220 (2CN), 3100-3400 (NH$_2$, 2NH$_2$); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.14 (s, 4H, J = 7.04 Hz, 2CH$_2$), 4.26 (s, 2H, J = 4.10 Hz, 2CHNH), 8.65(s,4H,2NH$_2$), 9.04(s,2H,2NH), 7.01-8.2(m,8H,aromatic protons). ^{13}CNMR (DMSO-d_6, ppm): δ_C= 49.68, 61.59, 84, 113.6,115.91, 47,154.27,160.9,196.58,122.68,125.77,129.26,150.98; MS, *m*/*z* (%):599 (M+, 83). Anal. Calcd. for $C_{28}H_{20}Cl_2N_{10}O_2$ (599.43); required C, 56.10; H, 3.36; Cl, 11.83; N, 23.37; found: C, 56.13; H, 3.38; Cl, 11.87; N, 23.40.

General Synthesis of compounds (3a-c): Equimolar amounts of compounds (**2a–c)** (0.01 mol) and ethylcyano acetate (0.02 mol) in ethanol were mixed together and irradiated in a microwave oven. The reaction mixture was monitored by TLC. Then the mixture was cooled and 50mL of ethanol was added. The formed precipitate was filtered and washed by ethanol and recrystallized from ethanol solvent to yield the corresponding compounds (**3a–c**).

6,14-diamino-1,7,9,15-tetraoxo-3,11-bis(phenylamino)-1,2,3,4,7,7a,9,10,11,12,15,15a-dodeca hydropyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-2,5,10,13-tetracarbonitrile(3a): Yield 80 %; mp. 230-232 °C; IR (KBr): ν_{max}/cm^{-1} =1695, 1659 (4CO), 2220 (4CN),3100-3400 (NH_2, $2NH_2$); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.16 (s, 4H, J = 8.04 Hz, $2CH_2$),4.13(s,2H,2NH) , 4.48 (s, 2H, J = 4.10 Hz, 2CHN), 5.36(S,2H,2CHCN), 8.45 (brs, 4H, NH_2),7.02-8.1(m,10H,aromatic protons) . ^{13}C NMR (DMSO-d_6, ppm): δ_C= 56.56, 61.66,66.82,85.64,113.27,113.69,116.03, 116.3,123.07,129.64, 149.63,154.31, 165.84, 196.61,; MS, *m/z* (%):664 (M+, 50). Anal. Calcd. for $C_{34}H_{24}N_{12}O_4$ (664.63); required C, 61.44; H, 3.64; N, 25.29; found: C, 61.47; H, 3.65; N, 25.32.

6,14-diamino-3,11-bis((4-hydroxyphenyl)amino)-1,7,9,15-tetraoxo-1,2,3,4,7,7a,9,10,11,12,15,15a-dodecahydropyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-2,5,10,13-tetracarbonitrile (3b): Yield 86 %; mp. 215-217 °C; IR (KBr): ν_{max}/cm^{-1} =1695, 1659 (4CO), 2217 (4CN), 3100-3400 (NH_2, $2NH_2$),3450(2OH); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.05 (s, 4H, J = 8.04 Hz, $2CH_2$), 4.13 (s,2H, 2NH), 4.48 (s, 2H, J = 4.10 Hz, 2CHN), 5.36(S,2H,2CHCN),5.68(s,2H,2OH), 8.45 (brs, 4H, $2NH_2$),7.02-8.1(m,8H,aromatic protons) . ^{13}C NMR (DMSO-d_6, ppm): δ_C= 56.56, 61.66,66.82, 85.64, 113.27, 113.69, 116.03,116.3,123.07,129.64,149.63,154.31,165.84,196.61,; MS, *m/z* (%):696 (M+, 55). Anal. Calcd. for $C_{34}H_{24}N_{12}O_6$ (696.63); required C, 58.62; H, 3.47; N, 24.13; found: C, 58.64; H, 3.50; N, 24.17.

6,14-diamino-3,11-bis((4-chlorophenyl)amino)-1,7,9,15-tetraoxo-1,2,3,4,7,7a,9,10,11,12,15,15a-dodecahydropyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-2,5,10,13-tetracarbonitrile (3c): Yield 89 %; mp. 245-247°C; IR (KBr): ν_{max}/cm^{-1} =1695 - 1659 (4CO), 2219 (4CN),3400-3100 ($2NH_2$, 2NH); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.26 (s, 4H, J = 8.04 Hz, $2CH_2$),4.27(s,2H,2NH), 4.58 (s, 2H, J = 4.10 Hz, 2CHN), 5.56(S,2H,2CHCN),58.85 (brs, 4H, $2NH_2$),7.02-8.1(m,8H,aromatic protons). ^{13}C NMR (DMSO-d_6, ppm): δ_C= 56.56, 61.66, 66.82, 85.64, 113.27, 113.69, 116.03, 116.3, 123.07, 129.64,149.63,154.31,165.84,196.61; MS, *m/z* (%):733 (M+, 46). Anal. Calcd. for $C_{34}H_{22}Cl_2N_{12}O_4$ (733.52); required C, 55.67; H, 3.02; Cl, 9.67; N, 22.91; found: C, 55.69; H, 3.05; Cl, 9.70; N, 22.94.

4,9-diamino-2,7-dimethyl-5,10-dioxo-1,5,5a,6,10,10a-hexahydropyrido[2,3-g]quinoline-3,8-dicarboxylic acid(4): 20 mL of glacial acetic acid was added to a solution of compound (**1)** (0.01 mol) in the presence of few drops of concentrated HCl solution. The reaction mixture was refluxed at 150°C for 7-8 h and its progress was controlled by TLC. After the reaction was complete, the solvent was removed by vacuum and the residues were rendered alkaline by adding sufficient amount of sodium hydroxide solution (10%).The formed precipitate was then collected by filtration and recrystallized from methanol. yield 65%; Mp: >300°C; IR (KBr, Cm−1): ν~ 1665-1645 (4C=O),3100 - 3400 (NH, NH_2), 3450 (2OH); ^{1}H-NMR δ (ppm): 1.59 (d. S, 6H, $2CH_3$), 5.78 (brs, 4H, $2NH_2$), δ 7.01 - 8.01 (m, 2H, Ar-H), δ 9.32 (brs, 2H, 2NH),11.02(brs.,2H,2OH); ^{13}C-NMR δ (ppm): 174.09 (C=O), 168.4 (C=O), 153.04 (carbon which attach by CH_3), 119.4 (carbon which attach by COOH), 136.5 (carbon which attach by NH_2) 96 - 138 (C=C), 64.02 (C-N), 20.03 (CH_3), 163.05 (2C=O); MS, *m/z* (%):360(M+, 50). Anal. Calcd. for $C_{16}H_{16}N_4O_6$(360.32) required C, 53.33; H, 4.48; N, 15.55:found : C, 53.34; H, 4.50; N, 15.58.

General Synthesis of Azo dye4,9-diamino-5,10-dioxo-2,7-bis-(phenyl,4-hydroxy phenyl,4-chloro phenyl)diazenyl)methyl)-1,5,5a,6,10,10a-hexahydropyrido [2,3-g]quinoline-3,8-dicarboxylic acid (5a-c) from compound (4): The synthesized compound (**4)** (0.01 mol) was dissolved in ethanol in a round bottom flask. To this solution, 0.02 mol of diazonium salts (aniline, 4-Aminophenol and 4-

Chloroaniline) was added. The reaction mixture was stirred for a period of 1hat 0°C. The progress of the reaction was carried out using TLC. After completion of the reaction, the mixture was poured into ice-cold water and the precipitates was filtrated and recrystallized from methanol to give the desired compounds(**5a-c**).

4,9-diamino-5,10-dioxo-2,7-bis-phenyldiazenyl)methyl)-1,5,5a,6,10,10a-hexahydropyrido[2,3-g] quinoline-3,8-dicarboxylic acid(5a): Yield 65 %; mp. 220-222°C; IR (KBr): ν_{max}/cm^{-1} =1500-1521(2N=N), 1696-1667 (4CO),3100 - 3400 (2NH, 2NH$_2$), 3450 (2OH); ^{1}H NMR (DMSO-d_6, ppm): δ_H = 2.14 (s, 4H, *J* = 7.04 Hz, 2CH$_2$), , 4.26 (s, 2H, *J* = 4.10 Hz, 2CHNH), 7.65 (brs, 4H, 2NH$_2$),7.02-8.1 (m,12H,aromatic protons),11.04(brs.,2H,OH). ^{13}C NMR (DMSO-d$_6$, ppm): δ_C=49.54, 61.55, 84, 113.4, 115.82, 47.08,154.21,160.4,196.51,122.63,125.72,129.22,150.90; MS, *m/z* (%):568(M+, 35) Anal. Calcd. for $C_{28}H_{24}N_8O_6$(568.54) required C, 59.15; H, 4.25; N, 19.71: found: C, 59.18; H, 4.29; N, 19.75.

4,9-diamino-2,7-bis-(4-hydroxyphenyl)diazenyl)methyl)-5,10-dioxo-1,5,5a,6,10,10a-hexahydro-pyrido[2,3-g]quinoline-3,8-dicarboxylic acid(5b): Yield 45 %; mp. 280-281°C; IR (KBr): ν_{max}/cm^{-1} =1501-1523(2N=N), 1696- 1665 (4CO), 3100 - 3400 (2NH, 2NH$_2$),3500-3435 (4OH); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.18 (s, 4H, J = 7.04 Hz, 2CH$_2$), , 4.29 (s, 2H, J = 4.10 Hz, 2CHNH), 5.41 (brs, 2H,2OH), 8.45 (brs, 4H, 2NH$_2$),9.04(s,2H,2NH),7.1-8.2(m,8H,aromatic protons), 11.04(brs,2H,2OH). ^{13}C NMR (DMSO-d6, ppm): δC =49.67, 61.59, 84,113.6, 115.91, 47,154.27, 160.9, 196.58,122.68, 125.77, 129.26, 150.98; MS, *m/z* (%):600 (M+, 50). Anal. Calcd. for $C_{28}H_{24}N_8O_8$ (600.54); required :C, 56.00; H, 4.03; N, 18.66; found: C, 56.21; H, 4.16; N, 18.69.

4,9-diamino-2,7-bis-(4-chlorophenyl)diazenyl)methyl)-5,10-dioxo-1,5,5a,6,10,10a-hexahydro pyrido [2,3-g]quinoline-3,8-dicarboxylic acid(5c): Yield 60 %; mp. 250-253°C; IR (KBr): ν_{max}/cm^{-1} =1502-1525(2N=N),3100 - 3400 (2NH, 2NH$_2$),3500-3450 (2OH), 1698-1670 (4CO); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.19 (s, 4H, J = 7.04 Hz, 2CH$_2$), 4.30 (s, 2H, J = 4.10 Hz, 2CHNH), 8.68(s,4H,2NH$_2$), 9.08 (s, 2H,2NH),7.01-8.2(m,8H,aromatic protons), 11.04(brs,2H,2OH). ^{13}C NMR (DMSO-d_6, ppm): δ_C= 49.68, 61.59,84,113.6,115.91, 47, 154.27, 160.9, 196.58, 122.68, 125.77, 129.26, 150.98; MS, *m/z* (%):636 (M+, 55). Anal. Calcd. for $C_{28}H_{22}Cl_2N_8O_6$ (637.43); required C, 52.76; H, 3.48; Cl, 11.12; N, 17.58; found: C, 52.79; H, 3.51; Cl, 11.14; N, 17.62.

Synthesis of compounds (6a-c): Equimolar amounts of compounds (**5a–c)** (0.01 mol) and ethylcyano acetate (0.02 mol) in ethanol were mixed together and irradiated in a microwave oven. The reaction mixture was heated (monitored by TLC). Then the mixture was cooled and 50mL of ethanol was added. The formed precipitate was filtered and washed by ethanol and recrystallized from the ethanol solvent to yield the corresponding compounds **(6a–c)**.

6,14-diamino-2,10-dicyano-1,7,9,15-tetraoxo-3,11-bis(phenylamino)-1,2,3,4,7,7a,9,10,11,12,15, 15a-dodecahydropyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-5,13-dicarboxylic acid (6a): Yield 85 %; mp. 220-222°C; IR (KBr): ν_{max}/cm^{-1} =1695-1659 (6CO), 3100- 3400 (2NH, 2NH$_2$), 3500-3450(2OH); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.16 (s, 4H, J = 8.04 Hz, 2CH$_2$), 4.13 (s, 2H,2NH) , 4.48 (s, 2H, J = 4.10 Hz, 2CHN), 5.36(S,2H,2CHCN), 8.45 (brs, 4H, 2NH$_2$),7.02-8.1(m,12H,aromatic protons), 11.12(brs.,2H,2OH). ^{13}C NMR (DMSO-d_6, ppm): δ_C= 56.56, 61.66, 66.82, 85.64, 113.27, 113.69, 116.03, 116.3, 123.07, 129.64, 149.63, 154.31, 165.84,196.61; MS, *m/z* (%):702 (M+, 60). Anal. Calcd. for $C_{34}H_{26}N_{10}O_8$ (702.63); required C, 58.12; H, 3.73; N, 19.93; found: C, 58.15; H, 3.77; N, 19.96.

6,14-diamino-2,10-dicyano-3,11-bis((4-hydroxyphenyl)amino)-1,7,9,15-tetraoxo-1,2,3,4, 7,7a,9, 10,11,12,15,15a-dodecahydropyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-5,13-dicarboxylic acid (6b): Yield 88 %; mp. 235-237°C; IR (KBr): ν_{max}/cm^{-1} =1695-1659 (6CO), 3100-3400 (2NH, 2NH$_2$), 3500-3450(4OH); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.05 (s, 4H, J = 8.04 Hz, 2CH$_2$), 4.13 (s,2H, 2NH), 4.48 (s, 2H, J = 4.10 Hz, 2CHN), 5.36(S,2H,2CHCN),5.68(s,2H,2OH), 8.45

(brs, 4H, $2NH_2$),7.02-8.1(m,8H,aromatic protons),11.18(brs.,2H,2OH) . ^{13}C NMR (DMSO-d_6, ppm): δ_C= 56.56, 61.66, 66.82,85.64, 113.27, 113.69,116.03, 116.3, 123.07,129.64, 149.63, 154.31, 165.84, 196.61; MS, *m/z* (%):734 (M+, 45). Anal. Calcd. for $C_{34}H_{26}N_{10}O_{10}$ (734.63); required C, 55.59; H, 3.57; N, 19.07; found: C, 55.63; H, 3.59; N, 19.12.

6,14-diamino-3,11-bis((4-chlorophenyl)amino)-2,10-dicyano-1,7,9,15-tetraoxo-1,2,3,4,7,7a,9,10, 11,12,15,15a-dodecahydropyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-5,13-dicarbo xylic acid (6c): Yield 90 %; mp. 290-292°C; IR (KBr): ν_{max}/cm^{-1} =2219, 1695- 1659 (6CO), 3100-3400 (2NH, $2NH_2$),3500-3450(2OH); ^{1}H NMR (DMSO-d_6, ppm): δ_H= 2.26 (s, 4H, J = 8.04 Hz, $2CH_2$), 4.27 (s,2H, 2NH), 4.58 (s, 2H, J = 4.10 Hz, 2CHN), 5.56(S,2H,2CHCN),58.85 (brs, 4H, NH_2),7.02-8.1 (m, 8H, aromatic protons) ,11.23(brs.,2H,2OH). ^{13}C NMR (DMSO-d_6, ppm): δ_C= 56.56, 61.66, 66.82, 85.64, 113.27,113.69,116.03,116.3,123.07,129.64,149.63,154.31,165.84,196.61,; MS, *m/z* (%):**770** (M+, 45). Anal. Calcd. for $C_{34}H_{24}Cl_2N_{10}O_8$ (771.52); required C, 52.93; H, 3.14; Cl, 9.19; N, 18.15; found: C, 52.95; H, 3.17; Cl, 9.19; N, 18.19.

Antimicrobial assessment Methodology: The antibacterial and antifungal activities of the synthesized compounds (**3a-c**) and (**6a-c**) were tested against different strains using a modified Bauer-Kirby diffusion protocol [29]. These strains included Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus* and Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. The antifungal activities of these compounds were also tested against *Aspergillus flavus* and *Candida albicans* using the standard Amphotericin B antifungal agent. Many media are available according to the literature surveys, but NCCL recommended Mueller–Hinton agar medium for bacteria due to it result in good batch-to-batch reproducibility. Disk diffusion method for filamentous fungi was tested using approved standard method (M38-A) and developed by evaluating the susceptibility of filamentous fungi to antifungal agents [30]. Disk diffusion method for yeasts was developed using approved standard method (M44-P) [31]. Briefly, 100 µL of the test bacteria/fungi was grown in 10 mL of fresh media until reaching a count of 108 cells mL^{-1} for bacteria and approximately 105 cells mL^{-1} for fungi [32-34].Then, one hundred microliter of microbial suspension was spread onto the Mueller–Hinton agar medium. Then, paper disks (Schueicher and Schuel, Spain) with a diameter of 8 mm were impregnated with10 µL of each compound. In this study, plates inoculated with filamentous fungi as *Aspergillus flavus* at 25°C for 48 h, gram (+) bacteria as Staphylococcus aureus, *Bacillus subtilis*; gram (–) bacteria as *Escherichia coli* and *Pseudomonas aeuroginosa* were incubated at 35-37°C for 24-48 h and yeast as Candida albicans was incubated at 30°C for 24-48 h. Standard disk of ampicillin (antibacterial agent), amphotericin B (antifungal agent) were used as positive controls for antimicrobial activity and filter disks impregnated with 10 µL of solvent (mixture of distilled water, chloroform and DMSO) were used as negative controls. All experiments were repeated and carried out in triplicate in the case of significant difference in the results and mean values were reported. The inhibition zone diameters were measured in mm mg^{-1} sample and the results are listed in Table 2 and graphically illustrated in Figures 1 and 2.

RESULTS AND DISCUSSION

Due to the intriguing biological activities of quinolones, cyclization reactions that make possible direct transformation of quinolones to pyrazino and pyrido-quinolones through green routes are considered an important and ongoing challenge. The synthesis procedure of azodyes (**2a-c**) involves a double addition from both sides of compound (**1)** with two equivalent different diazonium salts of aromatic amine (aniline, 4-Aminophenol, 4-Chloroaniline). The structures of compounds (**2a-c**) were established and confirmed based on their spectral data (IR, ^{1}H-NMR and ^{13}C-NMR and MS) and elemental analysis, (cf. Experimental section). The IR spectrum of these compounds presented the characteristic stretching vibration absorption bands of azodyes at the range of 1500-1526cm^{-1}.

In this study, the pyrazino pyrido quinolones (**3a-c**) (Scheme 1) were prepared through the reaction of azodyes compounds (**2a-c**) with ethyl cyanoacetate in ethanol in microwave oven. The

reaction occurred by ratio (1:2) to form 6,14-diamino-1,7,9,15-tetraoxo-3,11-bis((4-substitutedphenyl) amino)-1,2, 3, 4, 7, 7a,9, 10,11,12,15,15a-dodecahydropyrazino[1,2-a]pyrazino[1',2':1,6]pyrido[2,3-g]quinoline-2,5,10,13-tetracarbonitrile (**3a-c**) which crystallized from ethanol. These reactions were made possible due to the activation of the exocyclic methyl group at C2 by the cyano group at C3, which leads to easy formation of azodye (**2a-c**) [**20**]**.** Short reaction times (25-35 s) and good yields varying from 80 to 89 %were obtained in the absence of catalyst (Table 1).

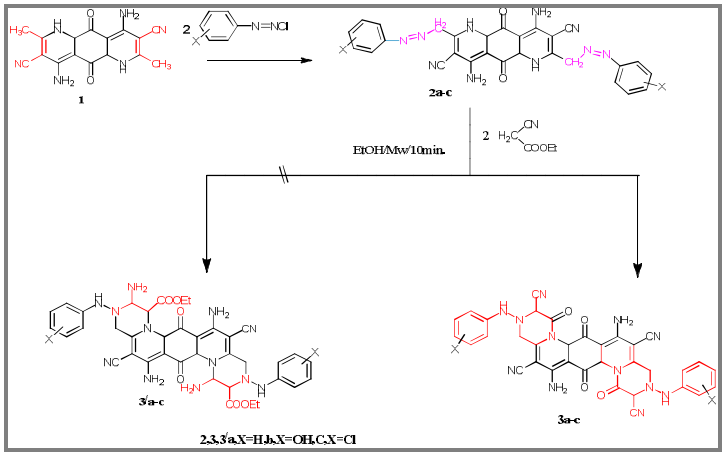
Table 1. Physicochemical data of the synthesized compounds (**3a-c**, **6a-c**)

Compound No.	X	Molecular formula	Crystallization	%Yield	Reaction Time (micro wave)
(3a)	H	$C_{34}H_{24}N_{12}O_4$	EtOH	**80**	35 s
(3b)	OH	$C_{34}H_{24}N_{12}O_6$	EtOH	**86**	30 s
(3c)	Cl	$C_{34}H_{22}Cl_2N_{12}O_4$	EtOH	**89**	25 s
(6a)	H	$C_{34}H_{26}N_{10}O_8$	EtOH	**85**	38 s
(6b)	OH	$C_{34}H_{26}N_{10}O_{10}$	EtOH	**88**	34 s
(6c)	Cl	$C_{34}H_{24}Cl_2N_{10}O_8$	EtOH	**90**	29 s

The IR spectrum of compounds (**3a-c**) showed absorption bands at 1695, 1659 cm^{-1} for (4 CO), 2220,2217,2219 cm^{-1} for (4 CN), and 3100-3400cm^{-1} for (2 NH, 2 NH_2) and 3450 cm^{-1} for (2OH) Their ^{1}H NMR spectrums declared singlet peaks at δ 4.48, 4.58 corresponding to CHN protons.

Additionally, the ^{1}H-NMR spectrum of the compounds (**3a-c**) in DMSO sign to the absence of the singlet for the ester group. Hence, the structures of compounds (**3a-c**) were considered more favorable than those of (**3′a-c**) (Scheme 1). The structures of compounds (**3′a-c**) were excluded on the basis of their spectral data. The mass spectrum of compounds (**3a-c**) displayed intense peaks at, 664 (M^+, 50) 696(M^+, 55), 773(M^+, 46) corresponding to the expected molecular formula $C_{34}H_{24}N_{12}O_4$ (M_{wt}=664.63), $C_{34}H_{24}N_{12}O_6$ (M_{wt}=696.63), $C_{34}H_{22}Cl_2N_{12}O_4$ (M_{wt}=733.52) g mol^{-1} (cf. Experimental section).

The reactivity of compounds (**3a-c**) was investigated, it was found that compound (**3a**) (in which X = H) took longer time than compounds (**3b**) (in which X = OH) and (**3c**) (in which X=Cl). This behavior was ascribed to the low activity of phenyl group in comparison with phenyl substituted in para position by hydroxyl and chloride groups/functions. The elemental analysis, IR, ^{1}H-NMR and ^{13}C NMR spectra and mass spectra support further the formation of compounds (**3a-c**).



Scheme 1. Synthesis of azodyes (**2a-c)** and pyrazino pyrido quinolone (**3a-c**).

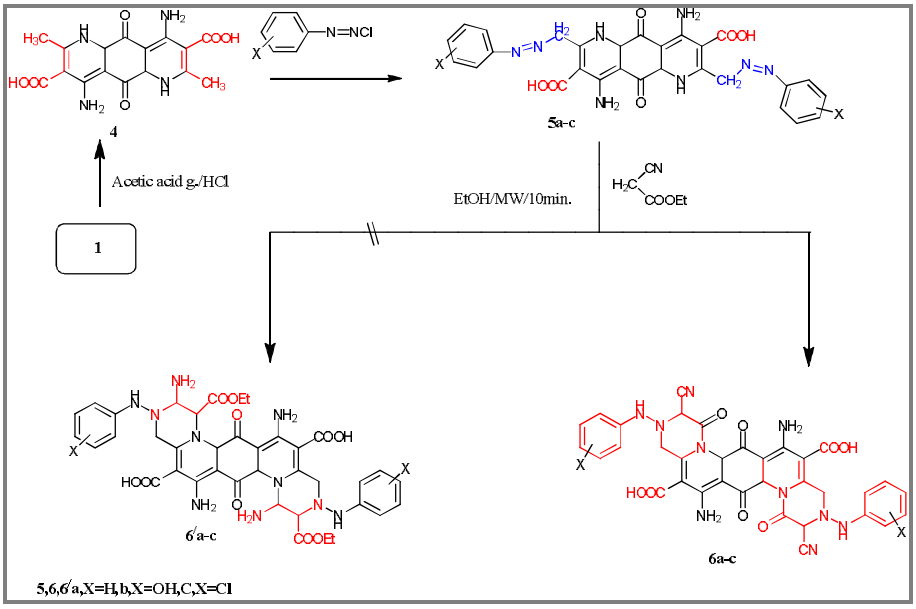
To enlarge the scope of our study, the two cyano groups present at compound (**1**) in positions 3 and 8 were converted to carboxylic groups via the reaction of compound (**1**) with a mixture of glacial acetic acid and few drops of concentrated hydrochloric acid. Subsequently, the reaction mixture was heated under reflux for a period of time that varies from 7 to 8 h. (Scheme 2).

The reaction of conversion of cyano group to carboxylic involves an acid hydrolysis [**23**] that yields the corresponding 4,9-diamino-2,7-dimethyl-5,10-dioxo-1,5,5a,6,10,10a-hexahydropyrido [2,3-g]quinoline-3,8-dicarboxylic acid (**4**) with molecular formula $C_{16}H_{16}N_4O_6$ (M_{wt}= 360.32 g mol^{-1}). The IR spectra of compound (**4**) depicted the presence of absorption bands for two hydroxyl group (2OH) at 3450 cm^{-1}. Besides, ^{1}H NMR spectra revealed the presence of different peaks at δ 1.59, singlet for $2CH_3$ and 11.02 brs for 2OH groups. All the spectral data and elemental analysis ascertain further the formation of compound (**4**). (cf. Experimental section).

In a similar manner, the reactivity of compound (**4**) towards different diazonium salts of aromatic amine, including aniline, 4-Aminophenol and4-Chloroaniline was investigated with ethyl cyano acetate in ethanol in microwave oven. Short reaction times (29-38 s) and good yields varying from 85 to 90 % were found in the absence of catalyst (Table 1).

The structures of the synthesized compounds (**5a-c**) were established by elemental analysis, IR, ^{1}H-NMR ,^{13}C NMR spectra and mass spectra (cf. Experimental section).It is worth mentioning that compounds (**5a-c**) have active methylene groups that render them available for the reaction with ethyl cyanoacetate to give rise to pyrazino pyrido quinolone carboxylic acid (**6a-c**) instead of (**6′a-c**) (Scheme 2). The structures of compounds (**6′a-c**) were excluded since their ^{1}H-NMR spectra showed the absence of signals signed to the ester group. The IR spectrum of compounds (**6a**) showed absorption bands at 1695-1659, 3400-3100 $cm^{-1}$and 3500-3450 cm^{-1} corresponding to (6 CO), (2 NH_2, 2 NH), (2 OH) functions and their ^{1}H NMR spectrum declared peaks at δ_H =2.16 as doublets signals/ singlets corresponding to CH_2 protons.

The mass spectrum of compounds **(6a)** displayed a peak corresponding to $C_{34}H_{26}N_{10}O_8$ (M_{wt} = 702.63 g mol^{-1}), the ^{13}C NMR spectra of these compounds are consistent with cyclization of azodyes structures. Consequently, the structures of compounds (**6a**) were in agreements with the obtained elemental and spectral data. Similarly, the structures of both compounds (**6b**) and (**6c**) were confirmed by elemental analysis, IR, ^{1}H-NMR, ^{13}C NMR, and mass spectra (cf. Experimental section).



Scheme 2.Synthesis of compounds (**4**), (**5a-c**) and (**6a-c**).

Antimicrobial Activities: The newly synthesized compounds pyrazinopyridoquinolinetetracarbo-nitrile and pyrazinopyridoquinoline-dicarboxylic acid (**3a-c**) and (**6a-c**) were screened by Kirby-Bauer method [**34**] for their antibacterial activity against four species of bacteria, including gram-positive, *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative, *Escherichia coli*, *Pseudomonas*

aeruginosa bacteria. The antifungal activities of these compounds were also tested against *Aspergillus flavus* and *Candida albicans* using the standard Amphotericin B antifungal agent.

Table 2. Antimicrobial evaluation of the newly synthesized compounds (**3a-c**) and (**6a-c**)

Sample	Inhibition zone diameter (mm mg^{-1} sample)					
	Bacterial species				Fungi	
	Gram-positive		Gram-negative			
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus flavus*	*Candida albicans*
Control (DMSO)	0	0	0	0	0	0
Ampicillin	26	21	25	26	–	–
Amphotericin B	–	–	–	–	15	19
1	10	10	10	10	0	10
(3a)	27	25	26	28	22	28
(3b)	16	16	16	16	0	12
(3c)	11	11	11	11	0	11
(6a)	17	16	16	15	0	12
(6b)	13	13	13	12	10	12
(6c)	15	13	10	13	0	12

From table 2 and figure 1 and 2, it is clear that compound (**3a)** showed the highest antimicrobial activity against both gram-positive and gram-negative bacterial strains and its activity was found to be considerably higher than the standard Ampicillin antibacterial agent. Compound (**3a**) also exhibited higher antifungal activities against both *Aspergillus flavus* and *Candida albicans* relevant to the antifungal agent Amphotericin B. On the other hand, compounds (**3b**), (**6a**) and (**6c**) presented moderate to low antibacterial activities. The antibacterial activities of compound (**3c**) were found to be the lowest among all the tested compounds and their activities was practically higher than the starting material (**1**). All compounds except (**3a**) and (**6b**) did not display antifungal activities against *Aspergillus flavus*in in comparison with Amphotericin B. Moreover, all the analyzed compounds except compound (**3a**) showed moderate antifungal activities.

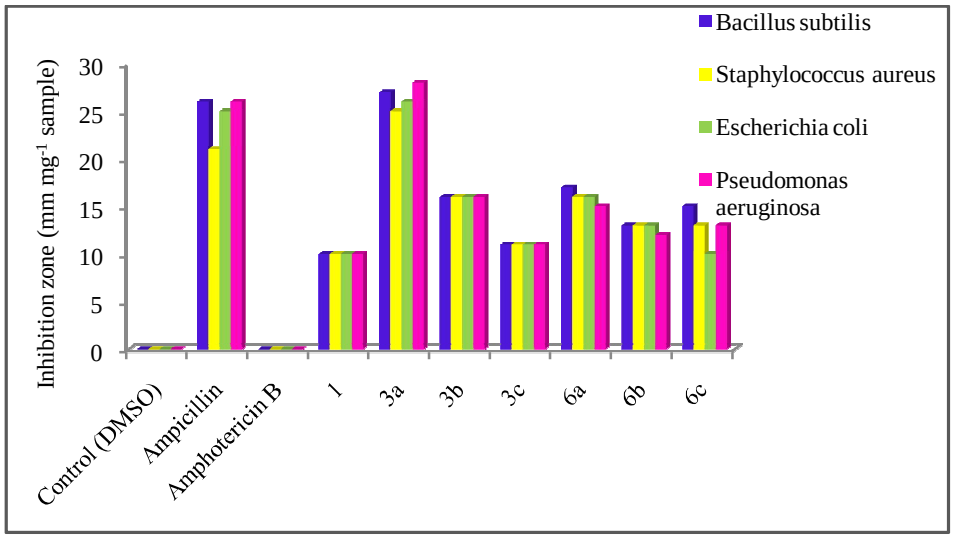


Figure 1. Antimicrobial activities of compounds (**3a-c**) and (**6a-c**).

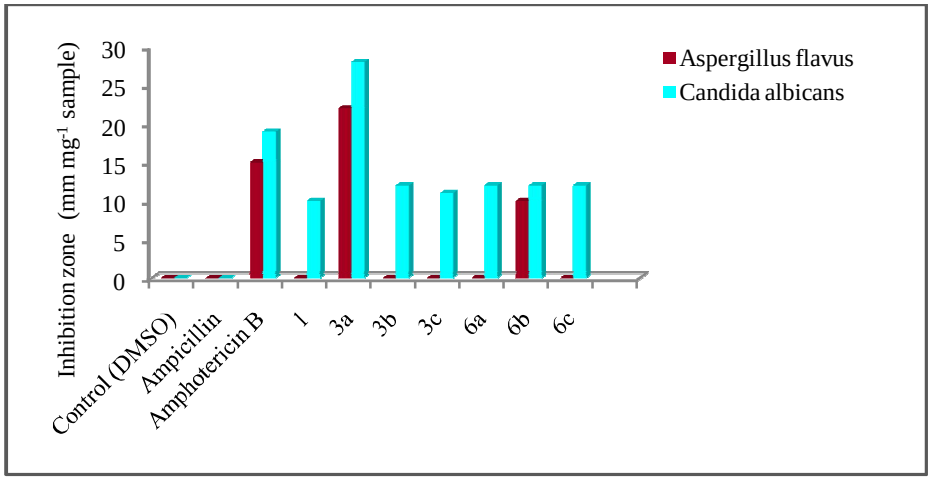


Figure 2. Antifungal activities of compounds (**3a-c**) and (**6a-c**).

APPLICATION

The advantage of this method concerned with decrease reaction time, better yield, and decrease in byproduct, biological application.

CONCLUSION

Novel pyrazino pyrido quinolone derivatives (**3a-c**) and (**6a-c**) were successfully synthesized via a simple, inexpensive and environmentally benign procedure, starting from azo dyes (**2a-c**) and (**5a-c**) respectively. The structures of the new compounds were confirmed by spectral and elemental analyses studies and were evaluated for *in vitro* antibacterial and antifungal activities. Among the entire six compounds, compound (**3a**) demonstrated the highest antibacterial and antifungal activities; however, the rest of the synthesized compounds exhibited moderate biological activities. The proposed green procedure is catalyst free and provided (**3a-c**) and (**6a-c**) compounds in high yields varying from 80 to 90 % over a short reaction time following a feasible process. Further studies of these new pyrazino pyrido quinolone derivatives are in progress related to their inflammatory and cytotoxic activities and will be reported elsewhere.

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Competing Interests: The authors declare no conflict of financial, academic, commercial, political, or personal interests.

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