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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Microwave solvent-free condition synthesis and pharmacological evaluation of pyrano[3,2-*c*]quinolines

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Abstract The microwave-induced three-component one-pot synthesis of 2-amino-3-carbethoxy-4-phenylpyrano[3,2-*c*]quinolin-5(6*H*)-ones (**4a–l**) from 4-hydroxyquinolin-2(1*H*)-ones, aromatic aldehydes and ethyl cyanoacetate was described. The detailed synthesis, structure analysis, and antimicrobial screening for the title compounds were also reported. Pharmacological screen studies like anti-inflammatory and antibacterial activities of newly synthesized quinoline derivatives were evaluated against carrageenan-induced rat paw edema model and Gram-positive and Gram-negative bacteria, respectively.

Keywords Multicomponent · Pyranoquinolines · 4-Hydroxy-quinolin-2(1*H*)-ones · Microwaves · Triethylamine

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Introduction

One-pot multicomponent reactions (Tietze and Lieb, 1998) (MCRs) represent nearly ideal cases of syntheses which can be used to construct compound libraries (Armstrong *et al.*, 1996; Li, 2005) because of their high degree of atom economy, convergence and productivity, ease of extraction, and excellent yield (Khurana and Kumar, 2009). The variation of two or more components of the reaction can make available a large number of compounds and increase the chemical diversity. Moreover, the possibility of performing multicomponent reactions under solventless conditions with heterogeneous catalysts could enhance their efficiency from an economic as well as an ecological point of view.

Naturally occurring compounds such as flindersine, simulenoline, melicobisquinolone A, melicobisquinolone B, zanthodioline, khaplofoline, huajiaosimulime, etc., were found to possess the pyranoquinoline ring system (Ulubelen *et al.*, 1994; Shwu Jen and Ih Sheng, 1993; Barr *et al.*, 1995). Structures incorporating this moiety show marked psychotropic, antiallergenic, anti-inflammatory, antihistaminic, and estrogenic activities and are thus of prime interest for biological applications (Chen *et al.*, 1994). Further, these compounds are also used as synthetic precursors for the preparation of other dimeric quinoline alkaloids and polyheterocylces (Ramesh *et al.*, 1984). Hence, the novel and efficient synthesis of such compounds still represents a highly pursued target (Huffman and Hsu, 1972; Ye *et al.*, 1999; de Groot and Jansen, 1975).

4-Hydroxyquinolin-2(1*H*)-ones, occur in *Rutaceae* plants (Lee *et al.*, 2001; Majumdar and Mukhopadhyay 2003, are attractive reactive intermediates that could be readily converted to several quinoline alkaloids. In recent years, the application of microwave irradiation in organic synthesis has attracted much interest, due to the resulting

shorter reaction time and operational simplicity with higher conversion and high degree of selectivity (Caddick, 1995; Varma, 1999). As part of our continued interest (Thamarai Selvi *et al.*, 2006; Nadaraj and Thamarai Selvi, 2007; Nadaraj *et al.*, 2006, 2009) in developing solvent-free methodologies for the synthesis of quinoline derivatives, we herein describe a simple and facile MCR synthesis of pyrano[3,2-*c*]quinoline derivatives.

Materials and methods

All chemicals purchased were of analytic grade and used without further purification unless mentioned otherwise. The melting points (Mps) were determined using a Boetieus micro heating table and are uncorrected. The infrared spectra were recorded on a Shimadzu-8201 spectrophotometer as pellets on KBr disks. The ^{1}H- and ^{13}C-NMR spectrum was recorded on a Bruker AMX-400 spectrometer in DMSO-d_6 using TMS as an internal reference unless otherwise stated. The elemental analyses were performed on a Perkin Elmer CHN-analyzer. The mass spectra (MS) were recorded on a Shimadzu GCMS-QP5050A (70 eV) mass spectrometer. The reactions were monitored by thin layer chromatography (TLC) using glass plates coated with Silica gel-G containing 13% calcium sulfate as a binder. Column chromatography was performed on silica gel (60–120 mesh). All reactions were performed in a microwave reactor modified for synthesis (RG 31L, India).

General procedure for the synthesis of 2-amino-3-carbethoxy-4-phenylpyrano-[3,2-*c*]quinolin-5(6*H*)-ones (**4a–l**)

A mixture of 4-hydroxyquinolin-2(1*H*)-one (0.6 mmol, 0.100 g) or 6-methyl-4-hydroxyquinolin-2(1*H*)-one (0.6 mmol, 0.105 g), substituted aryl aldehyde (0.6 mmol) and ethyl cyanoacetate (0.6 mmol, 0.03 ml) was placed in a 100 ml beaker. Then, a ca. two drop of triethylamine was added and the reaction mixture was irradiated with microwaves at a power of 250 W at 120°C for the specified time (5–12 min.). The reaction was monitored at intervals of 30 s by TLC and the mixture was poured into ice. The solid obtained was filtered, dried and purified by column chromatography using a mixture of petroleum ether and ethyl acetate (3:1) as the eluant.

*Ethyl 2-amino-4-(2-chlorophenyl)-5,6-dihydro-9-methyl-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4a**)*

IR (KBr, cm^{-1}): 3325 (NH_2, stretching, broad), 3215 (NH, asym, medium), 3050 (NH, sym, medium), 1685 (>C=O, ring carbonyl, strong), 1660 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.49 (s, 1H, NH), 7.81 (d, 1H, C_{10}–H), 7.78 (s, 2H, NH_2), 7.39–7.11 (m, 6H, Ar–H), 5.12 (s, 1H, C_4–H), 3.96–3.94 (q, 2H, COO$\underline{CH_2}$CH$_3$, $J_1 = 4.15$ Hz, $J_2 = 2.90$ Hz, $J_3 = 4.00$ Hz), 2.40 (s, 3H, C_9–CH$_3$), 1.09–1.06 (t, 3H, COOCH$_2\underline{CH_3}$, $J_{ortho} = 7.10$ Hz, $J_{ortho} = 7.00$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.9, 161.3, 160.4, 151.9, 143.3, 136.7, 133.7, 133.4, 133.1, 131.8, 130.2, 128.4, 127.3, 122.2, 116.0, 112.7, 111.4, 76.8, 59.6, 34.6, 21.5, 15.1; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 410; Anal. Calcd. For $C_{22}H_{19}N_2O_4Cl$: C, 64.31; H, 4.66; N, 6.82. Found: C, 64.37; H, 4.64; N, 6.80.

*Ethyl 2-amino-4-(3-chlorophenyl)-5,6-dihydro-9-methyl-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4b**)*

IR (KBr, cm^{-1}): 3310 (NH_2, stretching, broad), 3220 (NH, asym, medium), 3060 (NH, sym, medium), 1688 (>C=O, ring carbonyl, strong), 1660 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.54 (s, 1H, NH), 7.78 (d, 1H, C_{10}–H), 7.75 (s, 2H, NH_2), 7.39–7.12 (m, 6H, Ar–H), 5.14 (s, 1H, C_4–H), 3.92–3.96 (q, 2H, COO$\underline{CH_2}$CH$_3$, $J_1 = 4.14$ Hz, $J_2 = 2.91$ Hz, $J_3 = 4.04$ Hz), 2.39 (s, 3H, C_9–CH$_3$), 1.08–1.06 (t, 3H, COOCH$_2\underline{CH_3}$, $J_{ortho} = 7.11$ Hz, $J_{ortho} = 7.00$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.7, 161.2, 160.2, 152.0, 143.5, 136.6, 133.8, 133.3, 133.0, 131.5, 130.6, 128.5, 127.2, 122.1, 116.5, 112.4, 111.2, 76.7, 59.7, 34.6, 21.5, 15.2; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 410; Anal. Calcd. For $C_{22}H_{19}N_2O_4Cl$: C, 64.31; H, 4.66; N, 6.82. Found: C, 64.36; H, 4.69; N, 6.81.

*Ethyl 2-amino-4-(4-chlorophenyl)-5,6-dihydro-9-methyl-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4c**)*

IR (KBr, cm^{-1}): 3305 (NH_2, stretching, broad), 3215 (NH, asym, medium), 3030 (NH, sym, medium), 1687 (>C=O, ring carbonyl, strong), 1661 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.50 (s, 1H, NH), 7.77 (d, 1H, C_{10}–H), 7.74 (s, 2H, NH_2), 7.38–7.10 (m, 6H, Ar–H), 5.08 (s, 1H, C_4–H), 3.93–3.90 (q, 2H, COO$\underline{CH_2}$CH$_3$, $J_1 = 4.14$ Hz, $J_2 = 2.91$ Hz, $J_3 = 4.02$ Hz), 2.38 (s, 3H, C_9–CH$_3$), 1.22–1.15 (t, 3H, COOCH$_2\underline{CH_3}$, $J_{ortho} = 7.20$ Hz, $J_{ortho} = 7.05$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.8, 161.4, 160.5, 151.9, 143.2, 136.6, 133.5, 133.3, 133.0, 131.9, 130.3, 128.3, 127.2, 122.1, 116.3, 112.6, 111.3, 76.7, 59.7, 34.7, 21.6, 15.2; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 410; Anal. Calcd. For $C_{22}H_{19}N_2O_4Cl$: C, 64.31; H, 4.66; N, 6.82. Found: C, 64.34; H, 4.67; N, 6.84.

*Ethyl 2-amino-5,6-dihydro-4-(4-hydroxyphenyl)-9-methyl-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4d**)*

IR (KBr, cm^{-1}): 3300 (NH_2, stretching, broad), 3225 (NH, asym, medium), 3100 (NH, sym, medium), 2980 (OH, sharp), 1688 (>C=O, ring carbonyl, strong), 1663 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.63 (s, 1H, NH), 10.72 (S, 1H, OH), 7.87 (d, 1H, C_{10}–H), 7.74 (s, 2H, NH_2), 7.37–7.22 (m, 6H, Ar–H), 4.90 (s, 1H, C_4–H), 3.94–3.90 (q, 2H, COO$\underline{CH_2}$$CH_3$, $J_1 = 4.14$ Hz, $J_2 = 2.90$ Hz, $J_3 = 4.01$ Hz), 2.40 (s, 3H, C_9–CH_3), 1.10–1.06 (t, 3H, COO$CH_2\underline{CH_3}$, $J_{ortho} = 7.08$ Hz, $J_{ortho} = 7.01$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.4, 161.2, 160.3, 151.6, 143.2, 136.5, 133.8, 133.3, 133.0, 131.1, 130.4, 128.6, 127.2, 122.1, 116.4, 112.6, 111.7, 76.7, 59.5, 34.7, 21.4, 15.0; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 392; Anal. Calcd. For $C_{22}H_{20}N_2O_5$: C, 67.34; H, 5.14; N, 7.14. Found: C, 67.30; H, 5.11; N, 7.10.

*Ethyl 2-amino-5,6-dihydro-9-methyl-4-(3-nitrophenyl)-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4e**)*

IR (KBr, cm^{-1}): 3320 (NH_2, stretching, broad), 3205 (NH, asym, medium), 3050 (NH, sym, medium), 1688 (>C=O, ring carbonyl, strong), 1662 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.42 (s, 1H, NH), 7.95 (s, 1H, C_{10}–H), 7.79 (s, 2H, NH_2), 7.37–7.10 (m, 6H, Ar–H), 5.04 (s, 1H, C_4–H), 3.99–3.93 (q, 2H, COO$\underline{CH_2}$$CH_3$, $J_1 = 4.10$ Hz, $J_2 = 2.92$ Hz, $J_3 = 4.01$ Hz), 2.35 (s, 3H, C_9–CH_3), 1.14–1.12 (t, 3H, COO$CH_2\underline{CH_3}$, $J_{ortho} = 7.10$ Hz, $J_{ortho} = 7.00$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.4, 161.3, 160.6, 151.8, 143.3, 136.8, 133.8, 133.4, 133.0, 131.8, 130.1, 128.3, 127.3, 122.0, 116.1, 112.4, 111.2, 76.7, 59.6, 34.7, 21.5, 15.2; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 421; Anal. Calcd. For $C_{22}H_{19}N_3O_6$: C, 62.70; H, 4.54; N, 9.97. Found: C, 62.68; H, 4.51; N, 9.92.

*Ethyl 2-amino-5,6-dihydro-9-methyl-5-oxo-4-phenyl-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4f**)*

IR (KBr, cm^{-1}): 3305 (NH_2, stretching, broad), 3115 (NH, asym, medium), 3005 (NH, sym, medium), 1685 (>C=O, ring carbonyl, strong), 1662 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.62 (s, 1H, NH), 7.81 (d, 1H, C_{10}–H), 7.78 (s, 2H, NH_2), 7.35–7.10 (m, 7H, Ar–H), 4.82 (s, 1H, C_4–H), 4.00–3.96 (q, 2H, COO$\underline{CH_2}$$CH_3$, $J_1 = 4.13$ Hz, $J_2 = 2.90$ Hz, $J_3 = 4.01$ Hz), 2.40 (s, 3H, C_9–CH_3), 1.12–1.00 (t, 3H, COO$CH_2\underline{CH_3}$, $J_{ortho} = 7.11$ Hz, $J_{ortho} = 7.09$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.1, 161.2, 160.4, 151.8, 143.4, 136.8, 133.7, 133.3, 133.0, 131.6, 130.1, 128.2, 127.1, 122.2, 116.1, 112.7, 111.3, 76.6, 59.6, 34.5, 21.5, 15.2; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 376; Anal. Calcd. For $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 70.18; H, 5.32; N, 7.47.

*Ethyl 2-amino-4-(2-chlorophenyl)-5,6-dihydro-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4g**)*

IR (KBr, cm^{-1}): 3300 (NH_2, stretching, broad), 3205 (NH, asym, medium), 2995 (NH, sym, medium), 1685 (>C=O, ring carbonyl, strong), 1660 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.70 (s, 1H, NH), 7.97–7.95 (d, 1H, C_{10}–H, $J_{ortho} = 7.50$ Hz), 7.79 (s, 2H, NH_2), 7.58–7.22 (m, 7H, Ar–H), 5.03 (s, 1H, C_4–H), 3.96–3.90 (q, 2H, COO$\underline{CH_2}$$CH_3$), 1.12–1.08 (t, 3H, COOCH$_2\underline{CH_3}$, $J_{ortho} = 7.10$ Hz, $J_{ortho} = 7.06$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.1, 163.3, 161.5, 156.6, 143.1, 137.9, 130.4, 130.1, 129.9, 129.4, 128.2, 127.5, 126.8, 125.2, 122.7, 116.8, 99.5, 76.5, 60.8, 39.2, 15.0; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 396; Anal. Calcd. For $C_{21}H_{17}N_2O_4Cl$: C, 63.56; H, 4.32; N, 7.06. Found: C, 63.57; H, 4.30; N, 7.05.

*Ethyl 2-amino-4-(3-chlorophenyl)-5,6-dihydro-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4h**)*

IR (KBr, cm^{-1}): 3299 (NH_2, stretching, broad), 3150 (NH, asym, medium), 3015 (NH, sym, medium), 1685 (>C=O, ring carbonyl, strong), 1663 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.72 (s, 1H, NH), 7.94–7.90 (d, 1H, C_{10}–H, $J_{ortho} = 7.48$ Hz), 7.82 (s, 2H, NH_2), 7.36–7.10 (m, 7H, Ar–H), 4.98 (s, 1H, C_4–H), 3.94–3.90 (q, 2H, COO$\underline{CH_2}$$CH_3$), 1.15–1.11 (t, 3H, COOCH$_2\underline{CH_3}$, $J_{ortho} = 7.08$ Hz, $J_{ortho} = 7.06$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.1, 162.8, 161.5, 156.7, 143.1, 137.9, 130.3, 130.1, 129.5, 129.0, 128.2, 127.6, 126.5, 125.1, 122.4, 116.4, 99.6, 76.5, 60.6, 39.4, 15.1; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 396; Anal. Calcd. For $C_{21}H_{17}N_2O_4Cl$: C, 63.56; H, 4.32; N, 7.06. Found: C, 63.59; H, 4.34; N, 7.04.

*Ethyl 2-amino-4-(4-chlorophenyl)-5,6-dihydro-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4i**)*

IR (KBr, cm^{-1}): 3315 (NH_2, stretching, broad), 3205 (NH, asym, medium), 3070 (NH, sym, medium), 1688 (>C=O, ring carbonyl, strong), 1662 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.52 (s, 1H, NH), 7.97–7.96 (d, 1H, C_{10}–H, $J_{ortho} = 7.5$ Hz), 7.82 (s, 2H, NH_2), 7.57–7.12 (m, 7H, Ar–H), 5.13 (s, 1H, C_4–H), 3.97–3.93 (q, 2H, COO$\underline{CH_2}$$CH_3$), 1.09–1.06 (t, 3H, COOCH$_2\underline{CH_3}$, $J_{ortho} = 7.07$ Hz, $J_{ortho} = 7.00$ Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.2, 163.0, 161.3,

156.6, 143.1, 137.6, 130.5, 130.0, 129.7, 129.1, 128.0, 127.4, 126.3, 125.2, 122.1, 116.4, 99.6, 76.5, 60.7, 39.7, 14.9; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 396; Anal. Calcd. For $C_{21}H_{17}N_2O_4Cl$: C, 63.56; H, 4.32; N, 7.06. Found: C, 63.61; H, 4.29; N, 7.01.

*Ethyl 2-amino-5,6-dihydro-4-(4-hydroxyphenyl)-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4j**)*

IR (KBr, cm^{-1}): 3295 (NH_2, stretching, broad), 3175 (NH, asym, medium), 3065 (NH, sym, medium), 3000 (OH, sharp), 1687 (>C=O, ring carbonyl, strong), 1665 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.60 (s, 1H, NH), 10.56 (s, 1H, OH), 7.92–7.88 (d, 1H, C_{10}–H, J_{ortho} = 7.48 Hz), 7.72 (s, 2H, NH_2), 7.38–7.11 (m, 7H, Ar–H), 4.88 (s, 1H, C_4–H), 3.99–3.92 (q, 2H, $COO\underline{CH_2}CH_3$), 1.16–1.11 (t, 3H, $COOCH_2\underline{CH_3}$, J_{ortho} = 7.11 Hz, J_{ortho} = 7.06 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.1, 163.2, 161.3, 156.4, 143.1, 137.4, 130.5, 130.0, 129.2, 129.0, 128.1, 127.4, 126.2, 125.0, 122.2, 116.7, 99.4, 76.2, 60.6, 39.4, 15.0; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 378; Anal. Calcd. For $C_{21}H_{18}N_2O_5$: C, 66.66; H, 4.79; N, 7.40. Found: C, 66.61; H, 4.77; N, 7.43.

*Ethyl 2-amino-5,6-dihydro-4-(3-nitrophenyl)-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4k**)*

IR (KBr, cm^{-1}): 3295 (NH_2, stretching, broad), 3155 (NH, asym, medium), 3025 (NH, sym, medium), 1685 (>C=O, ring carbonyl, strong), 1660 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.68 (s, 1H, NH), 7.87–7.84 (d, 1H, C_{10}–H, J_{ortho} = 7.48 Hz), 7.72 (s, 2H, NH_2), 7.48–7.12 (m, 7H, Ar–H), 4.87 (s, 1H, C_4–H), 3.93–3.89 (q, 2H, $COO\underline{CH_2}CH_3$), 1.26–1.20 (t, 3H, $COOCH_2\underline{CH_3}$, J_{ortho} = 7.04 Hz, J_{ortho} = 7.00 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.3, 163.0, 161.4, 156.4, 143.2, 137.5, 130.2, 130.0, 129.6, 129.0, 128.2, 127.4, 126.2, 125.1, 122.2, 116.3, 99.2, 76.2, 60.6, 39.4, 15.0; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 407; Anal. Calcd. For $C_{21}H_{17}N_3O_6$: C, 61.91; H, 4.21; N, 10.31. Found: C, 61.87; H, 4.22; N, 10.30.

*Ethyl 2-amino-5,6-dihydro-5-oxo-4H-pyrano[3,2-c]quinoline-3-carboxylate (**4l**)*

IR (KBr, cm^{-1}): 3295 (NH_2, stretching, broad), 3205 (NH, asym, medium), 3085 (NH, sym, medium), 1685 (>C=O, ring carbonyl, strong), 1662 (>C=O, carbethoxy carbonyl, strong); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 11.73 (s, 1H, NH), 7.98–7.96 (d, 1H, C_{10}–H, J_{ortho} = 7.85 Hz), 7.78 (s, 2H, NH_2), 7.58–7.22 (m, 8H, Ar–H), 4.81 (s, 1H, C_4–H), 4.03–3.99 (q, 2H, $COO\underline{CH_2}CH_3$), 1.15–1.12 (t, 3H, $COOCH_2\underline{CH_3}$, J_{ortho} = 7.10 Hz, J_{ortho} = 7.05 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 168.0, 163.2, 161.4, 156.7, 143.0, 137.8, 130.5, 130.1, 129.8, 129.2, 128.2, 127.6, 126.6, 125.3, 122.4, 116.6, 99.8, 76.6, 60.7, 39.6, 15.2; EI-MS (70 eV, *m/e*, $M^{+\cdot}$): 362; Anal. Calcd. For $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.01; N, 7.73. Found: C, 69.62; H, 4.97; N, 7.71.

Antibacterial activity studies

Antibacterial activity screening studies of the pyrano[3,2-*c*]quinoline derivatives were carried out by the paper disk method (Collins and Lyne, 1970). 5 ml of nutrient agar (NA) medium (pH 6.8) was poured into sterilized plates and allowed to solidify. The plates were inoculated with spore suspensions of gram-positive (*Staphylococcus aureus)* and gram-negative (*Escherichia coli*) bacteria and, by using a sterilized cork borer, paper disks (6 mm in diameter) were inserted into the culture plates. 50 µl of bacteria was used for the test. Different concentrations (50 and 100 µg/ml) of the test pyrano[3,2-*c*]quinoline derivatives solution were prepared in DMSO and 10 µl of the test solution was added to the disks. The plates were incubated at 27°C for 24 h. The inhibition zone that appeared around the disk was measured and recorded as the antibacterial effect of the title compound. DMSO was used as a solvent control. The antibacterial test was repeated three times to determine the reproducibility. To calculate the antibacterial activity of these newly prepared pyrano[3,2-*c*]quinoline derivatives statistically, we used the standard Eq. 1 to measure the inhibition zone.

$$\bar{x} \pm ts/(\sqrt{n}) \quad (1)$$

where, n is the number of observations, s is the measured standard deviation, x is the measured mean value of the inhibition zone, and t is the student's t value (t = 2.775 at 95% confidence level).

Anti-inflammatory activity

All the title compounds were tested for anti-inflammatory activity by carrageenan induced rat paw edema model employing Plethysmometer to measure the paw thickness. Albino rats of either sex, weighing between 200 and 250 g were divided into fourteen groups of six animals each. All groups were fasted for overnight and allowed water *ad libitium.* Inflammation was induced by injecting 0.05 ml of 1% carrageenan suspension subcutaneously into subplantar region of the right hind paw and 0.05 ml of saline was injected into subplantar region of left hind paw for all the ten groups. One hour prior to carrageenan injection, the groups III to XI treated with title compounds **4a–l** (100 mg/kg). 1% Sodium carboxy methyl cellulose (CMC)

gel (1 ml/kg) was given to group-I used as carrageenan treated control and the standard drug saline (2 mg/kg) was administered to group-II. The paw volume of each rat was measured before 1 and 3 h of carrageenan treatment with the help of a plethysmometer. The percent anti-inflammatory activity was calculated according to the formula given below:

$$\text{Percentage of inhibition of edema} = (1 - V_t/V_c) \times 100$$

where V_t and V_c are paw volume of rats of the treated and control group, respectively. Results obtained were statistically analyzed.

Results and discussion

In the search for an inexpensive and easily available catalyst, triethylamine (TEA), piperidine, *p*-toluenesulfonic acid (PTSA), and cetrimide were examined separately in the microwave assisted reaction of 4-hydroxyquinolin-2(1*H*)-one (**1**), *o*-chlorobenzaldehyde (**2**) and ethyl cyanoacetate (**3**) at 120°C. All of the reactions were carried out in a microwave reactor at a power of 250 W, interestingly, in all of the reactions, a single product was obtained in high yield and the shortest reaction time was observed in the case of TEA (Table 1, entry 1). Therefore, TEA was chosen as the catalyst to synthesize a library of pyrano[3,2-*c*]quinolines.

To optimize the reaction temperature, the reaction of **1**, **2**, and **3** was carried out using TEA at temperatures ranging from 80 to 140°C with an increase of 20°C each time. It was found that the yield of the product was high when the temperature of the reaction was maintained at 120°C (Table 2, entry 3). Since there was little difference in the reaction time, we chose a reaction temperature of 120°C and a microwave power of 250 W as the optimum conditions.

Hence, in a typical experimental procedure, equimolar amounts of **1**, **2**, and **3** were mixed thoroughly with a catalytic amount of TEA and the reaction mixture was irradiated with microwave irradiation at a power of 250 W at 120°C for 6 min, which afforded exclusively a single product in 80% yield. Since this reaction can lead to the formation of more than one product, viz. **4** or **5** and/or **6** or **7**, the structure of the product was carefully examined (Scheme 1).

Table 1 Effect of catalyst on the synthesis of pyranoquinoline under microwave irradiation at 120°C

Entry	Catalyst	Time (min)	Yield (%)
1	TEA	5	80
2	Piperidine	12	76
3	PTSA	16	60
4	Cetrimide	17	63
5	None	No reaction	–

Table 2 Effect of temperature on the synthesis of pyranoquinoline under microwave irradiation in the presence of TEA

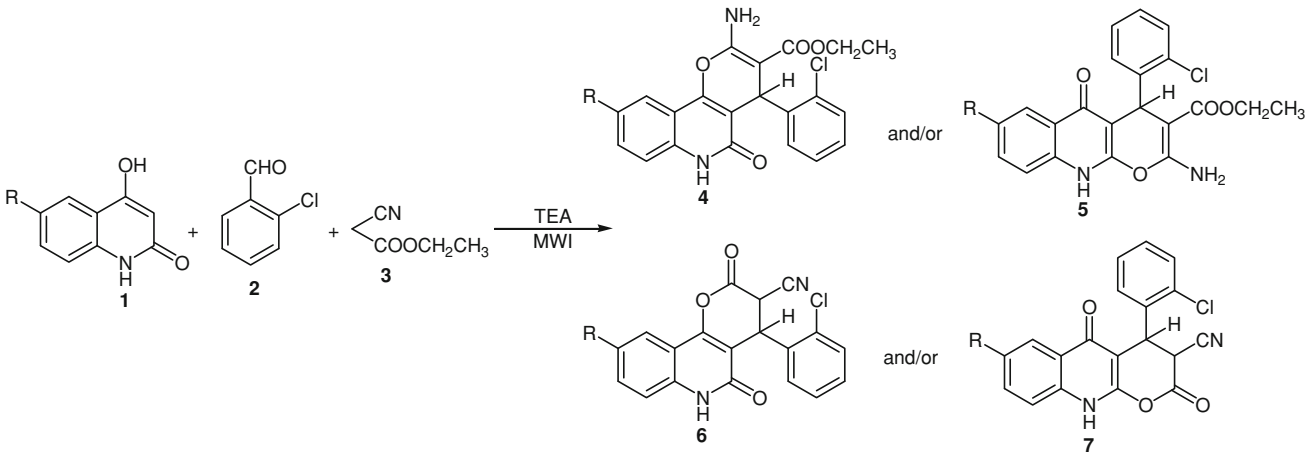
Entry	T (°C)	Time (min)	Yield (%)
1	80	11	74
2	100	8	76
3	120	5	80
4	140	5	79

The infrared spectrum of the product (**4a**) showed absorption at 1685 cm^{-1} for the ester functional group. The NMR spectral data also confirmed the presence of the $–COOCH_2CH_3$ group, as the ^{1}H-NMR spectrum showed a two proton quartet at δ 3.94–3.96 and a three proton triplet at δ 1.06–1.09 and the ^{13}C-NMR spectrum showed peaks at δ 21.5 & 34.6 for the $–CH_3$ & $–CH_2$ groups, respectively. This evidence eliminates the possibility of the formation of **6** and **7**. The characteristic upfield signal of C_{10}–H (δ 7.81) in the ^{1}H-NMR spectrum (Nadaraj *et al.*, 2011) and quinolinone carbonyl carbon peak at δ 161.3 in the ^{13}C-NMR spectrum (Manikandan *et al.*, 2002) also favored the formation of an angular product rather than a linear one. Furthermore, the IR spectrum showed absorption at 1660 cm^{-1} corresponding to the 2-quinolinone group. Conclusive evidence came from mass spectrum which showed a molecular ion peak at *m/z* 410 ($M^{+\cdot}$). The fragmentation pattern of the mass spectrum showed a peak at *m/z* 256 (100%) (Scheme 2), which is formed by the removal of the –CONH group. This is only possible if the 2-quinolinone moiety is present in the compound. Hence, the formed product is confirmed to be **4** rather than **5**.

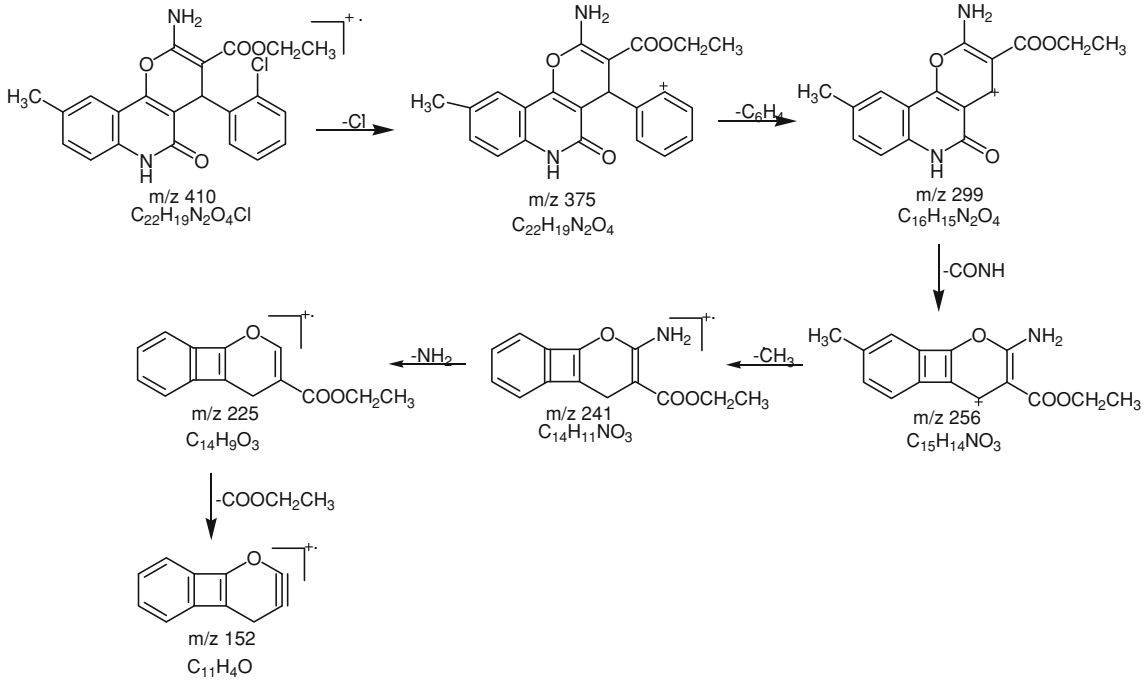
To explore this reaction in a synthetically useful context, we further examined the reactions of **1a,b** with various aldehydes (**2a–l**) and **3**, which afforded the products **4a–l** exclusively (Scheme 3; Table 3). This reaction may occur via a Michael addition, elimination and cyclization mechanism. The structures of the products (**4a–l**) were completely characterized by their spectral (IR, ^{1}H- & ^{13}C-NMR and Mass) and analytical data.

Antimicrobial studies

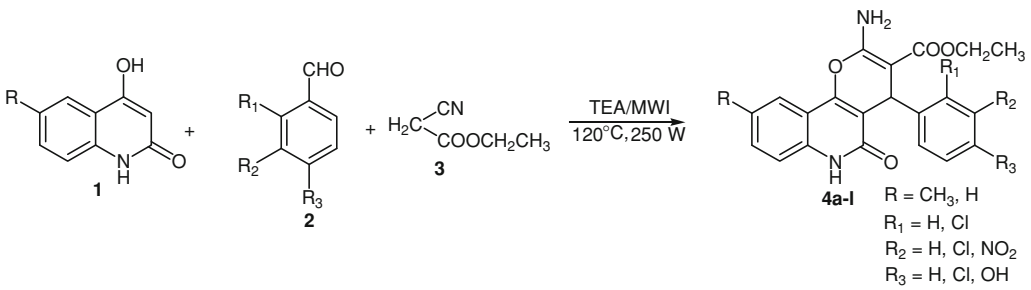
The in vitro antibacterial screening of the pyrano[3,2-*c*] quinoline derivatives was carried out against one gram-positive (*Staphylococcus aureus*) and one gram-negative (*Escherichia coli*) bacteriocide using the paper disk method (Collins and Lyne, 1970). *Ofloxacin* and *Streptomycin* were



Scheme 1 Synthetic scheme of the expected products **4** or **5** and/or **6** or **7**



Scheme 2 Mass fragmentation pathways of **4a**



Scheme 3 General synthetic scheme for the preparation of 2-amino-3-carbethoxy-4-(substituted phenyl)pyrano[3,2-*c*]quinolin-5(6*H*)-ones (**4a–l**)

Table 3 Microwave promoted preparation of 2-amino-3-carb-eth-oxy-4-(substituted phenyl) pyrano[3,2-*c*]quinolin-5(6*H*)-ones (**4a–l**)

Compound	R	R_1	R_2	R_3	Time (min)	Yield (%)	Mp (°C)
4a	CH_3	Cl	H	H	6	80	248–250
4b	CH_3	H	Cl	H	5	75	220–222
4c	CH_3	H	H	Cl	5	80	210–211
4d	CH_3	H	H	OH	5	81	196–197
4e	CH_3	H	NO_2	H	12	81	160–161
4f	CH_3	H	H	H	10	81	192–193
4g	H	Cl	H	H	7	82	200–201
4h	H	H	Cl	H	7	81	185–186
4i	H	H	H	Cl	8	83	240–242
4j	H	H	H	OH	5	80	228–229
4k	H	H	NO_2	H	8	80	165–166
4l	H	H	H	H	5	84	206–207

employed as reference standards. Table 4 shows the antibacterial activity data of the title compounds and revealed that almost all of the derivatives in this series were effective against both bacteria at dose levels of 50 and 100 μg/ml. Compounds **4a**, **4d**, **4e**, and **4k** possessed the maximum activity, which is probably due to the electronic effects of the substituents at the various positions of the aryl rings. This reveals the importance of such groups for favorable antibacterial activity. A few of the compounds were inactive against these two organisms at particular concentration, and it is thought that the variation in the effectiveness of the different compounds against different organisms depends either on the impermeability of the cells of the microbes or the differences in the ribosomes of the microbial cells (Daniel Thangadurai *et al.*, 2010). Normally, the inhibition activity of compounds increases with increasing concentration of the solution. However, even though the newly prepared derivative compounds possessed antibacterial activity, their effectiveness did not match that of the standard drugs, *Ofloxacin* and *Streptomycin*.

Anti-inflammatory studies

The anti-inflammatory activity of title compounds have been evaluated by using carrageenan-induced rat paw edema method (Winter *et al.*, 1962). The results of evaluation have been viewed by taking saline as the standard drug (Table 5). The results of anti-inflammatory activity revealed that the synthesized compounds exhibited moderate to considerable activity when compared with reference standard saline, but at a higher dose level as the standard drug was tested at 25 mg/kg, whereas the compounds were tested at a dose of 100 mg/kg. Compounds **4g**, **4i**, and **4k** possessed maximum activity. Other compounds tested in this present study also showed some degree of anti-inflammatory activity.

Table 4 Antibacterial activity of the title compounds (**4a–l**)

Compound	Inhibition zone (mm)[a] + Paper disk width (mm)[b]			
	Staphylococcus aureus (Gram positive)		*Escherichia coli* (Gram negative)	
	50 μg	100 μg	50 μg	100 μg
Ofloxacin	21.2 ± 0.1	24.3 ± 0.3	–	–
Streptomycin	–	–	20.1 ± 0.1	25.2 ± 0.2
Control	–	–	–	–
4a	16.1 ± 0.1	17.3 ± 0.3	15.4 ± 0.2	17.3 ± 0.4
4b	14.6 ± 0.2	15.7 ± 0.4	14.1 ± 0.5	15.2 ± 0.1
4c	15.8 ± 0.1	18.5 ± 0.3	15.4 ± 0.4	–
4d	17.6 ± 0.3	19.7 ± 0.5	16.2 ± 0.1	18.2 ± 0.2
4e	18.1 ± 0.1	19.2 ± 0.3	16.5 ± 0.3	17.4 ± 0.4
4f	14.7 ± 0.4	–	15.7 ± 0.1	16.2 ± 0.3
4g	15.2 ± 0.1	16.3 ± 0.3	13.7 ± 0.2	15.0 ± 0.2
4h	15.4 ± 0.5	15.7 ± 0.5	14.1 ± 0.4	–
4i	16.3 ± 0.4	16.7 ± 0.5	15.2 ± 0.3	16.2 ± 0.4
4j	16.9 ± 0.3	17.6 ± 0.3	15.7 ± 0.4	17.0 ± 0.5
4k	17.3 ± 0.5	–	16.1 ± 0.2	17.0 ± 0.3
4l	15.6 ± 0.2	16.7 ± 0.3	15.2 ± 0.4	16.2 ± 0.2

[a] $x \pm ts/(\sqrt{n})$, $n = 5$ at 95% confidence level, [b] paper disk width of 6 mm

Table 5 Anti-inflammatory activity of the title compounds (**4a–l**)

Compound	Dose (mg/kg)	Circumference of the hind paws ± Mean value		
		After 1 h %ROV	After 2 h %ROV	After 3 h %ROV
Control	0.5 ml	–	–	–
Saline	25	66.24 ± 0.15	79.34 ± 0.10	95.65 ± 0.01
4a	100	58.12 ± 0.21	68.11 ± 0.14	74.16 ± 0.02
4b	100	56.35 ± 0.24	69.72 ± 0.21	75.51 ± 0.14
4c	100	59.44 ± 0.19	70.31 ± 0.12	77.65 ± 0.16
4d	100	57.13 ± 0.11	70.43 ± 0.13	69.15 ± 0.13
4e	100	54.27 ± 0.13	70.34 ± 0.15	75. 45 ± 0.15
4f	100	56.23 ± 0.22	71.14 ± 0.16	75.24 ± 0.18
4g	100	55.61 ± 0.26	69.36 ± 0.19	80.61 ± 0.17
4h	100	57.56 ± 0.21	68.52 ± 0.20	75.87 ± 0.30
4i	100	56.21 ± 0.17	66.12 ± 0.18	80.65 ± 0.22
4j	100	58.43 ± 0.19	63.35 ± 0.22	75.17 ± 0.20
4k	100	53.51 ± 0.22	69.01 ± 0.17	79.32 ± 0.23
4l	100	54.17 ± 0.16	61.93 ± 0.14	75.17 ± 0.26

Conclusion

In conclusion, we demonstrated a simple and convenient synthesis of novel pyrano[3,2-*c*]quinoline derivatives in good yield under microwave irradiation. The advantages of the present protocol are its one-pot reaction conditions and shorter reaction times, as well as the fact that the reactive components used in the above reaction including the catalyst are commercially available. Noteworthy is the complete selectivity in the angular cyclization, which makes this method a highly practical way to produce pharmaceutically important heterocycles. The present pharmacological studies showed that all of the derivative compounds exhibited significant antibacterial and anti-inflammatory activity. However, further studies are required to establish the mechanism of action of the title compounds. The physico-chemical properties leading to their antimicrobial activity need to be established by detailed QSAR studies, which may provide insight into the structural requirements of this class of molecules. Currently, we are investigating the mechanisms of various in vitro cytotoxic activities of these newly prepared pyrano[3,2-*c*]quinoline derivatives and the results will be published in due course.

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