Synthesis and anticoccidial activities of eight novel ethyl 7-alkyl-6-(2-aryloxyethoxy)-4-hydroxyquinoline-3-carboxylates

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Eight novel ethyl 7-alkyl-6-(2-aryloxyethoxy)-4-hydroxyquinoline-3-carboxylates were synthesised and their structures were identified by ^{1}H NMR, MS, and IR spectra. The anticoccidial activities of these compounds were evaluated according to the ACI (the anticoccidial index) method. The results showed that two of these compounds exhibited anticoccidial activities against *Eimeria tenella* in the chicken' diet with a dose of 27 mg kg^{-1}.

Keywords: ethyl 7-alkyl-6-(2-aryloxyethoxy)-4-hydroxyquinoline-3-carboxylates, anticoccidial activity, synthesis, *Eimeria tenella*

Coccidiosis is an intestinal infection caused by protozoan parasites of the genus *Eimeria* resulting in intestinal lesions, diarrhoea, enteritis and death,[1,2] which causes serious economic loss in poultry industry.[3] Drugs play an important role in controlling coccidiosis. However, the coccidia inevitably develop resistance to the drugs which have been used for a long time.[4,5] The drug resistance is a very serious problem that hinders the effective control of coccidiosis,[6] and has developed in almost all of the anticoccidial drugs introduced so far.[5] Therefore, it is necessary to develop new drugs.

Hydroxyquinolinecarboxylates with general structure (Fig. 1, compound **1**) exhibit good anticoccidial activities for chickens and little toxicity for human.[9,10] Among them, decoquinate (Fig. 1, compound **2**) has high anticoccidial activity when it is administered to chicken in the diet with a 27 mg kg^{-1} concentration.[11–13] However, the use of this drug has also declined because of the development of drug resistance.[5]

We hope to find more novel compounds with anticoccidial activities, because the coccidia have developed resistance to the old structural compounds. In this paper, eight novel ethyl 7-alkyl-6-(2-aryloxyethoxy)-4-hydroxyquinoline-3-carboxylates with new structure (Fig. 1, compound **3**) were designed and synthesised. The new compounds have increased the coordination atom in the side-chain of the 6 position of quinoline ring. The synthesis route is shown in Scheme 1.

With increased coordination atoms, the recognition between the molecules and enzymes in the coccidia bodies and the bio-activity will be changed. The anticoccidial activities of the new compounds were evaluated according to the Anticoccidial Index (ACI) method. The preliminary results showed that two new structural compounds had anticoccidial activities against *Eimeria tenella* in the chickens' diet with a dose of 27 mg kg^{-1}.

Experimental

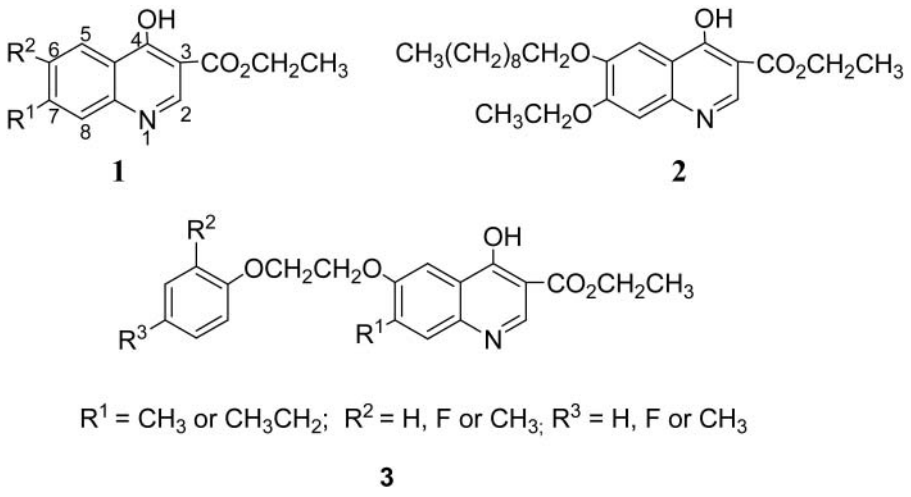
Solvents and reagents were obtained from commercial sources and used without further purification. Melting point was recorded on XRC-1 apparatus and the thermometer was uncorrected. Proton NMR spectra were recorded on a Varian Unity Inova-400 spectrometer with d_6-$CDCl_3$ or d_6-DMSO as the solvent and TMS as the internal standard. Mass spectra were recorded with Agilent 6210 (DOF-MAS) spectrometer using the ESI method. IR spectra were recorded with Perkin-Elmer 16PC-FT instrument. The HPLC analysis was conducted on a Shimadza HPLC with a model SPD-M_{20}A diode array detector at 265 nm. A Shim-pack VP-ODS C18 column (150 L * 4.6) was used at room temperature. The mobile phase was CH_2Cl_2/CH_3OH (9:1, v/v) with a flow rate of 0.5 mL min^{-1}. Analytical TLC was carried out on precoated plates (silica gel GF_{254}), and spots were visualised with UV light.

General procedure for preparation of **5a–b**

The compounds were synthesised by a literature method.[14]

General procedure for preparation of **6a–b** *and* **7a–b**

A sample of 4-nitroso-2-alkylphenol (compound **5**) (128 mmol) was reduced by 5% Pd/C (0.98 g) catalysed hydrogenation in ethyl acetate (200 mL) at the experimental value of pressure. The reaction finished



1 **2** **3**

Fig. 1 The structures of compounds **1**, **2** and **3**.

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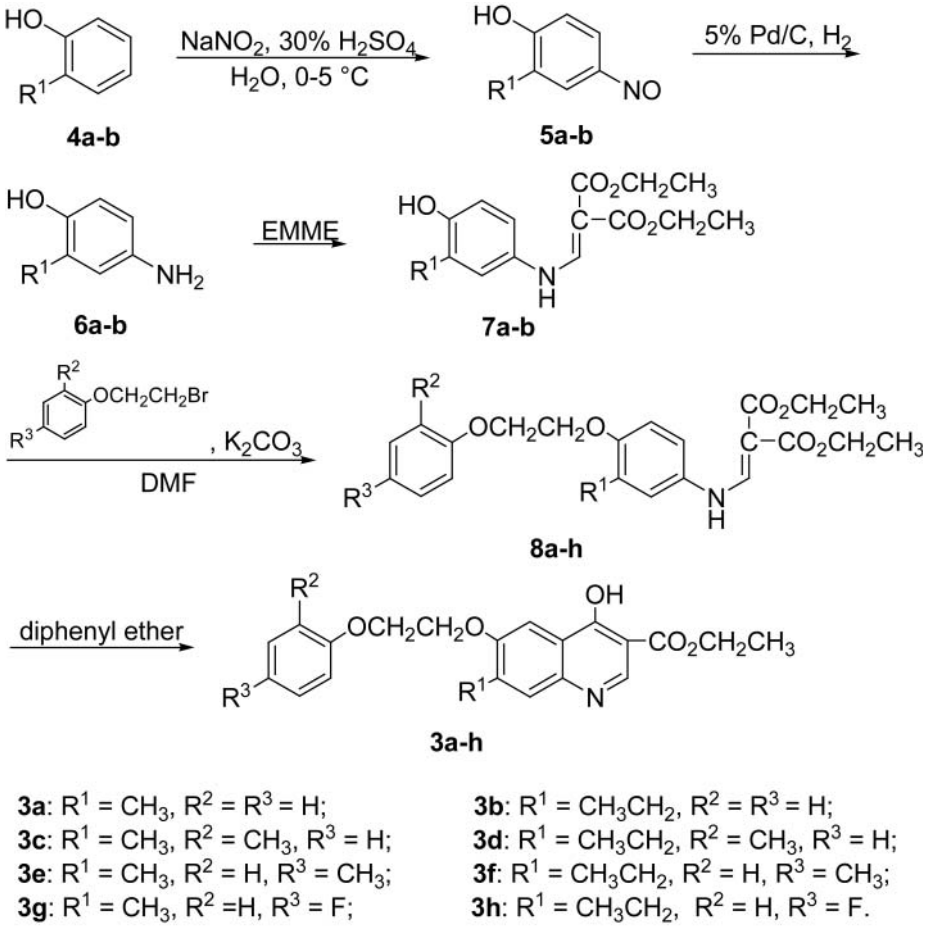

Scheme 1 General synthesis route for compounds **3a–h.**

after 6–8 hours. The solution was directly used for the next step without further processing.

Then, EMME (ethoxymethylenemalonic diethyl ester) (27.65 g, 128 mmol) was added dropwise, and the mixture was heated under reflux for 4h. After the mixture was cooled and filtered off, the filtrate was concentrated under reduced pressure to afford a solid. The crude material was recrystallised in ethanol, and dried to obtain **7a–b** in 85–90% yield.

*Diethyl (4-hydroxy-3-methylphenyl)aminomethyl*enemal*onate* (**7a**): Yellow solid; yield: 89%; m.p. 140–141 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.94 (d, *J* = 13.6 Hz, 1H), 8.42 (d, *J* = 13.6 Hz, 1H), 6.92 (s, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 5.19 (s, 1H), 4.32–4.22 (m, 4H), 2.26 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3424, 3244, 2978, 2926, 1672, 1629, 1521, 1415, 1381, 1283, 1240, 1115, 1031, 859, 809, 619, 550. HR-MS (ESI): Calcd for $C_{15}H_{20}NO_5$ (MH^+): 294.1336; Found: 294.1339.

Diethyl (4-hydroxy-3-ethylphenyl)aminomehylenemalonate (**7b**): Dark-brown solid; yield: 86%; m.p. 109–110 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.97 (d, *J* = 13.6 Hz, 1H), 8.44 (d, *J* = 13.6 Hz, 1H), 6.91 (s, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 5.20 (s, 1H), 4.32–4.22 (m, 4H), 2.64 (q, *J* = 7.2 Hz, 2H), 1.37 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3274, 2973, 2932, 1670, 1635, 1517, 1416, 1381, 1322, 1276, 1227, 1103, 1031, 986, 867, 811, 619, 559. HR-MS (ESI): Calcd for $C_{16}H_{22}NO_5$ (MH^+): 308.1492; Found: 308.1496.

General procedure for preparation of **8a–h**

Compound **7** (9.8 mmol), 1-(2-bromoethoxy)arene (9.8 mmol), TBAB (tetrabutylammonium bromide) (0.3 g) and DMF (*N, N*-dimethylformamide) (20 mL) were added into a three-necked flask with a thermometer and a condenser. Then, the resulting mixture was stirred and refluxed for 1h. After the mixture was cooled to room temperature, water was added and the mixture was extracted with 3 × 30 mL diethyl ether. The combined organic layer was washed with 5% of the sodium hydroxide and water, and dried over anhydrous $MgSO_4$. After evaporation of solvent, the crude products were obtained and were recrystallised from ethanol to provide intermediates **8a–h** in 80–85 % yield.

Diethyl [3-methyl-4-(2-phenoxyethoxy)phenyl]aminomethylenemalonate (**8a**): Primrose yellow solid; yield: 84%; m.p. 109–111 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.96 (d, *J* = 14.0 Hz, 1H), 8.44 (d, *J* = 14.0 Hz, 1H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.00–6.85 (m, 6H), 4.34–4.22 (m, 8H), 2.24 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3437, 2985, 2926, 1689, 1641, 1615, 1502, 1427, 1384, 1344, 1228, 1174, 1092, 1025, 993, 935, 875, 800, 757, 692, 617. HR-MS (ESI): Calcd for $C_{23}H_{28}NO_6$ (MH^+): 414.1911; Found: 414.1912.

Diethyl [3-ethyl-4-(2-phenoxyethoxy)phenyl]aminomethylenemalonate (**8b**): Brown solid; yield: 82%; m.p. 77–78 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.99 (d, *J* = 14.0 Hz, 1H), 8.46 (d, *J* = 14.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.00–6.86 (m, 6H), 4.33–4.22 (m, 8H), 2.64 (q, *J* = 7.6 Hz, 2H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3447, 2986, 2933, 1685, 1640, 1611, 1585, 1502, 1453, 1407, 1382, 1309, 1229, 1114, 1032, 947, 801, 751, 619. HR-MS (ESI): Calcd for $C_{24}H_{30}NO_6$ (MH^+): 428.2068; Found: 428.2070.

Diethyl {3-methyl-4-[2-(2-methylphenoxy)ethoxy]phenyl}aminomethylenemalonate (**8c**): Yellow solid; yield: 85%; m.p. 91–92 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.96 (d, *J* = 14.0 Hz, 1H), 8.44 (d, *J* = 14.0 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 2H), 6.96–6.91 (m, 3H), 6.88 (d, *J* = 7.6 Hz, 2H), 4.34–4.22 (m, 8H), 2.24 (s, 3H), 2.23 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3437, 2981, 2925, 1685, 1639, 1614, 1503, 1453, 1406, 1381, 1232, 1121, 1032, 945, 800, 750, 618. HR-MS (ESI): Calcd for $C_{24}H_{30}NO_6$ (MH^+): 428.2068; Found: 428.2071.

Diethyl {3-ethyl-4-[2-(2-methylphenoxy)ethoxy]phenyl}aminomethylenemalonate (**8d**): Dark-brown solid; yield: 80%; m.p. 80–81 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.99

(d, *J* = 13.6 Hz, 1H), 8.46 (d, *J* = 13.6 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 2H), 6.96–6.87 (m, 5H), 4.34–4.22 (m, 8H), 2.65 (q, *J* = 7.6 Hz, 2H), 2.22 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3438, 2976, 2932, 1685, 1640, 1614, 1589, 1500, 1456, 1409, 1380, 1346, 1235, 1121, 1034, 993, 946, 881, 802, 755, 618, 584. HR-MS (ESI): Calcd for $C_{25}H_{32}NO_6$ (MH^+): 442.2224; Found: 442.2229.

Diethyl {3-methyl-4-[2-(4-methylphenoxy)ethoxy]phenyl}aminomethylenemalonate (**8e**): Yellow solid; yield: 83%; m.p. 90–92 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.96 (d, *J* = 14.0 Hz, 1H), 8.44 (d, *J* = 14.0 Hz, 1H), 7.10 (d, *J* = 7.6 Hz, 2H), 6.94 (d, *J* = 12.0 Hz, 2H), 6.86 (d, *J* = 7.6 Hz, 3H), 4.33–4.22 (m, 8H), 2.30 (s, 3H), 2.24 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3444, 2925, 1683, 1638, 1611, 1586, 1510, 1445, 1404, 1383, 1346, 1276, 1231, 1120, 1030, 936, 801, 619, 509. HR-MS (ESI): Calcd for $C_{24}H_{30}NO_6$ (MH^+): 428.2068; Found: 428.2069.

Diethyl {3-ethyl-4-[2-(4-methylphenoxy)ethoxy]phenyl}aminomethylenemalonate (**8f**): Brown solid; yield: 83%; m.p. 94–96 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.99 (d, *J* = 13.6 Hz, 1H), 8.46 (d, *J* = 13.6 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 6.94 (d, *J* = 6.0 Hz, 2H), 6.86 (d, *J* = 8.0 Hz, 3H), 4.33–4.22 (m, 8H), 2.64 (q, *J* = 7.6 Hz, 2H), 2.30 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3433, 2975, 2933, 1684, 1643, 1613, 1586, 1509, 1456, 1407, 1383, 1262, 1229, 1114, 1034, 949, 874, 798, 619. HR-MS (ESI): Calcd for $C_{25}H_{32}NO_6$ (MH^+): 442.2224; Found: 442.2229.

Diethyl {3-methyl-4-[2-(4-fluorophenoxy)ethoxy]phenyl}aminomethylenemalonate (**8g**): Buff solid; yield: 82%; m.p. 87–88 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.96 (d, *J* = 14.0 Hz, 1H), 8.44 (d, *J* = 14.0 Hz, 1H), 7.01–6.84 (m, 7H), 4.33–4.22 (m, 8H), 2.23 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3436, 2984, 2924, 1682, 1637, 1612, 1586, 1509, 1448, 1402, 1381, 1346, 1228, 1122, 1032, 849, 826, 800, 748, 619, 512. HR-MS (ESI): Calcd for $C_{23}H_{27}FNO_6$ (MH^+): 432.1817; Found: 432.1820.

Diethyl {3-ethyl-4-[2-(4-fluoro-phenoxy)ethoxy]phenyl}aminomethylenemalonate (**8h**): Buff solid; yield: 81%; m.p. 92–94 °C; ^{1}H NMR spectrum (400 MHz; d_6-$CDCl_3$; TMS): δ (ppm) = 10.98 (d, *J* = 14.0 Hz, 1H), 8.45 (d, *J* = 14.0 Hz, 1H), 7.01–6.85 (m, 7H), 4.33–4.22 (m, 8H), 2.63 (q, *J* = 7.6 Hz, 2H), 1.38 (t, *J* = 7.6 Hz, 3H), 1.33 (t, *J* = 7.6 Hz, 3H), 1.18 (t, *J* = 7.6 Hz, 3H); IR (KBr, cm^{-1}) 3436, 2979, 2931, 2866, 1692, 1655, 1615, 1508, 1456, 1410, 1384, 1347, 1269, 1235, 1111, 946, 834, 799, 748, 619. HR-MS (ESI): Calcd for $C_{24}H_{29}FNO_6$ (MH^+): 446.1973; Found: 446.1975.

General procedure for preparation of **3a–h**

Diphenyl ether (10 ml) was heated to 260 °C and compounds **5a–h** (3 g) were added. The reaction mixture was stirred at 255 °C for 6–9 minutes, and then cooled to room temperature. Petroleum ether (50 mL) was added to the mixture and the obtained solid was filtrated, washed with petroleum ether and dried. The yields of the products **3a–h** were 60–75 %. The physical and spectra data of the compounds **3a–h** are as follows.

Ethyl4-hydroxy-7-methyl-6-(2-phenoxyethoxy)quinoline-3-carboxylate (**3a**): Yellow solid; yield: 62%; m.p. 230–232 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (d, *J* = 6.8 Hz, 1H), 8.44 (d, *J* = 6.8 Hz, 1H), 7.59 (s, 1H), 7.41 (s, 1H), 7.33–7.29 (m, 2H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.95 (t, *J* = 7.2 Hz, 1H), 4.41–4.39 (m, 4H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.26 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3432, 3035, 2921, 2846, 1705, 1621, 1586, 1529, 1481, 1383, 1295, 1247, 1184, 1154, 1091, 1038, 939, 897, 803, 751, 694, 620; HR-MS (ESI): Calcd for $C_{21}H_{22}NO_5$ (MH^+): 368.1498; Found: 368.1508. HPLC: R_t = 6.0 min; Purity: 100%

Ethyl 4-hydroxy-7-ethyl-6-(2-phenoxyethoxy)quinoline-3-carboxylate (**3b**): Buff solid; yield: 65%; m.p. 226–228 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (d, *J* = 6.4 Hz, 1H), 8.45 (d, *J* = 6.4 Hz, 1H), 7.59 (s, 1H), 7.41 (s, 1H), 7.30 (t, *J* = 7.6 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 2H), 6.94 (t, *J* = 7.2 Hz, 1H), 4.40 (s, 4H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.66 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 6.8 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3432, 3168, 3051, 2967, 2924, 1712, 1619, 1584, 1530, 1478, 1388, 1294, 1246, 1182, 1149, 1090, 1043, 937, 900, 802, 749, 691, 621; HR-MS (ESI): Calcd for $C_{22}H_{24}NO_5$ (MH^+): 382.1654; Found: 382.1650. HPLC: R_t = 6.0 min; Purity: 100%

Ethyl 4-hydroxy-7-methyl-6-[2-(2-methylphenoxy)ethoxy]quinoline-3-carboxylate (**3c**): Yellow solid; yield: 67%; m.p. 228–231 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (d, *J* = 6.4 Hz, 1H), 8.44 (d, *J* = 6.4 Hz, 1H), 7.60 (s, 1H), 7.41 (s, 1H), 7.18–7.13 (m, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.85 (t, *J* = 7.2 Hz, 1H), 4.41 (d, *J* = 9.6 Hz, 4H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.27 (s, 3H), 2.13 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3433, 3166, 3046, 2923, 1708, 1620, 1586, 1530, 1481, 1382, 1294, 1246, 1186, 1155, 1123, 1092, 1038, 900, 803, 747, 730, 623; HR-MS (ESI): Calcd for $C_{22}H_{24}NO_5$ (MH^+): 382.1654; Found: 382.1654. HPLC: R_t = 6.0 min; Purity: 100%

Ethyl 4-hydroxy-7-ethyl-6-[2-(2-methylphenoxy)ethoxy]quinoline-3-carboxylate (**3d**): Ivory-white solid; yield: 65%; m.p. 224–225 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (d, *J* = 6.8 Hz, 1H), 8.45 (d, *J* = 7.2 Hz, 1H), 7.61 (s, 1H), 7.41 (s, 1H), 7.18–7.13 (m, 2H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.85 (t, *J* = 7.2 Hz, 1H), 4.41 (dd, J_1 = 5.2 Hz, J_2 = 4.8 Hz, 4H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.68 (q, *J* = 7.2 Hz, 2H), 2.13 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.18 (t, *J* = 7.6 Hz, 3H); IR (KBr, cm^{-1}) 3430, 3061, 2967, 2919, 1699, 1620, 1587, 1535, 1475, 1384, 1291, 1248, 1188, 1150, 1245, 1087, 1038, 943, 886, 807, 749, 694; HR-MS (ESI): Calcd for $C_{23}H_{26}NO_5$ (MH^+): 396.1811; Found: 396.1807. HPLC: R_t = 5.9 min; Purity: 100%

Ethyl 4-hydroxy-7-methyl-6-[2-(4-methylphenoxy)ethoxy]quinoline-3-carboxylate (**3e**): Yellow solid; yield: 63%; m.p. 226–228 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (s, 1H), 8.44 (s, 1H), 7.58 (s, 1H), 7.41 (s, 1H), 7.10 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.4 Hz, 2H), 4.36 (dd, J_1 = J_2 = 5.2 Hz, 4H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.27 (s, 3H), 2.24 (s, 3H), 1.26 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3427, 3167, 3043, 2922, 1708, 1619, 1586, 1531, 1481, 1450, 1382, 1294, 1245, 1184, 1154, 1092, 1039, 945, 900, 802, 745, 701, 622; HR-MS (ESI): Calcd for $C_{22}H_{24}NO_5$ (MH^+): 382.1654; Found: 382.1646. HPLC: R_t = 6.0 min; Purity: 100%

Ethyl 4-hydroxy-7-ethyl-6-[2-(4-methylphenoxy)ethoxy]quinoline-3-carboxylate (**3f**): Buff solid; yield: 70%; m.p. 225–227 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (d, *J* = 6.8 Hz, 1H), 8.45 (d, *J* = 6.4 Hz, 1H), 7.58 (s, 1H), 7.41 (s, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8.0 Hz, 2H), 4.37 (dd, J_1 = 4.8 Hz, J_2 = 4.4 Hz, 4H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.67 (q, *J* = 7.2 Hz, 2H), 2.33 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3419, 2921, 2852, 1712, 1621, 1584, 1532, 1477, 1390, 1295, 1245, 1182, 1151, 1093, 1042, 939, 902, 804, 620; HR-MS (ESI): Calcd for $C_{23}H_{26}NO_5$ (MH^+): 396.1811; Found: 396.1804. HPLC: R_t = 6.0 min; Purity: 100%

Ethyl 4-hydroxy-7-methyl-6-[2-(4-fluoro-phenoxy)ethoxy]quinoline-3-carboxylate (**3g**): Yellow solid; yield: 68%; m.p. 239–240 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (d, *J* = 6.4 Hz, 1H), 8.44 (d, *J* = 6.4 Hz, 1H), 7.58 (s, 1H), 7.41 (s, 1H), 7.13 (t, *J* = 8.8 Hz, 2H), 7.03 (t, *J* = 8.4 Hz, 2H), 4.38 (s, 4H), 4.20 (q, *J* = 6.8 Hz, 2H), 2.26 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3425, 3162, 2922, 2854, 1701, 1620, 1584, 1531, 1508, 1481, 1450, 1383, 1295, 1253, 1208, 1186, 1155, 1095, 1038, 900, 827, 803, 750, 700, 620; HR-MS (ESI): Calcd for $C_{21}H_{21}FNO_5$ (MH^+): 386.1404; Found: 386.1402. HPLC: R_t = 5.9 min; Purity: 100%

Ethyl 4-hydroxy-7-ethyl-6-[2-(4-fluoro-phenoxy)ethoxy]quinoline-3-carboxylate (**3h**): Ivory-white solid; yield: 72%; m.p. 232–235 °C; ^{1}H NMR spectrum (400 MHz; d_6-DMSO; TMS): δ (ppm) = 12.20 (d, *J* = 6.8 Hz, 1H), 8.45 (d, *J* = 6.8 Hz, 1H), 7.59 (s, 1H), 7.41 (s, 1H), 7.15–7.11 (m, 2H), 7.04–7.00 (m, 2H), 4.38 (s, 4H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.66 (q, *J* = 7.2 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3430, 3166, 2966, 2877, 1694, 1617, 1584, 1552, 1531, 1507, 1477, 1389, 1335, 1292, 1249, 1183, 1148, 1093, 1038, 936, 901, 807, 755, 695, 625; HR-MS (ESI): Calcd for $C_{22}H_{23}FNO_5$ (MH^+): 400.1560; Found: 400.1556. HPLC: R_t = 5.9 min; Purity: 100%

Biological assay

The anticoccidial activities of the compounds **3a–h** were evaluated according to the anticoccidial index method,[15–17] using decoquinate and diclazuril as reference drug. Briefly, the chickens used to test the anticoccidial activity of compounds were fed to 12-days of age by the feedstuff without using any anticoccidial drugs.

Groups of the chickens were randomly housed, with 10 in each cage, 12 cages were randomly assigned by tier. Groups 1–10 of 13-day-old chickens were fed the basal starter diet with the compounds **3a–h** or decoquinate in 27 mg kg^{-1}, or diclazuril in 1 mg kg^{-1}

Table 1 Data for anticoccidial activities of compounds **3a–h**, decoquinate and diclazuril against *Eimeria tenella*

Test groups	Test compounds/ mg kg^{-1}	Rate of relative body weight gain	Survival rate/%	Lesion scores	Oocyst scores	ACI[a]
1	**3a** (27)	85.07	90	38	40	97
2	**3b** (27)	77.93	90	36	40	92
3	**3c** (27)	86.07	70	27.5	20	109
4	**3d** (27)	87.93	90	28	20	130
5	**3e** (27)	73.55	90	33.5	40	90
6	**3f** (27)	80.44	90	24.5	20	126
7	**3g** (27)	77.68	80	32.5	40	85
8	**3h** (27)	78.31	70	29	20	99
9	Decoquinate (27)	98.98	90	10	10	169
10	Diclazuril (1)	90	86.95	30	20	127
11	ING[b]	78.96	60	38	40	61
12	NNG[c]	100	100	0	0	200

[a]Anticoccidial activity index.
[b]Infected non-medicated group.
[c]Non-infected non-medicated group.

until the end of the test. Groups 1–11 of 14-day-old chickens were infected artificially with the *Eimeria tenella* spores with 100,000 oocysts. They were held on observation for 9 days after infection, the weight gain, mortality, lesion scores and oocysts scores of the chicken were recorded, and the ACI calculated. Results of the test are given in Table 1.

Results and discussion

Synthesis

The intermediate 6 is unstable in the air, so the EMME was added directly to the reaction solution when the intermediate 6 was synthesised.

The last cycle-closing reaction was carried out by heating the intermediate **8** in diphenyl ether at 255°C for 6–9 minutes. The pure and well dried intermediate **8** was necessary. Otherwise, a lot of by-products formed and the purification process of product became very difficult. If compound **8** contained too much moisture or other impurity, cycle-closing reaction did not take place and no product could be obtained.

Biological activity

The data for anticoccidial activities of the compounds **3a–h** are shown in Table 1. ACI are calculated on rate of relative body weight gain, survival rate, and the lesion, oocyst scores data. In the positive control group, the coccidiosis in chickens was obvious with an ACI of 61. In the negative control group, no coccidiosis in chickens occurred. So the control was set up. The inference drawn from Table 1 revealed that two of these compounds exhibited anticoccidial activities against *Eimeria tenella* with ACI 130 and 126 respectively.

Conclusion

In conclusion, eight novel ethyl 7-alkyl-6-(2-aryloxyethoxy)-4-hydroxyquinoline-3-carboxylates have been synthesised and the anticoccidial activities of these compounds were evaluated according to the ACI method. The results indicate that compounds **3d** and **3f** have anticoccidial activities against *E. tenella* at a dose of 27 mg kg^{-1}. Further studies on the structural optimisation and anticoccidial activities of the 4-hydroxyquinoline-3-carboxylate analogues are in progress.

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