Synthesis and anticoccidial activities of substituted ethyl 4-hydroxy-11-oxo-11*H*-chromeno[2,3-g]quinoline-3-carboxylates

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A series of substituted ethyl 4-hydroxy-11-oxo-11*H*-chromeno[2,3-g]quinoline-3-carboxylates were designed and synthesised as anticoccidial drugs, and the structures were characterised by ^{1}H NMR, MS, IR spectra and elementary analysis. The anticocciadial activities of the new compounds were assessed according to the anticoccidial index method. The results demonstrated that four of these compounds exihibited effective anticoccidial activities against *Eimeria tenella* in chicken's diet with a dose of 27 mg kg^{-1}.

Keywords: anticoccidial activities, coccidiosis, *Eimeria tenella*

Coccidiosis, which is caused by protozoan parasites of the genus *Eimeria*, causes morbidity and mortality of animals[1] and seriously hinders the development of the poultry industry. Anticoccidial drugs were used to control this disease successfully in the past. However, when one drug was used for a period of time, the coccidia inevitably developed resistance to it.[2] Now the majority of the drugs used have lower activity than before, and some, such as salinomycin, dinitolmide, maduramicin and amprolium[3], have completely lost their anticoccidial activity.

In order to control coccidiosis successfully, many scientists are trying to develop novel anticoccidial drugs with new structures. In 1998, a new series of frenolicin B analogues were synthesised and tested for activity against parasitic protozoa of the genus *Eimeria* [4]. Five years later, a set of 1-phenyl-4-pyridyl-butadienes were found to exhibit *in vitro* activity against *Eimeria tenella* in a cell-based assay.[5] Since then, a lot of new compounds with anticoccidial activity were synthesised, such as 2-(4-fluorophenyl)-3-pyrimidine-4-ylimidazo[1,2-a]piperidine derivatives,[6] 2-(4-fluorophenyl)-3-(4-pyridinyl)-5-substituted pyrroles,[7] diarylimidazo[1,2-a] pyridine derivatives,[8] imidazo[1,2-a]pyridine,[9,10] and 5,6-diarylimidazo[2,1-b][1,3]thiazoles.[11] Since 2005, our group have synthesised many novel compounds with anticoccidial acitivity, such as ethyl 6-aryl-methoxy-7-alkoxy-4-hydroxy-3-quinolinecarboxylates[12], 4-(2-methoxyphenyl)-2-oxobutylquinazolinone derivatives,[13] 3-(2-(2-methoxyphenyl)-2-oxoethyl) quinazolinone derivatives,[14] ethyl 6-substituted benzyloxy-7-alkoxy-4-hydroxy-quinolinecarboxylates,[15] ethyl 7-alkoxy-6-(2-aryloxyethoxy)-4-hydroxy-quinolinecarboxylates,[16] and ethyl 6-alkoxy-7-phenyl-4-hydroxy-3-quinolinecarboxylates.[17]

In order to prepare more compounds with novel chemical structures and aniticoccidial activity, a series of ethyl 4-hydroxy-11-oxo-11*H*-chromeno[2,3-g]quinoline-3-carboxylates have been synthesised and are now reported for the first time. Target compounds were prepared as shown in Scheme 1.

Experimental

The 2-chloro-5-nitro-benzoic acid sample was prepared by known procedures.[18] Other solvents and reagents were obtained from commercial sources and used without further purification. The melting points were recorded with a XRC-1 apparatus (Sichuan University Instrument Inc., Chengdu, China), and the thermometer was uncorrected. Proton NMR spectra were recorded on a Varian unity Inova-400 spectrometer (Varian Inc., Palo Alto, CA, USA) with d_1-chloroform and d_6-DMSO as the solvents and TMS as the internal standard. The mass spectra were recorded with an Agilent 6210 (DOF-MAS) spectrometer (Agilent Inc., Santa Clara, CA, USA) using the electrospray ionisation (ESI) method. The IR spectra were recorded with a Perkin-Elmer 16PC-FT instrument (Perkin-Elmer Inc., Norwalk Conn, CA, USA). Elemental analyses were carried out by a Euro EA 3000 instrument (Euro Vector S.P.A., Italy). Analytical TLC was carried out on precoated plates (silica gel GF_{254}), and the spots were visualised with UV light.

Preparation of **1a–i**;[19] *general procedure*

A mixture of 2–chloro-5-nitrobenzoic acid (2.98 g, 14.8 mmol), K_2CO_3 (2.91 g, 21.1 mmol) and DMF (N, N-dimethylformamide) (50 mL) was stirred at 120–125 °C for 1–2 h, then the reaction temperature was cooled down to 80–90 °C and substituted phenols (16.3 mmol) were added. The mixture was stirred for 4–5 h and then the temperature was raised to 120–125 °C and maintained for 7–9 h. The pH of the mixture was adjusted to 1–2 with 36.5% hydrochloric acid, and water (50 mL) was added to afford a yellow powder **1a–i** by filtration (81–85% yield following drying). The compounds **1a–i** were directly used for the next step without any further purification.

2-(2-Chloro-phenoxy)-5-nitro-benzoic acid (**1a**): Light yellow powder; yield: 85%; m.p. 173–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.21 (d, J = 2.8 Hz, 1H), 8.55 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 8.36 (d, J = 9.2 Hz, 1H), 7.75 (m, 2H), 7.63 (m, 2H); IR (KBr, cm^{-1}) 3400, 1736, 1658, 1623, 1564, 14687, 1306; HR-MS (ESI): Calcd for $C_{13}H_9ClNO_5$ [M+H]$^+$: 294.0170. Found: 294.0172.

2-(4-Methyl-phenoxy)-5-nitro-benzoic acid (**1b**): Light yellow powder; m.p. 181–182 °C; yield: 81%; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.05 (d, J = 2.8 Hz, 1H), 8.27 (dd, J_1 = 2.8Hz, J_2 = 9.0 Hz, 1H), 7.52 (s, 1H), 7.05 (d, J = 8.4 Hz, 2H), 7.00 (s, 1H), 7.39 (d, 1H), 2.59 (s, 3H); IR (KBr, cm^{-1}) 3413, 2925, 1710, 1682, 1612, 1582, 1513, 1477, 1253, 1137, 836; HR-MS (ESI): Calcd for $C_{14}H_{12}NO_5$ [M+H]$^+$: 274.0716. Found: 274.0685.

2-(4-Chloro-phenoxy)-5-nitro-benzoic acid (**1c**): Light yellow powder; yield: 83%; m.p. 179–181 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 8.98 (d, J = 2.4 Hz, 1H), 8.32 (dd, J_1 = 2.4 Hz, J_2 = 8.8 Hz, 1H), 7.44 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 9.2 Hz, 1H); IR (KBr, cm^{-1}) 3432, 3098, 1707, 1615, 1521, 1479, 1344, 747; HR-MS (ESI): Calcd for $C_{13}H_9ClNO_5$ [M+H]$^+$: 294.0170. Found: 294.0185.

2-(2-methyl-phenoxy)-5-nitro-benzoic acid (**1d**): Yellow powder; yield: 84%; m.p. 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.23 (d, J = 2.8 Hz, 1H), 8.57 (dd, J_1 = 2.6 Hz, J_2 = 9.4 Hz, 1H), 8.20 (J = 9.2 Hz, 1H), 7.65–7.71 (m, 2H), 7.36–7.39 (m, 2H), 2.60 (s, 3H); IR (KBr, cm^{-1}) 3400, 2936, 1715, 1645, 1603, 1598, 1436, 1265; HR-MS (ESI): Calcd for $C_{14}H_{12}NO_5$ [M+H]$^+$: 274.0716. Found: 274.0711.

2-(2-Methoxy-phenoxy)-5-nitro-benzoic acid (**1e**): Yellow powder; yield: 85%; m.p. 175–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.03 (d, J = 3.2 Hz, 1H), 8.28 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.09–7.11 (m, 2H), 6.99–7.01 (m, 2H), 6.90 (d, J = 9.2 Hz, 1H), 3.86 (s, 3H); IR (KBr, cm^{-1}) 3072, 2974, 1688, 1615, 1580, 1507, 1454, 1351, 1298, 1175, 743; HR-MS (ESI): Calcd for $C_{14}H_{12}NO_6$[M+H]$^+$: 290.0665. Found: 290.0643.

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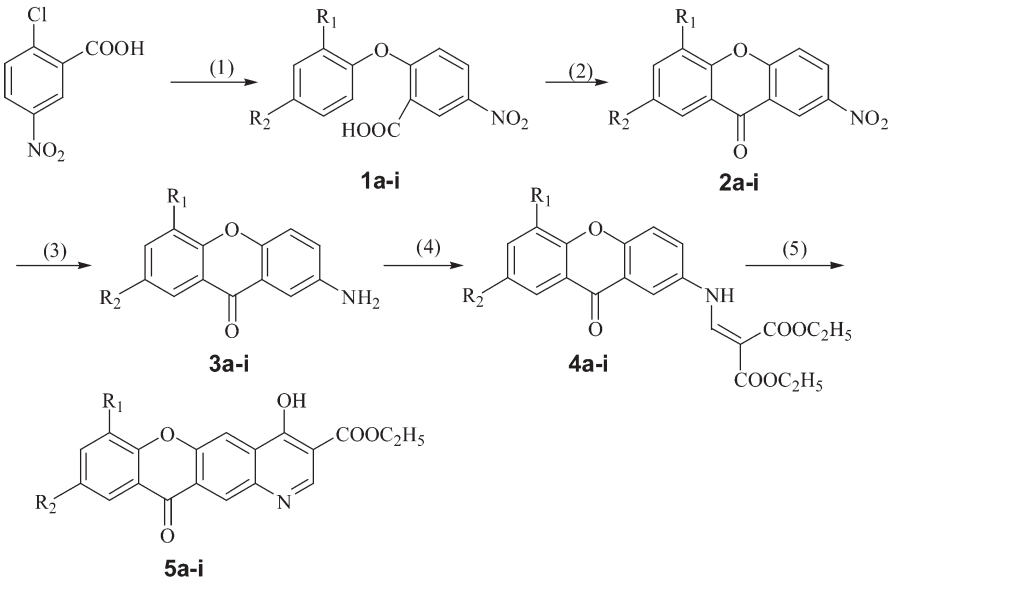

a: R_1 = Cl, R_2 = H b: R_1 = H, R_2 = CH_3 c: R_1 = H, R_2 = Cl d: R_1 = CH_3, R_2 = H e: R_1 = CH_3O, R_2 = H

f: R_1 = H, R_2 = CH_3O g: R_1 = H, R_2 = $(CH_3)_3C$ h: R_1 = H, R_2 = H i: R_1 = H, R_2 = F

Scheme 1 General synthetic route. Reagents and conditions:(1):(a) DMF, K_2CO_3, 120–125 °C, 1h; (b) substituted phenol, 80–90 °C, 4h; (c) 120–125 °C, 7–9h; (2): PPA, 100–105 °C, 2h; (3) EtOAc, 5% Pd/C, 45 °C, 7–8h; (4) EtOAc, EMME, 7–8h; (5): diphenyl ether, 255 °C, 6–9min.

2-(4-Methoxy-phenoxy)-5-nitro-benzoic acid (**1f**): Light yellow powder; yield: 85%; m.p. 173–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.05 (d, J = 3.2 Hz, 1H), 8.26 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.34–7.38 (m, 2H), 7.07–7.10 (m, 2H), 6.84 (d, J = 9.2 Hz, 1H), 3.80 (s, 3H); IR (KBr, cm^{-1}) 3443, 2963, 1706, 1613, 1508, 1477, 1344, 1239, 1182, 1033, 918, 838, 803; HR-MS (ESI): Calcd for $C_{14}H_{12}NO_6$ [M+H]$^+$: 290.0665. Found: 290.0652.

2-(4-Tert-butyl-phenoxy)-5-nitro-benzoic acid (**1g**): Light yellow powder; yield: 85%; m.p. 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.06 (d, J = 2.8 Hz, 1H), 8.29 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.10 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 1.273 (s, 9H); IR (KBr, cm^{-1}) 3437, 3076, 2965, 1710, 1611, 1582, 1515, 1478, 1426, 1374, 1259, 1204, 803; HR-MS (ESI): Calcd for $C_{17}H_{18}NO_5$ [M+H]$^+$: 316.1186. Found: 316.1192.

2-Phenoxy-5-nitro-benzoic acid (**1h**): Bright yellow powder; yield: 83%; m.p. 155–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.03 (d, J = 2.8 Hz, 1H), 8.29 (dd, J_1 = 3.2 Hz, J_2 = 9.2 Hz, 1H), 7.48–7.52 (m, 2H), 7.33–7.37 (m, 1H), 7.15–7.17 (m, 2H), 6.92 (d, J = 9.6 Hz, 1H); IR (KBr, cm^{-1})= 3399, 2986, 1705, 1655, 1613, 1588, 1454, 1155; HR-MS (ESI): Calcd for $C_{13}H_{10}NO_5$[M+H]$^+$: 260.0560. Found: 260.0557.

2-(4-Fluoro-phenoxy)-5-nitro-benzoic acid (**1i**): Light yellow powder; yield: 82%; m.p. 170–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.06 (d, J = 2.8 Hz, 1H), 8.29 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.50 (t, J = 8.8 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.8 Hz, 1H); IR (KBr, cm^{-1}) 3431, 3080, 1707, 1615, 1584, 1502, 1478, 1348, 1267, 1183, 835; HR-MS (ESI):Calcd for $C_{13}H_9FNO_5$[M+H]$^+$: 278.0466. Found: 278.0470.

Preparation of **2a–i**;[20] *general procedure*

PPA (polyphosphoric acid) (10.58 g) was stirred and heated to 100–105 °C in a three-necked flask, then **1a–i** (7.9 mmol) was added, the mixture was kept 2–3h at this temperature, then water (50 mL) was poured off. After filtration, the obtained solid was washed with saturated sodium bicarbonate (3×20 mL), water (20 mL) and was dried. The yields of the products were 92–94%. The obtained intermediates **2a–i** were directly used for the next step without further purification.

5-Chloro-2-nitro-xanthen-9-one (**2a**): Grey powder; yield: 93%; m.p. 215–217 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.25 (d, J = 2.8 Hz, 1H), 8.54 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 8.14 (d, J = 2.8 Hz, 1H), 7.63 (m, 2H), 7.47 (d, J = 9.2 Hz, 1H); IR (KBr, cm^{-1}) 3100, 2969, 1678, 1635, 1534, 1458, 1304; HR-MS (ESI): Calcd for $C_{13}H_7ClNO_4$ [M+H]$^+$: 276.0064. Found: 276.0095.

2-Methyl-7-nitro-xanthen-9-one (**2b**): Grey powder; yield: 94%; m.p. 209–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.06 (d, J = 2.8 Hz, 1H), 8.27 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.29–7.38 (m, 3H), 7.08 (m, 1H), 6.76 (d, J = 9.6 Hz, 1H), 2.21 (s, 3H); IR (KBr, cm^{-1}) 3106, 2919, 1658, 1620, 1527, 1468, 1341, 1299, 1135, 828; HR-MS (ESI): Calcd for $C_{14}H_{10}NO_4$[M+H]$^+$: 256.0611. Found: 256.0603.

2-Chloro-7-nitro-xanthen-9-one (**2c**): Grey powder; yield: 92%; m.p. 213–215 °C ; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.28 (d, J = 2.8 Hz, 1H), 8.65 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 8.35 (d, J = 2.8 Hz, 1H), 7.89 (m, 2H), 7.56 (d, J = 9.2 Hz, 1H); IR (KBr, cm^{-1}) 3013, 2934, 1687, 1676, 1488, 1456, 1330; HR-MS (ESI): Calcd for $C_{13}H_7ClNO_4$ [M+H]$^+$: 276.0064. Found: 276.0051.

5-Methyl-2-nitro-xanthen-9-one (**2d**): Grey powder; yield: 92%; m.p. 214–216 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.23 (d, J = 2.8 Hz, 1H), 8.57(dd, J_1 = 2.4 Hz, J_2 = 9.4 Hz, 1H), 8.37 (d, J = 9.2 Hz, 1H), 7.80–7.84 (m, 1H), 7.57 (d, J = 9.6 Hz, 1H), 7.47–7.50 (m, 1H), 2.10 (s, 3H); IR (KBr, cm^{-1}) 3030, 2998, 1670, 1611, 1535, 1469, 1428, 1306; HR-MS (ESI): Calcd for $C_{14}H_{10}NO_4$[M+H]$^+$: 256.0611. Found: 256.0601.

5-Methoxy-2-nitro-xanthen-9-one (**2e**): Grey powder; yield: 92%; m.p. 220–221 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.23 (d, J = 3.2 Hz, 1H), 8.57 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.92 (d, J = 9.2 Hz, 1H), 7.76 (d, J = 9.6 Hz, 1H), 7.48–7.42 (m, 2H), 4.06 (s, 3H); IR (KBr, cm^{-1}) 3103, 2924, 1667, 1608, 1491, 1446, 1339, 756; HR-MS (ESI): Calcd for $C_{14}H_{10}NO_5$ [M+H]$^+$: 272.0560. Found: 272.0567.

2-Methoxy-7-nitro-xanthen-9-one (**2f**): Grey powder; yield: 92%; m.p. 223–224 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.24 (d, J = 2.8 Hz, 1H), 8.55 (dd, J_1 = 2.8 Hz, J_2 = 9.0 Hz, 1H), 7.71 (d, J = 3.2 Hz, 1H), 7.64 (d, J = 9.2 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.41 (dd, J_1 = 3.0 Hz, J_2 = 9.0 Hz, 1H), 3.95 (s, 3H); IR (KBr, cm^{-1}) 3039, 2997, 1660, 1621, 1525, 1471, 1430, 1346, 1294, 1146; HR-MS (ESI): Calcd for $C_{14}H_{10}NO_5$ [M+H]$^+$: 272.0560. Found: 272.0554.

2-Tert-butyl-7-nitro-xanthen-9-one (**2g**): Grey powder; yield: 94%; m.p. 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.05 (J = 2.8 Hz, 1H), 8.21 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.23 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 3.2 Hz, 1H), 1.26 (s, 9H); IR (KBr, cm^{-1}) 3049, 1689, 1615, 1589, 1545, 1424, 1305; HR-MS (ESI): Calcd for $C_{17}H_{16}NO_4$ [M+H]$^+$: 298.1080. Found: 298.1042.

2-Nitro-xanthen-9-one (**2h**): Grey powder; yield: 93%; m.p. 200–202 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.00 (d, J = 2.8 Hz, 1H), 8.31 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.11–7.21 (m, 4H), 6.90 (d, J = 9.2 Hz, 1H); IR (KBr, cm^{-1}) 3079, 1669, 1605, 1574, 1532, 1464, 1314, 770; HR-MS (ESI): Calcd for $C_{13}H_8NO_4$[M+H]$^+$: 242.0454. Found: 242.0470.

2-Fluoro-7-nitro-xanthen-9-one (**2i**): Grey powder; yield: 93%; m.p. 238–239 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 9.22 (d, *J* = 2.8 Hz, 1H), 8.59 (dd, J_1 = 3.2 Hz, J_2 = 9.2 Hz, 1H), 7.99–8.01 (m, 1H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.52–7.61 (m, 1H), 6.56 (d, *J* = 9.6 Hz, 1H); IR (KBr, cm^{-1}) 3089, 1662, 1624, 1527, 1470, 1342, 1291, 839; HR-MS (ESI): Calcd for $C_{13}H_7FNO_4$ $[M+H]^+$: 260.0360. Found: 260.0350.

Preparation of **3a–i** *and* **4a–i***; general procedure*

The compounds **2a–i** (6.7 mmol) were reduced by 5% Pd/C (0.46 g) in a catalysed hydrogenation in EtOAc (ethyl acetate) (18.6 mL) at atmospheric pressure for 7–9 h. Then EMME (ethoxymethylene diethyl malonate) (1.45 g, 6.7 mmol) was added, and the mixture was refluxed for another 7–8 h and filtered at a lower temperature. The filtrate was evaporated under reduced pressure condition to afford the crude products, which were recrystallised with ethanol/dichloromethane (50–80 mL) (1:1) and dried to obtain **4a–i** in 81–83% yield.

4-(5-Chloro-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4a**): Yellow powder; yield: 81%; m.p. 217–218 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 11.01 (d, *J* = 13.2 Hz, 1H), 8.89 (d, *J* = 13.2 Hz, 1H), 8.37 (d, *J* = 3.2 Hz, 1H), 8.02 (m, 3H), 7.93 (dd, J_1 = 2.8 Hz, J_2 = 8.8 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 1H), 4.36 (m, 4H), 1.36 (m, 6H); IR (KBr, cm^{-1}) 3137, 1639, 1404, 1279, 1224, 1064, 807; HR-MS (ESI): Calcd for $C_{21}H_{19}ClNO_6$ $[M+H]^+$: 416.0902. Found: 416.0891.

3-(7-Methyl-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4b**): Yellow powder; yield: 82%; m.p. 205–206°C ; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 11.16 (d, *J* = 13.2 Hz, 1H), 8.69 (d, *J* = 13.2 Hz, 1H), 8.08 (d, *J* = 2.4 Hz, 1H), 7.89 (dd, J_1 = 2.4 Hz, J_2 = 8.8 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.13 (m, 2H), 6.56 (d, *J* = 3.2 Hz, 1H), 4.36 (m, 4H), 2.63 (s, 3H), 1.39(m, 6H); IR (KBr, cm^{-1}) 3999, 3066, 2982, 1720, 1663, 1619, 1473, 1412, 1377, 1292, 1079, 802; HR-MS (ESI): Calcd for $C_{22}H_{22}NO_6$[M+H]$^+$: 396.1448. Found: 396.1434.

3-(7-Chloro-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4c**): Yellow powder; yield: 83%; m.p. 225–226 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 11.22 (d, *J* = 13.2 Hz, 1H), 8.56 (d, *J* = 13.2 Hz, 1H), 7.60–7.95 (m, 1H), 7.46–7.60 (m, 3H), 4.21–4.37 (m, 4H), 1.31–1.40 (m, 6H); IR (KBr, cm^{-1}) 3400, 1678, 1615, 1245, 1164; HR-MS (ESI): Calcd for $C_{21}H_{19}ClNO_6$[M+H]$^+$: 416.0902. Found: 416.0901.

3-(5-Methyl-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4d**): Yellow powder; yield: 83%; m.p. 181–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 11.22 (d, *J* = 13.2 Hz, 1H), 8.59 (d, *J* = 13.2 Hz, 1H), 8.21 (d, *J* = 2.8 Hz, 1H), 8.08 (m, 2H), 7.99 (dd, J_1 = 2.4 Hz, J_2 = 8.8 Hz, 1H), 7.54 (*J* = 3.2 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 4.56 (m, 4H), 2.35 (s, 3H), 1.45(m, 6H); IR (KBr, cm^{-1}) 3400, 2981, 1636, 1486, 1407, 1381, 1278, 1222, 1064, 807; HR-MS (ESI): Calcd for $C_{22}H_{22}NO_6$[M+H]$^+$: 396.1448. Found: 396.1443.

3-(5-Methoxy-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4e**): Yellow powder; yield: 83%; m.p. 223–224 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 11.01 (d, *J* = 13.6 Hz, 1H), 8.58(d, *J* = 13.6 Hz, 1H), 8.08 (d, *J* = 2.4 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.66 (d, *J* = 8.8 Hz, 1H), 7.49–7.51 (m, 1H), 7.27–7.35 (m, 2H), 4.25–4.35 (m, 4H), 4.05 (s, 3H), 1.33–1.41 (m, 6H); IR (KBr, cm^{-1}) 3430, 3154, 2979, 1692, 1662, 1640, 1495, 1420, 1312, 1267, 1220, 798; HR-MS (ESI): Calcd for $C_{22}H_{22}NO_7$[M+H]$^+$: 412.1397. Found: 412.1390.

3-(7-Methoxy-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4f**): Yellow powder; yield: 82%; m.p. 226–228 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 11.23 (d, *J* = 13.6 Hz, 1H), 8.61 (d, *J* = 13.6 Hz, 1H), 8.11 (s, 1H), 7.71 (s, 1H), 7.46–7.54 (m, 3H), 7.38 (s, 1H), 4.29–4.34 (m, 4H), 3.95 (s, 3H), 1.37–1.41 (m, 6H); IR (KBr, cm^{-1}) 3411, 3188, 2983, 1724, 1659, 1620, 1482, 1413, 1300, 1077, 815; HR-MS (ESI): Calcd for $C_{22}H_{22}NO_7$[M+H]$^+$: 412.1397. Found: 412.1396.

3-(7-Tert-butyl-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4g**): Yellow powder; yield: 82%; m.p. 208–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 10.89 (d, *J* = 14.0 Hz, 1H), 8.35 (d, *J* = 14.0 Hz, 1H), 8.25 (d, *J* = 3.2 Hz, 1H), 8.01 (dd, J_1 = 2.8 Hz, J_2 = 8.2 Hz, 1H), 7.83–7.90 (m, 2H), 7.50 (d, *J* = 3.2 Hz, 1H), 7.21 (d, *J* = 3.2 Hz, 1H), 4.22 (m, 4H), 1.28 (m, 15H); IR (KBr, cm^{-1}) 3401, 3198, 2993, 1734, 1669, 1660, 1452, 1433, 1296; HR-MS (ESI): Calcd for $C_{25}H_{28}NO_6$ $[M+H]^+$: 438.1917. Found: 438.1910.

3-(9-Oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4h**): Yellow powder; yield: 83%; m.p. 190–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 10.78 (d, *J* = 14.0 Hz, 1H), 8.43 (d, *J* = 14.0 Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.04 (d, *J* = 2.8 Hz, 1H), 7.93 (m, 2H), 7.70 (m, 2H), 7.50 (t, *J* = 7.2 Hz, 1H), 4.22 (q, *J* = 7.6 Hz, 2H), 4.14(q, *J* = 7.6 Hz, 2H), 1.26 (m, 6H); HR-MS (ESI): IR (KBr, cm^{-1}) 3149, 2989, 1680, 1620, 1460, 1403, 1272, 1076; Calcd for $C_{21}H_{20}NO_6$ $[M+H]^+$: 382.1291. Found: 382.1290.

3-(7-Fluoro-9-oxo-9H-xanthen-2-ylamino)-acrylic acid ethyl ester (**4i**): Yellow powder; yield: 83%; m.p. 220–221 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 11.21 (d, *J* = 13.2 Hz, 1H), 8.58 (d, *J* = 13.2 Hz, 1H), 8.08 (d, *J* = 2.4 Hz, 1H), 7.99 (dd, J_1 = 2.8 Hz, J_2 = 8.0 Hz, 1H), 7.53 (m, 4H), 4.31–4.35 (m, 4H), 1.35–1.40 (m, 6H); IR (KBr, cm^{-1}) 3139, 2979, 1690, 1644, 1480, 1403, 1272, 1223, 1066, 803; Calcd for $C_{21}H_{19}FNO_6$ $[M+H]^+$: 400.1197. Found: 400.1182.

Preparation of **5a–i***; general procedure*

Diphenyl ether (13 mL) was heated to 120–130 °C for 0.5–1 h to evaporate a trace of water, and then the temperature was increased to 255 °C, compounds **4a–i** (6.03 mmol) were added and stirred for 7–9 min. Then petroleum ether (50 mL) was added to the mixture at the temperature of 100–110 °C to obtain crude products by filtration. The products were washed with ethyl acetate (3×20 mL) and dried, and the yields of the products were 63–70%.

Ethyl 7-chloro-4-hydroxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5a**): Orange solid; yield: 63%; m.p. 244–246 °C; ^{1}H NMR (400 MHz; DMSO-d_6, TMS): δ (ppm) = 12.42 (s, 1H), 8.55 (s, 1H), 8.05 (s, 1H), 7.91–7.97 (m, 3H), 7.76 (s, 1H), 4.26 (q, *J* = 6.4 Hz, 2H), 1.31 (t, *J* = 6.4 Hz, 3H); IR (KBr, cm^{-1}) 3433, 3089, 2925, 1716, 1667, 1598, 1534, 1473, 1443, 768; HR-MS (ESI): Calcd for $C_{19}H_{13}ClNO_5$ $[M+H]^+$: 370.0479. Found: 370.0473; Anal. Calcd for $C_{19}H_{12}ClNO_5$: C, 61.72; H, 3.27; N, 3.79. Found: C, 61.71; H, 3.24; N, 3.81%.

Ethyl 9-methyl-4-hydroxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5b**): Yellow solid; yield: 63%; m.p. 221–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.38 (s, 1H), 8.54 (s, 1H), 8.06 (s, 1H), 7.90–7.96 (m, 2H), 7.74 (s, 1H), 7.60 (s, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 1.24 (s, 3H), 1.06 (t, 3H, *J* = 7.2 Hz); IR (KBr, cm^{-1}) 3435, 3078, 2923, 1716, 1618, 1566, 1509, 1470, 758; HR-MS (ESI): Calcd for $C_{20}H_{16}NO_5$ $[M+H]^+$: 350.1079. Found: 350.1021; Anal. Calcd for $C_{20}H_{15}NO_5$: C, 68.76; H, 4.33; N, 4.01. Found: C, 68.71; H, 4.25; N, 4.02%.

Ethyl 9-chloro-4-hydroxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5c**): Yellow solid; yield: 64%; m.p. 242–244 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.42 (s, 1H), 8.55 (s, 1H), 8.05 (s, 1H), 7.97 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.77 (s, 1H), 4.26 (q, 2H, *J* = 6.4 Hz), 1.32 (t, 3H, *J* = 6.4 Hz); IR (KBr, cm^{-1}) 3431, 3084, 2956, 1717, 1666, 1601, 1533, 1461, 787; HR-MS (ESI): Calcd for $C_{19}H_{13}ClNO_5$ $[M+H]^+$: 370.0479. Found: 370.0476; Anal. Calcd for $C_{19}H_{12}ClNO_5$: C, 61.72; H, 3.27; N, 3.79. Found: C, 61.70; H, 3.20; N, 3.75%.

Ethyl 7-methyl-4-hydroxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5d**): Yellow solid; yield: 66%; m.p. 221–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.40 (s, 1H), 8.54 (s, 1H), 8.23 (s, 1H), 7.88–8.02 (m, 2H), 7.70 (s, 2H), 4.27 (q, *J* = 6.4 Hz, 2H), 1.32 (s, 3H), 1.06 (t, *J* = 6.4 Hz, 3H); IR (KBr, cm^{-1}) 3428, 3085, 2977, 1716, 1665, 1596, 1533, 1467, 1420, 809; HR-MS (ESI): Calcd for $C_{20}H_{16}NO_5$ $[M+H]^+$: 350.1079. Found: 350.1020; Anal. Calcd for $C_{20}H_{15}NO_5$: C, 68.76; H, 4.33; N, 4.01. Found: C, 68.74; H, 4.30; N, 4.05%.

Ethyl 4-hydroxy-7-methoxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5e**): Yellow; yield: 68%; m.p. 214–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.43 (s, 1H), 8.60 (s, 1H), 8.03 (s, 1H), 7.96 (s, 1H), 7.71 (s, 1H), 7.51–7.61 (m, 2H), 3.85 (s, 3H), 3.41 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3436, 3075, 2983, 1688, 1615, 1594, 1568, 1486, 785; HR-MS (ESI): Calcd for $C_{20}H_{16}NO_6$ $[M+H]^+$: 366.0979. Found: 366.0971; Anal. Calcd for $C_{20}H_{15}NO_6$: C, 65.75; H, 4.14; N, 3.83. Found: C, 65.79; H, 4.19; N, 3.81%.

Ethyl 4-hydroxy-9-methoxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5f**): Yellow solid; yield: 70%; m.p. 242–243 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.61 (s, 1H), 8.65 (s, 1H), 8.06 (s, 1H), 7.74 (s, 1H), 7.54 (s, 1H), 7.40–7.51 (m, 2H), 4.29 (q, *J* = 7.2 Hz, 2H), 4.02 (s, 3H), 1.31 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3446, 3085, 2982, 1698, 1616, 1597, 1569, 1597, 1569, 1499, 1445, 778; HR-MS (ESI): Calcd for $C_{20}H_{16}NO_6$ $[M+H]^+$: 366.0979. Found: 366.0965; Anal. Calcd for $C_{20}H_{15}NO_6$: C, 65.75; H, 4.14; N, 3.83. Found: C, 65.76; H, 4.11; N, 3.78%.

Ethyl 9-tert-butyl-4-hydroxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylates (**5g**): Yellow solid; yield: 69%; m.p. 226–228 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.40 (s, 1H), 8.54 (s, 1H), 7.90–7.96 (m, 3H), 7.63 (s, 1H), 7.61 (s, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H), 1.25 (s, 9H); IR (KBr, cm^{-1}) 3435, 3082, 2960, 1716, 1663, 1602, 1530, 1492, 1466, 787; HR-MS (ESI): Calcd for $C_{23}H_{22}NO_5$ $[M+H]^+$: 392.1479. Found: 392.1489; Anal. Calcd for $C_{23}H_{21}NO_5$: C, 70.58; H, 5.41; N, 3.58. Found: C, 70.55; H, 5.31; N, 3.59%.

Ethyl 4-hydroxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5h**) : Yellow solid; yield: 65%; m.p. 190–192 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.42 (s, 1H), 8.64 (s, 1H), 8.11–8.18 (m, 1H), 8.04 (s, 1H), 7.90–7.97 (m, 2H), 7.67–7.75 (m, 1H), 7.50 (s, 1H), 4.30 (q, *J* = 7.2 Hz, 3H), 1.33 (t, *J* = 7.2 Hz, 2H); IR (KBr, cm^{-1}) 3427, 3080, 2976, 1716, 1664, 1603, 1532, 1461, 757; HR-MS (ESI): Calcd for $C_{19}H_{14}NO_5$ $[M+H]^+$: 336.0879. Found: 336.0869; Anal. Calcd for $C_{19}H_{13}NO_5$: C, 68.06; H, 3.91; N, 4.18. Found: C, 68.01; H, 3.89; N, 4.15%.

Ethyl 9-fluoro-4-hydroxy-11-oxo-11H-chromeno[2,3-g]quinoline-3-carboxylate (**5i**): Yellow solid; yield: 64%; m.p. 238–239 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ (ppm) =12.41 (s, 1H), 8.16 (s, 1H), 7.61 (s, 1H), 7.51 (s, 1H), 7.30–7.45 (m, 3H), 3.83 (q, *J* = 7.2 Hz, 2H), 0.87 (t, *J* = 7.2 Hz, 3H); IR (KBr, cm^{-1}) 3428, 3081, 2925, 1718, 1666, 1605, 1533, 1466, 815; HR-MS (ESI): Calcd for $C_{19}H_{13}FNO_5$ $[M+H]^+$: 354.0779. Found: 354.0770; Anal. Calcd for $C_{19}H_{12}FNO_5$: C, 64.59; H, 3.42; N, 3.96. Found: C, 64.54; H, 3.41; N, 3.92%.

Biological asssay

The anticoccidial activities of the compounds **5a–d** and **5e–i** were evaluated according to the anticoccidial index (ACI) method[21], using decoquinate as reference drug. The chickens used to test anticoccidial activities of the compounds were 12-day old broilers, which were fed normally without any anticoccidial drug and drank clean water. Groups of the chickens were randomly housed, with 15 in each cage, and 15 cages were randomly assigned by tier. Groups 1–5 and 8–13 of 13–days chicken were fed starter diet with the compounds **5a–i** and decoquinate in 27mg kg–1. Groups 1–6 and 8–14 of 14–days chicken were artificially infected with the *Eimeria tenell* spores with 100 000 oocysts. On observing the chicken after infection for 7 days, we recorded the weight gain, mortality, dropping scores, lesion scores and oocyst scores of the chicken and calculated the ACI. The results of test are given in Table 1.

Results and discussion

The nucleophilic substitution reaction in the first step was finished at 120–125 °C within 7–9 h to obtain yellow products that were directly used for the next step. The compounds **2a–i** were easily synthesised according to the literature[19] and did not need to be purified.

Compounds **3a–i** and **4a–i** can be easily produced under the referred conditions. Compounds **3a–i** were unstable in air so they were directly used for the next step without further purification. Pure and well dried intermediates **4a–i** were necessary. Otherwise, many by-products would form and the purification process of **5a–i** would become very difficult in the next step.

The last ring-closing reaction was carried out by heating the intermediates **4a–i** in diphenyl ether above 255 °C for 6–9 minutes. Time and temperature of the reaction must be strictly controlled to improve the purity of crude products because the products of this step could not dissolve in any solvent and were very difficult to purify.

The data for anticoccidial activities of the tested compounds **5a–i** are shown in Table 1. In the positive control group, the ACI was obviously 69 and 94. In the negative control group, no coccidiosis happened in the chickens and the ACI was 200. So the control was set up. Compounds **5a**, **5b**, **5c** and **5d** showed anticoccidial activities against the *Eimeria tenella* with the ACI 147, 123, 133, 148 respectively.

Table 1 Data for anticocciadial activity of the compounds **5a–i** and decoquinate against *Emeria tellena*

Group	Test compounds /mg kg^{-1}	Rate of relative weight gain /%	Survival rate/%	Lesion value	Oocyst value	ACI
1	**5a**	9	93	21	20	147
2	**5b**	83	100	19	40	123
3	**5c**	91	100	18	40	133
4	**5d**	87	100	19	40	148
5	Decoquinate	76	93	19	20	130
6	Infected-untreated	64	97	28	40	69
7	Uninfected-untreated	100	100	0	0	200
8	**5e**	20	75	34	40	21
9	**5f**	62	80	18	40	84
10	**5g**	69	90	28	30	100
11	**5h**	50	95	32	40	73
12	**5i**	49	75	24	40	60
13	Decoquinate	76	93	19	20	130
14	Infected-untreated	64	100	30	40	95
15	Uninfected-untreated	100	100	0	0	200

Conclusion

In summary, for the first time nine substituted ethyl 4-hydroxy-11-oxo-11*H*-chromeno[2,3-g]-quinoline-3-carboxylates were designed and synthesised as new anticoccidial drugs. The results indicate that the compounds **5a**, **5b**, **5c** and **5d** have anticoccidial activities against *Eemeria tenella* at a dose of 27 mg kg^{-1} and they might be developed as available anticoccidial drugs. Further research work is still in progress.

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