Synthesis and anticoccidial activity of 3-(2-(benzofuran)-2-yl)-2-oxoethylquinazolinone derivatives

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In order to develop novel and effective anticoccidial compounds, a series of 3-(2-(benzofuran)-2-yl)-2-oxoethylquinazolinone derivatives were designed, synthesised and evaluated as potential anticoccidial drugs. The structures of these compounds were characterised by ^{1}H NMR, IR, HRMS spectra and elemental analysis. These compounds were tested for anticoccidial activities against *Eimeria tenella* according to the anticoccidial index method. 6-Chloro-3-(2-(benzofuran-2-yl)-2-oxoethyl) quinazolin-4-(3H)-one exhibited significant anticoccidial activities in the chicken's diet with a dose of 18 mg kg^{-1}.

Keywords: 3-(2-(benzofuran)-2-yl)-2-oxoethylquinazolinone, anticoccidial activity, anticoccidial index method, *Eimeria tenella*

Coccidiosis is an intestinal infection caused by protozoan parasites of the genus *Eimeria*, which can invade the intestinal mucosa and induce a degree of epithelial cell damage and inflammation. Intestinal lesions, diarrhoea, enteritis and death can occur.[1] Many anticoccidial drugs play an important role in controlling the disease. However, because of drug-resistance caused by both using anticoccidial medicine extensively and the heredity of coccidia, the coccidial populations have been a constant threat to the continued success of chemotherapy.[2] Therefore, we have investigated the development of novel and effective compounds.

Dichroa Febrifuge had been used as a traditional medicine for the treatment of malaria and coccidiosis in China. Its active components are confirmed as the quinazoline alkaloids, such as febrifugine (**1**) and isofebrifugine (**2**) (Fig. 1).[3] However, it has not been used in anticoccidial drugs because of its side effects of diarrhoea, vomiting and liver toxicity.[4] Halofuginone (**3**) (Fig. 1) (commercial name Stenorol) was designed and synthesised based on the structure of febrifugine (**1**), It has been a good anticoccidiostat at a dose of 3 mg kg^{-1} for a long time.[5] In contrast, the drug-resistance which led to the decline in the effectiveness of halofuginone (**3**) has also been found.[6] Furthermore, the synthesis of halofuginone (**3**) is very complicated requiring both the synthesis of the quinazolinone ring and the piperidine ring,[7] resulting in expensive production costs and limitations for broad-usage.

Our groups have been trying to find new and potential anticoccidial compounds,[8–12] some of which were quinazolinone derivatives. Here we describe the synthesis of six 3-(2-(benzofuran) -2-yl) -2 -oxoethylquinazolinone derivatives having structures related to halofiginone (**3**) with the benzofuran ring taking the place of piperidine ring. The synthetic route to the target compounds (**4a–f**) is shown in Scheme 1. The anticoccidial activities of the novel compounds were evaluated according to the anticoccidial index (ACI) method.[13,14]

Experimental

Solvents and reagents were obtained from commercial sources without further purification except salicylaldehyde which was purified through redistilling and then kept at 0 °C. 1-(Benzofuran-2-yl)-ethanone (**1**) was synthesised according to the literature.[15] Quinazolin-4-(3H)-one derivatives (**3a–f**) were synthesised according to the literature.[9,16–18] Melting points were recorded using an XRC-1 apparatus and the thermometer was uncorrected. Proton NMR spectra were recorded on a Varian Unity Inova-400 spectrometer with $CDCl_3$ as the solvent and TMS as the internal standard, and the chemical shift values (δ) were given in ppm. Mass spectra were recorded with an Agilent 6210 (DOF-MAS) spectrometer using ESI. IR spectra were recorded with a Perkin-Elmer 16PC-FT instrument. Elemental analyses were carried out by the Euro EA 3000 instrument (Euro Vector S.P.A., Italy). Analytical TLC was determined with silica gel GF254, and spots were visualised with UV light.

*Synthesis of 2-bromo-1-(benzofuran-2-yl)-ethanone (***2***)*[19]

A mixture of 1-(benzofuran-2-yl)-ethanone (**1**) (12.8 g, 80 mmol) in CH_2Cl_2 (50 mL) was stirred vigorously at room temperature. A solution of bromine (12.8 g, 80 mmol) in CH_2Cl_2 (50 mL) was added in small portions in such a way that the colour of bromine completely disappeared before the next portion was added. Then HBr gas was neutralised by the addition of sodium hydroxide solution. The reaction mixture was adjusted to pH 7–8 with saturated $NaHCO_3$ and the organic layer was separated out and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic phases were washed with saturated NaCl (3×40 mL), dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give a crude product as a brown yellow oil. A yellow crude solid was obtained by addition of petroleum ether (20 mL) to the oil and yellow crystals were produced by recrystallisation from petroleum ether. Yield: 80–85%; m.p. 83 °C (84.3 °C); ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ = 7.74 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.61–7.59 (m, 1H), 7.55–7.50 (m, 1H), 7.37–7.33 (m, 1H), 4.45 (s, 2H).

*Synthesis of 3-(2-(benzofuran)-2-yl)-2-oxoethylquinazolinone derivatives (***4a–f***); general procedure*

In a three-necked flask with a thermometer and a condenser, a mixture of (**3a–f**) (5 mmol), KI (0.37 g, 2.25 mmol) and NaH (0.24 g, 10 mmol)

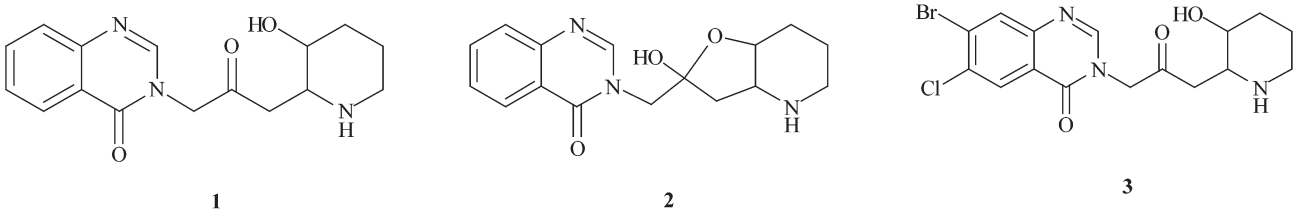

Fig. 1 Structure of febrifugine (**1**), isofebrifugine (**2**) and halofuginone (**3**).

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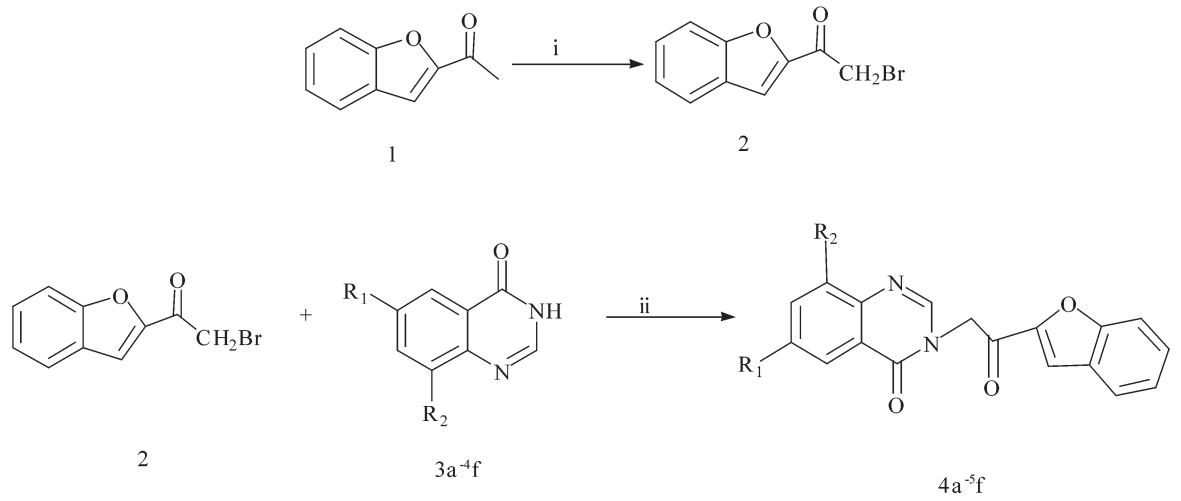

a: R_1=H, R_2=H; b: R_1=F, R_2=H; c: R_1=F, R_2=Br;

d: R_1=Cl, R_2=H; e: R_1=Cl, R_2=Br; f: R_1=Br, R_2=H;

Reagents and conditions: i) Br_2, room temperature

ii) DMSO, NaH, KI, 75 °C, 2^{-4} h.

Scheme 1 General synthesis route for compounds **4a–f**.

in DMSO (10 mL) was heated to 75 °C with stirring. Then a solution of intermediate (**2**) (1.20 g, 5 mmol) in DMSO (10 mL) was added dropwise. After 2–4 h, the mixture was cooled to room temperature and washed with water (3 × 100 mL) giving much solid which was dried and recrystallised from anhydrous ethanol to give (**4a–f**).

3-(2-(Benzofuran-2-yl)-2-oxoethyl) quinazolin-4-(3H)-one (**4a**): Dark grey solid; yield: 40%; m.p. 204–206 °C; ^{1}H NMR spectrum (400 MHz,$CDCl_3$) δ = 8.31 (d, *J* = 8.0 Hz, 1H), 8.04 (s, 1H), 7.82–7.73 (m, 4H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.57–7.51 (m, 2H), 7.42–7.32 (m, 1H), 5.44 (s, 2H); IR (KBr, cm^{-1}) 3377, 3029, 2939, 1698, 1669, 1608, 1560, 1472, 1359, 1165, 1135, 782, 752. Anal. Calcd for $C_{18}H_{12}N_2O_3$: C, 71.05; H, 3.97; N, 9.21. Found: C, 71.01; H, 3.99; N, 9.24%. HR-MS (ESI): Calcd for $C_{18}H_{12}N_2O_3$ (M + H^+): 305.0926; found: 305.0926.

6-Fluoro-3-(2-(benzofuran-2-yl)-2-oxoethyl) quinazolin-4-(3H)-one (**4b**): Light pink flocculent solid; yield: 65.8%; m.p. 204–206 °C; ^{1}H NMR spectrum (400 MHz, $CDCl_3$) δ = 8.01 (s, 1H), 7.94 (dd, J_1 = 2.8 Hz, J_2 = 8.4 Hz, 1H), 7.80–7.76 (m, 2H), 7.73(s, 1H), 7.62(d, *J* = 8.4 Hz, 1H), 7.58-7.50 (m, 2H), 7.37 (t, $J_1 = J_2$ = 7.6 Hz, 1H), 5.44 (s, 2H); IR (KBr, cm^{-1}): 3432, 3024, 2944, 1701, 1672, 1606, 1560, 1482, 1163, 1134, 843, 761. Anal. Calcd for $C_{18}H_{11}FN_2O_3$: C, 67.08; H, 3.44; N, 8.69. Found: C, 67.09; H, 3.48; N, 8.72%; HR-MS (ESI): Calcd for $C_{18}H_{11}FN_2O_3$ (M + H^+): 323.0832, 324.0866; found: 323.0827, 324.0860 (5:1).

8-Bromo-6-fluoro-3-(2-(benzofuran-2-yl)-2-oxoethyl) quinazolin-4-(3H)-one (**4c**): Silver flocculent solid; yield: 68.5%; m.p. 240–241 °C; ^{1}H NMR spectrum (400 MHz,$CDCl_3$) δ = 8.09 (s, 1H), 7.95 (dd, J_1 = 2.8 Hz, J_2 = 7.6 Hz, 1H), 7.85 (dd, J_1 = 2.8 Hz, J_2 = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.73 (s, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.56 (t, $J_1 = J_2$ = 7.6 Hz, 1H), 7.37 (t, $J_1 = J_2$ = 7.6 Hz, 1H), 5.44 (s, 2H); IR (KBr, cm^{-1}): 3425, 3079, 2935, 1693, 1667, 1608, 1554, 1461, 1185, 1017, 862, 749. Anal. Calcd for $C_{18}H_{10}BrFN_2O_3$: C, 53.89; H, 2.51; N, 6.98. Found: C, 53.93; H, 2.53; N, 6.95%; HR-MS (ESI): Calcd for $C_{18}H_{10}BrFN_2O_3$ (M + H^+, isotopic peak): 400.9937, 401.9971, 402.9917, 403.9950; found: 400.9935, 401.9971, 402.9917, 403.9949 (5:1:5:1).

6-Chloro-3-(2-(benzofuran-2-yl)-2-oxoethyl) quinazolin-4-(3H)-one (**4d**): White grey flocculent solid; yield: 60.2%; m.p. 229–230 °C; ^{1}H NMR spectrum (400 MHz,$CDCl_3$) δ = 8.27 (d, *J* = 1.2 Hz, 1H), 8.01 (s, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.73–7.72 (m, 3H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.58–7.52 (m, 1H), 7.40–7.36 (t, $J_1 = J_2$ = 7.2 Hz, 1H),5.26 (s, 2H); IR (KBr, cm^{-1}): 3428, 3071, 2932, 1692, 1669, 1609, 1551, 1448, 748. Anal. Calcd for $C_{18}H_{11}ClN_2O_3$: C, 63.82; H, 3.27; N, 8.27. Found: C, 63.79; H, 3.30; N, 8.31%; HR-MS (ESI): Calcd for $C_{18}H_{11}ClN_2O_3$ (M + H^+, isotopic peak): 339.0536, 341.0507; found: 339.0529, 341.0506 (3:1).

8-Bromo-6-chloro 3-(2-(benzofuran-2-yl)-2-oxoethyl) quinazolin-4-(3H)-one (**4e**): Light grey flocculent solid; yield: 70.5%; m.p. 229–230 °C. ^{1}H NMR spectrum (400 MHz,$CDCl_3$) δ = 8.25 (d, *J* = 2.4 Hz, 1H), 8.10 (s, 1H), 8.05 (d, *J* = 2.4 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 0.8 Hz, 1H), 7.64–7.56 (m, 2H), 7.38–7.36 (m, 1H), 5.44 (s, 2H); IR (KBr, cm^{-1}): 3428, 3077, 2932, 1692, 1669, 1609, 1551, 1448, 748. Anal. Calcd for $C_{18}H_{10}BrClN_2O_3$: C, 51.77; H, 2.41; N, 6.71. Found: C, 51.81; H, 2.44; N, 6.68%; HR-MS (ESI): Calcd for $C_{18}H_{10}BrClN_2O_3$ (M + H^+, isotopic peak): 416.9642, 418.9621, 418.9612; found: 416.9628, 418.9616, 418.9607 (4:3:1).

6-Bromo-3-(2-(benzofuran-2-yl)-2-oxoethyl) quinazolin-4-(3H)-one (**4f**): Light grey flocculent solid; yield: 45%; m.p. 212–214 °C; ^{1}H NMR spectrum (400 MHz,$CDCl_3$) δ = 8.47 (d, *J* = 2.4 Hz, 1H), 8.21 (s, 1H), 7.92 (dd, $J_1 = J_2$ = 2.4 Hz, 1H), 7.80–7.72 (m, 3H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.60–7.54 (m, 1H), 7.42–7.38 (m, 1H), 5.51 (s, 2H); IR (KBr, cm^{-1}): 3436, 3072, 2929, 1694, 1670, 1609, 1549, 1465, 1175, 1029, 745. Anal. Calcd for $C_{18}H_{11}BrN_2O_3$: C, 56.42; H, 2.89; N, 7.31. Found: C, 56.44; H, 2.93; N, 7.28%; HR-MS (ESI): Calcd for $C_{18}H_{11}BrN_2O_3$ (M + H^+, isotopic peak): 383.0031, 385.0011; found: 383.0027, 385.0009 (1:1).

Biological assay

The anticoccidial activities of the new compounds were evaluated according to the anticoccidial index method, using the decoquinate and diclazuril as reference drugs at a dose of 27 mg kg^{-1} and 1 mg kg^{-1}, respectively. Decoquinate is a highly sensitive drug to the coccidia, but the diclazuril had been regarded as a good anticoccidiostat prior to its use. The chickens that were used for testing were fed to 12-days-old by a feedstuff that did not contain any anticoccidial drug.

Groups of these chickens were randomly housed in 10 cages with 15 in each. Groups 1–6 of 13-day-old chickens were fed the basal starter diet with the compounds (**4a–f**) as 18 mg kg^{-1} until the end of the test. Chickens in groups 7–8 were fed the basal starter diet with decoquinate or diclazuril until the end of the test. Groups 1–9 of 14-day-old chickens were infected artificially with the *Eimeria tenella* spores of the oocysts 100,000 and kept under observation for 9 days

Table 1 Data for anticoccidial activities of compounds **4a–f** against *Eimeria tenella*

Test groups	Test compounds /mg kg^{-1}	Survival rate/%	Rate of relative body weight gain	Lesion values	Oocyst values	OPG values[d] $\times 10^6$	ACI[a]
1	**4a** (18[e])	93.33	53.97	17.14	1.00	0.14	129.16
2	**4b** (18[e])	100.00	50.00	15.00	1.00	0.80	134.00
3	**4c** (18[e])	93.33	65.48	14.64	10.00	1.25	134.17
4	**4d** (18[e])	100.00	72.22	14.29	10.00	1.16	147.93
5	**4e** (18[e])	93.33	53.17	18.00	10.00	1.16	118.50
6	**4f** (18[e])	93.33	60.00	23.00	20.00	6.45	110.33
7	Decoquinate(27[e])	100.00	71.65	13.00	0	0.01	158.65
8	Diclazuril (1[e])	93.33	53.54	20.00	20.00	6.22	106.87
9	ING[b]	80.00	62.99	28.00	40.00	19.34	74.99
10	NNG[c]	100.00	100.00	0	0	0	200.00

[a] Anticoccidial activity index.
[b] Infected non-medicated group.
[c] Non-infected non-medicated group.
[d] Occyst per gram.
[e] The dose of tested compounds 18 mg kg^{-1}, 27 mg kg^{-1}, 1mg kg^{-1}.

after infection. The weight gain, mortality, lesion scores and oocysts' counts of the chickens were recorded and then the ACI was calculated. Results of the tests are shown in Table 1.

Results and discussion

When the chickens were infected by coccidia, their survival rates would reduce, bodyweight gain would diminish, lesions would be found in the cecum and the oocyst of the next generation would be produced. The ACI was calculated as follows:

$$\text{ACI} = (\text{relative rate of weight gain} + \text{rate of survival}) - (\text{lesion value} + \text{oocyst value})$$

Based on the literature of Johnson and Reid,[14] the lesion of chickens was ranked in five classes and the related lesion score was recorded as 0-4. The lesion score of each group was the average value for chickens in each team. And the lesion value was calculated as below:

$$\text{Lesion value} = \text{lesion score} \times 10$$

Table 2 shows the relation between oocyst counts of per gram and the oocyst value.[20]

The data for anticoccidial activities of the compounds (**4a–f**) are shown in Table 1. In the positive control group, the coccidiosis in chickens is serious with an ACI of 74.99. By contrast, the negative control group showing no coccidiosis in chickens has an ACI of 200. Thus the control was set up.

Lesion values could evaluate the effect of *Eimeria* to chicken's caecum. The lesion values in all groups of compounds (**4a–f**) were 17.14, 15.00, 14.64, 14.29, 18.00 and 23.00 which are lower than the value 28.00 of the infected non-medicated group. It clearly indicated that all the tested compounds could control the effect of *Eimeria tenella* on the chickens' caecum.

The oocyst value data ranges from 0 to 40 reflecting the amount of oocysts per gram, which are produced by the elder generation of coccidian. The oocyst value data in Table 1 are 1.00 (**4a, 4b**), 10.00 (**4c, 4d, 4e**) and 20.00 (**4f),** respectively, and they are significantly lower than the value 40.00 of the infected-untreated group. They showed that all the tested compounds could effectively suppress the generation of oocysts.

Survival rate and the relative rate of weight gain indicated the affect of drugs or coccidia on the birds' growth. The survival rate data are 93.33 (**4a, 4c, 4e, 4f**) and 100.00 (**4b, 4d**).

Table 2 The relation between OPG value and oocyst value

OPG value $\times 10^6$	0–0.1	0.1–1.0	2.0–5.0	6.0–10.0	≥11.0
Oocyst value	0	1	10	20	40

Compared with 80.00 (the infected non-medicated group), all the tested drugs could obviously protect the chickens from death. Compared with the infected non-medicated group, two of the tested compounds were advantageous for the growth of the chickens. According to the infected non-medicated group (62.99), the relative rate of weight gain is better when (**4c**) (65.48) and (**4d**) (72.22) were used respectively.

Briefly, three of these compounds exhibited higher anticoccidial activities against *Eimeria tenella* with ACI of 134.00 (**4b**), 134.17 (**4c**) and 147.93 (**4d**), and other compounds (**4a**) (129.16), (**4e**) (118.50) and (**4f**) (110.33) exhibited certain anticoccidial activities. We selected only one concentration at random to test the anticoccidial activity of the synthesised compounds, without considering the optimal concentration to obtain the best ACI.

Conclusion

Six novel 3-(2-(benzofuran)-2-yl)-2-oxoethylquinazolinone derivatives have been synthesised and the anticoccidial activities of these compounds were evaluated according to the ACI method. The results indicated that all of compounds have biological activities. Especially, (**4d**) (ACI of 147.93) had significant anticoccidial activities against *Eimeria tenella* at a dose of 18 mg kg^{-1} and might be developed as an anticoccidial drug when the coccidian-increased resistance to older compounds, such as decoquinate, increases.

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