Synthesis and anticoccidial activity of 4-(2-methoxyphenyl)-2-oxobutylquinazolinone derivatives

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Abstract

A series of 4-(2-methoxyphenyl)-2-oxo-butyl-quinazolinones were designed and synthesized based on the structure of febrifugine. The structures of the new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR spectra and HRMS. The biological activity test results indicated that they exhibited anticoccidial activities against *Eimeria tenella* in the chicken diet with a dose of 9 mg/kg. Compared with halofuginone, these compounds have the advantages of shorter synthetic routes and lower cost.

Keywords: Anticoccidial index method, anticoccidial activities, *Eimeria tenella*, 4-(2-methoxyphenyl)-2-oxo-butyl-quinazolinones, synthesis

Introduction

Coccidiosis is an intestinal infection caused by protozoan parasites of the genus Eimeria, especially for broilers and turkeys, which occurs all over the world and leads to extensive loss in the poultry industry.[1] Anticoccidial drugs play a very important role in controlling chicken coccidiosis. But the drug resistance of *Eimeria* against anticoccidial drugs is increasing because many drugs have been used for a long time.[2] Thus, the discovery of new and effective anticoccidial drugs is urgently needed.

In China, Dichroa febrifuga is a well-known medicine for the treatment of malaria. Febrifugine (Figure 1, Compound 1) and its stereoisomer isofebrifugine (Figure 1, Compound 2) have been identified as the active components.[3] The potent anticoccidial activity of febrifugine in poultry was discovered in the 1960's. Because of side effects, such as diarrhea, vomiting,[4] and liver toxicity,[5] it has been precluded as an anticoccidial drug. In 1967 halofuginone (Figure 1, Compound 3) (commercial name Stenorol) was designed and synthesized based on the structure of febrifugine by the American Cyanamid Company.[6,7] Halofuginone is a broad-spectrum anticoccidial medicine with low toxicity and no cross-resistance.[8] Compared to other anticoccidial drugs, halofuginone has a higher anticoccidial activity when administered to chickens in the diet with a concentration of 3 mg/kg. However, the synthetic process of to

produce halofuginone is complicated (Scheme 1),[9] which leads to a high cost and limits its application.

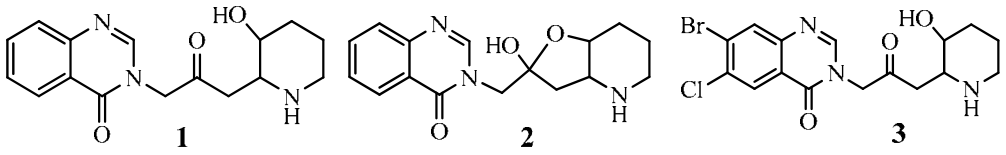
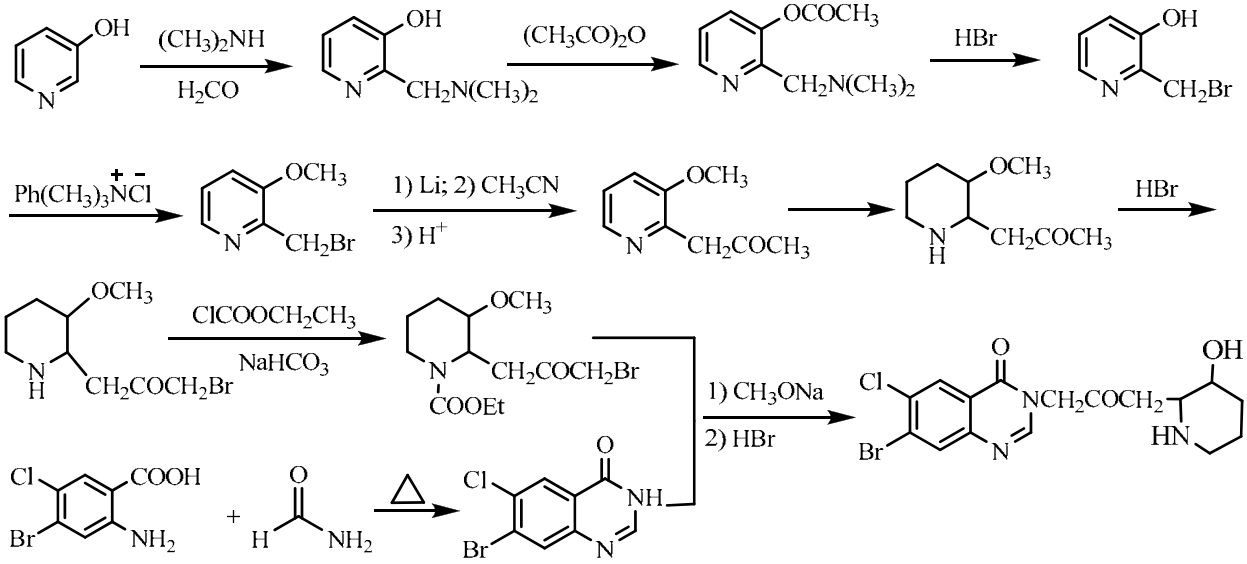


Figure 1. Structure of Febrifugine (**1**), Isofebrifugine (**2**) and Halofuginone **(3)**.



Scheme 1. Synthesis route for halofuginone.

In order to find new and effective anticoccidial drugs with a simpler structure and lower cost, eight 4-(2-methoxyphenyl)-2-oxobutylquinazolinones were designed and synthesized according to the structure of febrifugine (Figure 2). Compared with the structure of halofuginone, the 4-(2'-methoxyphenyl)-2-oxo-butyl moiety in the target compounds was easier to be introduced from salicylaldehyde and acetone. The 6' and 8' substitution of quinazolines is easier than the 6' and 7' substitution because the 3' and 5'-substitution of anthranilic acid can be performed more easily. Instead of a hydroxyl function in halofuginone with a methoxy at C-2″, the stability of the target compounds was improved. The anticoccidial activities of the new compounds were also evaluated according to the Anticoccidial Index (ACI) method.[10-12]

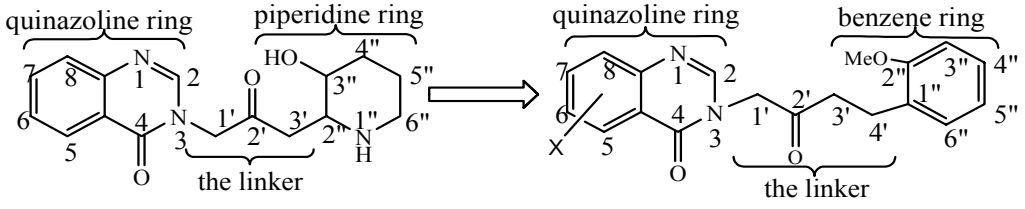
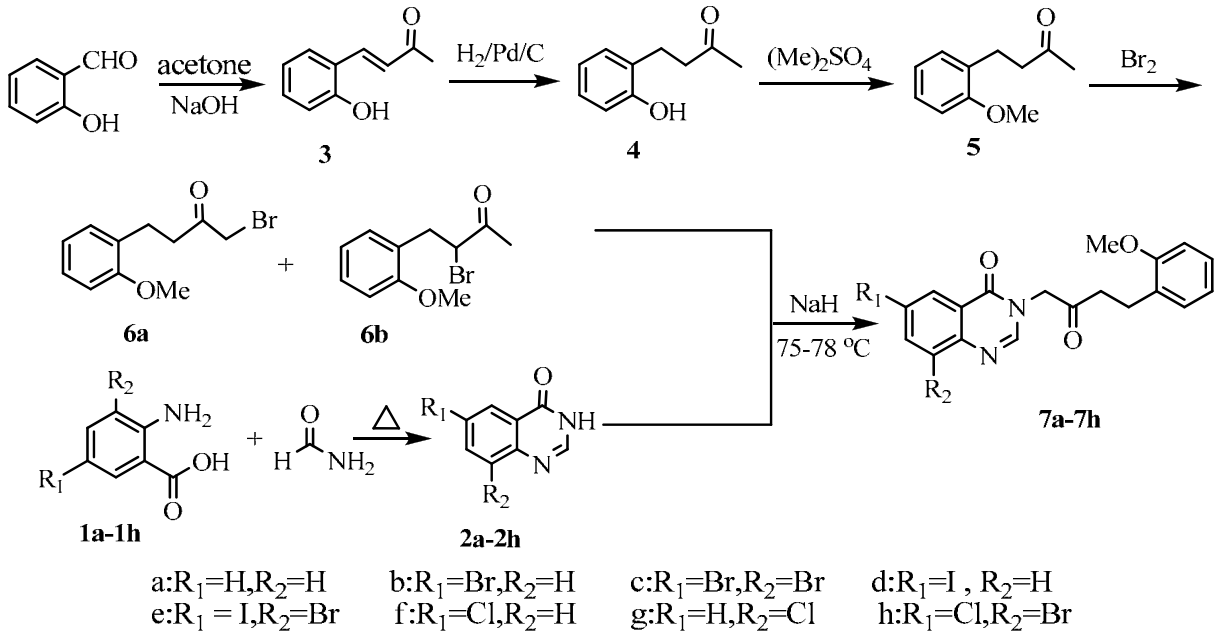


Figure 2. Structures of 4-(2-methoxyphenyl) -2-oxo-butyl-quinazolinone derivatives.

Results and Discussion

Synthesis

As shown in Scheme 2, the important intermediates **(2a - 2h)** were synthesized by the reaction of formamide and substituted anthranilic acids **(1a - 1h)**. The halogen groups could reduce the activity of anthranilic acid, which requires a high temperature in this step. 4-(2-Hydroxyphenyl) butan-2-one can be synthesized by reduction of (E)-4-(2-hydroxyphenyl) but-3-en-2-one **(3)** with H_2/Pd/C (5 %) in ethyl acetate. As the hydroxyl is unstable, the product becomes pink. The reaction temperature and solvent are very important in the reaction of compound **5** with bromine. According to the ^{1}H NMR analysis, we found that if methanol was used as solvent, the ratio of **6a** and **6b** is 5:1 and 3:1 at room temperature and reflux, respectively. If acetic acid was used as solvent, **6b** was the main product. Because of the different activity of **6a** and **6b**, the mixture could be used in the next reaction without further purification. Subsequently, intermediate **6**, NaH and quinazolinone compounds **2a - 2h** were stirred for 1 h at 75 - 78 °C to give the target compounds **7a - 7h** in 40 - 65 % yields (Scheme 2). In this reaction, we found that the reaction time could be shortened by adding KI.



Scheme 2. General synthesis route for compounds **7a - 7h**.

Biological activity

The data for anticoccidial activities of the compounds **7a - 7h** are presented in Table 1. There are four aspects of changes when the chickens are infected by coccidia: their bodyweight gain would decrease, the survival rate might decline sometimes, lesion would be found in cecum and the oocyst of a new generation would be produced in the intestine. The data in the infected non-medicated group were 69.9, 100, 15 and 30 respectively. Compared to the non-infected non-

medicated group, the coccidiosis was obviously occurring in the infected non-medicated group, and the control was set up.

Table 1. Data for anticoccidial activities of compounds **7a - 7h** against *Eimeria tenella*

Test groups	Test compounds	RWG[a](%)	Survival rate (%)	Lesion value	Oocyst value	ACI[b]
1	7a	84.1	95	37	40	**102.1**
2	7b	100	100	7	20	**173**
3	7c	15.5	100	22	20	**73.5**
4	7d	99.0	100	15	30	**154**
5	7e	107	100	17	20	**170**
6	7f	78.6	100	16	30	**132.6**
7	7g	75.0	75	23	20	**107**
8	7h	101.9	100	11	30	**160.9**
9	decoquinte*****	101.3	100	12	1	188.3
10	ING[c]	69.9	100	15	30	124.9
11	NNG[d]	100	100	0	0	200

[a]Rate of relative bodyweight gain. [b]Anticoccidial activity index.
[c]Infected non-medicated group. [d]Non-infected non-medicated group.
*****27mg/Kg (Halofuginone is a highly effective anticoccidial medicine, but many *Eimeria tenella* strains in China developed resistance to it. According to literature, the Anticoccidial Index of halofuginone against eight *Eimeria tenella* strains derived from Heilongjiang, Beijing, Sichuan, Gansu province and Zhongshan, Xinhui, Dongguan and Jiangchun (Guangzhou suburb) of Guangdong province are 186.91, 155.63, 125.23, 175.23, 195.60, 180.13, 173.06 and 69.90 respectively. If halofuginone would be used as a control in the biological activity test, it is likely that the ACI of halofuginone is very low. This situation is therefore not effective to estimate the relative activity of the new synthetic compounds. Therefore, decoquinate was used as a control. Decoquinate is highly effective anticoccidial medicine at present and is widely applied in China at a dose of 27 mg/Kg).

A lesion value (0 - 40) could reflect the effect of *Eimeria* to chicken's cecum. The lesion values in the groups of compounds **7b** (7) and **7h** (11) were lower than the infected non-medicated group (15). This exhibited that compounds **7b** and **7h** could effectively reduce the effect of *Eimeria* to chickens' cecum. The oocyst value (0 - 40) reflected the amount of oocysts produced by the elder generation of coccidia. The oocyst values in the groups treated with compounds **7b** (20), **7c** (20), **7e** (20) and **7g** (20) were lower than the infected non-medicated group (30). This shows that compounds **7b**, **7c**, **7e** and **7g** could effectively suppress the generation of oocysts. Compared with the infected non-medicated group, the lesion and oocyst value of **7b** was reduced from 15 to 7 and 30 to 20, respectively. This indicates that compound

7b has an obvious anticoccidial efficacy against *E. tenella*. In the safe concentration for chickens, all the activity parameters might be improved with the increase of the concentration.

The weight gain and survival rate reflected the affection of drugs or coccidia on the growth of chickens. Compared with the infected non-medicated group, the weight gain was obviously improved from 69.9 to 100, 99, 107 and 101.9 by using compounds **7b**, **7d**, **7e** and **7h,** respectively. But the weight gain of chicken by adding compound **7c** was 15.5, which was lower than the infected non-medicated group. The reason might be that compound **7c** was toxic to chickens, which influenced their growth. The survival rates were 100 % in the test groups except **7a** (95) and **7h** (75). This result indicates that with a dose of 9 mg/kg in the chicken diet, the application of compounds **7b**, **7d**, **7e** and **7h** didn't affect the growth of chickens.

The anticoccidial index (ACI) could reflect the comprehensive anticoccidial ability of drugs, which was calculated from the growth rate, the survival rate, the lesion value and the oocyst value.

ACI = (weight gain + survival rate) × 100 - (Lesion value + oocyst value)

The ACI indicated that the compound **7b** (173) and **7e** (170) have obvious anticoccidial efficacy against *E. tenella* and compound **7d** (154.0) and **7h** (160.9) exhibited certain anticoccidial activities. The anticoccidial activity of the other compounds was not obvious. We selected only one concentration at random to test whether the synthesized compounds had anticoccidial activity without considering selecting the optimal concentration to obtain the best anti-coccidia index.

Analyzing the structures and activity of the 4-(2-methoxyphenyl)-2-oxo-butyl-quinazolinone derivatives, febrifugine and halofuginone, we found that (1) the quinazoline ring might play a vital role in the anticoccidial activity; (2) the introduction of halogen groups might change the anticoccidial activity; (3) the anticoccidial activity of these compounds probably have a relationship with the 2′-carbonyl and 3″-hydroxyl or 2″-methoxy.

Experimental Section

General Procedures. Substituted anthranilic acid **(1a - 1h)** were prepared according to the literature.[13-17] (E)-4-(2-hydroxyphenyl) but-3-en-2-one **(3)** was prepared according to the literature.[18] Other solvents and reagents were obtained from commercial sources and used without further purification. Melting Points were recorded on a XRC-1 apparatus (Sichuan University Instrument Inc, Chengdu, China) and the values are uncorrected. Proton NMR spectra and ^{13}C NMR spectra were recorded on Bruker AV - 400 MHz spectrometer (Bruker company, Switzerland) with $CDCl_3$ or d_6-dimethyl sulfoxide as the solvent. Chemical shift values (δ) were given in ppm and were downfield from internal tetramethylsilane. Mass spectra were recorded with Agilent 6210 (DOF-MAS) spectrometer (Agilent Inc, Santa Clara, CA, USA) using the EI method. IR spectra were recorded with a Perkin-Elmer 16PC-FT instrument (Perkin-Elmer Inc,

Norwalk Conn, CA, USA). Analytical TLC was performed on silica gel GF_{254}, and spots were visualized with ultraviolet light.

Synthesis of quinazoline and its derivatives (2a - 2h). General Procedure

A mixture of **1a - 1h** (0.06 mo1) and formamide 10.8 g (0.24 mo1) was stirred at 135 - 165 °C for 4 h, then 15 ml water was added and a lot of solid appeared immediately. The mixture was cooled to 60 °C slowly, and then 30 ml water was added again. The mixture was stirred for another 30 minutes. The resulting precipitate was filtered and recrystallized with ethanol to give **2a - 2h**.

Quinazolin-4(3H)-one (2a). White flocculent solid. Melting point: 214-215 °C (215.5-216. 5 °C). [19] Yield: 82.2 %. ^{1}H NMR ($CDCl_3$, 400M H_Z) δ: 10.41(br, NH, 1H), 8.32(d, C_5-H, J=8.0H_Z, 1H), 8.09(s, C_2-H, 1H), 7.80(m, C_7-H, C_8-H, 2H), 7.55(dd, C_6-H, J_1=7.6H_Z, J_2=6.8H_Z, 1H).

6-Bromoquinazolin-4(3H)-one (2b). White flocculent solid. Melting point: 267-269 °C (267 °C). [20] Yield: 62 %. ^{1}H NMR ($CDCl_3$, 400M H_Z) δ: 10.26 (br, NH, 1H); 8.44 (d, C_5-H, J=2.0 H_Z, 1H); 8.06 (s, C_2-H, 1H); 7.88 (dd, C_7-H, J_1=2.4 H_z, J_2=8.0 H_Z, 1H); 7.64 (d, C_8-H, J=8.8 H_Z, 1H).

6,8-dibromoquinazolin-4(3H)-one (2c). White flocculent solid. Melting point: 311-312 °C (>300 °C). [21] Yield: 69 %. ^{1}H NMR ($CDCl_3$, 400M H_Z) δ: 12.77 (br, NH, 1H); 8.37 (d, C_5-H, J=2.0H_Z, 1H); 8.26 (d, C_2-H, J=3.6Hz, 1H); 8.20 (d, C_6-H, J=2.0Hz, 1H).

6-Iodoquinazolin-4(3*H*)-one (2d). Purple solid. Melting point: 267-269 °C (270-272 °C). [22] Yield: 43.5 %. ^{1}H NMR (DMSO, 400M HZ) δ: 12.41 (br, NH, 1H); 8.38 (d, C_5-H, J=1.6Hz, 1H); 8.13 (s, C_2-H, 1H); 8.10 (dd, C_7-H, J_1=1.2Hz, J_2=5.6 Hz, 1H); 7.46(d, C_8-H, J=5.6 Hz, 1H).

8-Bromo-6-iodoquinazolin-4(3*H*)-one (2e). Brown solid. Melting point: 317-319 °C (316-317 °C). [22] Yield: 56.5 %. ^{1}H NMR (DMSO, 400M H_Z) δ: 12.66 (br, NH, 1H); 8.36 (s, C_5-H, 1H); 8.25 (d, C_2-H, J=2.4 Hz, 1H); 8.20 (s, C_7-H, 1H).

6-Chloroquinazolin-4(3*H*)-one (2f). White solid. Melting point: 259-260 °C (259-261 °C). [20] Yield: 70 %. ^{1}H NMR (DMSO, 400M H_Z) δ: 12.45 (br, NH, 1H); 8.13 (d, C_2-H, J=3.6Hz, 1H); 8.06 (d, C_5-H, J=2.4Hz, 1H); 7.85 (dd, C_7-H, J_1=2.4 Hz, J_2=8.4 Hz, 1H); 7.70 (d, C_8-H, J=8.4 Hz, 1H).

8-Chloroquinazolin-4(3*H*)-one (2g). White flocculent solid. Melting point: 300-302 °C (302 °C). [21] Yield: 65 %. ^{1}H NMR (DMSO, 400M H_Z) δ: 12.53(br, NH, 1H); 8.22 (s, C_2-H, 1H); 8.09 (dd, C_5-H, J_1=1.6 H_Z, J_2=8.0 H_Z, 1H); 7.98 (dd, C_7-H, J_1=1.6 H_Z, J_2=8.0 H_Z, 1H); 7.50(dd, C_6-H, J_1=7.6 H_Z, J_2=8.0 H_Z, 1H).

8-bromo-6-chloroquinazolin-4(3*H*)-one (2h). White solid. Melting point: 339-341 °C (341 °C). [22] Yield: 50 %. ^{1}H NMR (DMSO, 400M H_Z) δ: 12.69 (br, NH, 1H); 8.26 (d, C_5-H, J=2.4 Hz, 1H); 8.23 (s, C_2-H, 1H); 8.15 (d, C_7-H, J=2.4 Hz, 1H).

Synthesis of 4-(2-Hydroxyphenyl) butan-2-one (4)

7.2 g Pd/C was added to a water-warmed solution (50 °C) of **3** (58.50 g, 0.36 mol) in ethyl acetate (450 ml), the mixture was charged with hydrogen under stirring at 50 °C. When the

absorbing of hydrogen was stopped, the reaction was quenched and cooled to RT. The solid was filtered out; the organic layer was dried with anhydrous Na_2SO_4 and concentrated to give **4** as colorless oil. Yield: 98 %. ^{1}H NMR ($CDCl_3$, 400M Hz) δ: 7.68 (s, -OH, 1H), 7.09 (m, ArH, 2H), 6.85 (m, ArH, 2H), 2.86 (t, -CH_2CH_2-, *J*=7.6 Hz, 2H), 2.81 (t, -CH_2CH_2-, *J*=7.2 Hz, 2H), 2.13(s, 3H).

Synthesis of 4-(2-methoxyphenyl) butan-2-one (5)

A solution of 21.9 g (0.55 mol) of sodium hydroxide in 150 ml water was cooled to 0 °C and 54.0 g (0.33 mol) **4** was added under stirring. Then 45.8 g (0.36 mol) dimethyl sulfate was slowly added and the mixture was kept at 35 - 45 °C. The reaction mixture was allowed to stir at this temperature for another 0.5 h and was heated to 95 - 100 °C for 2 h, then cooled to room temperature and 80 ml water was added. The mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic phase was washed with 3 % NaOH until the aqueous layer was colorless. The organic layer was separated out, and dried over anhydrous Na_2SO_4, concentrated and distilled under reduced pressure (8 mmHg, 110 °C) to give 29.82 g of **5** as colorless oil. Yield: 50.7 %. ^{1}H NMR ($CDCl_3$, 400M Hz) δ: 7.19 (m, ArH, 2H), 6.85 (m, ArH, 2H), 3.81 (s, -OCH_3, 3H), 2.87 (t, -CH_2CH_2-, *J*=7.6 Hz, 2H), 2.72 (t, -CH_2CH_2-, *J*=7.2 Hz, 2H), 2.13(s, 3H).

Synthesis of 1-bromo-4-(2-methoxyphenyl) butan-2-one (6a)

A mixture of 26.8 g (0.167 mol) of bromine and 10 mL methanol was added dropwise to a solution of 29.82 g (0.167 mol) **5** in 100 mL methanol while stirring at 0 - 7 °C. The reaction mixture was stirred at room temperature until the color of Br_2 disappeared. Methanol was removed by distillation under reduced pressure. The residue was dissolved with 50 ml ethyl acetate, washed with 40 mL saturated $NaHCO_3$, 40 ml saturated NaCl and 40 ml water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a light yellow residue 41.55 g. The crude product was chromatographed on a silica gel column using petroleum ether–ethyl acetate (4:1 v/v) as the mobile phase to give **6a** and **6b** as yellow oil (5:1)**.** Yield: 80.6 %. **6a:** ^{1}H NMR ($CDCl_3$, 400M Hz) δ: 7.19 (m, ArH, 2H), 6.85 (m, ArH, 2H), 3.87 (s, -CH_2Br, 2H), 3.81 (d, -OCH_3, *J*=4 Hz, 3H), 2.93 (s, -CH_2CH_2-, 4H); **6b:** ^{1}H NMR ($CDCl_3$, 400M Hz) δ: 7.19 (m, ArH, 2H), 6.85 (m, ArH, 2H), 4.64 (t, -CHBr, *J*=7.2 Hz, 1H), 3.87 (s, -OCH_3, 3H), 3.44 (m, PhCH_2-, 1H), 3.15 (q, PhCH_2-, *J*=7.6 Hz, 1H), 2.32 (s, -CH_3CO-, 3H).

Synthesis of 4-(2-methoxyphenyl)-2-oxo-butyl-Quinazolinone derivatives (7a - 7h)

General procedure

A solution of 4.94 g **6** (0.0192 mol) in 10 mL DMSO was added dropwise to a mixture of **2a - 2h** (0.016 mol), 1.0 g KI, 0.45 g (0.018 mol) NaH and 30 ml DMSO under stirring at 75 °C. After 1 h the mixture was cooled to RT, and then 100 ml water and 70 ml ethyl acetate was added. The organic layer was separated, washed with 70 ml water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a crude product, which was chromatographed on a silica gel column using petroleum ether–ethyl acetate (8:1 v/v) as the mobile phase to give **7a - 7h.**

3-(4-(2-Methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7a). White flocculent solid, yield: 55.0 %. Melting point: 201-203 °C. ^{1}H NMR ($CDCl_3$, 400 M Hz) δ:8.29(dd, C_5-H, J_1=1.6 Hz, J_2=8.0 Hz, 1H), 8.05(s, C_2-H, 1H), 7.81(m, C_7-H, C_8-H, 2H), 7.55(td, C_6-H, J_1=2.4 Hz, J_2=8.0 Hz, 1H), 7.21(td, ArH, J_1=1.2 Hz, J_2=7.6 Hz, 1H), 7.15(dd, ArH, J_1=7.6 Hz, J_2=1.2 Hz, 1H), 6.89(m, ArH, 2H), 4.78(s, -CH_2-, 2H), 3.84(s, -OCH_3, 3H), 2.98(t, -CH_2CH_2-, *J*=7.2 Hz, 2H), 2.92(t, -CH_2CH_2-, *J*=7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): *δ* 201.77, 160.90, 156.54, 148.21, 146.26, 134.52, 132.80, 130.77, 130.39, 127.66, 127.43, 126.74, 120.87, 112.66, 112.01, 55.51, 54.11, 40.26, 24.47; IR(KBr) ν: 3432, 3064, 2933, 2833, 1722, 1671, 1615, 1490, 780cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{18}N_2O_3$: 323.1351 (M + H^+), found: 323.1390.

6-Bromo-3-(4-(2-methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7b). White flocculent solid, yield: 52.1 %. Melting point: 160-162 °C. ^{1}H NMR ($CDCl_3$, 400 M Hz) δ:8.41(d, C_5-H, *J*=2.4 Hz, 1H), 7.85(dd, C_7-H, J_1=2.4 Hz, J_2=8.8 Hz, 1H), 7.74(s, C_2-H, 1H), 7.60(d, C_8-H, *J*=8.4Hz, 1H), 7.21(td, ArH, J_1=1.6 Hz, J_2=7.6 Hz, 1H), 7.15(dd, ArH, J_1=1.6 Hz, J_2=7.2 Hz, 1H), 6.90(m, ArH, 2H), 4.73(s, -CH_2-, 2H), 3.84(s, -OCH_3, 3H), 2.98(t, -CH_2CH_2-, *J*=7.6 Hz, 2H), 2.92(t, -CH_2CH_2-, *J*=7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): *δ* 201.60, 159.13, 157.37, 147.34, 147.28, 140.56, 140.51, 130.19, 128.99, 128.23, 127.95, 124.10, 123.50, 120.89, 110.42, 55.28, 54.44, 40.28, 25.11; IR(KBr) ν: 3409, 3072, 2931, 2825, 1725, 1691, 1610, 1493, 750cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{17}BrN_2O_3$: 401.0417, 403.0397 (M + H^+, Isotopic Peak), found: 401.0427, 403.0410 (1:1).

6, 8-Dibromo-3-(4-(2-methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7c). White flocculent solid, yield: 50.0 %. Melting point: 170-172 °C. ^{1}H NMR ($CDCl_3$, 400 M Hz) δ:8.37(d, C_5-H, *J*=1.6 Hz, 1H), 8.16(d, C_7-H, *J*=2.0 Hz, 1H), 7.85(s, C_2-H, 1H), 7.21(td, ArH, J_1=1.6 Hz, J_2=7.6 Hz, 1H), 7.14(dd, ArH, J_1=1.6 Hz, J_2=7.2 Hz, 1H), 6.89(m, ArH, 2H), 4.69(s, -CH_2-, 2H), 3.84(s, -OCH_3, 3H), 2.98(t, -CH_2CH_2-, *J*=6.8 Hz, 2H), 2.90(t, -CH_2CH_2-, *J*=6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): *δ* 201.58, 159.14, 157.38, 147.33, 144.97, 140.52, 130.20, 129.00, 128.15, 127.99, 124.12, 123.49, 120.74, 120.66, 110.42, 55.28, 54.43, 40.28, 25.13; IR (KBr) ν: 3434, 3072, 2928, 2831, 1725, 1692, 1611, 1492, 749cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{16}Br_2N_2O_3$: 478.9523, 480.9503, 482.9483 (M+H^+, Isotopic Peak); found: 478.9525, 480.9501, 482.9489 (1:2:1).

6-Iodo-3-(4-(2-methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7d). Greenish brown solid, yield: 65.0 %. Melting point: 180-182 °C. ^{1}H NMR (DMSO, 400 M Hz) δ:8.41(d, C_5-H, *J*=2.0 Hz, 1H), 8.25(s, C_2-H, 1H), 8.15(dd, C_7-H, J_1=2.0 Hz, J_2=8.8 Hz, 1H), 7.50(d, C_8-H, *J*=8.4 Hz, 1H), 7.20(m, ArH, 2H), 6.96(d, ArH, *J*=8.4 Hz, 1H), 6.89(td, ArH, J_1=0.8 Hz, J_2=7.6 Hz, 1H), 4.98(s, -CH_2-, 2H), 3.79(s, -OCH_3, 3H), 2.88(t, -CH_2CH_2-, *J*=6.8 Hz, 2H), 2.80(t, -CH_2CH_2-, *J*=6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): *δ* 201.97, 159.45, 157.42, 147.52, 146.82, 143.23, 135.64, 130.22, 129.44, 128.28, 127.94, 123.50, 120.65, 110.44, 91.89, 55.27, 54.22, 40.28, 25.13; IR (KBr) ν: 3396, 3065, 2927, 2825, 1729, 1679, 1603, 1489, 753cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{17}IN_2O_3$: 449.0317 (M + H^+), found: 449.0374.

8-Bromo-6-iodo-3-(4-(2-methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7e). Heliotrope solid, yield: 40.0 %. Melting point: 164-166 °C. ^{1}H NMR ($CDCl_3$, 400M Hz) δ:8.37(d, C_5-H,

J=2.0 Hz, 1H), 8.16(d, C_7-H, *J*=2.0 Hz, 1H), 7.87(s, C_2-H, 1H), 7.22(td, ArH, J_1=1.2 Hz, J_2=8.0 Hz, 1H), 7.13(dd, ArH, J_1=1.6 Hz, J_2=7.6 Hz, 1H), 6.90(m, ArH, 2H), 4.69(s, -CH_2-, 2H), 3.84(s, -OCH_3, 3H), 2.97(t, -CH_2CH_2-, *J*=6.8 Hz, 2H), 2.91(t, -CH_2CH_2-, *J*=6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): δ 201.57, 159.16, 157.38, 147.31, 145.84, 140.54, 135.29, 130.21, 129.02, 128.15, 127.99, 124.13, 123.49, 120.67, 110.42, 55.28, 54.44, 40.28, 25.14; IR (KBr) ν: 3437, 3070, 2931, 2831, 1725, 1692, 1611, 1493, 794cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{16}BrIN_2O_3$: 526.9384, 528.9364 (M + H^+, Isotopic Peak), found: 526.9386, 528.9368 (1:1).

6-Chloro-3-(4-(2-methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7f). Yellow-white solid, yield: 45.0 %. Melting point: 130-132 °C. ^{1}H NMR ($CDCl_3$, 400M Hz) δ:8.24(d, C_5-H, *J*=1.6 Hz, 1H), 7.82(s, C_2-H, 1H), 7.71(m, C_7-H, C_8-H, 2H), 7.21(td, ArH, J_1=1.6 Hz, J_2=7.6 Hz, 1H), 7.14(dd, ArH, J_1=1.2 Hz, J_2= 7.6 Hz, 1H), 6.89(m, ArH, 2H), 4.78(s, -CH_2-, 2H), 3.84(s, -OCH_3, 3H), 2.98(t, -CH_2CH_2-, *J*=6.8 Hz, 2H), 2.91(t, -CH_2CH_2-, *J*=6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): δ 202.02, 159.88, 157.41, 146.70, 146.49, 134.90, 133.27, 130.25, 129.31, 128.25, 127.96, 126.17, 122.95, 120.64, 110.40, 55.26, 54.23, 40.28, 25.14; IR (KBr) ν: 3441, 3065, 2935, 2838, 1724, 1677, 1611, 1491, 747cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{17}ClN_2O_3$: 357.1007, 359.0972 (M + H^+, Isotopic Peak), found: 357.0971, 359.0948 (3:1).

8-Chloro-3-(4-(2-methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7g). White flocculent solid, yield: 48.0 %. Melting point: 168-170 °C. ^{1}H NMR ($CDCl_3$, 400 M Hz) δ:8.20(dd, C_5-H, J_1=1.6 Hz, J_2=8.0 Hz, 1H), 7.88(s, C_2-H, 1H), 7.86(dd, C_7-H, J_1=1.6 Hz, J_2=8.0 Hz, 1H), 7.44(t, C_6-H, J_1=7.6 Hz, J_2=8.0 Hz, 1H), 7.22(td, ArH, J_1=1.2 Hz, J_2=7.6 Hz, J_3=8.0 Hz, 1H), 7.13(dd, ArH, J_1=1.2 Hz, J_2=7.6 Hz, 1H), 6.89(m, ArH, 2H), 4.71(s, -CH_2-, 2H), 3.84(s, -OCH_3, 3H), 2.98(t, -CH_2CH_2-, *J*=6.8 Hz, 2H), 2.90(t, -CH_2CH_2-, *J*=6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): δ 201.88, 160.24, 157.38, 147.09, 144.88, 134.81, 131.87, 130.17, 128.25, 127.91, 127.48, 125.62, 123.45, 120.61, 110.38, 55.23, 54.38, 40.25, 25.06; IR (KBr) ν: 3422, 3078, 2936, 2831, 1722, 1688, 1608, 1494, 761cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{17}ClN_2O_3$: 357.1007, 359.0972 (M + H^+, Isotopic Peak), found: 357.0973, 359.0948 (3:1).

8-Bromo-6-chloro-3-(4-(2-methoxyphenyl)-2-oxobutyl) quinazolin-4(3*H*)-one (7h). White flocculent solid, yield: 57.1 %. Melting point: 172-174 °C. ^{1}H NMR ($CDCl_3$, 400M Hz) δ:8.15(d, C_5-H, *J*=2.0 Hz, 1H), 7.95(d, C_7-H, *J*=2.4 Hz, 1H), 7.78(s, C_2-H, 1H), 7.15(td, ArH, J_1=1.2 Hz, J_2=7.6 Hz, J_3=8.0 Hz, 1H), 7.06(dd, ArH, J_1=1.2 Hz, J_2=7.2 Hz, 1H), 6.82(m, ArH, 2H), 4.60(s, -CH_2-, 2H), 3.78(s, -OCH_3, 3H), 2.91(t, -CH_2CH_2-, *J*=6.8 Hz, 2H), 2.83(t, -CH_2CH_2-, *J*=6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400M Hz): δ 201.56, 159.27, 157.39, 147.19, 144.65, 137.94, 133.24, 130.18, 128.19, 127.97, 125.82, 123.78, 123.37, 120.67, 110.45, 55.27, 54.39, 40.29, 25.11; IR (KBr) ν: 3437, 3073, 2961, 2927, 1725, 1695, 1613, 1493, 748cm^{-1}; HRMS (ESI) Calculated for $C_{19}H_{16}BrClN_2O_3$: 435.0028, 437.0008, 439.9979 (M + H^+, Isotopic Peak), found: 435.0073, 437.0046, 439.0039 (3:4:1).

Biological assay

The anticoccidial activities of the compounds **7a - 7h** were evaluated according to the ACI method. Briefly, the chickens used to test the anticoccidial activity of compounds were 12-day-old broiler chickens bought from the Institute of Animal Science, Guangdong Province Academy of Agricultural Sciences. All chickens were fed by the feedstuff without any anticoccidial drugs and drank clean water.

Groups of these chickens were randomly housed in 11 cages with 20 in each. Groups 1 - 8 of 13-day-old chickens were fed the basal starter diet with the compounds **7a-7h** in 9 mg/Kg until the end of the test. Chicks in group 9 were fed the basal starter diet with decoquinte in 27 mg/Kg until the end of the test. Groups 1 - 10 of 14-day-old chickens were infected artificially with the Eimeria tenella spores of the oocysts 100 000. Held on observation for 7 days after infection, recorded the weight gain, survival rate, lesion scores, and oocysts scores of the chicken, and calculated the ACI.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 20372052).

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