Synthesis and Anticoccidial Activities of Quinoline Carboxylate Derivatives with Methyl (*E*)-2-(3-methoxy)acrylate Moiety

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A series of novel quinoline carboxylate derivatives with methyl (*E*)-2-(3-methoxy) acrylate group were designed and synthesized as anticoccidial medicines. The structures were confirmed by ¹H NMR, IR and HR-MS spectra. The biological activities were primarily evaluated according to the anticoccidial index method. The results indicated that these compounds (**7c**, **7d**, **7e**, **7g**) exhibited anticoccidial activities against *Eimeria tenella*. In particular, the anticoccidial index of 6-decyloxy-7-ethoxy-4-{6-[2-(2-methoxy-1-methoxycarbonyl-vinyl)phenoxy]pyrimidin-4-yloxy}-quinoline-3-carboxylic acid ethyl ester (**7e**) was 168.7, which indicated that the compound has a good anticoccidial activity.

Key Words: Quinoline carboxylate derivatives, Methyl (*E*)-2-(3-methoxy)acrylate, Anticoccidial activity, Identification, Synthesis.

INTRODUCTION

Coccidiosis is one of the most prevalent diseases in world's poultry industry that causes poor growth rate, high morbidity and high mortality rates^{1,2}. It is an infection of intestinal epithelium caused by protozoan parasite of the genus *Eimeria*^{3,4}. Anticoccidial agents are extensively used to control coccidiosis, but the coccidia was very easy to develop resistance to all of the drugs that have been introduced^{5,6}. Since there are no ways to restrain drug-resistance at present, the research work on new anticoccidial drugs should be continued.

Quinoline carboxylate is a kind of potent anticoccidial active substance against protozoan parasite genus *Eimeria*⁷. But the anticoccidial effects of quinoline carboxylate had declined in the poultry industry due to the development of drug resistance⁸. In this article quinoline carboxylate moiety, which was the key factor for the biological activity, was reserved and β-methoxy acrylic acid was introduced to synthesize new derivatives. The structure of the new compounds is different from traditional quinoline carboxylate, might be sensitive to drug -resistance coccidiosis.

Eight of novel ethyl quinoline carboxylate derivatives with methyl (*E*)-2-(3-methoxy)acrylate group at C-4 were designed and synthesized. The general synthetic methods of the target compounds (**7a-7h**) was shown in **Scheme-I**. The biological activities of these compounds (**7a-7h**) were primarily evaluated according to the anticoccidial index method^{9,10}. These compounds have good anticoccidial activities and can be used as anticoccidial drugs in the fowl.

EXPERIMENTAL

N,N-Dimethyl formamide was dried and distilled over anhydrous magnesium sulfate. Melting points were resolved using XRC-1 melting point apparatus (Sichuan University Instrument Inc., Chengdu, China) without being corrected. Analytical thin-layer chromatography was performed on silica plates GF254, the spots on TLC were visualized with ultra-violet (UV) light. Mass spectra (MS) were obtained with the Agilent 6210 (DOF-MAS) spectrometer (Agilent Inc., Santa Clara, CA, USA) using the electrospray ionization (ESI) method. ¹H NMR spectra were performed in CDCl₃ solution on a Varian Unity Inova-400 spectrometer (Varian Inc., Palo Alto, CA, USA) with TMS as the internal standard. A Perkin-Elmer 16PC-FT instrument (Perkin-Elmer Inc., Norwalk Conn, CA, USA) was used to determine the IR spectra. Intermediate **5e** was commercially acquirable. Intermediate **1** was synthesized according to the literature¹¹. Intermediate **5h** was synthesized according to the literature¹². Intermediates **5a-5d** and Intermediates **5f-5g** were synthesized according to the literature¹¹. Intermediate **6** was synthesized according to the literature¹³.

Synthesis

Synthesis of compound 3a¹⁴: A sample of 4-nitroso-2-methylphenol (intermediate **1**) (3.52 g, 25.6 mmol) was reduced by 10 % Pd/C (0.35 g) catalyzed hydrogenation in ethyl acetate (40 mL). The reaction was stirred at 40 °C for 5 h, cooled and directly used to react with diethylethoxymethylene-malonate

Scheme-I: Synthetic route of compounds 7a-7h

(EMME; 5.54 g, 25.6 mmol). The mixture was heated under reflux for 2 h. After the mixture was cooled and filtered off, the filtrate was purified through recrystallization in ethanol and dried to form intermediate **3** (yield 72-81 %).

Synthesis of intermediate 4a-d and intermediate 4f-h:

A mixture of intermediate **3** (0.79 g, 2.70 mmol), K₂CO₃ (0.41 g, 2.97 mmol) and bromoalkane (R-Br, 2.7 mmol) in dry DMF (5 mL) was stirred at 80 °C for 2 h. After the mixture was cooled to room temperature, water was added cooled. The resulted solids was filtered off, recrystallized in ethanol and dried to give intermediate **4** (yield 83-92 %).

Synthesis of Intermediates 5a-5d and intermediates 5f-5h¹²: Diphenyl ether (10 mL) was heated to 250 °C, then intermediate **4** was added and the mixture was kept at 250 °C for 8-12 min and then cooled to room temperature. Petroleum ether (20 mL) was added to the mixture and the resulted solid was filtrated, washed with petroleum ether and dried. The crude material was recrystallized in DMF and dried to give intermediate **5** (yield 43-49 %).

Synthesis of intermediate 6¹³: 2-[2-(6-Chloro-pyrimidin-4-yloxy)-phenyl]-3-methoxy-acrylic acid was prepared according to the literature¹³. In the synthesis of 2-[2-(6-chloro-pyrimidin-4-yloxy)-phenyl]-3-methoxy-acrylic acid methyl ester, sodium methoxide was freshly synthesized by sodium and methanol. And the crude product was chromatographed on silica gel using petroleum ether/ethyl acetate (4:1) as eluent to afford a pure product (yield 48 %). Intermediate **6** is known compound reported in literature¹³ and were identified by melting point test, ¹H NMR and IR, all data was the same with the literature¹³.

Synthesis of compound 7a-7h: A solution of intermediate **6** (0.51 g, 1.27 mmol) in 3 mL DMF was added dropwise to a mixture of intermediate **5a-h** (1.06 mmol), 0.06 g KI,

0.18 g (1.38 mmol) K₂CO₃ and 25 mL DMF under stirring at 90 °C. After 10 h the mixture was cooled to the room temperature and then 30 mL water was added. The solid was filtered off, the crude product was chromatographed on a silica gel column using petroleum ether/ethyl acetate (2:1 v/v) as the mobile phase to give the target molecule (yield 40-45 %).

4-{6-[2-(2-Methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-7-methyl-6-tetradecyloxy-quinoline-3-carboxylic acid ethyl ester (7a) : Light yellow solid; yield: 43 %; m.p. 135-138 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm.) = 0.876 (3H, t, *J* = 6.8 Hz), 1.261 (24H, m), 1.834 (3H, t, *J* = 6.8 Hz), 2.298 (3H, s), 3.612 (3H, s), 3.774 (3H, s), 4.103 (2H, t, *J* = 6.8 Hz), 4.398 (2H, q, *J* = 6.8 Hz), 6.957 (1H, s), 7.22-7.54 (6H, m), 7.820 (1H, s), 8.712 (1H, s), 8.861 (1H, s).

IR (KBr, ν_{max}, cm⁻¹): 3054, 2925, 2853, 1730, 1618, 1583, 1465, 1376, 1256, 1217, 1132, 1097, 1026, 943, 859, 802, 748, 689, 617, 550. HR-MS (ESI): Calcd. for C₄₂H₅₄N₃O₈ [M+H]⁺: 728.3911. Found: 728.3902.

6-Hexadecyloxy-4-{6-[2-(2-methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-7-methyl-quinoline-3-carboxylic acid ethyl ester (7b) : Light yellow solid; yield: 40 %; m.p. 136-138 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm.) = 0.871 (3H, t, *J* = 6.4 Hz), 1.252 (28H, m), 1.833 (3H, t, *J* = 6.0 Hz), 2.300 (3H, s), 3.610 (3H, s), 3.776 (3H, s), 4.099 (2H, t, *J* = 6.8 Hz), 4.402 (2H, q, *J* = 6.8 Hz), 6.983 (1H, s), 7.22-7.52 (6H, m), 7.817 (1H, s), 8.729 (1H, s), 8.858 (1H, s). IR (KBr, ν_{max}, cm⁻¹): 3054, 2962, 2924, 2853, 1710, 1619, 1551, 1464, 1395, 1262, 1217, 1097, 1022, 867, 801, 694, 621. HR-MS (ESI): calcd. for C₄₄H₅₈N₃O₈ [M+H]⁺: 756.4224. Found: 756.4200.

6-Decyloxy-4-{6-[2-(2-methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-7-methyl-quinoline-3-

TABLE-1
DATA FOR ANTICOCCIDIAL ACTIVITIES OF COMPOUNDS **7a-7h** AND THE
COMPOUND DECOQUINATE AGAINST *Eimeria tenella* AT 27 mg/Kg

Test groups	Test compounds (mg/Kg)	Rate of relative body weight gain	Survival rate (%)	Lesion scores	Oocyst scores	ACI ^a
1	7a (27)	75.8	85	30	20	110.8
2	7b (27)	70.3	85	30	20	105.3
3	7c (27)	87.7	90	20	20	137.7
4	7d (27)	86.7	90	20	20	137.7
5	7e (27)	88.7	100	10	10	168.7
6	7f (27)	72.8	85	30	20	107.8
7	7g (27)	73.4	90	20	20	123.4
8	7h (27)	73.8	85	20	20	108.8
9	Decoquinate (27)	91.3	100	10	1	180.3
10	ING ^b	60.0	80	30	30	80
11	NNG ^c	100.0	100	0	0	200

^aAnticoccidial activity index; ^bInfected non-medicated group; ^cNon-infected non-medicated group

carboxylic acid ethyl ester (7c): Light yellow solid; yield: 42 %; m.p. 132-134 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) = 0.884 (3H, t, *J* = 6.4 Hz), 1.278 (16H, m), 1.838 (3H, t, *J* = 6.4 Hz), 2.306 (3H, s), 3.617 (3H, s), 3.782 (3H, s), 4.107 (2H, t, *J* = 6.8 Hz), 4.408 (2H, q, *J* = 6.8 Hz), 6.999 (1H, s), 7.26-7.52 (6H, m), 7.824 (1H, s), 8.723 (1H, s), 8.866 (1H, s). IR (KBr, ν_{max}, cm⁻¹): 3054, 2958, 2925, 2853, 1706, 1618, 1550, 1449, 1395, 1262, 1217, 1096, 1023, 942, 874, 801, 687, 614, 551. HR-MS (ESI): calcd. for C₃₈H₄₆N₃O₈ [M+H]⁺: 672.3285. Found: 672.3264.

4-{6-[2-(2-Methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-7-methyl-6-octyloxy-quinoline-3-carboxylic acid ethyl ester (7d) : Light yellow solid; yield: 45 %; m.p. 130-132 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) = 0.886 (3H, t, *J* = 6.4 Hz), 1.248 (12H, m), 1.833 (3H, t, *J* = 6.8 Hz), 2.297 (3H, s), 3.608 (3H, s), 3.772 (3H, s), 4.100 (2H, t, *J* = 6.8 Hz), 4.398 (2H, q, *J* = 6.4 Hz), 6.955 (1H, s), 7.22-7.44 (6H, m), 7.817 (1H, s), 8.712 (1H, s), 8.858 (1H, s). IR (KBr, ν_{max}, cm⁻¹): 3052, 2963, 2926, 2855, 1707, 1617, 1551, 1449, 1398, 1262, 1217, 1022, 866, 801, 692, 613. HR-MS (ESI): calcd. for C₃₆H₄₂N₃O₈ [M+H]⁺: 644.2972. Found: 644.2950.

6-Decyloxy-7-ethoxy-4-{6-[2-(2-methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-quinoline-3-carboxylic acid ethyl ester (7e) : Light yellow solid; yield: 40 %; m.p. 140-142 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) = 0.873 (3H, t, *J* = 7.2 Hz), 1.264 (16H, m), 1.407 (3H, t, *J* = 6.8 Hz), 1.450 (3H, t, *J* = 6.8 Hz), 3.573 (3H, s), 3.761 (3H, s), 3.961 (2H, q, *J* = 6.8 Hz), 4.124 (2H, t, *J* = 6.4 Hz), 4.396 (2H, q, *J* = 7.2 Hz), 6.875 (1H, s), 6.994 (1H, s), 7.249 (1H, s), 7.32-7.47 (4H, m), 7.863 (1H, s), 8.706 (1H, s), 8.863 (1H, s). IR (KBr, ν_{max}, cm⁻¹): 3054, 2927, 2854, 1707, 1620, 1553, 1507, 1451, 1382, 1270, 1226, 1131, 1097, 1025, 992, 952, 877, 801, 723, 691, 621, 592, 560. HR-MS (ESI): calcd. for C₃₉H₄₇N₃O₉Na [M+Na]⁺: 724.3216. Found: 724.3210.

6-Dodecyloxy-4-{6-[2-(2-methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-7-methyl-quinoline-3-carboxylic acid ethyl ester (7f) : Light yellow solid; yield: 42 %; m.p. 135-136 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) = 0.881 (3H, t, *J* = 6.4 Hz), 1.228 (20H, m), 1.838 (3H, t, *J* = 6.8 Hz), 2.303 (3H, s), 3.616 (3H, s), 3.779 (3H, s), 4.105 (2H, t, *J* = 6.4 Hz), 4.406 (2H, q,

J = 6.8 Hz), 6.964 (1H, s), 7.22-7.45 (5H, m), 7.489 (1H, s), 7.823 (1H, s), 8.718 (1H, s), 8.864 (1H, s). IR (KBr, ν_{max}, cm⁻¹): 3055, 2926, 2853, 1706, 1618, 1551, 1450, 1395, 1267, 1218, 1131, 1098, 1025, 992, 943, 881, 802, 764, 723, 688, 615. HR-MS (ESI): calcd. for C₄₀H₅₀N₃O₈ [M+H]⁺: 700.3598. Found: 700.3573.

6-Benzyloxy-4-{6-[2-(2-methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-7-methyl-quinoline-3-carboxylic acid ethyl ester (7g): Light yellow solid; yield: 40 %; m.p. 156-158 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) = 1.424 (3H, t, *J* = 7.2 Hz), 2.374 (3H, s), 3.621 (3H, s), 3.788 (3H, s), 4.419 (2H, t, *J* = 7.2 Hz), 4.402 (2H, q, *J* = 6.8 Hz), 5.231 (2H, s), 7.000 (1H, s), 7.22-7.54 (11H, m), 7.950 (1H, s), 8.752 (1H, s), 8.870 (1H, s). IR (KBr, ν_{max}, cm⁻¹): 3060, 2936, 1732, 1707, 1627, 1558, 1489, 1447, 1384, 1252, 1213, 1168, 1134, 1101, 1021, 946, 855, 804, 758, 699, 622, 588, 461. HR-MS (ESI): calcd. for C₃₅H₃₁N₃O₈Na [M+Na]⁺: 644.2009. Found: 644.2009.

6-Benzyloxy-7-methoxy-4-{6-[2-(2-methoxy-1-methoxycarbonyl-vinyl)-phenoxy]-pyrimidin-4-yloxy}-quinoline-3-carboxylic acid ethyl ester (7h): Light yellow solid; yield: 40 %; m.p. 184-187 °C; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) = 1.412 (3H, t, *J* = 6.4 Hz), 3.573 (3H, s), 3.778 (3H, s), 3.818 (3H, s), 4.410 (2H, q, *J* = 7.2 Hz), 5.265 (2H, s), 6.911 (1H, s), 7.059 (1H, s), 7.22-7.50 (10H, m), 7.964 (1H, s), 8.733 (1H, s), 8.874 (1H, s). IR (KBr, ν_{max}, cm⁻¹): 3059, 2952, 1621, 1586, 1551, 1450, 1387, 1265, 1218, 1132, 1098, 1024, 944, 857, 802, 696, 462. HR-MS (ESI): calcd. for C₃₅H₃₁N₃O₉Na [M+Na]⁺: 660.1958. Found: 660.1959.

Biological assay: The anticoccidial activities of **7a-7h** were evaluated according to the anticoccidial index method, using decoquinate as a reference drug^{15,16}. The chickens used to test the anticoccidial activity of compounds were 15-day-old broiler chickens, which fed by the feedstuff without any anticoccidial drugs and drank clean water by Guangdong province academy of agricultural sciences. Then these chickens were randomly divided into 11 groups, 20 in each group. Groups 1-9 of these chickens were fed the diet with the compounds **7a-7h** or decoquinate in 27 mg/Kg. Groups 1-10 of these chickens were infected factitiously with the *Eimeria tenella*. After 7 days, recorded the weight gain, mortality,

dropping scores, lesion scores and oocysts scores of the chickens to calculate the anticoccidial index.

RESULTS AND DISCUSSION

Synthesis: The previous steps were almost synthesized according to the literatures, the final and most important step is to synthesize target compounds. Primarily NaH, quinoline carboxylates and intermediate 6 were stirred at 90 °C for 5 h in order to give the target compounds, but formed lots of by-products and the purification process was very difficult. Subsequently, K₂CO₃ was used in place of NaH to get target compounds, formed little by-products and the purification process become easy.

Temperature was another principal influencing element. The synthetic reaction of **7a-h** ran slowly at room temperature. The elevation of temperature could accelerate the process, 90~95 °C was the optimal condition.

The amount of intermediate **6** should be excessive to intermediate **5**, because intermediate **5** was difficult to scrub out. The ratio of intermediate **5a-5h** : K₂CO₃: intermediate **6** was set at 1:1.3:1.2.

In this reaction, we found that the reaction time could be shortened by adding KI as a catalyze.

Biological activity: The results of anticoccidial activities of the compounds **7a-7h** are listed in Table-1. The results showed that four of the target compounds exhibited good anticoccidial activities with a dose of 27 mg/Kg. Among them, compound **7e** showed strong anticoccidial activity with anticoccidial index 168.7, might be developed as available anticoccidial drugs. Meanwhile compounds **7c**, **7d** and **7g** exhibited obvious anticoccidial activities, with anticoccidial index 137.7, 137.7 and 123.4.

Conclusion

A series of novel quinoline carboxylate derivatives with methyl(*E*)-2-(3-methoxy) acrylate group were designed and

synthesized as anticoccidial medicines. The anticoccidial activity results indicated that compound **7e** showed a high anticoccidial activity at a dose of 27 mg/Kg, might be a new anticoccidial drug.

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