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Synthesis and Anticoccidial Activities of Novel N-(2-Aminophenyl)-2-quinazolinone-acetamide Hydrochloride

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Eight novel N-(2-aminophenyl)-2-quinazolinone-acetamide hydrochloride were synthesized and their structures were identified by ^{1}H NMR, MS and IR spectra. Seven of the new compounds were chosen for anticoccidial activity test and the results showed that N-(2-aminophenyl)-2-(6-methyl-8-bromo quinazolinone)acetamide hydrochloride (**3h**) exhibited anticoccidial activity against *Eimeria tenella* in the chicken' diet with a dose of 18 mg/Kg.

Key Words: N-(2-Aminophenyl)-2-quinazolinone-acetamide hydrochloride, Anticoccidial activity, *Eimeria tenella*.

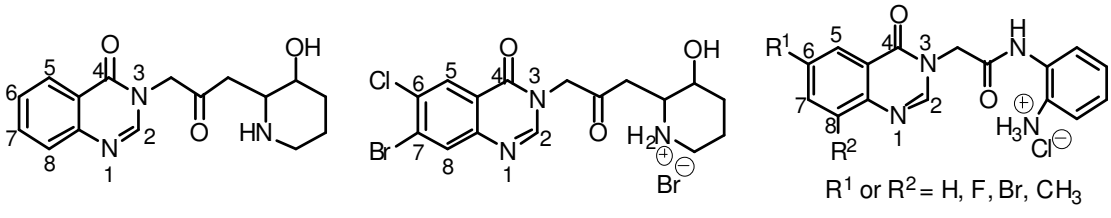
INTRODUCTION

Coccidiosis is an intestinal infection caused by protozoan parasites of the genus Eimeria resulting in intestinal lesions, diarrhoea, enteritis and death[1,2], which occurs all over the world and leads to extensive loss in the poultry industry[3]. Many drugs are effective in the prevention and treatment of coccidiosis, but the coccidia are inevitably to develop resistance to them[4,5]. And the drug resistance has developed almost to all of the anticoccidial drugs introduced so far[5]. Therefore, the search and discovery of new anticoccidial activity drugs to be needed urgently.

Our team designed and synthesized some anticoccidial compounds based on the structures of hydroxyquinoline-carboxylates and febrifugine, respectively. And we found some of them have anticoccidial activities[6-13].

Febrifugine (Fig. 1, compound **1**) have anticoccidial activity which is the effective component of Dichroa febrifuga. American Cyanamid Company designed and synthesized a anticoccidial drug---halofuginone based on the structure of febrifugine in 1967[14,15]. Halofuginone (Fig. 1, compound **2**) is a high anticoccidial activity, broad-spectrum and low toxicity anticoccidial drug. However, the synthetic route of halofuginone is long and the raw material is hard to synthesized, which leads to a high cost and limits its application.

In order to find novel and high anticoccidial activities drugs with easy synthesis and lower cost, You *et al.*[11,12], Zhan *et al.*[13] designed and synthesized some quinazolinone derivatives, respectively based on the structure of febrifugine. The anticoccidial activities of these compounds were evaluated and the results indicated that these compounds exhibited anticoccidial activities against *Eimeria tenella*. In this paper, eight novel N-(2-aminophenyl)-2-quinazolinone-acetamide hydrochloride (Fig. 1, compound **3**) were designed and synthesized according to the structure of febrifugine. The synthesis route is showed in **Scheme-I**. The structure characteristics of



1 **2** **3**

Fig. 1. Structures of compounds **1-3**

the new compounds are as follows: first, the quinazolinone ring of febrifugine is retained; second, the piperidine ring-acetone is replaced by N-(2-aminophenyl)-acetamide. The structures of the new compounds are similar with halofuginone. Therefore, the recognitions between molecules and enzymes in the coccidia bodies should also be similar with halofuginone. The new compounds would have anticoccidial activities. Seven of them were chosen for anticoccidial activity test according to the ACI method.

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 DMSO-d_6 as the solvent and TMS as the internal standard and mass spectra on Agilent 6210 Series Time of Flight LC/MS using the ESI source. IR spectra were recorded with Perkin-Elmer 16PC-FT instrument. Analytical thin-layer chromatography was carried out on precoated plates (silica gel GF_{254}) and spots were visualized with ultraviolet light.

General procedure for preparation of 4[16]: Bromoacetyl chloride (7.12 g, 45.2 mmol) was added dropwise to a solution of *o*-nitroaniline (5.80 g, 42.0 mmol) in dichloromethane (60 mL) at 0 ºC. Then, pyridine (3.32 g, 42.0 mmol) was slowly added dropwise at 0 ºC. The resulting mixture was stirred for 5 h at room temperature. And the mixture was washed with 3 mL × 20 mL brine and 3 mL × 20 mL water and dried over anhydrous $MgSO_4$. After evaporation of solvent, the crude products were obtained and were recrystallized in a mixed solution of ethanol and water to provide intermediates **4** in 74.1 % yield.

General procedure for preparation of 5a-h: The compounds **5a-h** were synthesized using the similar reaction conditions in reference[11].

8-Fluoroquinazolin-4(3*H*)-one (5a): Brown solid; m.p. 271-273 ºC (lit.[17] 272-273 ºC).

6-Bromo-8-fluoroquinazolin-4(3*H*)-one (5b): Dark brown solid; m.p. > 300 ºC; HR-MS (ESI): Calcd. (%) for $C_8H_9BrFN_2O^+$ $(M + H)^+$: 242.9557, 244.9543 (1:1). The mass spectrum data correspond to lit.[18].

6-Fluoroquinazolin-4(3*H*)-one (5c): Brown solid; m.p. 248-249 ºC (lit.[19] 250 ºC).

6-Fluoro-8-bromoquinazolin-4(3*H*)-one (5d): Yellowish-brown solid; m.p. > 300 ºC; HR-MS (ESI): Calcd. (%) for $C_8H_9BrFN_2O^+$ $(M + H)^+$: 242.9565, 244.9547 (1:1). The mass spectrum data correspond to lit.[20].

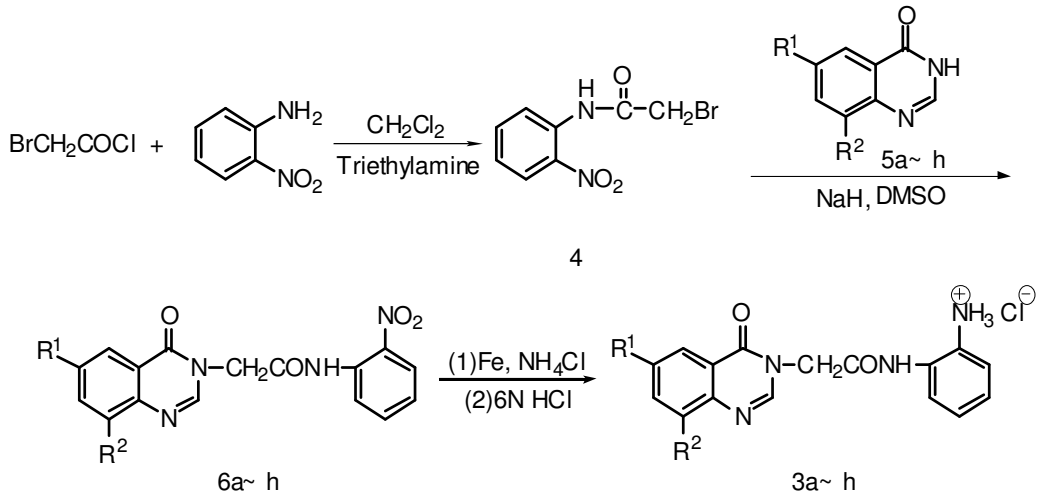
8-Methylquinazolin-4(3*H*)-one (5e): Yellow solid; m.p. 247-249 ºC (lit.[19] 249 ºC).

6-Bromo-8-methylquinazolin-4(3*H*)-one (5f): Brown solid; m.p. > 300 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 12.47 (s, 1H), 8.17 (s, 1H), 8.04 (d, J = 2.0 Hz, 1H), 7.89 (s, 1H), 2.53 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3364, 3199, 3079, 2884, 2631, 2362, 1945, 1861, 1777, 1683, 1609, 1456, 1403, 1326, 1271, 1220, 1162, 1048, 1002, 947, 888, 837, 797, 699, 595, 508, 480; HR-MS (ESI): Calcd. (%) for $C_9H_8BrN_2O^+$ $(M + H)^+$: 238.9815; found (%): 238.9816, 240.9798(1: 1).

6-Methylquinazolin-4(3*H*)-one (5g): Ivory-white solid; m.p. 253-255 ºC (lit.[19] 256 ºC).

8-Bromo-6-methylquinazolin-4(3*H*)-one (5h)[21]: Yellow brown solid; m.p. > 300 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 12.43 (s, 1H), 8.16 (s, 1H), 8.010 (s, 1H), 7.93 (s, 1H), 2.43 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3359, 3184, 3063, 2919, 2624, 1944, 1777, 1679, 1606, 1543, 1459, 1397, 1321, 1272, 1250, 1213, 1144, 1086, 1038, 904, 870, 798, 741, 605, 566, 530, 442; HR-MS (ESI): Calcd. (%) for $C_9H_8BrN_2O^+$ $(M + H)^+$: 238.9815; found (%): 238.9808, 240.9794(1: 1).

General procedure for preparation of 6a-h: A solution of **4** (11.7 mmol) in 15 mL DMSO was added dropwise to a mixture of **5a-h**[11] (9.33 mmol), KI (0.5 g), NaH (0.28 g, 11.7 mmol) and 40 mL DMSO under stirring at 75 ºC. The reaction mixture was stirred at this temperature for 1.5-3.0 h and then cooled to room temperature. Water (50 mL) was added to the



3a: R^1 = H, R^2 =F; 3b: R^1 = Br, R^2 = F; 3c: R^1 = F, R^2 = H; 3d: R^1 = F, R^2 = Br;
3e: R^1 = H, R^2 = CH_3; 3f: R^1 = Br, R^2 = CH_3; 3g: R^1 = CH_3, R^2 = H; 3h: R^1 = CH_3, R^2 = Br.

Scheme-I: General synthesis route for compounds **3a-h**

mixture and the obtained solid was filtrated and recrystallized with ethanol to give **6a-h** in 81-87 % yield. The physical and spectra data of the compounds **6a-h** are as follows.

N-(2-Nitrophenyl)-2-(8-fluoro-quinazolinone)acetamide (6a): Light-yellow solid; yield: 84 %; m.p. 220-221 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.82 (s, 1H), 8.72 (d, *J* = 8.4 Hz, 1H), 8.25 (d, *J* = 7.6 Hz, 1H), 8.14-8.11 (m, 2H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.57-7.47 (m, 2H), 7.24 (d, *J* = 8.0 Hz, 1H), 4.88 (s, 2H); IR (KBr, ν_{max}, cm^{-1}) 3439, 3282, 1676, 1609, 1520, 1479, 1366, 1338, 1284, 1254, 1152, 1035, 947, 788, 763; HR-MS (ESI): Calcd. (%) for $C_{16}H_{12}FN_4O_4^+$ (M + H)$^+$: 343.0837; found (%): 343.0830.

N-(2-Nitrophenyl)-2-(6-bromo-8-fluoro-quinazolinone)-acetamide (6b): Light-yellow solid; yield: 86 %; m.p. 208-209 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.81 (s, 1H), 8.71 (d, *J* = 8.4 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 2H), 8.13 (s, 1H), 7.67 (t, *J* = 7.2 Hz, 2H), 7.28-7.24 (m, 1H), 4.88 (s, 2H); IR (KBr, ν_{max}, cm^{-1}) 3433, 3298, 1693, 1608, 1507, 1469, 1363, 1331, 1276, 1250, 1177, 1046, 966, 867, 797, 745, 714; HR-MS (ESI): Calcd. (%) for $C_{16}H_{11}BrFN_4O_4^+$ (M + H)$^+$: 420.9942; found (%): 420.9950, 422.9928(1 :1).

N-(2-Nitrophenyl)-2-(6-fluoro-quinazolinone)acetamide (6c): Light-yellow solid; yield: 83 %; m.p. 196-198 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.81 (s, 1H), 8.72 (d, *J* = 8.4 Hz, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 8.10 (s, 1H), 7.97 (dd, J_1 = 2.8 Hz, *J* 2 = 8.4 Hz, 1H), 7.80 (dd, J_1 = 4.4 Hz, J_2 = 4.8 Hz, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.53 (td, J_1 = 2.8 Hz, J_2 = 8.4 Hz, 1H), 7.24 (d, *J* = 7.6 Hz, 1H), 4.88 (s, 2H); IR (KBr, ν_{max}, cm^{-1}) 3432, 3317, 1673, 1613, 1587, 1544, 1510, 1483, 1404, 1349, 1280, 1158, 964, 847, 787, 743, 708, 621; HR-MS (ESI): Calcd. (%) for $C_{16}H_{12}FN_4O_4^+$ (M + H)$^+$: 343.0837; found (%): 343.0841.

N-(2-Nitrophenyl)-2-(6-fluoro-8-bromo-quinazolinone)-acetamide (6d): Yellow solid; yield: 87 %; m.p. 251-252 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.81 (s, 1H), 8.71 (d, *J* = 8.4 Hz, 1H), 8.25 (dd, J_1 = 1.6 Hz, J_2 = 8.4 Hz, 1H), 8.17 (s, 1H), 7.97 (dd, J_1 = 2.8 Hz, J_2 = 8.0 Hz, 1H), 7.88 (dd, J_1 = 2.8 Hz, J_2 = 8.4 Hz, 1H), 7.67 (t, *J* = 8.4 Hz, 1H), 7.27-7.23 (m, 1H), 4.88 (s, 2H); IR (KBr, ν_{max}, cm^{-1}) 3434, 3298, 3074, 1687, 1611, 1552, 1513, 1465, 1386, 1440, 1282, 1164, 961, 878, 795, 739, 630; HR-MS (ESI): Calcd. (%) for $C_{16}H_{11}BrFN_4O_4^+$ (M + H)$^+$: 420.9942; found (%): 420.9936, 422.9920(1:1).

N-(2-Nitrophenyl)-2-(8-methyl-quinazolinone)-acetamide (6e): Light-yellow solid; yield: 80 %; m.p. 210-212 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.81 (s, 1H), 8.72 (d, *J* = 8.4 Hz, 1H), 8.24-8.18 (m, 2H), 8.12 (s, 1H), 7.67-7.63 (m, 2H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 4.87 (s, 2H), 2.65 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3433, 3271, 1688, 1609, 1550, 1514, 1371, 1337, 1283, 1154, 962, 775, 734; HR-MS (ESI): Calcd. (%) for $C_{17}H_{15}N_4O_4^+$ (M + H)$^+$: 339.1088; found (%): 339.1089.

N-(2-Nitrophenyl)-2-(6-bromo-8-methyl-quinazolinone)-acetamide (6f): Yellow solid; yield: 82 %; m.p. 236-238 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.80 (s, 1H), 8.71 (d, *J* = 8.4 Hz, 1H), 8.31 (d, *J* = 2.0 Hz, 1H), 8.24 (dd, J_1 = 1.2 Hz, J_2 = 8.4 Hz, 1H), 8.16 (s, 1H), 7.76 (d, J_1 = 1.6Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.24-7.22 (m, 1H), 4.88 (s, 2H), 2.63 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3432, 3309, 1701, 1610, 1555, 1513, 1459, 1337, 1283, 1162, 962, 877, 796, 741, 633; HR-MS (ESI): Calcd. (%) for $C_{17}H_{14}BrN_4O_4^+$ (M + H)$^+$: 417.0193; found (%): 417.0180, 419.0165(1 :1).

N-(2-Nitrophenyl)-2-(6-methyl-quinazolinone)acetamide (6g): Ivory-white solid; yield: 78 %; m.p. 232-234 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.81 (s, 1H), 8.72 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 8.13 (s, 1H), 8.09 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 2H), 7.23 (t, *J* = 8.0 Hz, 1H), 4.87 (s, 2H), 2.51 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3429, 3238, 1689, 1609, 1522, 1382, 1340, 1283, 1166, 962, 838, 738; HR-MS (ESI): Calcd. (%) for $C_{17}H_{15}N_4O_4^+$ (M + H)$^+$: 339.1088; found (%): 339.1083.

N-(2-Nitrophenyl)-2-(6-methyl-8-bromo-quinazolinone)-acetamide (6h): Light-yellow solid; yield: 81 %; m.p. 261-263 ºC; ^{1}H NMR spectrum (400 MHz; $CDCl_3$-d_6; TMS): δ (ppm) = 10.81 (s, 1H), 8.72 (d, *J* = 8.4 Hz, 1H), 8.25 (s, 1H), 8.23 (s, 1H), 8.10 (s, 1H), 7.93 (s, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 4.89 (s, 2H), 2.49 (s, 3H); IR (KBr, ν_{max}, cm-1) 3431, 3287, 2920,, 1686, 1610, 1511, 1466, 1367, 1339, 1281, 1169, 958, 855, 795, 738; HR-MS (ESI): Calcd. (%) for $C_{17}H_{14}BrN_4O_4^+$ (M + H)$^+$: 417.0193; found (%): 417.0182, 419.0167(1 :1).

General procedure for preparation of 3a-h: A mixture of **6a-h** (1.66 mmol), Fe (0.28 g, 5.00 mmol)), NH_4Cl (0.80 g, 15.00 mmol), tetrabutylammonium bromide (0.30 g), ethyl alcohol (25 mL) and water (5 mL) was stirred and refluxed at 70 ºC for 0.5-2.0 h. The mixture was filtered after cooled to room temperature then red-brown solid was obtained. This solid was dissolved in DMSO (10-20 mL) and then filtered. Yellow filtrate was retained. Water (100 mL) was added to the filtrate and a lot of solid appeared immediately. The resulting precipitate was filtered and acidified with HCl to give **3a-h** in 69-81 % yield. The physical and spectra data of the compounds **3a-h** are as follows.

N-(2-Aminophenyl)-2-(8-fluoro-quinazolinone)acetamide hydrochloride (3a): Light-yellow solid; yield: 72 %; m.p. 207-210 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 10.59 (s, 1H), 8.47 (s, 1H), 7.98 (d, *J* = 7.6 Hz, 1H), 7.78-7.73 (m, 1H), 7.57 (dt, J_1 = 4.8 Hz, J_2 = 8.0 Hz,1H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.30-7.15 (m, 3H), 4.98 (s, 2H), 6.00-3.60 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3421, 3232, 2986, 2823, 2591, 2021, 1910, 1800, 1720, 1654, 1618, 1544, 1498, 1423, 1391, 1366, 1344, ,1296, 1251, 1218, 1185, 1175, 1155,1109, 1024, 963, 934, 908, 878, 839, 795, 745, 707, 654, 610, 574, 549, 501, 466; HR-MS (ESI): Calcd. (%) for $C_{16}H_{14}FN_4O_2^+$ (M - Cl)$^+$: 313.1095; found (%): 313.1091.

N-(2-Aminophenyl)-2-(6-bromo-8-fluoro-quinazolinone)-acetamide hydrochloride (3b): Ivory-white solid; yield: 74 %; m.p. 221-224 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 10.38 (s, 1H), 8.52 (s, 1H), 8.12-8.09 (m, 2H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.20-7.00 (m, 3H), 4.97 (s, 2H), 5.70-3.30 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3430, 3266, 3117, 3078, 2828, 2542, 1949, 1898, 1687, 1611, 1560, 1530, 1478, 1393, 1360, 1332, 1286, 1254, 1212, 1169, 1111, 1054, 966, 911, 867, 808, 788, 760, 680, 613, 570, 548, 493, 451; HR-MS (ESI): Calcd. (%) for $C_{16}H_{13}BrFN_4O_2^+$ (M-Cl)$^+$: 391.0200; found (%): 391.0193, 393.0177 (1 :1).

N-(2-Aminophenyl)-2-(6-fluoro-quinazolinone)acetamide hydrochloride (3c): Yellow solid; yield: 69 %; m.p. 217-220 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 10.71 (s, 1H), 8.44 (s, 1H), 7.86-7.75 (m, 3H), 7.46-7.42 (m, 1H), 7.34-7.26 (m, 3H), 5.00 (s, 2H), 6.20-4.40 (s, 3H); IR (KBr, ν_{max}, cm-1) 3420, 3206, 2824, 2589, 1716, 1666, 1620, 1586, 1560,, 1532, 1491, 1457, 1391, 1361, 1294, 1272, 1195, 1151, 1121, 1056, 1007, 966, 905, 840, 769, 744, 699, 643, 569, 544, 522, 493, 453, 424, 403; HR-MS (ESI): Calcd. (%) for $C_{16}H_{14}FN_4O_2^+$ (M - Cl)$^+$: 313.1095; found (%): 313.1092.

N-(2-Aminophenyl)-2-(6-fluoro-8-bromo-quinazolinone)-acetamide hydrochloride (3d): Light-yellow solid; yield: 74 %; m.p. 226-230 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 9.75 (s, 1H), 8.49 (s, 1H), 8.26 (dd, J_1 = 2.8 Hz, J_2 = 8.4 Hz, 1H), 7.90 (dd, J_1 = 2.8 Hz, J_2 = 8.4 Hz, 1H), 7.15 (d, J = 7.6 Hz, 1H), 6.98 (t, J = 7.2 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.63 (t, J = 7.2 Hz, 1H), 4.90 (s, 2H), 4.20-3.10 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3428, 3249, 1690, 1656, 1612, 1542, 1499, 1462, 1399, 1328, 1111, 963, 878, 799, 750, 579; HR-MS (ESI): Calcd. (%) for $C_{16}H_{13}BrFN_4O_2^+$ (M - Cl)$^+$: 391.0200; found (5): 391.0195, 393.0179 (1 :1).

N-(2-Aminophenyl)-2-(8-methyl-quinazolinone)-acetamide hydrochloride (3e): Yellow solid; yield: 72 %; m.p. 236-240 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 10.82 (s, 1H), 8.47 (s, 1H), 8.01 (d, J = 7.2 Hz, 1H), 7.73 (d, J = 7.2 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.42-7.28 (m, 3H), 9.00-6.00 (s, 3H), 5.00 (s, 2H), 2.57 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3421, 2961, 2579, 1712, 1658, 1621, 1587, 1544, 1493, 1459, 1384, 1358, 1334, 1293, 1225, 1194, 1156, 1130, 1064, 1034, 990, 915, 863, 761, 733, 802, 670, 599, 540, 488, 459; HR-MS (ESI): Calcd. (%) for $C_{17}H_{17}N_4O_2^+$ (M - Cl)$^+$: 309.1346; found (%): 309.1343.

N-(2-Aminophenyl)-2-(6-bromo-8-methyl-quinazolinone)-acetamide hydrochloride (3f): Light-yellow solid; yield: 70 %; m.p. 210-213 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 10.70 (s, 1H), 8.50 (s, 1H), 8.08 (d, J = 2.0 Hz, 1H), 7.94 (d, J = 1.2 Hz, 1H), 7.43-7.20 (m, 4H), 7.20-6.00 (s, 3H), 4.99 (s, 2H), 2.56 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3424, 2801, 2580, 1716, 1658, 1617, 1577, 1540, 1493, 1459, 1385, 1358, 1331, 1289, 1221, 1195, 1159, 1129, 1040, 1001, 964, 872, 794, 764, 702, 665, 601, 544, 488, 456; HR-MS (ESI): Calcd. (%) for $C_{17}H_{16}BrN_4O_2^+$ (M - Cl)$^+$: 387.0451; found (%): 387.0445, 389.0429 (1: 1).

N-(2-Aminophenyl)-2-(6-methyl-quinazolinone)-acetamide hydrochloride (3g): Ivory-white solid; yield: 81 %; m.p. 229-232 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 10.63 (s, 1H), 8.41 (s, 1H), 7.97 (s, 1H), 7.70 (dd, J_1 = 1.6 Hz, J_2 = 8.4 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 7.42-7.40 (m, 1H), 7.30-7.24 (m, 3H), 6.00-4.00 (s, 3H), 4.97 (s, 2H), 2.47 (s, 3H); IR (KBr, ν_{max}, cm-1) 3421, 3267, 2979, 2960, 2481, 2039, 1922, 1798, 1711, 1671, 1613, 1579, 1535, 1493, 1454, 1379, 1309, 1266, 1224, 1198, 1176, 1137, 1027, 981, 901, 868, 825, 783, 757, 696, 663, 619, 538, 496, 454, 423; HR-MS (ESI): Calcd. (%) for $C_{17}H_{17}N_4O_2^+$ (M - Cl)$^+$: 309.1346; found (%): 309.1346.

N-(2-Aminophenyl)-2-(6-methyl-8-bromo-quinazolinone)-acetamide hydrochloride (3h): Yellow solid; yield: 75 %; m.p. 193-195 ºC; ^{1}H NMR spectrum (400 MHz; DMSO-d_6; TMS): δ (ppm) = 9.92 (s, 1H), 8.43 (s, 1H), 8.06 (d, J = 1.6 Hz, 1H), 7.97 (s, 1H), 7.20 (d, J = 7.2 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.89 (d, J = 7.6 Hz, 1H), 6.80-7.70 (m, 1H), 4.90 (s, 2H), 4.20-3.20 (s, 3H), 2.46 (s, 3H); IR (KBr, ν_{max}, cm^{-1}) 3433, 3249, 2922, 2535, 1669, 1612, 1528, 1465, 1399, 1127, 754, 616; HR-MS (ESI): Calcd. (%) for $C_{17}H_{16}BrN_4O_2^+$ (M - Cl)$^+$: 387.0451; found (%): 387.0447, 389.0431 (1: 1).

Biological assay: The anticoccidial activities of the compounds **3a-d** and **3f-h** were evaluated according to the anticoccidial index method[22-24], using decoquinate and diclazuril as reference drug. Briefly, the chickens used to test the anticoccidial activity of compounds were brought and fed to 12-day-old by the feed stuff without using any anticoccidial drugs.

Groups of the chickens were randomly housed, with 15 in each cage and 11 cages were randomly assigned by tier. Group 1-9 of 13-day-old chickens were fed the basal starter diet with the compounds **3a-d** (18 mg/Kg) or **3f-h** (18 mg/Kg) or decoquinate (27 mg/Kg), or diclazuril (1 mg/Kg) until the end of the test. Group1-10 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, mortality, lesion scores and oocysts scores of the chicken and calculated the anticoccidial index (ACI). Results of test are given in Table-1.

RESULTS AND DISCUSSION

As shown in **Scheme-I**, intermediates **6a-h** were synthesized by the reaction of intermediate **4** and **5a-h** with the NaH.

TABLE-1
DATA FOR ANTICOCCIDIAL ACTIVITIES OF COMPOUNDS **3a-d**, **3f-h**, DECOQUINATE AND DICLAZURIL AGAINST *Eimeria tenella*

Test groups	Test compounds (mg/Kg)	Rate of relative body weight gain	Survival rate (%)	Lesion scores	Oocyst scores	ACI[a]
1	**3a** (18)	46.8	100	30	40	76.8
2	**3b** (18)	76.6	100	28	40	108.6
3	**3c** (18)	34.1	100	20	20	94.1
4	**3d** (18)	63.9	93.3	25	40	92.2
5	**3f** (18)	59.6	100	28	40	91.6
6	**3g** (18)	46.8	100	29	40	77.8
7	**3h** (18)	68.1	100	26	20	122.1
8	Decoquinate (27)	71.7	100	13	0	158.7
9	Diclazuril (1)	53.5	93.3	20	20	106.8
10	ING[b]	63.0	86.7	28	40	81.7
11	NNG[c]	100	100	0	0	200

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

To ensure the intermediates **5a-h** were reacted completely, the intermediate **4** must be in excess. And the overdose of intermediate **4** will to be removed by the washing with ethanol or ethyl acetate. Using of this method, we can obtain the intermediates **6a-h** with good purity and high yield.

Biological activity: The data for anticoccidial activities of the compounds **3a-d** and **3f-h** were 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 obviously with ACI 81.7. And in the negative control group, no coccidiosis in chickens was occurred. So the control was set up. The result revealed that N-(2-aminophenyl)-2-(6-methyl-8-bromo quinazolinone)acetamide hydrochloride (**3h**) has anticoccidial activity against *Eimeria tenella* with ACI 122.1. And the compound **3h** exhibited effective anticoccidial activity compare with the positive control group.

Conclusion

Eight novel N-(2-aminophenyl)-2-quinazolinone-acetamide hydrochloride (1:1) were designed and synthesized. And seven of these compounds were chosen for anticoccidial activity test according to the ACI. The results indicated that N-(2-aminophenyl)-2-(6-methyl-8-bromo quinazolinone)-acetamide hydrochloride (**3h**) has anticoccidial activity against *Eimeria tenella* with ACI 122.1 and exhibited effective anticoccidial activity compared with the positive control group. Further structural optimization and anticoccidial activities about the quinazolinone derivatives are still in progress.

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