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Synthesis and anthelmintic activity of coumarin–imidazole hybrid derivatives against *Dactylogyrus intermedius* in goldfish

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ABSTRACT

With an intention to find more potent anti-parasite agents, four bromoalkane substituted coumarin derivatives (**1**–**4**) and twenty coumarin–imidazole hybrid derivatives were synthesized and screened for their anthelmintic activity and the acute toxicity. Anti-parasites results confirmed that most coumarin derivatives retained their anthelmintic activity against *Dactylogyrus intermedius* at the dose range from 1 to 10 mg/L. Among the candidates, compound **23** showed the best anthelmintic activity than other compounds against *D. intermedius* infestation with EC_{50} value of 0.85 mg/L. The structure–activity relationship analysis confirmed that the anthelmintic activities of derivatives were determined by the length of '*linker*' (R^1substitute position) and the substitute group in R^2 position. The active data confirmed that six carbon atoms length of '*linker*' and benzimidazole substitute group can increased the anthelmintic activity of compound, significantly. On the basis of these results, compound **23** can be used as a potential lead compound for the development of commercial drug against *D. intermedius*.

In the past couple of decades, aquaculture is considered to be the fastest growing industry in the food-production sphere, and continues to dominate all other animal food-producing industry in terms of its growth, growing at an average annual rate of 8.9% since 1970.[1] However, the continuing growth of fish aquaculture worldwide has been seriously menaced by the increasing diseases caused by bacterial, viral or parasitic infection. Monogenean parasites, such as *Dactylogyrus intermedius*, which are endemic ectoparasites in Asia, Central Europe, Middle East and North America, cause serious economic losses in aquaculture industry in these regions.[2] They are usually attached to the gills of freshwater fish causing irritation, excessive mucus production, accelerated respiration and mixed infection with other pathogen and leading to a serious damage to the host such as lower growth performance and high mortalities.[3,4] At present, the insecticides and animal-use medicines, such as praziquantel, toltrazuril, mebendazole, trichlorfon and formalin were frequently used in aquaculture industry for the control of monogenean parasites disease. However, those drugs had limited efficacy due to the raised drug-resistance, risk of residue, environmental contamination, and toxicity to host caused by the frequent and excessive use, which have stimulated the search for new control strategies.[5–9]

Botanicals have been used to treat aquatic animal parasitic disease for hundreds of years and play an increasingly important role in drug discovery and development.[10,11] Natural products with a coumarinic moiety have been found in many plant species[12,13] and their derivatives have attracted considerable attention due to their extensively biological activities such as anti-bacterial,[14,15] anti-viral,[16] anti-coagulant,[17] anti-inflammatory,[18] anti-cancer,[19] anti-oxidant[20] and anti-parasite[21–23] properties. Chinese medicinal herb is one of the largest sources of bioactive molecules, such as coumarins and flavonoids, has received more and more concerns in recent years. For instance, *Artemisia annua* L., the Asteraceae family, showed significant activity against parasites of *Sarotherodon melanotheron*.[24] In our previous studies, some naturally occurring coumarin, such as isopsoralen, psoralidin and osthol, were isolated from the fruit of *Psoraleacorylifolia* and *Fructuscnidii*, the anti-parasites test confirmed that those compounds exhibit significant effects on against *Ichthyophthirius multifiliis* or *D. intermedius*.[25,26]

Multitudinous efforts have been devoted not only towards the isolation and purification of naturally occurring biological coumarins from a variety of plants, animals and microorganisms, but also towards the developments of artificial synthetic coumarin derivatives as potential drugs. Various new derivatives with coumarin ring including the furanocoumarins, pyrano coumarins and coumarin sulfamates have been found to be useful in anti-bacterial and anti-parasite therapy.[27–31] In recent years, many hybrid

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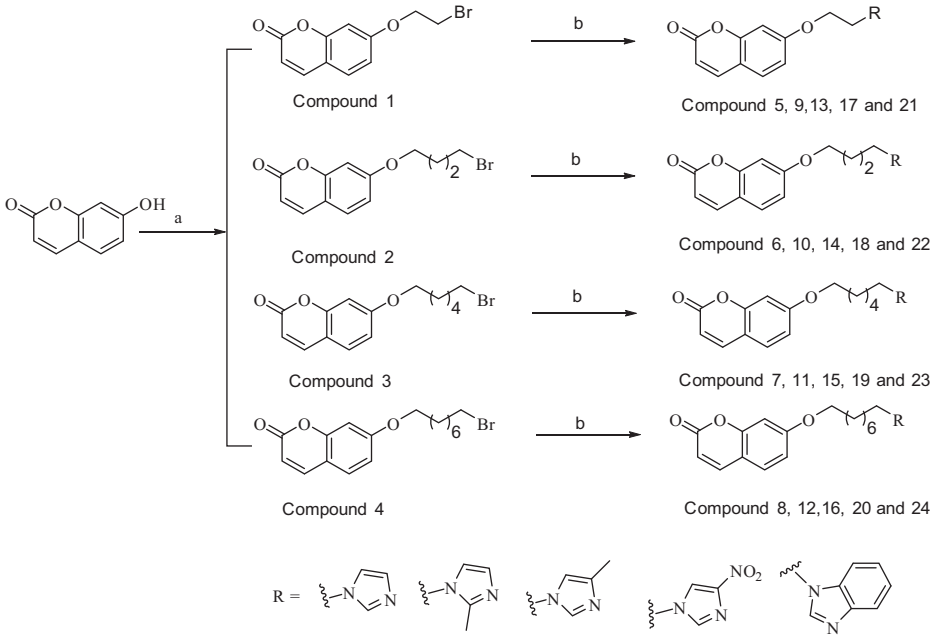
molecules with coumarin based ring systems have been synthesized utilizing novel synthetic methodologies. Some coumarins derivatives conjugated with nitrogen-containing heterocyclic moieties, such as triazole, pyridine and pyrimidines, were synthesized and proved to possess anti-parasite bioactivity.[32,33] Imidazole and its derivatives are of great importance in medicinal chemistry and can be used for the synthesis of myriad heterocyclic compounds with different biological activities such as antiviral, antibacterial, antifungal, anti-tuberculosis, anticancer.[34–40] Moreover, imidazole based compounds such as mebendazole, levamisole, flubendazole and thiabendazole are very important anti-parasite medicines for clinical treatment.[41–44] Thus, the design and synthesis of novel coumarin-imidazole hybrid derivatives is the prospective direction for the development of novel anti-parasite agents with better efficacy, as well as lower toxicity. In an attempt to develop novel anthelmintic agents, a series of novel coumarin-imidazole hybrid derivatives were synthesized. All the analogues were evaluated for their anti-parasitic activity in vivo against the *D. intermedius* and the acute toxicity tests of the compounds were undertaken for goldfish simultaneously.

As shown in Scheme 1, the general procedures for the preparation of the halogen substituted coumarin derivatives (**1**–**4**) and coumarin-imidazole hybrid derivatives (**5**–**24**) were efficiently synthesized according to previous report.[45] Compounds **1**–**4**, which are crucial to the synthesis of all coumarin–imidazole hybrid derivatives, were synthesized from 7-hydroxy coumarin by reacting with corresponding α, ω-dibromoalkanes and triethylamine in anhydrous acetone at reflux condition. Compounds **5**–**24** were synthesized in 60–90% yield by treatment of **1**–**4** compounds with imidazole derivatives and anhydrous potassium carbonate in acetonitrile at room temperature. The structures of a total 24 synthesized compounds were confirmed by ESI-MS, ^{1}H and ^{13}C NMR (see the Supplementary data).

In order to measure the contribution of the alkyl bromide or imidazole derivatives motifs to the anti-parasite activity, all of the coumarin derivatives were evaluated using in vivo bioassays. The EC_{50} values of the compounds against *D. intermedius* are given in Table 1. In our previous study, the initial reactant, 7-hydroxy coumarin showed no anthelmintic efficacy at the concentration of 26.0 mg/L, which mortality of the goldfish was firstly occurred. However, most of the derivatives showed an obvious insecticide activity against *D. intermedius* in goldfish. Its indicated that the alkyl bromide or imidazole derivatives substituent group could increase the anti-parasite activity of coumarin. As indicated in the Table 1, compound **23** showed the highest activity against the parasites, with the EC_{50} value of 0.85 mg/L, which are slightly higher than those standard drugs, mebendazole and praziquantel, with the EC_{50} values of 1.25 and 2.84 mg/L, respectively. Taking account of the toxicity of the coumarin derivatives against fish, compounds **1**–**4**, **7**, **11**, **15** appears to be the suitable candidates in the prepared derivatives of coumarin, with EC_{50} values ranging from 2.42 to 2.92 mg/L, which were close to the value of praziquantel (EC_{50} = 2.84 mg/L). It's worth noting that compound **23** exhibited about two to threefold anti-parasitic activity than praziquantel. However, unfortunately, the boosted activity of this compound was accompanied with sharply increased toxicity. In the anti-parasitic test of compound **23**, the mortalities were observed before the anthelmintic efficacy reached 100%. In contrast, the toxicity of the analogues **1**–**4**, **7**, **11** and **15** were lower than the control drugs, mebendazole and praziquantel.

According to the results obtained in the anthelmintic effects test, the test compounds exhibited similar tendency of activity against *D. intermedius* in goldfish. As shown in Table 1, we can find that the anthelmintic effects of those compounds were affected by the length of '*linker*' (R^1substituted group). For example, the compounds **21**–**24**, with the same R^2 substituted group but different in R^1 substituted group, exhibited a significant difference in anti-parasitic activity (Fig. 1). Compound **23**, with the '*linker*' of six carbon atoms, shows the highest activity against *D. intermedius* with the EC_{50} value of 0.85 mg/L. Otherwise, the EC_{50} values were increased to 3 –4 mg/L, if the length of the linker is two, four or eight carbon atoms. This rule also can observe in the other four groups of compounds, such as compound **5**–**8** and **17**–**20**. The activities exhibited by the derivatives demonstrated that the length of the '*linker*' plays an important role in the anthelmintic activity of compound, and the biological activity of a compound



Scheme 1. Synthetic route of coumarin derivatives. Reagents and conditions: (a) alkyl dibromide, K_2CO_3, triethylamine, dry acetone, reflux, 20–24 h; (b) imidazole/2-methylimidazole/4-methylimidazole/4-nitroimidazole/ benzimidazole, K_2CO_3, CH_3CN, rt, 20–24 h.

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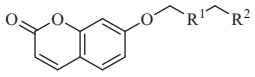
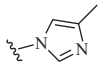
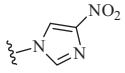
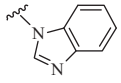
Table 1
The structures and anthelmintic efficacy, acute toxicity values of coumarin derivatives



Compound	Substituent group		Anthelmintic efficacy	Acute toxicity	Therapeutic index (LC_{50}/EC_{50})
	R^1	R^2	EC_{50} (mg/L;95%CL)	LC_{50} (mg/L;95%CL)	
1		Br	2.71 (2.36–3.04)	12.46 (11.44–13.53)	4.60
2	$-C_2H_4-$		2.58 (2.15–2.99)	8.16 (7.64–8.66)	3.16
3	$-C_4H_8-$		2.42 (2.13–2.79)	5.15 (4.35–5.76)	2.13
4	$-C_6H_{12}-$		2.85 (2.33–3.34)	9.54 (9.02–10.03)	3.35
5			5.52 (4.51–6.33)	13.42 (12.58–14.42)	2.43
6	$-C_2H_4-$		4.12 (3.92–4.32)	12.67 (12.19–13.13)	3.08
7	$-C_4H_8-$		2.82 (2.32–3.23)	7.28 (7.16–7.38)	2.58
8	$-C_6H_{12}-$		4.94 (4.56–5.33)	14.76 (13.88–15.59)	2.99
9			5.05 (4.83)	13.07 (12.62–13.50)	2.59
10	$-C_2H_4-$		4.41 (3.41–5.40)	13.43 (13.17–13.69)	3.05
11	$-C_4H_8-$		2.73 (2.48–3.01)	9.39 (8.55–10.64)	3.44
12	$-C_6H_{12}-$		4.85 (4.29–5.53)	9.43 (8.99–10.07)	1.94
13			5.46 (4.84)	12.77 (12.15–13.36)	2.34
14	$-C_2H_4-$		4.23 (3.74–4.68)	13.06 (12.47–13.68)	3.09
15	$-C_4H_8-$		2.92 (2.51–3.35)	9.08 (8.11–10.23)	3.11
16	$-C_6H_{12}-$		4.52 (4.07–4.95)	9.48 (9.23–9.74)	2.10
17			—	14.02 (13.32–14.63)	—
18	$-C_2H_4-$		8.70 (7.08–12.66)	15.32 (14.14–16.45)	1.76
19	$-C_4H_8-$		5.72 (4.86–8.37)	10.34 (9.29–10.94)	1.81
20	$-C_6H_{12}-$		7.72 (7.41–8.09)	12.28 (11.70–12.90)	1.59
21			4.03 (3.39–4.66)	10.66 (10.07–11.22)	2.65
22	$-C_2H_4-$		2.92 (1.31–3.78)	11.64 (11.27–12.05)	3.99
23	$-C_4H_8-$		0.85 (0.80–0.91)	1.21 (1.12–1.39)	1.42
24	$-C_6H_{12}-$		3.67 (3.44–3.88)	8.18 (7.90–8.43)	2.23
Mebendazole[a]			1.25 (1.09–1.57)	3.55 (3.33–3.98)	2.84
Praziquantel[a]			2.84 (2.62–3.20)	6.47 (6.03–6.92)	2.28
7-Hydroxy coumarin[b]			—	27.55 (25.26–30.17)	—

"—" Means the mortalities were observed before the anthelmintic efficacy reached 50%.
CL confidence level.
[a] Positive control.
[b] Initial reactant.

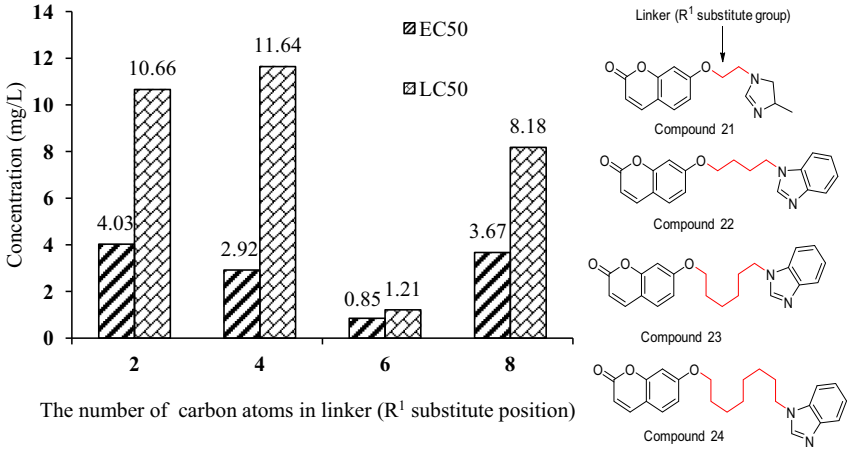


Figure 1. The EC_{50} and LC_{50} values of compounds **21**–**24**, which were different in the linker length of alkyl side chain (R^1 substituted group). The numbers of carbon atoms in the 'linker' are 2, 4, 6 and 8, corresponding to compounds **21**–**24**, respectively.

may be sharply increased when it contains a suitable length of '*linker*'. In our test, the '*linker*' consisting of six carbon atoms was confirmed to be the optimal length to link the coumarin and imidazole derivatives.

Through the simple analysis of the structure–activity relationship, we found that the types of imidazole derivatives (R^2 substituted group) also occupy an important place in the anthelmintic activity of compound. We synthesized six compounds with the same 'linker' (R^1 substituted group) but different in R^2 substituted position and the compounds displayed various anti-parasitic activities. As shown in Figure 2, the compounds **3**, which contain a bromine atom in R^2 position, with the EC_{50} values of 2.42 mg/L. However, there is significant variation in anthelmintic activity of compound, if we turned bromine into imidazole derivatives. For

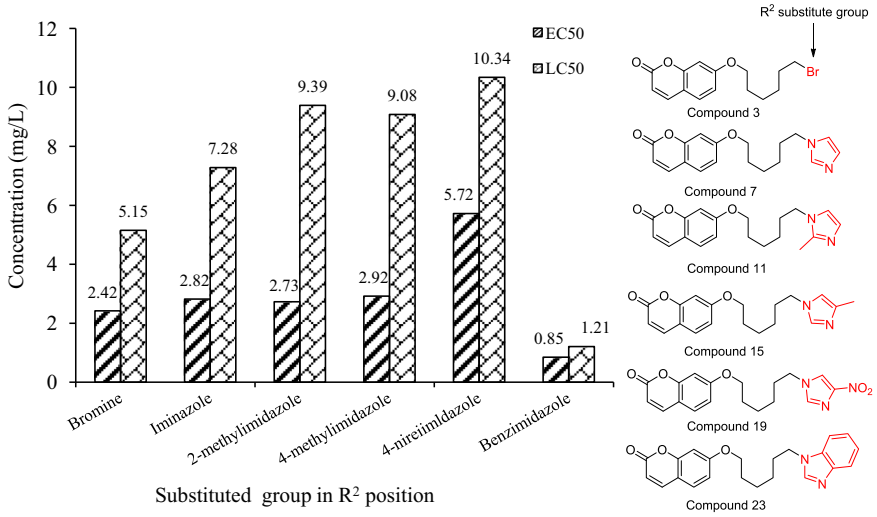


Figure 2. The EC_{50} and LC_{50} values of compounds **3**, **7**, **11**, **15**, **19** and **23**, which were different in the imidazole derivative substituent groups (R^2 substituted group). The substituted group, bromine, iminazole, 2-methyliminazole, 4-methyliminazole, 4-nireiiminazole and benzimidazole, are corresponding to compounds **3**, **7**, **11**, **15**, **19** and **23**, respectively.

example, compound **19** which contain a 4-nireiimidazole group, with the EC_{50} value of 5.72 mg/L. Moreover, by the active data analysis we found that an electron-withdrawing group in imidazole ring can obviously decrease the anthelmintic activity whereas an electron-donating group can increase the anti-parasite activity. For example, compound **19** containing a 4-nireiimidazole group and compound **23** with a benzimidazole group, the EC_{50} value of two compounds were 5.72 and 0.85 mg/L, respectively.

Interestingly, the length of the '*linker*' was indistinctive affected on the anthelmintic activity of compounds **1**–**4**, which has the bromine atom in R^2 substituted position but different in R^1 substituted group (Fig. 3). Theoretically speaking, compounds **1**–**4** can release a certain amount of Br anion through hydrolyzation in water, if assume that a definite concentration of Br anion can get rid of parasites from the goldfish, it's could explain why the length of '*linker*' shows a indistinctive impacts on the anthelmintic activity of compounds **1**–**4**. However, our additional test, using NaBr as testee, the anthelmintic results demonstrated that Br anion has no anthelmintic activity against *D. intermedius* at a dose of 10 mg/L. This result implied that coumarin bromides may evaded the drastic change of biological activity caused by the different length of the '*linker*' through other methods, such as influence on cell membrane, induces oxidative stress and DNA damage or inhibit the activity of related enzymes in parasites.

The synthesized compounds and two positive control drugs were evaluated an aqueous static 48 h bioassay. The 48 h LC_{50} values of goldfish against the compounds are listed in Table 1. The LC_{50} values of compounds shows that the toxicity of the test compounds influenced by the length of '*linker*' (R^1 substitute position) and the substitute group in R^2 position, similarly. As shown in Table 1, if the compounds with the same R^1 substitute group, those compounds with the benzimidazole group in R^2 position shows more toxic to goldfish than others. For example, compounds **7**, **11**, **15** and **19**, with the same R^1 substituted group, but different in R^2 substituted position (Fig. 2), shows the LC_{50} values ranging from 7 to 10 mg/L. However, the LC_{50} value is sharply decreased to 1.21 mg/L, if a benzimidazole group appeared in the R_2 position. These results indicated that the benzimidazole group could enhance the toxicity of the coumarin derivatives against fish.

Furthermore, toxicity test showed the toxic effects of the compounds also relies with the difference of the R^1 substituted group. If the compounds with same R^2 substitute group, those compounds

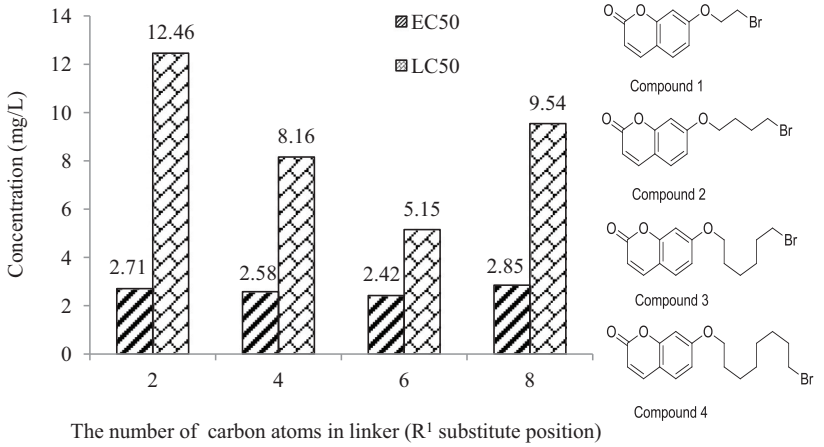


Figure 3. The EC_{50} and LC_{50} values of compounds **1**–**4**, which were different in the linker length of alkyl side chain (R^1 substituted group). The numbers of carbon atoms in the 'linker' are 2, 4, 6 and 8, corresponding to compounds **1**–**4**, respectively.

with six carbon atoms length of linker may exhibited higher toxicity to goldfish than the others (Table 1). For example, the compounds **1**–**4**, with the same R^2 substituted group but different in R^1 substituted group, exhibited a widely variable ranges of LC_{50} values ranging from 5 to 13 mg/L (Fig. 3). Compound **3**, with a six carbon atoms length of linker, shows the highest toxic to goldfish with the LC_{50} value of 5.15 mg/L. Otherwise, the LC_{50} values were increased to 8–13 mg/L, if the length of the linker is two, four or eight carbon atoms. This pattern was also displayed in other compounds, especially the compound **23**, which has the six carbon atoms length of linker and benzimidazole group simultaneously, shows the highest toxicity to goldfish in all test compounds, with the LC_{50} value of 1.21 mg/L. Compared with control groups, the toxic of compound **23** was nearly three to five times higher than mebendazole or praziquantel, which with the LC_{50} values of 3.55 and 6.47 mg/L, respectively. These results suggested that the length of linker may play an important role in the enhancement of coumarin compound toxicity.

In summary, a series of coumarin bromides and coumarin-imidazole hybrid derivatives were designed, synthesized and evaluated for anthelmintic efficacy against *D. intermedius* in vivo. Our results suggest that compound **23** is the most potential anti-parasite candidate in all the coumarin derivatives. Compound **23** exhibited the highest activity in the anthelmintic efficacy against *D. intermedius*. Nevertheless, compound **23** also showed strong toxicity to goldfish. Further structural modifications of benzimidazole substitute group need to be conducted in order to decrease the toxicity of compound **23**.

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Supplementary data

Supplementary data (experimental procedures and characterization data of compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.08.090.

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