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REGULAR ARTICLE

Developing a highly potent anthelmintic: study of catalytic application of L-proline derived aminothiourea in rapid synthesis of biscoumarins and their *in vitro* anthelmintic essay

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Abstract. Due to serious side effects of benzimidazoles such as apoptosis and mitotic arrest, development of alternative anthelmintic drugs with comparable efficacy to target the acetylcholine receptors of parasites is considered very important to control this parasitic disease. Here we have developed an excellent method for synthesis of biscoumarins by employing a mild and efficient proline derived bifunctional thiourea catalyst bearing pyrrolidine and thiourea catalytic sites and tested their anthelmintic activity against helminth parasites *Raillietina echinobothrida* and *Syphacia obvelata*. The compounds **2a**, **2j**, **2k** and **2o** demonstrated much stronger anthelmintic activity against *Raillietina echinobothrida* in comparison to the standard drug, Praziquantel. Molecular docking simulations of the optimized compounds with β-tubulin showed excellent binding interactions with several amino acid residues of the active site and the docking scores with β-tubulin were found to be comparable to the *in vitro* anthelmintic activity.

Keywords. L-Proline thiourea; biscoumarin; anthelmintic; *Raillietina echinobothrida*; docking.

1. Introduction

Helminth infection, a major parasitic disease that affects millions of people on the earth, often damage blood vessels leading to liver, eyes, urinary tract, etc. Although the helminth infections are localized mainly in the intestinal tract, they can enter the blood circulation, heart, liver, lungs and muscles. Ideally, the anthelmintic drugs are designed to exterminate the helminths from the host organism by damaging of neuromuscular coordination of the helminths, their energetic processes and enzymatic system, laying of eggs, etc. Helminth parasites employ acetylcholine receptors for fast-synaptic transmission in their neuromusculature to experience the outside world and respond to it. Although many drugs targeting the acetylcholine receptor are developed, their efficacy is inferior to benzimidazole family.[1] Since some of benzimidazoles have shown serious side effects such as apoptosis and mitotic arrest,[2,3] development of efficient drugs with comparable efficacy to benzimidazoles to target the acetylcholine receptors of parasites is considered very important to control of this parasitic disease.

Coumarin or 2*H*-chromen-2-one is a 'privileged' structural motif with diverse pharmacological resume.[4] Among the coumarin derivatives, biscoumarins have demonstrated unique biological properties such as anticancer,[5–9] anticoagulant,[10,11] enzyme inhibitory activity,[12–14] antioxidant,[15,16] antimicrobial,[17] and many more.[18–20] In addition, they also exhibit optical and fluorescence properties which have also been studied.[20–23] Recently, we reported acetylcholine esterase inhibitory activity of coumarin based unsymmetrical trisubstituted methanes as a potential candidate for the treatment of Alzheimer's

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disease.[24] Since inhibition of acetylcholine receptors are one of the major modes of action of anthelmintic drugs, we employed those unsymmetrical trisubstituted methanes (**A**, Figure 1) for treatment against helminth parasites, *Raillietina echinobothrida* and *Syphacia obvelata* and noted that some of the compounds demonstrated better activity than commonly used anthelmintic drugs such as albendazole which is very significant for a drug inhibiting the acetylcholine receptor. Given the portfolio of biscoumarins, we proposed to study the effect of the presence of two coumarin units in a symmetrical trisubstituted methane system.

Many homogeneous and heterogeneous catalysts under varying reaction conditions are reported to catalyze the synthesis of coumarin based symmetrical trisubstituted methanes by a multicomponent reaction of aldehyde with 4-hydroxycoumarinin the presence of a host of aldehydes.[25–40] Most of the reported methods employ acid catalysis to affect this transformation. Generally, acid-catalyzed reactions are often difficult due to their poor functional group compatibility, susceptibility towards moisture and air, and hazardous to handle. In industries, acid catalysts damage the plant through their corrosiveness and lead to large volumes of toxic and corrosive wastes. Therefore, the use of organothiourea as a mild alternative to Lewis/Bronsted acids has taken a great stride in recent years. Especially, the bifunctional thioureas have found numerous applications as organocatalysts due to their ability to activate the substrates bearing anions and carbonyl groups through double-hydrogen bonding.[41–48] Recently, we have observed that L-proline derived bifunctional amidothiourea[49,50] catalyzed synthesis of bis(indolyl)methanes *via* a nucleophilic addition-substitution pathway rather than the conventional route, i.e., by a domino Knoevenagel–Michael reaction pathway. This new pathway has the potential to generate asymmetric trisubstituted methanes under the optimized conditions. Given the growing applications of L-proline and its derivatives in organocatalysis[51–53] and our recent finding on the efficacy of the catalyst in carbon–carbon bond-forming reactions, we planned to apply the catalyst **1** for the multicomponent synthesis of the proposed biscoumarin compounds (Scheme 1) and study their anthelmintic activity. Our study led to the development of an extremely efficient protocol for the synthesis of biscoumarin under environmentally benign multicomponent reaction conditions in water medium bereft of any organic solvent and chromatographic purification.

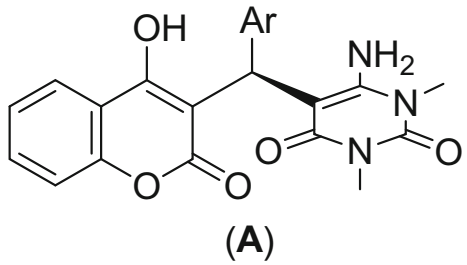


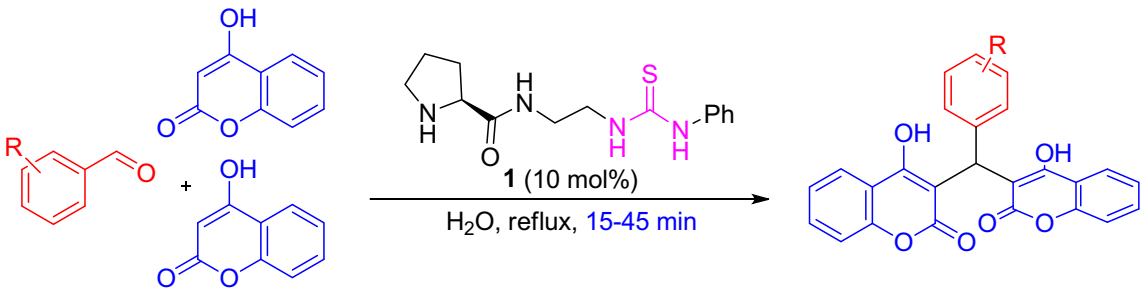
Figure 1. Potent anthelmintic against *Syphacia obvelata*.

2. Experimental

2.1 *Chemistry*

2.1a *General.* Unless otherwise mentioned, all the chemicals and reagents were available commercially and were used without further purification. The products were characterized by IR, ^{1}H NMR, ^{13}C NMR. The IR spectra were recorded on a Perkin Elmer spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Bruker AC-400 using $CDCl_3$ as solvent and TMS as an internal standard. The catalyst, **1** was synthesized by following the methods reported by Basumatary *et al.*[49]

2.1b *General procedure for the synthesis of bis-coumarin.* A suspension of the aldehyde (0.5 mmol), 4-hydroxy coumarin (1 mmol) and the catalyst **1** (10 mol%) in H_2O (5 mL) in a 50 mL round bottom



Scheme 1. Organocatalytic synthesis of biscoumarins.

flask fitted with condenser was placed in a heating mantle and heated to reflux. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature to observe the formation of the precipitate. The precipitate was then washed with water (2 × 5 mL) and ethanol (2 × 5 mL) to remove any residual unreacted starting materials and the catalyst. The precipitate was then recrystallised from ethanol to obtain a highly pure product in excellent yield.

2.1c *3,3′-(Phenylmethylene)bis(2-hydroxy-4H-chromen-4-one).*[27] White solid; Yield: 96% (396 mg); M.p. 230–232 °C (228–230 °C); Rf = 0.5 (EtOAc/Hex: 6.8/3.8 v/v); IR (KBr) 3449, 3078, 1674, 1613, 1099, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.10 (s, 1H, –CH), 7.21–7.42 (m, 11H, ArH), 7.61–7.65 (m, 1H, ArH), 8.06–8.08 (m, 1H, ArH), 11.3 (s, 1H, Ar-OH), 11.5 (s, 1H, Ar-OH).^{13}C NMR (100 MHz, $CDCl_3$) δ 36.4, 116.9, 124.8, 125.1, 126.7, 127.1, 128.9, 133.1, 135.4, 152.5, 152.8, 164.8, 166.0, 167.1 ppm; ESI-MS (*m/z*): 435 $[M + Na]^+$, 413 $[M + H]^+$. Anal. calcd. for $C_{25}H_{16}O_6$ (412.39 g/mol): C, 72.81; H, 3.91; found: C, 72.80.72; H, 3.92.

2.1d *3,3′-(p-Tolylmethylene)bis(2-hydroxy-4H-chromen-4-one)).*[30] White solid; Yield 92% (392 mg); M.p. 267–268 °C (268–269 °C); Rf = 0.6 (EtOAc/Hex: 8/2 v/v); IR (KBr); 3050, 2995, 2915, 1673, 1608, 1093, 764 cm^{-1}.^1H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H), 6.06 (s, 1H, –CH), 7.11 (m, 4H, ArH), 7.41–7.40 (m, 4H, ArH), 7.64–7.06 (m, 2H, ArH), 8.06 (m, 2H, ArH), 11.2 (s, 1H, Ar-OH), 11.5 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.8, 36.7, 106.6, 117.5, 125.2, 125.7, 127.2, 130.2, 132.9, 133.7, 137.3, 153.1, 153.4, 165.4, 166.6 ppm; ESI-MS (*m/z*): 459 $[M + Na]^+$, 427 $[M + H]^+$; Anal. calcd. for $C_{26}H_{18}O_6$ (426.11 g/mol): C, 73.23; H, 4.25; found: C, 73.21; H, 4.26.

2.1e *3,3′-((4-methoxyphenyl)methylene)bis(4-hydroxy-2H-chromen-2-one).*[34] White solid; Yield 95% (420 mg); M.p. 247–249 °C (247–249 °C); Rf = 0.5 (EtOAc/Hex: 8/2 v/v); IR (KBr): 3452, 3072, 1672, 1611, 1563, 1507, 1451, 1350, 1307, 1256, 760 cm^{-1}; ^1H NMR ($CDCl_3$): δ3.83 (s, 3H, CH_3O), 6.08 (s, 1H, CH), 6.87–8.08 (m, 12H), 11.32 (s, 1H, OH), 11.54 (s, 1H, OH) ppm; ^{13}C NMR ($CDCl_3$): δ 35.5, 55.2, 114.0, 116.6, 124.3, 124.8, 126.9, 127.6, 132.7, 158.4 ppm; ESI-MS (*m/z*): 465 $[M + Na]^+$, 443 $[M + H]^+$; Anal. calcd. for $C_{26}H_{18}O_7$ (442.10 g/mol): C, 70.58; H, 4.10; found: C, 70.54; H, 4.13.

2.1f *3,3′-((2-Methoxyphenyl)methylene)bis(2-hydroxy-4H-chromen-4-one).*[30] White solid; Yield 93% (411 mg); M.p. 218–220 °C (217–219 °C); Rf = 0.5 (EtOAc/Hex:7.5/2.5 v/v); IR (KBr): 3075, 2729, 1666, 1094, 765 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.79 (s, 3H), 6.04 (s, 1H, –CH), 6.86 (d, J = 8.8 Hz, 2H), 7.13 (d, J = 8 Hz, 2H,, ArH), 7.39–7.41 (m, 6H, ArH), 7.60–7.64 (m, 1H, ArH), 8.00–8.05 (m, 1H, ArH), 11.3 (s, 1H, Ar-OH), 11.5 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.7, 55.5, 104.4, 106.0, 114.2, 116.8, 124.6, 125.1, 127.1, 127.8, 133.0, 152.5, 152.7, 158.6, 164.8, 165.9, 169.5 ppm; ESI-MS (*m/z*): 465 $[M + Na]^+$, 443 $[M + H]^+$; Anal. calcd. for $C_{26}H_{18}O_7$ (442.10 g/mol): C, 70.58; H, 4.10; found: C, 70.57; H, 4.09.

2.1g *3,3′-((3,4-Dimethoxyphenyl)methylene)bis(4-hydroxy-2H-chromen-2-one).*[30] Colourless powder; Yield: 90% (425 mg); Rf: 0.35 (*n*-Hexane/EtOAc: 1/9 v/v). M.p. 258–260 °C (256–258 °C); IR (KBr): *v* 3490, 3068, 1644, 1615, 1560, 1261, 1095, 760 $cm^{-1;}$ ^1H NMR (400 MHz, DMSO-d6): δ 3.50 (s, 3H,-OCH_3), 3.67 (s, 3H,-OCH_3), 5.14 (s, 1H, –CH–), 6.19 (s, 1H), 6.64–6.61 (m, 2H), 6.74 (d, J = 7.9 Hz, 1H), 7.25–7.20 (m, 4H), 7.50 (t, J = 8.5 Hz, 2H), 7.80 (d, J = 8.5 Hz, 2H), 17.59 (s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d6): δ35.7, 55.4, 57.5, 103.6, 111.5, 115.4, 118.8, 119.9, 122.8, 130.8, 134.9, 146.5, 148.1, 152.4, 164.5, 168.0 ppm; ESI-MS (*m/z*): 495 $[M + Na]^+$, 473 $[M + H]^+$; Anal. calcd. for $C_{27}H_{20}O_8$ (472.11 g/mol): C, 68.64; H, 4.27; found: C, 68.64; H, 4.28.

2.1h *3,3′-(Benzo[d][1,3]dioxol-5-ylmethylene)bis(2-hydroxy-4H-chromen-4-one).*[30] White solid; Yield 94% (429 mg); M.p. 256–257 °C (254–257 °C); Rf = 0.6 (EtOAc/Hex: 8/2 v/v); IR (KBr): *v* 3445, 2898, 1660, 1613, 1100, 1039, 765 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.93 (s, 2H) 6.01 (s, 1H, –CH), 6.74–6.67 (m, 3H, ArH), 7.40 (m, 4H, ArH), 7.62 (t, J = 8 Hz, 2H, ArH), 8.04 (m, 2H, ArH), 11.2 (s, 1H, Ar-OH), 11.5 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.1, 101.4, 107.4, 108.5, 116.8, 119.8, 124.6, 125.1, 129.1, 133.1, 146.7, 148.4, 152.7, 164.8, 165.9, 167, 169.4 ppm; ESI-MS (*m/z*): 479 $[M + Na]^+$, 457 $[M + H]^+$; Anal. calcd. for $C_{26}H_{16}O_8$ (456.08 g/mol): C, 68.42; H, 3.53; found: C, 68.40; H, 3.55.

2.1i *3,3′-((3-Nitrophenyl)methylene)bis(2-hydroxy-4H-chromen-4-one).*[36] White solid; Yield 97% (443 mg); M.p. 238–240 °C (238–239 °C); Rf = 0.6 (EtOAc/Hex: 8/2 v/v); IR (KBr): *v* 3069, 2728, 1672, 1107, 762 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.12 (s, 1H, –CH), 7.58–7.38 (m, 6H, ArH), 7.66 (m, 2H, ArH), 8.00 (d, J = 8 Hz, 1H, ArH), 8.09–8.06 (m, 2H,

ArH), 8.15 (d, J = 8 Hz, 1H, ArH), 11.3 (s, 1H, Ar-OH), 11.5 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.4, 104.8, 117.1, 122.0, 122.4, 124.74, 125.4, 129.8, 133.0, 133.6, 138.2, 148.9, 152.5, 152.8, 167.2, 169.4 ppm; ESI-MS (m/z): 480 $[M + Na]^+$, 458 $[M + H]^+$; Anal. calcd. for $C_{25}H_{15}NO_8$ (457.07 g/mol): C, 65.65; H, 3.31; N, 3.06 found: C, 65.62; H, 3.30; N, 3.09.

2.1j *3,3′-(2-Nitrophenyl)methylene)-bis(4-hydroxy-2H-chromen-2-one).*[27] Yield: 95% (434 mg); M.p. 202–205 °C (202–205 °C); Rf = 0.5 (EtOAc/Hex: 8/2 v/v); IR (KBr): ν 3433, 3076, 2722, 1652, 1618, 1561, 1532, 1450, 1352, 1309, 1099, 761 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 6.64 (s, 1H, CH), 7.27–8.01 (m, 12H, ArH), 11.55 (bs, 2H, OH) ppm; ^{13}C NMR ($CDCl_3$): δ_C 33.86, 103.71, 116.66, 124.57, 125.03, 128.19, 129.49, 131.94, 133.20, 149.76 ppm; ESI-MS (m/z): 481 $[M + Na]^+$, 459 $[M + H]^+$; Anal. calcd. for $C_{25}H_{15}NO_8$ (457.37 g/mol): C, 65.65; H, 3.31; N, 3.06 found: C, 65.64; H, 3.32; N, 3.10.

2.1k *3,3′-(2-Hydroxyphenyl)methylene)-bis(4-hydroxy-2H-chromen-2-one).*[35] White solid, 90% yield (385 mg); M.p. 160–161 °C (ref = 160–254 °C); Rf = 0.5 (EtOAc/Hex: 6.8/3.8 v/v); IR (KBr): ν 3441, 3075, 1672, 1611, 1097, 755 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6): δ 17.10 (s, 1H), 8.22 (d, J = 4.9 Hz, 1H), 8.11 (s, 2H), 7.73 (dd, J = 7.6, 2.1 Hz, 2H), 7.50 (dd, J = 7.9, 2.1 Hz, 1H), 7.46–7.40 (m, 2H), 7.21–7.13 (m, 4H), 7.09 (d, J = 7.9 Hz, 1H), 7.04–6.98 (m, 1H), 6.2o (s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d6): δ 168.0, 165.0, 162.35, 153.0, 148.8, 136.2, 131.3, 124.6, 123.3, 121.4, 120.8, 120.5, 115.9, 103.8, 72.7, 39.9 ppm; ESI-MS (m/z): 451 $[M + Na]^+$, 429 $[M + H]^+$. Anal. calcd. for $C_{25}H_{16}O_7$ (428.08 g/mol): C, 70.09; H, 3.76; found: C, 70.11; H, 3.77.

2.1l *4-(Bis(2-hydroxy-4-oxo-4H-chromen-3-yl)methyl)-benzonitrile.*[30] White solid; Yield 96% (420 mg); M.p. 262–264 °C (261–263 °C); Rf = 0.5 (EtOAc/Hex: 7/3 v/v); IR (KBr): ν 3071, 2975, 2226, 1662, 1614, 1095, 790 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.09 (s,1H, –CH), 7.43–7.35 (m, 6H, ArH), 7.61–7.68 (m, 4H, ArH), 7.98–8.08 (m, 2H, ArH), 11.35 (s, 1H, Ar-OH), 11.55 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.7, 103.4, 116.4, 116.94, 118.9, 124.7, 125.4, 127.7, 132.7, 133.5, 141.5, 152.8, 165.0, 166.6, 167.2 ppm; ESI-MS (m/z): 460 $[M + Na]^+$, 438 $[M + H]^+$; Anal. calcd. for $C_{26}H_{15}NO_6$ (437.08 g/mol): C, 71.39; H, 3.46; N 3.20; found: C, 71.40; H, 3.45; N 3.23.

2.1m *3-((4-Fluorophenyl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)-4-hydroxy-2H-chromen-2-on.*[32] White solid; Yield: 95% (408 mg); Rf: 0.35 (*n*-Hexane/EtOAc: 1/9 v/v); M.p. 215–216 °C (215–216 °C); Rf = 0.5 (EtOAc/Hex: 8/2 v/v); IR (KBr): ν 3401, 3085, 1662, 1631, 1577, 1275, 1109, 774 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.02 (s, 1H, CH), 6.80–7.65 (m, 12H, ArH), 7.98 (s, 1H, OH), 8.05 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 36.1, 105.7, 116.4, 116.6, 124.4, 124.8, 126.4, 126.8, 128.6, 128.9 132.8, 135.1, 145.1, 162.9 ppm; ESI-MS (m/z): 453 $[M + Na]^+$, 431 $[M + H]^+$; Anal. calcd. for $C_{25}H_{15}FO_6$ (430.08 g/mol): C, 69.77; H, 3.51; found: C, 69.72; H, 3.54.

2.1n *3,3′-((4-Bromophenyl)methylene)bis(2-hydroxy-4H-chromen-4-one).*[27] White solid; Yield 93% (457 mg); M.p. 268–269 °C (ref 266–268 °C); Rf = 0.5 (EtOAc/Hex: 7/3 v/v); IR (KBr): ν 3072, 2981, 1672, 1607, 1099, 765 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.01 (s, 1H, –CH), 7.10 (d, J = 7.6 Hz, 2H), 7.36–7.44 (m, 6H, ArH), 7.61–7.66 (m, 2H, ArH), 7.98–8.07 (m, 2H, ArH), 11.3 (s, 1H, Ar-OH), 11.5 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.1, 116.9, 121.0, 124.7, 125.2, 128.6, 131.9, 133.3, 135.7, 152.5, 152.8, 164.9, 166.3, 167.1 ppm; ESI-MS (m/z): 514 $[M + Na]^+$, 492 $[M + H]^+$; Anal. calcd. for $C_{25}H_{15}BrO_6$ (490.00 g/mol): C, 61.12; H, 3.08; found: C, 61.09; H, 3.04.

2.1o *3,3′-((3-Bromophenyl)methylene)bis(4-hydroxy-2H-chromen-2-one).*[28] White powder. Yield: 94% (461 mg); M.p. 253–255 °C (253–254 °C); Rf = 0.5 (EtOAc/Hex: 8/2 v/v); IR (KBr): ν 3406, 3070, 1656, 1619, 1568, 1270, 1100, 761 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 6.41 (s, 1H, 4H), 7.14–7.95 (m,12H, Ar-H), 11.26 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6): δ 35.4, 103.7, 115.9, 116.6, 121.8, 123.8, 123.9, 125.5, 128.8, 129.1, 129.9, 132.1, 140.9, 151.9, 164.4, 165.3 ppm; ESI-MS (m/z): 514 $[M + Na]^+$, 492 $[M + H]^+$. Anal. calcd. for $C_{25}H_{15}BrO_6$ (490.00 g/mol): C, 61.12; H, 3.08; found: C, 61.11; H, 3.07.

2.1p *3,3′-((4-Chlorophenyl)methylene)bis(4-hydroxy-2H-chromen-2-one).*[27] White powder. Yield: 97% (433 mg); M.p.: 257–259 °C (258–259 °C); Rf = 0.5 (EtOAc/Hex: 8/2 v/v); IR (KBr): ν 3432, 3071, 1667, 1618, 1562, 1281, 1093, 766 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.97 (s, 1H, 4*H*), 7.08 (d, J = 8.0 Hz, 2H, Ar-H), 7.21 (m, 2H, Ar-H), 7.34 (m, 2H, Ar-H), 7.57 (m, 2H, Ar-H), 7.91 (m, 2H, Ar-H), 8.00 (m, 2H, Ar-H), 11.24 (s, 1H, OH), 11.46 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$):δ 34.74, 102.63, 104.16, 115.29, 115.62, 123.35, 123.94, 126.88, 126.93, 127.70, 131.64, 132.00, 132.82, 151.23, 151.44, 163.57, 164.96 ppm; ESI-MS (m/z): 470 $[M + Na]^+$,

448 $[M + H]^+$; Anal. calcd. for $C_{25}H_{15}ClO_6$ (446.05 g/mol): C, 67.20; H, 3.38; found: C, 67.21; H, 3.40.

2.1q *3,3′-(2-Chlorophenylmethylene)-bis(4-hydroxy-2H-chromen-2-one).*[27] White solid; Yield: 96% (428 mg); Rf: 0.45 (*n*-Hexane/EtOAc: 2/8 v/v); M.p. 202–204 °C (203–204 °C); Rf = 0.5 (EtOAc/Hex 8/2); IR (KBr): *v* 3069, 2720, 1649, 1556, 1498, 1444, 1340, 1097, 760 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.16 (s, 1H, CH), 7.27–8.04 (m, 12H, ArH), 10.93 (s, 1H, OH), 11.64 (s, 1H, OH) ppm; ^{13}C NMR ($CDCl_3$): δ 35.72, 116.59, 124.42, 124.87, 126.76, 128.59, 129.24, 130.81, 132.83, 133.49, 162.33 ppm; ESI-MS (*m/z*): 470 $[M + Na]^+$, 448 $[M + H]^+$; Anal. calcd. for $C_{25}H_{15}ClO_6$ (446.05 g/mol): C, 67.20; H, 3.38; found: C, 67.24; H, 3.41.

2.1r *3,3′-((4-Chloro-3-nitrophenyl)methylene)bis(2-hydroxy-4H-chromen-4-one).*[29] White solid; Yield 97% (476 mg); M.p. 250–252 °C (249–251 °C); Rf = 0.6 (EtOAc/Hex: 8/2 v/v); IR (KBr): *v* 3445, 2724, 1665, 1612, 1560, 1351, 1098, 789 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.05 (s, 1H, –CH), 7.51–7.37 (m, 6H, ArH), 7.65–7.71 (m, 3H, ArH), 8.00 (d, J = 8 Hz, 1H, ArH), 8.09 (d, J = 7.6 Hz, 1H, ArH), 11.38 (s, 1H, Ar-OH), 11.58 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 36.0, 103.2, 117.1, 124.3, 124.8, 125.5, 131.8, 132.3, 133.7, 136.7, 148.3, 152.6, 152.8, 165.1, 167.1, 169.3 ppm; ESI-MS (*m/z*): 515 $[M + Na]^+$, 493 $[M + H]^+$; Anal. calcd. for $C_{25}H_{14}ClNO_8$ (491.04 g/mol): C, 61.05; H, 2.87; N 2.85; found: C, 61.00; H, 2.84; N 2.90.

2.1s *3,3′-((3-Chloro-4-methoxyphenyl)methylene)-bis(2-hydroxy-4H-chromen-4-one).* White solid; Yield 93% (443 mg); M.p. 248–250 °C (NA); Rf = 0.6 (EtOAc/Hex: 7.5/2.5 v/v); IR (KBr): *v* 3449, 2731, 1665, 1614, 1565, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H), 6.01 (s,1H, –CH), 6.88 (d, J = 8.4 Hz, 1H, ArH), 7.08 (m, 1H, ArH), 7.18 (s, 1H, ArH), 7.38–7.42 (m, 4H, ArH), 7.63 (m, 2H, ArH), 8.07 (m, 2H, ArH), 11.29 (s, 1H, Ar-OH), 11.56 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.5, 56.4, 104.0, 105.5, 112.2, 116.9, 122.9, 124.7, 125.2, 126.1, 128.6, 133.2, 152.8, 154.1, 164.9, 166.2, 167.0 ppm; ESI-MS (*m/z*): 500 $[M + Na]^+$, 478 $[M + H]^+$; Anal. calcd. for $C_{26}H_{17}ClO_7$ (466.06 g/mol): C, 65.49; H, 3.59; found: C, 65.49; H, 3.58.

2.1t *3,3′-(Naphthalen-2-ylmethylene)bis(2-hydroxy-4H-chromen-4-one).*[32] White solid; Yield 95% (439 mg); M.p. 265–266 °C (264–265 °C); Rf = 0.5 (EtOAc/Hex: 8/2 v/v); IR (KBr): *v* 3449, 2713, 1661, 1611, 1089, 764 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.66 (s, 1H, –CH), 7.28–7.47 (m,10H,, ArH), 7.55–7.63 (m, 4H, ArH), 7.72 (d, J = 7.6 Hz, 1H, ArH), 7.85 (m, 3H, ArH), 7.97 (d, J = 7.6 Hz, 1H, ArH), 8.13 (d, J = 7.6 Hz, 1H, ArH), 11.1 (s, 1H, Ar-OH), 11.4 (s, 1H, Ar-OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4, 107.6, 116.8, 123.0, 124.7, 125.2, 125.8, 126.5, 128.8, 129.6, 131.0, 131.4, 133.1, 134.6, 152.4, 152.7, 164.8, 165.3, 167.2, 169.2 ppm; ESI-MS (*m/z*): 485 $[M + Na]^+$, 463 $[M + H]^+$; Anal. calcd. for $C_{29}H_{18}O_6$ (462.11 g/mol): C, 75.32; H, 3.92; found: C, 75.29; H, 3.90.

2.1u *3,3′-(Butane-1,1-diyl)bis(4-hydroxy-2H-chromen-2-one).*[34] White solid; Yield: 89% (336 mg); Rf: 0.35 (*n*-Hexane/EtOAc (1/9). M.p. 118–120 °C (119–123 °C); Rf = 0.5 (EtOAc/Hex: 8/2 v/v); IR (KBr): *v* 3400, 3074, 1650, 1620, 1565, 1266, 1100, 765 cm^{-1}; ^1H NMR (DMSO-*d6*): δ 0.90 (t, CH_3), 1.25–1.31 (m, CH_2), 2.13 (q, J = 7.2 Hz, CH_2), 4.96 (d, J = 8.0 Hz, CH), 7.38–7.41 (m, ArH), 7.63 (t, J = 7.2 Hz, ArH), 7.99 (d, J = 7.2 Hz, ArH), 11.97 (brs, 2H, OH) ppm; ESI-MS (*m/z*): 401 $[M + Na]^+$, 379 $[M + H]^+$; Anal. calcd. for $C_{22}H_{18}O_6$ (378.11 g/mol): C, 69.83; H, 4.79; found: C, 69.79; H, 7.76.

2.2 *Biology*

2.2a *Anthelmintic assay*: This study employed two species of helminth parasites, *Raillietina echinobothrida* (a poultry tapeworm) and *Syphacia obvelata* (a rodent pinworm). *R. echinobothrida* was collected from the intestines of domestic fowl that were freshly slaughtered at local abattoirs and *S. obvelata* was collected from the intestines of laboratory-maintained infection in Swiss mice. The adult worms were collected in 0.9% phosphate-buffered saline (PBS) at 37 ± 1 °C and washed several times before they were used for in vitro assays. The worms were divided in three groups (n = 5) and maintained in Petri dishes containing PBS at 37 ± 1 °C inside an incubator. Group I and II of worms served as a negative and a positive control, respectively, where the former received only PBS and the latter was given 800 µg/mL of a reference drug, praziquantel for tapeworms, and albendazole in case of nematodes. Group III of worms were exposed to 800 µg/mL concentrations of different biscoumarin compounds. Observations were made at regular intervals under a light microscope and the paralysis and mortality time of worms were recorded to adjudge the anthelmintic potency of compounds.[54]

2.2b *Statistical analysis*: The experiments were repeated three times and the results are represented as mean ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey test with $p < 0.05$ considered as statistically significant. All the statistical calculations were done using Origin version 8.0 SR6.

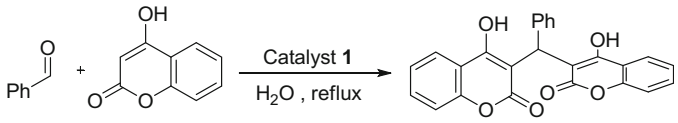
2.2c *Molecular docking simulation*: In our experiment, molecular docking studies of biscoumarins (**2a–2s**) with β-tubulin were performed to observe binding affinity and appropriate orientation of the compounds inside the protein receptor by using Auto Dock 4.2 program.[55] Autodock 4.2 is an interactive molecular graphics program employed to investigate the drug-protein interaction.[56] The crystal structure of β-tubulin (PDB ID: 1QSD) was obtained from the research collaboratory for structural bioinformatics (RCSB) protein data bank. The protein structure in pdb format was then prepared with the structure preparation tool available in Auto Dock Tools package version 1.5.4. All the water molecules and the native ligand had been removed from the crystal structure of protein and then polar hydrogen atoms were added for saturation, Gasteiger charges were computed and non-polar hydrogen atoms were merged. A grid box was built with a grid spacing of 0.414 Å and the dimension of 60 × 60 × 60 points along x, y and z axes around the active site of the protein receptor. This grid box carries the complete binding site of the active site of protein and gives sufficient space for the translational and rotational movement of compounds. After that ten possible docking runs were performed with step sizes of 2 Å for translation and 500 for rotation. A maximum number of energy evaluations are set to 25000 and a maximum number of 27000 GA operations were generated with an initial population of 150 individuals. The rate of gene mutation and crossover was set to be 0.02 and 0.80, respectively.

3. Results and Discussion

3.1a *Chemistry*. The investigation started with stirring a suspension of freshly distilled benzaldehyde (0.106 g, 1 mmol) with 4-hydroxycoumarin (0.324 g, 2 mmol) in the presence of the catalyst **1** (0.03 g, 0.1 mmol) in water under conventional heating at reflux temperature. To our surprise, the reaction was complete within 15 min as indicated by thin-layer chromatography (TLC) plate and gave 96% isolated yield (0.396 g) after recrystallization. Although we were elated with the result, we reasoned that if the reaction can be carried out under homogeneous reaction conditions rather than starting with a suspension, the reaction time could further be reduced. Therefore, we screen the reaction with a host of other solvents (Table 1) to observe that ethanol has almost similar efficacy as a solvent, while the other solvents such as methanol, acetonitrile, THF, and diethyl ether were not as effective in comparison We reason that the optimum efficacy of the reaction in water medium might be due to increased ion concentration of water at high temperature and "on water" rate acceleration. Ion concentration (H_3O^+ and OH^-) of water increases with the increase in temperature.[57] Under such circumstances water may act like a bifunctional catalyst that can accelerate the reaction rate by activating the electrophilic aldehyde and nucleophilic 4-hydroxycoumarin. Given the fact that the reaction is very slow at room temperature reaction conditions and did not give complete conversion even in 8 h, the temperature aided rate acceleration due to increased ionic strength is a clear possibility. On the other hand, the rate acceleration of the reactions in water medium may be due to "on water" conditions that give high yield and fewer side products when either the starting material, product, or both are relatively insoluble in water.[58] Such rate acceleration in aqueous suspension are attributed to high heat capacity (4.18 J cm^{-3} K), hydrogen bonding, charge stabilization and dipolar effect of water.[59] Since the reactants, products and the catalyst are insoluble in water, "on water" rate acceleration might have helped to reduce the reaction time.

The catalyst loading was also checked to observe that 10 mol% of the catalyst gives optimum yield under our reaction conditions while increasing the catalyst loading to 15 mol% and lowering till 5 mol% were not found optimum as reflected in Table 1 (entries 8–10). In the absence of the catalyst, the reaction gave only 14% yield upon heating the reaction mixture at reflux in water for 1 h. The model reaction was also screened for other organocatalysts such as bifunctional L-proline, H-bonding catalyst 1,3-diphenylthiourea, pyrrolidine and *p*-TsOH, among which L-proline (Entry 12) showed maximum efficacy to give 84% yield in 150 min of reflux in water. An equimolar mixture of L-proline and 1,3-diphenylthiourea bearing the catalytic site as those of the catalyst **1** was also found less efficient in comparison and took the same time as that of L-proline to give slightly higher yield (Entry 16). Therefore, the model reaction gave the optimum efficacy upon heating at reflux in water medium

Table 1. Optimization of reaction conditions[a].



Entry	Solvents	Cat (mol%)	Temp.	Time/min	% Yield[b]
1	H_2O	**1** (10)	Reflux	15	96
2	EtOH	**1** (10)	Reflux	30	92
3	MeOH	**1** (10)	Reflux	30	78
4	ACN	**1** (10)	Reflux	45	76
5	THF	**1** (10)	Reflux	45	79
6	Diethyl ether	**1** (10)	Reflux	45	52
7	H_2O	**1** (10)	RT	8[c]	68
8	H_2O	**1** (15)	Reflux	15	96
9	H_2O	**1** (5)	Reflux	30	84
10	H_2O	**1** (8)	Reflux	30	89
11	H_2O	–	Reflux	60	14
12	H_2O	L-Proline (10)	Reflux	150	84
13	H_2O	1,3-Diphenylthiourea (10)	Reflux	180	32
14	H_2O	Pyrrolidine (10)	Reflux	180	44
15	H_2O	*p*-TsOH (10)	Reflux	180	28
16	H_2O	L-Proline (10) + 1,3-diphenylthiourea(10)	Reflux	150	90

[a]Reaction conditions: benzaldehyde (1 mmol) and 4-hydroxycoumarin (2 mmol).

[b]Isolated yield.

[c]Time in hour.

for 15 min in the presence of 10 mol% of the catalyst **1**.

Different substituted aromatic aldehydes were condensed with 4-hydroxycoumarin under the optimized reaction condition to achieve 3,3′-arylidenebis-4-hydroxycoumarins in excellent yields within a very short reaction time (Table 2). The structure of these compounds was confirmed by mass spectral, IR, ^{1}H NMR and ^{13}CNMR analyses. The nature of substituents on the phenyl ring has hardly affected the reaction rate and yields. Due to the mild nature of the catalyst **1**, the acid-sensitive functional groups such as OMe, CN and methylenedioxy were not affected and gave excellent yields. The aliphatic aldehyde was found to be less reactive as evident from the reaction of butyraldehyde with 4-hydroxycoumarin that took 45 min for complete conversion to give 89% yield of the desired product (Entry 19).

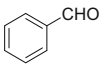
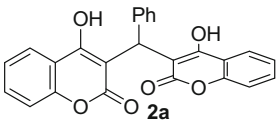
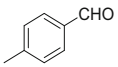
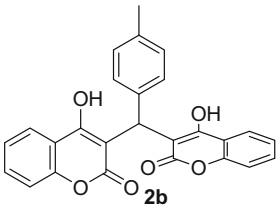
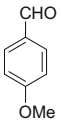
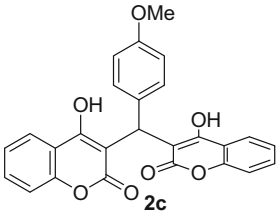
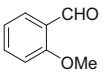
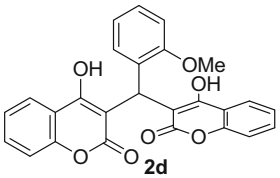
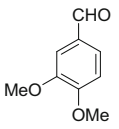
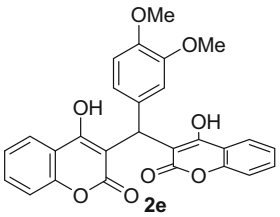
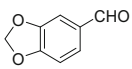
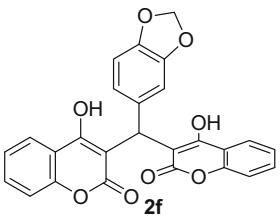
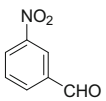
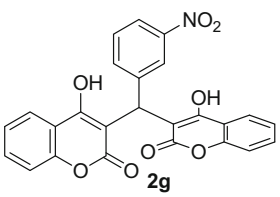
A plausible mechanistic route is proposed (Scheme 2). The catalyst **1** reacts with aldehyde to generate the iminium salt **A** which upon nucleophilic attack by 4-hydroxycoumarin forms the intermediate **B1**. Intermediate B may undergo keto-enol tautomerism to form energetically stable intermediates **B2** or may form **B3** by releasing the catalyst (1). The 1,4-addition of another molecule of 4-hydroxycoumarin to **B3** formed the bis-coumarin derivative.

3.1 *Biology*

The *in vitro* anthelmintic tests showed that the test compounds are comparatively more effective against *R. echonobothrida* than *S. obvelata* (Figure 2). While compounds showed excellent to moderate efficacy against *R. echonobothrida*, they were only moderately effective against *S. obvelata,* with only a few compounds that showed efficacy comparable to albendazole (ABZ). It also emerged out from this study that about half of the tested compounds possess a better efficacy against *R. echonobothrida* than the reference drug, praziquantel (PZQ). Notably, the compounds derived from 2-chlorobenzaldehyde (**2o**), 4-fluorobenzaldehyde (**2k**), 4-cyanobenzaldehyde (**2j**) and benzaldehydes (**2a**), took only about half the time for paralysis and mortality than that of the reference drug PZQ. This study also revealed that the structure of the compounds plays an important role in regulating their anthelmintic efficacy.

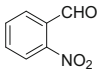
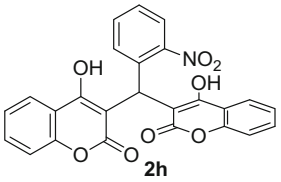
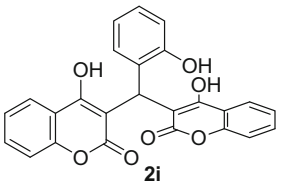
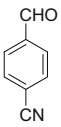
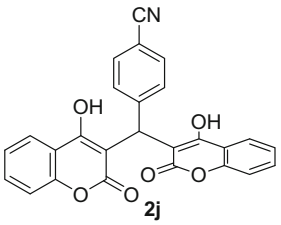
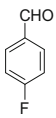
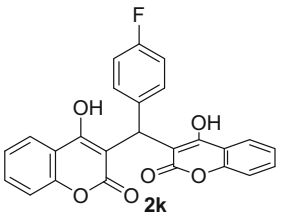
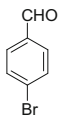
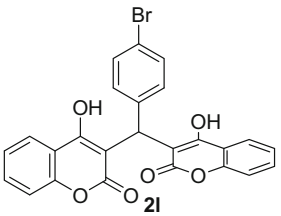
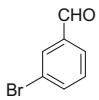
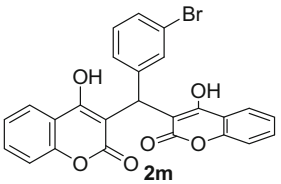
Initial testing of coumarin, uracil, biscoumarin and bis-uracil showed that, in particular, the biscoumarins

Table 2. Synthesis of biscoumarins *via* Scheme 1[a]

Entry	Aldehyde	Product	t/min	Yield[b]	M.p./°C	M.p.[ref.]
1		 **2a**	15	96	230-232	228-230 [27]
2		 **2b**	15	92	267-268	268-269 [37]
3		 **2c**	15	95	247-249	247-249 [27]
4		 **2d**	15	93	218-220	217-219 [27]
5		 **2e**	15	90	258-260	256-258 [37]
6		 **2f**	15	94	256-257	254-257 [37]
7		 **2g**	15	97	238-240	238-239 [36]

possess an excellent activity against both *Raillietina* and *Syphacia* parasites.[60] These finding led us to synthesize a series of biscoumarin derivatives, with different benzaldehydes substitutions. In the present study, almost all synthesized biscoumarins showed significant activity against *R. echinobothrida,* but only

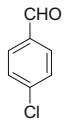
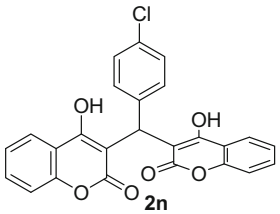
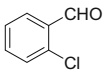
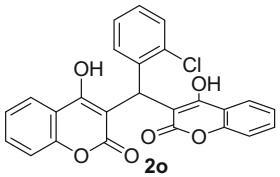
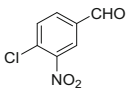
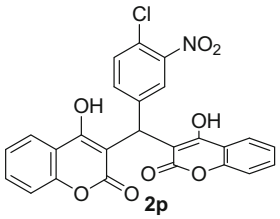
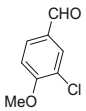
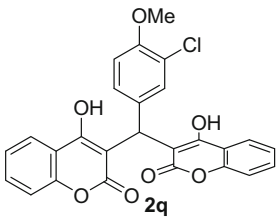
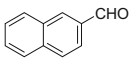
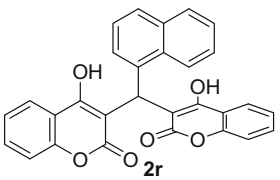
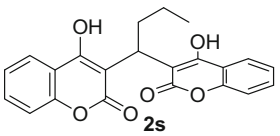
Table 2. continued

8			15	95	202-205	202-205 [27]
9			15	90	252-254	253-254 [35]
10			15	96	262-264	261-263 [37]
11			15	95	215-216	215-216 [39]
12			15	93	268-269	266-268 [27]
13			15	94	253-255	252-254 [36]

moderate to low efficacy against *S. obvelata*. The observed differences in the activity of compounds against two test parasites could be attributed to the differences in the drug diffusion capacity of the two helminth species. While *Raillietina,* which is a cestode, has an outer body surface called tegument which allows solutes to cross *via* trans-epithelial transport. On the other hand, *Syphacia* possesses a complex cuticle that usually hinders a free passage of large molecules. It is believed that the lipid barriers in the nematode cuticle act as a restriction for passive transport. While lipophilic moieties can passively diffuse through the membrane, the poor lipid solubility of ionised moieties are excluded from such diffusion.[61,62] This justification bodes well with our earlier observation,[60] where we noted that trisubstituted methane bearing 4-hydroxycoumarin and 1,3-dimethyl uracil were potent against *S. obvelata.*

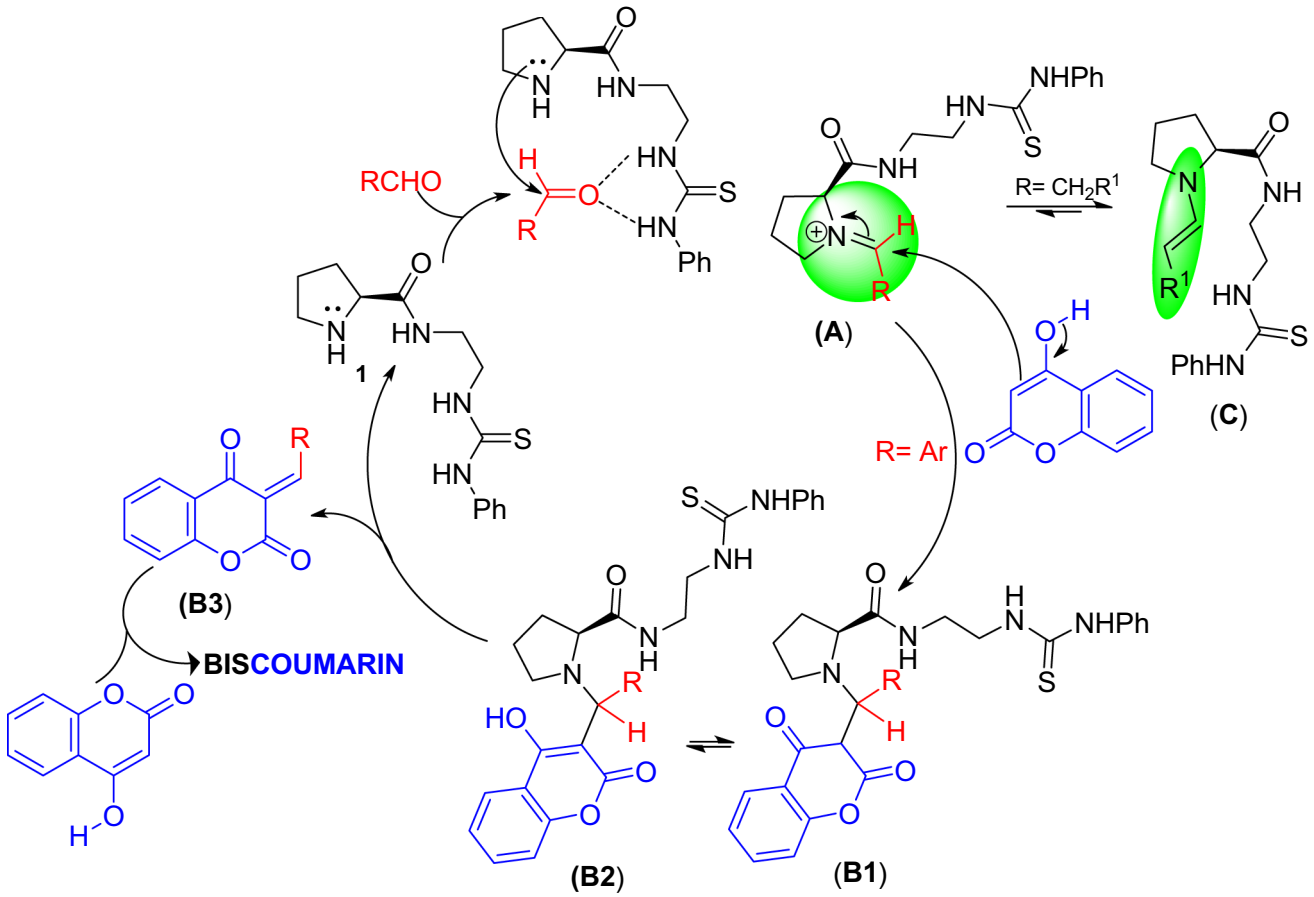
The structure–activity studies on synthesized compounds revealed that when substitution in the

Table 2. continued

14		 **2n**	15	97	257-259	258-259 [27]
15		 **2o**	15	96	202-204	203-204 [27]
16		 **2p**	15	97	250-252	249-251 [29]
17		 **2q**	15	93	248-250	NA
18		 **2r**	15	95	265-266	264-265 [39]
19		 **2s**	45	89	118-120	119-123 [38]

[a]Reaction conditions: aldehyde (1 mmol), 4-hydroxycoumarin (2 mmol) and **1** (0.1 mmol).
[b]Isolated yield.

compound is with an electron-withdrawing moiety, the activity of the compound got enhanced, while the presence of an electron-donating group led to a reduction in the efficacy of the compound. Likewise, the compounds with bromo, chloro, cyano, fluoro and nitro substituents, irrespective of their position in the phenyl ring, showed an efficacy that was almost comparable to that of the reference drug, i.e., the mortality of *Raillietina* worms in only about 2 h. In contrast, the substitution with a methoxy group showed a decrease in the efficacy of the compound. Further, a compound like **2q**, which has both an



Scheme 2. Proposed mechanism for the synthesis of biscoumarin using L-proline derivative as catalyst.

electron-withdrawing group and an electron-donating group in its architecture showed a shift in its activity towards the direction dictated by the donating group, thus decreasing the activity of the compound. It is also noteworthy here that the presence of two electron-withdrawing groups in the compound, as in compound **2p**, does not enhance the activity of compound than those in the compound with only one electron-withdrawing moiety in their structure.

3.2 *Molecular docking*

Molecular docking simulation is an attractive platform in the field of rational drug design and discovery. This method is widely used in the prediction of the ligand conformation and its orientation in the active site of the receptor. Molecular docking of anthelmintic drug with β-tubulin to study the activity by drug-tubulin interaction is already proven[63] because inhibition of β-tubulin of the helminths can severely affect their vital cellular functions such as mitosis, motility, and transport.[64–66] To rationalize the anthelmintic activity of the synthesized biscoumarins and understand their possible interactions, molecular docking simulation of all the compounds have been carried out against β-tubulin (pdb id: 1oj0).[63] Analysis of the molecular docking simulation shows that all the compounds (**2a**–**2s**) bind very strongly with the amino acid residue inside the active site of the β-tubulin (Table 3).

Among all the compounds, compound **2a** and **2j** show the highest binding interaction of −9.25 kcal/-mol and −9.03 kcal/mol with the protein receptor. Apart from **2j**, the compounds **2k**, **2l**, **2m**, **2n** and **2o** also show effective affinity toward the active site of β-tubulin with a docked score of −8.33, −8.74, −8.67, −8.03 and −8.30 kcal/mol, respectively, indicating that the compounds having electron-withdrawing group exhibits good efficacy. Whereas compounds with an electron-donating group show lower binding affinity as observed from our docking simulation **2b** (−7.11 kcal/mol) and **2s** (−7.27 kcal/mol). The docking results are in good agreement with the experimental study. The higher binding energy of these compounds towards target protein can be attributed to their sterically unhindered structure and

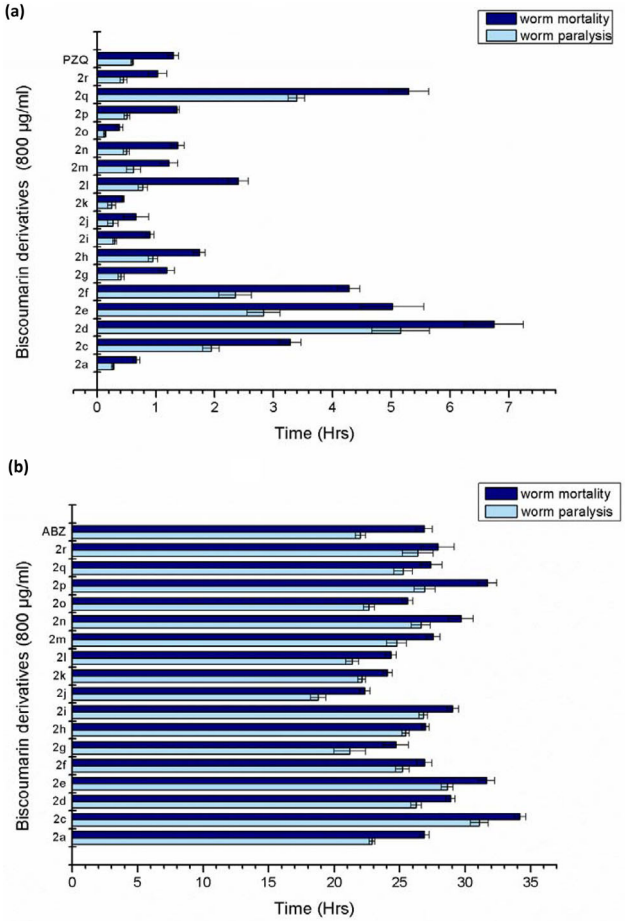


Figure 2. *In vitro* anthelmintic efficacy of biscoumarin derivatives against (a) *R. echinobothrida* and (b) *S. obvelata.* The paralysis and mortality time of control worm for respective experiments was (a) 49.38 ± 1.14 h and 52.2 ± 1.27 h; (b) 37.29 ± 2.45 h and 42.18 ± 2.13 h. Data are expressed as mean ± SEM; $p < 0.05$ compared with control groups; one-way ANOVA, followed by Tukey's test.

highly reactive functional groups like cyano, methoxy and hydroxyl groups which are involved in hydrogen bonding interaction. The binding modes of two of the active compounds **2a** and **2j** are shown in Figures 3 and 4.

Compound **2a** interacts with the amino acid residue threonine of chain A of (Thr64A) β-tubulin at a distance of about 2.56 Å. Other amino acid residue such as Thr58(B), Asp57(B), Arg60(B), Arg60(A), Asp57(A), Thr64(B), Thr58(A), Leu61(B) and Leu61(A) also play a significant role in binding with the compound **2a** in the active site of β-tubulin. In Figure 2, the docking output of the compound **2j** with the tubulin receptor is placed and found that amino acid residue Gln25(B), Tyr23(A), Tyr23(B), Tyr22(A), Glu26(B), Tyr22(B), Glu26(A) and Gln25(A) participate in binding with the compound.

Table 3. Docked score of the test compounds with the β-tubulin.

Compounds	Docked score in kcal/mol
2a	− 9.25
2b	− 7.11
2c	− 8.03
2d	− 8.13
2e	− 7.37
2f	− 8.49
2g	− 7.99
2h	− 7.50
2i	− 7.46
2j	− 9.03
2k	− 8.33
2l	− 8.74
2m	− 8.67
2n	− 8.03
2o	− 8.30
2p	− 8.32
2q	− 8.41
2r	− 8.84
2s	− 7.27

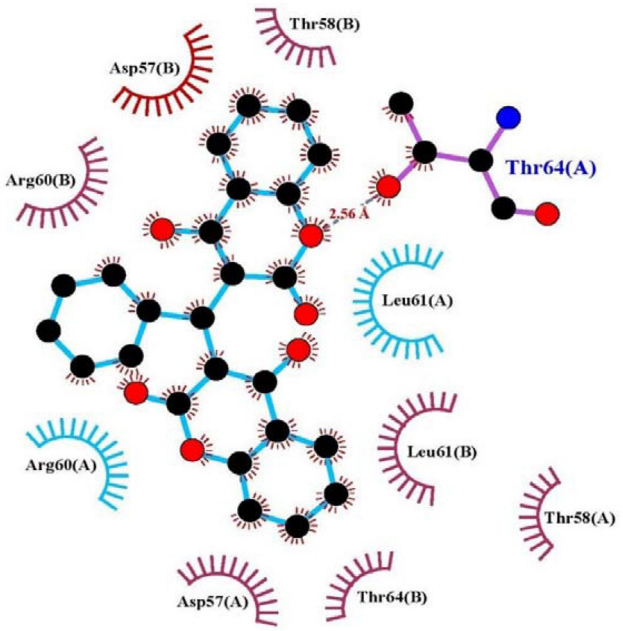


Figure 3. Best docking conformation of the compound **2a** in the active site of β-tubulin. H-bonding interaction of the compound with amino acid residue is shown.

Three hydrogen bonding interactions of **2j** with amino acid residues Gln25(B) (2.92 Å), Tyr22(A) (2.51 Å) and Gln25(A) (2.83 Å) have been observed.

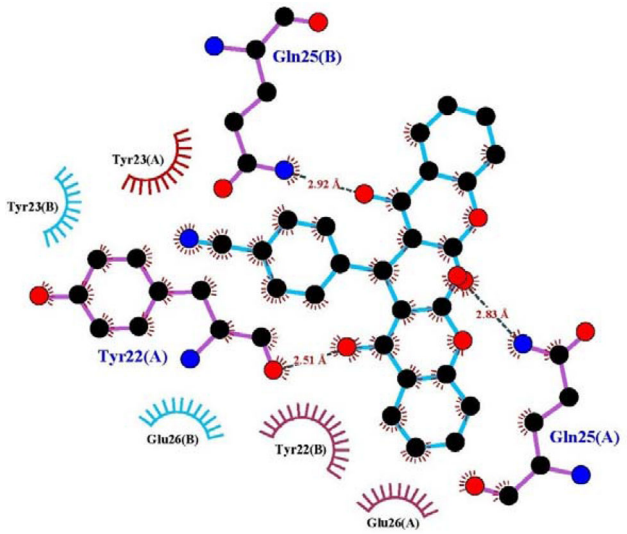


Figure 4. Best docking conformation of the compound **2j** in the active site of β-tubulin. H-bonding interaction of the compound with amino acid residue is shown.

4. Conclusions

We have established an excellent methodology for the synthesis of biscoumarins from the reaction of 4-hydroxycoumarin with aldehydes using L-proline derived amidoamine thiourea **1** as a catalyst and hence developed a viable alternative to mostly acid-catalyzed methods reported in the literature. The presence of both acidic and basic catalytic sites in the bifunctional catalyst facilitated the use of low catalyst loading (10 mol%), and short reaction time (15 min). The use of water, a green solvent, as a reaction medium, use of simple filtration to terminate the reaction and separate the product from the reaction mixture, chromatography free purification of the product *via* recrystallization and no use of hazardous organic solvents during entire process are some of the salient features.

Remarkably, the biscoumarins have been found to demonstrate excellent anthelmintic activity against the helminth *R. echinobothrida*. In the *in vitro* anthelmintic study of the biscoumarins, about half of the compounds showed a potential efficacy against cestode parasite which was comparable to the reference drug, praziquantel (PZQ). In particular, the compound **2o** showed 4 times more worm mortality in comparison to PZQ. The analogues with a halogen or other electro withdrawing groups displayed remarkable anthelmintic activity. The study reveals strong binding interaction of all the test compounds with several amino acid residues in the active site of β-tubulin. Among all, **2a** and **2j** show highest binding efficacy with a binding energy of -9.25 and -9.03 kcal/mol, hence can be a good inhibitor. The compounds **2k**, **2l**, **2m**, **2n** and **2o** are also observed to be a good inhibitor of β-tubulin which also agrees well with the experimental data. A detailed study on the mode of action of the biscoumarins and *in vivo* efficacy of the anthelmintic candidates are currently underway and will be reported in due course of time.

Supplementary Information (SI)

The ^{1}HNMR and ^{13}CNMR spectra of selected compounds. This material is available at www.ias.ac.in/chemsci

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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