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REGULAR ARTICLE

Coumarin-based Trisubstituted Methanes as Potent Anthelmintic: Synthesis, Molecular Docking and *in vitro* Efficacy

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Abstract. A series of coumarin-based trisubstituted methanes (TRSMs) having uracil scaffold was synthesised employing a green, chromatography-free, and a highly efficient sonochemical multicomponent reaction of diverse aldehydes with 1,3-dimethyl-6-aminouracil and 4-hydroxy-coumarin in the presence of a catalytic amount of DABCO at room temperature and tested their anthelmintic efficacy against helminth parasites, *Raillietina echinobothrida* and *Syphacia obvelata*. Some of the TRSMs with substituents in the para position of the phenyl ring showed excellent anthelmintic activity in comparison to the commonly used drugs such as albendazole and praziquantel. The docking study revealed the binding interaction of all the optimized compounds with several amino acid residues in the active site of β-tubulin. The compounds showing good docking score with β-tubulin showed comparable anthelmintic activity experimentally as well.

Keywords. Coumarin-based TRSMs; Sonochemical MCR; Anthelmintic; *Raillietina echinobothrida*; *Syphacia obvelata*; Molecular docking.

1. Introduction

Helminth infection is one of the most common gastrointestinal infection in human beings that vectors through the air, food, and water. These parasites secrete toxins and steal the vital nutrients from host bodies to create a disease state.[1] Ideally, a good anthelmintic should have a broad spectrum of action, high percentage of cure with a single therapeutic dose, besides free from toxicity to its host.[2] However, with only limited pharmacopoeia of anthelmintic available presently (e.g., benzimidazoles, nicotinic acetylcholine agonists, macrocyclic lactones), particularly against human helminths, not all drugs possess these desired attributes and some of them have serious side effects such as apoptosis and mitotic arrest.[3,4] Moreover, benzimidazoles (e.g., albendazole) are more effective against hookworm infection, but poor against whipworm T. trichiura.[5] On the other hand, the current nicotinic acetylcholine receptor (nAChR) agonists such as pyrantel and levamisole have much lower efficacy than the benzimidazoles.[6] Therefore, the development of a new nicotinic acetylcholine receptor (nAChR) agonists having comparable efficacy to that of benzimidazole bears critical significance. Nevertheless, since cases of emergence of resistance to some anthelmintic drugs have also been reported in recent years, development and screening of new drugs to identify leads with better anthelmintic efficacy with lower side effects is very important.[7]

Trisubstituted methanes (TRSMs)[8] occupy a special place in pharmacology due to their applications as anti-cancer,[9] antiproliferative,[10] and antitubercular.[11,12] Many coumarin-fused heterocycles have been reported to exhibit antifungal, anticoagulant, antimicrobial, antiasthmatic, antitumor, and anti-HIV activities.[13–17] Likewise, uracil derivatives have exhibited numerous pharmacological activities like analgesic, antiallergic, antibacterial, antifungal, antihypertensive, antimalarial, antitumor, bronchiodilator, cardiotonic,

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vasodilator, and CNS depressant properties.[18–22] Since coumarin derivatives are reported for acetylcholinesterase (AChE) inhibition, we wanted to observe the effect of the introduction of 6-amino-1,3-dimethyluracilyl on AChE inhibition in coumarin-based trisubstituted methane (TRSM). The result was extremely significant where the TRSM, 3-(6-amino-1,3-dimethyluracilyl)benzyl-4-hydroxycoumarin (**1a**) acted as a potent AChE inhibitor with IC_{50} value at 48.49 ± 5.6 nM which is much better than the reference drug donepezil (IC_{50} = 74.13 ± 8.3 nM).[23] This finding led us to explore if the coumarin and uracil containing chimeric TRSM that have shown extremely good AChE inhibition can act as potential anthelmintics. Given the poor efficacy of currently used nicotinic acetylcholine agonists,[6] we planned to synthesize a series TRSMs to study their anthelmintic efficacy.

For the synthesis of the chimeric TRSMs from the reaction of aldehyde, 4-hydroxycoumarin and 6-amino-1,3-dimethyluracil, Bharti *et al.,* carried out the reaction at reflux temperature in the presence of organocatalysts such as L-proline[24] and bifunctional thiourea.[25] Lu and Cai also reported the synthesis of such compounds *via* one-pot reaction at 80 °C using 20 mol% *p*-toluenesulfonic acid as catalyst in poly(-ethylene glycol)$_{200}$/H_2O as solvent.[26] In all the cases, the reaction took high catalyst loading (20 mol%) and long reaction time (4–5 h) even at reflux temperature. Given the recent emphasis on multicomponent reactions (MCRs) to design reactions,[27–30] development of energy-efficient protocols to compliment green chemistry requirements is a very important aspect. Sonochemical reactions have been proven to increase the reaction rate even at room temperature leading to a reduction of byproducts and increased energy efficacy.[31–33] Therefore, we planned to employ sonochemistry to bring better efficacy for the synthesis of these coumarin based TRSMs. Our effort led to the development of a simple multicomponent reaction of aldehydes, 1,3-dimethyl-6-aminouracil and 4-hydroxy-coumarin to synthesize a library of our target TRSMs (Scheme 1).

2. Experimental

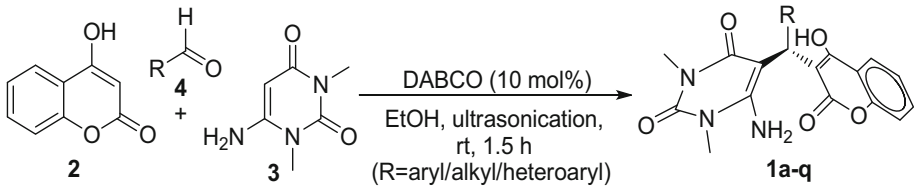
2.1 *Chemistry*

2.1a *General*: The starting materials were commercially available and were used without further purification. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. Single crystal was analyzed by single-crystal XRD. IR spectra were recorded on Perkin-Elmer Spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded either in $CDCl_3$ or in DMSO-d_6 and are expressed in parts per million (δ, ppm) downfield using Me_4Si as the internal standard on a Bruker AC-400. ^{1}H NMR data are reported in the order of chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), coupling constant (J) in hertz (Hz), and a number of protons. Mass spectra were obtained from Waters ZQ 4000 mass spectrometer by the ESI method. The reaction progress was monitored by thin-layer chromatography on silica gel G in ethyl acetate using iodine vapour as the detecting agent. Melting points are uncorrected and were determined by the melting point apparatus using a capillary tube method.

2.1b *Typical procedure for the synthesis of compounds (**1a–q**)*: To an equimolar solution of aldehyde (1 mmol), 6-amino-1, 3-dimethyluracil (1 mmol, 155 mg) and 4-hydroxycoumarin (1 mmol, 162 mg) in 1 mL of ethanol, 10 mol% DABCO (11 mg) was added and the resulting mixture was ultrasonicated at room temperature for 1.5 h. On completion of the reaction (monitored by TLC) a solid product precipitated out. The solid was filtered off and rinsed with ice-cold water to wash off the catalyst. The pure product was obtained by recrystallization from ethanol.

2.1c *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(phenyl)methyl)-1,3-dimethylpyrimidine -2,4(1H,3H)-dione (**1a**)*[26]: White Solid. M.p. 215–216 °C; IR (KBr): ν 3434, 3245, 2955, 1666, 1617, 1569, 1542, 706 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.14 (s, 3H, -NC$\underline{H}_3$), 3.38 (s, 3H, -NC$\underline{H}_3$), 5.63 (s, 1H, -C$\underline{H}$), 7.16–7.85 (m, 11H, Ar$\underline{H}$, -N$\underline{H}_2$), 14.00 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.25, 30.59, 36.07, 74.93, 86.82, 104.67, 116.17, 116.97, 123.74, 124.34, 125.71, 126.39, 128.13, 132.45, 138.31, 150.08, 151.98, 155.18, 163.85, 164.11, 165.86; ESI-MS (m/z): 428.0 $[M + Na]^+$, 406.0 $[M + H]^+$. Anal. Calcd for $C_{22}H_{19}N_3O_5$: C 65.18, H 4.72, N, 10.37. Found: C 65.08, H 4.75, N 10.42.

2.1d *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(2-nitro-phenyl)methyl)-1,3-dimethyl-pyrimidine-2,4(1H,3H)-dione (**1b**)*: Yellow Solid. M.p. 220–222 °C; IR (KBr): ν 3398, 3230, 2941, 1705, 1643, 1618, 1570, 1526, 1361 cm^{-1}; ^{1}H NMR (400 MHz, DMSO- d_6, δ ppm): 3.10 (s, 3H, -NC$\underline{H}_3$), 3.36 (s, 3H, -NC$\underline{H}_3$), 6.02 (s, 1H, -C$\underline{H}$), 7.35–7.86 (m, 10H, Ar$\underline{H}$, -N$\underline{H}_2$), 13.64 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.20, 30.68, 33.50, 86.20, 103.17, 116.22, 116.58, 123.84, 124.46, 124.06, 127.69, 129.17, 131.62, 131.88, 132.73, 149.41, 149.82, 151.91, 155.16, 163.92, 164.06, 165.31; ESI-MS (m/z): 451.0 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}N_4O_7$: C 58.67, H 4.03, N 12.44. Found: C 58.63, H 4.10, N 12.48.



Scheme 1. Synthesis of coumarin based unsymmetrical TRSMs.

2.1e *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(3-nitro-phenyl)methyl)-1,3-dimethyl-pyrimidine-2,4(1H,3H)-dione (**1c**)*: Yellow Solid. M.p. 230–231 °C IR (KBr): ν 3461, 3208, 2947, 1702, 1673, 1609, 1572, 1524, 1509, 1342 cm^{-1}; 1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.14 (s, 3H, -NC$\underline{H}_3$), 3.38 (s, 3H, -NC$\underline{H}_3$), 5.76 (s, 1H, -C$\underline{H}$), 7.38–8.07 (m, 10H, Ar$\underline{H}$, -N$\underline{H}_2$), 13.96 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.34, 30.70, 36.14, 86.04, 104.36, 116.29, 121.12, 121.26, 123.87, 124.49, 129.70, 132.73, 133.80, 141.40, 148.03, 150.10, 152.10, 155.41, 163.96, 164.15, 165.58. ESI-MS (m/z): 451.0 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}N_4O_7$: C 58.67, H 4.03, N 12.44. Found: C 58.65, H 4.08, N 12.46.

2.1f *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-nitro-phenyl)methyl)-1,3-dimethyl-pyrimidine-2,4(1H,3H)-dione (**1d**)*: Yellow Solid. M.p. 205–206 °C; IR (KBr):ν 3390, 3213, 2927, 1668, 1622, 1516, 1347 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.14 (s, 3H, -NC$\underline{H}_3$), 3.38 (s, 3H, -NC$\underline{H}_3$), 5.75 (s, 1H, -C$\underline{H}$), 7.38–8.11 (m, 10H, Ar$\underline{H}$, -N$\underline{H}_2$), 13.96 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.41, 30.73, 36.65, 86.43, 104.34, 116.30, 116.99, 123.31, 123.93, 124.50, 128.06, 132.76, 145.87, 147.42, 150.17, 152.14, 155.39, 164.12, 164.25, 165.76. ESI-MS (m/z): 473.0 $[M + Na]^+$. Anal. Calcd for $C_{22}H_{18}N_4O_7$: C 58.67, H 4.03, N 12.44. Found: C 58.71, H 4.05, N 12.51.

2.1g *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-fluoro-phenyl)methyl)-1,3-dimethyl-pyrimidine-2,4(1H,3H)-dione (**1e**)*[25]: White Solid. M.p. 246–248 °C; IR (KBr): ν 3429, 3241, 2953, 1694, 1649, 1620, 1570, 1503, 1219 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.14(s, 3H, -NC$\underline{H}_3$), 3.37 (s, 3H, -NC$\underline{H}_3$), 5.60 (s, 1H, -C$\underline{H}$), 7.02–7.85 (m, 10H, Ar$\underline{H}$, -N$\underline{H}_2$), 13.99 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.28, 30.61, 35.58, 86.73, 104.82, 114.63, 114.84, 116.19, 117.00, 123.78, 124.35, 128.36, 128.44, 132.51, 150.09, 152.02, 155.18, 163.85, 164.10, 165.77. ESI-MS (m/z): 424.2 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}FN_3O_5$: C 62.41, H 4.29, N 9.92. Found: C 62.38, H 4.28, N 9.94.

2.1h *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(2-chloro-phenyl)methyl)-1,3-dimethyl-pyrimidine-2,4(1H,3H)-dione (**1f**)*[25]: White crystalline solid. M.p. 227–229 °C; IR (KBr): ν 3368, 3218, 2953, 2838, 1667, 1620, 1570, 1508, 772 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.14(s, 3H, -NC$\underline{H}_3$), 3.38 (s, 3H, -NC$\underline{H}_3$), 5.62 (s, 1H, -C$\underline{H}$), 7.23–7.86 (m, 10H, Ar$\underline{H}$, -N$\underline{H}_2$), 13.46 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.38, 30.77, 35.99, 86.25, 104.93, 116.24, 116.94, 123.87, 124.49, 126.85, 128.21, 128.67, 130.17, 132.56, 132.82, 136.80, 150.17, 151.95, 154.82, 163.59, 164.15, 165.10; ESI-MS (m/z): 462.1 $[M + Na]^+$, 464.1 $[M + Na]^+$. Anal. Calcd for $C_{22}H_{18}ClN_3O_5$: C 60.07, H 4.12, N 9.55. Found: C 60.02, H 4.09, N 9.59.

2.1i *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-chlor-ophenyl)methyl)-1,3-dimethyl-pyrimidine-2,4(1H,3H)-dione (**1g**)*[26]: White crystalline solid. M.p. 219–220 °C; IR (KBr): ν 3368, 3218, 2952, 1667, 1571, 1509, 1449, 1354, 1195, 1105, 1068, 766 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.14(s, 3H, -NC$\underline{H}_3$), 3.37(s, 3H, -NC$\underline{H}_3$), 5.61 (s, 1H, -C$\underline{H}$), 7.17–7.36 (m, 6H, Ar$\underline{H}$, -N$\underline{H}_2$), 7.44 (d, 1H, J = 8 Hz, Ar$\underline{H}$), 7.65 (t, J = 8 Hz, 1H, Ar$\underline{H}$), 7.84 (d, J = 8 Hz, 1H, Ar$\underline{H}$), 13.99 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.30, 30.63, 35.76, 86.54, 104.59, 116.22, 123.81, 124.43, 128.02, 128.55, 130.37, 132.60, 137.62, 150.09, 152.03, 155.23, 163.87, 164.10, 165.73; ESI-MS (m/z): 440.1 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}ClN_3O_5$: C 60.07, H 4.12, N 9.55. Found: C 60.04, H 4.08, N 9.60.

2.1j *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(2-bromophenyl)methyl)-1,3-dimeth-ylpyrimidine-2,4(1H,3H)-dione (**1h**)*: White Solid. M.p. 210–212 °C; IR (KBr): ν 3365, 3210, 2972, 1665, 1570, 1443, 1176 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.29 (s, 3H, -NC$\underline{H}_3$), 3.55 (s, 3H, -NC$\underline{H}_3$), 5.67 (s, 1H, -C$\underline{H}$), 6.29 (s, 2H, -N$\underline{H}_2$), 7.08–7.60 (m, 7H, Ar$\underline{H}$), 8.03 (d, 1H, J = 8 Hz, Ar$\underline{H}$), 13.52 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.37, 30.76, 37.96, 79.27, 86.41, 104.73, 116.30, 116.91, 123.07, 123.89, 124.54, 127.40, 128.48, 129.78, 132.65, 133.66, 150.19, 151.97, 154.94, 163.70, 164.00, 165.06; ESI-MS (m/z): 508.0 $[M + H + Na]^+$, 510.0 $[M + H + Na]^+$. Anal. Calcd for $C_{22}H_{18}BrN_3O_5$: C 54.56, H 3.75, N 8.68. Found: C 54.49, H 3.75, N 8.72.

2.1k *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-bromophenyl)methyl)-1,3-dimethyl-pyrimidine-2,4(1H,3H)-dione (**1i**)* [25]: White crystalline solid. M.p.

236–238 °C; IR (KBr): ν 3355, 3210, 2972, 1667, 1570, 1443, 1176 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 3.14 (s, 3H, -NC$\underline{H}_3$), 3.37 (s, 3H, -NC$\underline{H}_3$), 5.59 (s, 1H, -C$\underline{H}$), 7.12–7.85 (m, 10H, Ar$\underline{H}$, -N$\underline{H}_2$), 13.98 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 28.30, 30.63, 35.82, 86.51, 104.50, 116.20, 116.95, 118.83, 123.79, 124.39, 128.94, 130.92, 132.56, 138.07, 150.07, 152.02, 155.23, 163.86, 164.09, 165.74. ESI-MS (m/z): 484.0 $[M + H]^+$, 486.0 $[M + H]^+$. Anal. Calcd for $C_{22}H_{18}BrN_3O_5$: C 54.56, H 3.75, N 8.68. Found: C 54.51, H 3.73, N 8.70.

2.1l *4-((6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)benzonitrile* ***(1j)***[25]: White crystalline solid. M.p. 221–223 °C; IR (KBr): ν 3424, 3192, 2961, 2225, 1710, 1666, 1619, 1572, 1512, 1198, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 3.32 (s, 3H, -NC$\underline{H}_3$), 3.57 (s, 3H, -NC$\underline{H}_3$), 5.74 (s, 1H, -C$\underline{H}$), 6.46 (s, 2H, -N$\underline{H}_2$), 7.31–7.37 (m, 4H, Ar$\underline{H}$), 7.57–7.62 (m, 3H, Ar$\underline{H}$), 7.97 (d, *J* = 8 Hz, 1H, Ar$\underline{H}$), 13.34 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 28.68, 30.05, 36.82, 88.39, 103.20, 109.99, 116.21, 117.05, 118.90, 124.48, 124.52, 127.19, 132.20, 132.64, 143.71, 150.38, 152.28, 154.95, 164.55, 165.37, 167.80. ESI-MS (m/z): 431.1 $[M + H]^+$. Anal. Calcd for $C_{23}H_{18}N_4O_5$: C 64.18, H 4.22, N 13.02. Found: C 64.14, H 4.19, N 13.07.

2.1m *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(2-methoxyphenyl)methyl)-1,3-dime-thyl-pyrimidine-2,4(1H,3H)-dione* ***(1k)***: Colourless crystal. M.p. 200–201 °C; IR (KBr): ν 3435, 3238, 2929, 1702, 1666, 1622, 1576, 1504, 757 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.15 (s, 3H, -NC$\underline{H}_3$), 3.38 (s, 3H, -NC$\underline{H}_3$), 3.55 (s, 3H, -OC$\underline{H}_3$), 5.60 (s, 1H, -C$\underline{H}$), 6.82–7.83 (m, 10H, Ar$\underline{H}$, -N$\underline{H}_2$), 13.55 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.24, 30.59, 33.00, 55.64, 86.80, 105.98, 111.18, 116.02, 117.09, 119.90, 123.62, 124.22, 126.90, 127.50, 128.15, 132.04, 150.05, 151.74, 154.46, 157.36, 162.70, 164.16, 165.22; ESI-MS (m/z): 436.0 $[M + H]^+$. Anal. Calcd for $C_{23}H_{21}N_3O_6$: C 63.44, H 4.86, N 9.65. Found: C 63.40, H 4.81, N 9.70.

2.1n *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-methoxyphenyl)methyl)-1,3-dime-thylpyrimidine-2,4(1H,3H)-dione* ***(1l)***[25]: Colourless solid. M.p. 180–181 °C; IR (KBr): ν 3368, 3224, 2968, 1725, 1709, 1623, 1607, 1573, 1518 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.33 (s, 3H, -NC$\underline{H}_3$), 3.56 (s, 3H, -NC$\underline{H}_3$), 3.77 (s, 3H, -OC$\underline{H}_3$), 5.69 (s, 1H, -C$\underline{H}$), 6.44 (s, 2H, -N$\underline{H}_2$), 6.81-8.00 (m, 8H, Ar$\underline{H}$), 13.43 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.66, 29.88, 35.83, 55.14, 89.44, 104.40, 113.74, 116.14, 117.37, 124.29, 124.48, 127.28, 129.18, 132.23, 150.60, 152.29, 154.59, 157.91, 164.61, 165.16, 168.01; ESI-MS (m/z): 458.3 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{21}N_3O_6$: C 63.44, H 4.86, N 9.65. Found: C 63.42, H 4.83, N 9.69.

2.1o *6-Amino-5-((4-chloro-3-nitrophenyl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione* ***(1m)***: Yellow Solid. M.p. 230–231 °C; IR (KBr): ν 3464, 3212, 2929, 1706, 1681, 1656, 1620, 1569, 1533, 1509, 1352, 1202, 768 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 3.33 (s, 3H, -NC$\underline{H}_3$), 3.58 (s, 3H, -NC$\underline{H}_3$), 5.71 (s, 1H, -C$\underline{H}$), 6.49 (s, 2H, -N$\underline{H}_2$), 7.35–7.98 (m, 7H, Ar$\underline{H}$), 13.40 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.41, 30.70, 35.94, 86.00, 104.28, 116.27, 117.07, 121.91, 123.70, 123.93, 124.47, 130.91, 132.18, 132.72, 140.64, 148.07, 150.22, 152.18, 155.45, 164.06, 164.24, 165.67. Anal. Calcd for $C_{22}H_{17}ClN_4O_7$: C 54.50, H 3.53, N 11.56. Found: C 54.47, H 3.49, N 11.60.

2.1p *6-Amino-5-((3-chloro-4-methoxyphenyl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione* ***(1n)***: White Solid. M.p. 228–230 °C; IR (KBr): ν 3468, 3203, 2948, 1698, 1677, 1651, 1622, 1571, 1504, 1465, 1354, 1062, 860, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 3.34 (s, 3H, -NC$\underline{H}_3$), 3.57 (s, 3H, -NC$\underline{H}_3$), 3.87 (s, 3H, -OC$\underline{H}_3$), 5.67 (s, 1H, -C$\underline{H}$), 6.40 (s, 2H, -N$\underline{H}_2$), 6.83 (d, *J* = 8,4 Hz, 1H, Ar$\underline{H}$), 7.03 (d, *J* = 8.4 Hz, 1H, Ar$\underline{H}$), 7.15 (s, 1H, Ar$\underline{H}$), 7.31–7.36 (m, 2H, Ar$\underline{H}$), 7.59 (t, *J* = 8.4 Hz, 1H, Ar$\underline{H}$), 7.99(d, *J* = 8 Hz, 1H, Ar$\underline{H}$), 13.43 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.42, 30.73, 35.37, 56.14, 86.78, 104.77, 112.47, 116.34, 117.08, 120.91, 123.90, 124.56, 126.46, 128.01, 131.67, 132.70, 150.26, 152.13, 152.78, 155.36, 164.03, 164.23, 165.93; ESI-MS (m/z): 492.0 $[M + Na]^+$, 494.0 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_6$: C 58.79, H 4.29, N 8.94. Found: C 58.76, H 4.48, N 8.97.

2.1q *6-Amino-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(pyridin-3-yl)methyl)-1,3-dimethylpyri-midine-2,4(1H,3H)-dione* ***(1o)***: White Solid. M.p. 223–221 °C; IR (KBr): ν 3382, 3189, 2948, 1682, 1622, 1575, 1511, 1191, 763 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 3.32 (s, 3H, -NC$\underline{H}_3$), 3.58 (s, 3H, -NC$\underline{H}_3$), 5.74 (s, 1H, -C$\underline{H}$), 6.57 (s, 2H, -N$\underline{H}_2$), 7.21–7.36 (m, 3H, Ar$\underline{H}$), 7.49-7.61 (m, 2H, Ar$\underline{H}$), 7.97 (d, *J* = 8 Hz, 1H, Ar$\underline{H}$), 8.45 (d, *J* = 6 Hz, 2H, Ar$\underline{H}$), 13.45 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 28.60, 30.12, 34.66, 87.98, 103.07, 116.15, 117.09, 123.17, 124.41, 132.49, 133.51, 134.34, 147.05, 147.99, 150.43, 152.26, 155.04, 164.48, 165.35, 167.75; ESI-MS (m/z): 429.0 [M + Na] + , 407.0 $[M + H]^+$. Anal. Calcd for $C_{21}H_{18}N_4O_5$: C 62.06, H 4.46, N 13.79. Found: C 62.09, H 4.50, N 13.84.

2.1r *6-Amino-5-(benzo[d][1, 3]dioxol-5-yl(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione* ***(1p)***: White Solid. M.p. 239–241 °C; IR (KBr): ν 3398, 3228, 2919, 1696, 1621, 1570, 1498, 1239, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 3.34 (s, 3H, -NC$\underline{H}_3$), 3.55 (s,

3H, -NC$\underline{H}_3$), 5.67 (s, 1H,-C$\underline{H}$), 5.93 (s, 2H –C$\underline{H}_2$-), 6.34 (s, 2H, -N$\underline{H}_2$), 6.64-6.73 (m, 3H, Ar$\underline{H}$), 7.30-7.35 (m, 2H, Ar$\underline{H}$), 7.57 (t, J = 8 Hz, 1H, Ar$\underline{H}$), 7.99 (d, J = 8 Hz, 1H, Ar$\underline{H}$), 13.40 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 28.43, 30.71, 36.00, 87.27, 100.96, 105.00, 107.45, 107.89, 116.34, 117.09, 119.30, 123.90, 124.57, 132.33, 132.69, 145.45, 147.59, 150.30, 152.11, 155.30, 164.02, 164.23, 166.02. ESI-MS (m/z): 450.1 $[M + H]^+$. Anal. Calcd for $C_{23}H_{19}N_3O_7$: C 61.47, H 4.26, N 9.35. Found: C 61.41, H 4.22, N 9.42.

2.1s *Ethyl 2-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetra-hydropyrimidin-5-yl)-2-(4-hydroxy-2-oxo-2H-chromen-3-yl)acetate (**1q**)*: Beige Solid. Mp. 195–197 °C; IR (KBr): ν 3355, 3218, 2952, 1739, 1667, 1571, 1195 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.23 (t, J = 7.2 Hz, 3H, -C$\underline{H}_3$), 3.38 (s, 3H, -NC$\underline{H}_3$), 3.51 (s, 3H, -NC$\underline{H}_3$), 4.23 (q, J = 7.2 Hz, 2H, -C$\underline{H}_2$-), 5.12 (s, 1H, -C$\underline{H}$), 6.30 (s, 2H, -N$\underline{H}_2$), 7.31 (t, J = 8 Hz, 2H, Ar$\underline{H}$), 7.55 (t, J = 8 Hz, 1H, Ar$\underline{H}$), 8.00 (d, J = 8 Hz, 1H, Ar$\underline{H}$), 12.98 (s, 1H, -O$\underline{H}$); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 14.06, 28.31, 30.56, 38.05, 61.07, 85.68, 102.12, 116.24, 116.60, 123.80, 124.55, 132.73, 149.94, 151.85, 154.42, 164.04, 164.39, 165.61, 169.07; ESI-MS (m/z): 424.1 $[M + Na] +$, 402.2 $[M + H]^+$. Anal. Calcd for $C_{19}H_{19}N_3O_7$: C 56.86, H 4.77, N 10.47. Found: C 56.78, H 4.72, N 10.53.

2.2 *Biochemical assays*

2.2a *Test helminth parasites*: A representative from each phylum of helminth parasites (Platyhelminthes and Nemathelminthes) was used in this study. *Raillietina echinobothrida* (Platyhelminthes) was collected from the intestine of freshly slaughtered domestic fowl at local abattoirs in Shillong, India. *Syphacia obvelata* (Nemathelminthes) was obtained from the intestine of freshly necropsied Swiss mice, in which the infection was maintained in the laboratory. The live adult specimens of these two parasites were first washed several times in 0.9% phosphate-buffered saline (PBS) at 37 ± 1°C before they were subjected to the test compounds.

2.2b *In vitro anthelmintic assay*: Compounds were quantified according to their ability to cause paralysis and eventually death of the test parasites. Every experimental setup was divided into four groups, with each group having five number of worms (n = 5) maintained in 0.9% PBS at 37 ± 1°C inside an incubator. Group I and II served as a negative and positive control, respectively. The worms in the former group were exposed to only PBS, while the worms in the latter group were exposed to 800 µg/mL concentration of a reference drug (praziquantel, PZQ or albendazole, ABZ). Parasites in group III were exposed to a low dose of 200 µg/mL concentration of test compounds, while a higher dose of 800 µg/mL of compounds was given to worms in group IV. The worms were observed at every hour and the anthelmintic efficacy was adjudged in terms of physical motility and mortality of parasites.[34]

2.2c *Statistical Analysis*: The experiments were repeated thrice and the results were represented as mean ± standard error of the mean (SEM). Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey test with $P < 0.001$ considered as statistically significant. All the statistical calculations were done using Origin version 8.0 SR6.

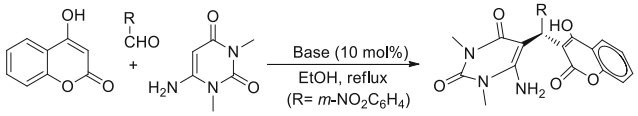
2.2d *Molecular docking simulation*: For molecular docking simulation, we have taken DFT optimized structure of all the compounds and crystal structure β-tubulin entitled 1oj0, obtained from Research Collaboratory for structural bioinformatics (RCSB) protein data bank.[29] An interactive molecular graphics program Autodock 4.2 is used to carry out molecular docking simulation.1 For docking simulation, we have first prepared the protein structure by structure preparation tool available in Auto Dock Tools package version 1.5.4. For preparing protein structure, all the water molecules and the residue have been removed from the crystal structure of β-tubulin and then polar hydrogen atoms are added for saturation, Gasteiger charges are computed and non-polar hydrogen atoms are merged. A grid box with a grid spacing of 0.375 Å and dimension of 60 × 60 × 60 grid points along x, y and z axes are built around the ligand-binding site. The grid box carries the complete binding site of the protein receptor and gives sufficient space for the ligand translational and rotational walk. Finally, ten possible docking runs are 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 are generated with an initial population of 150 individuals. The rate of gene mutation and crossover are set to 0.02 and 0.80, respectively.

3. Results and Discussion

3.1 *Chemistry*

Despite its apparent straightforwardness, the multi-component reactions (MCRs) often give side products unless the solvent, catalyst and order of addition are not thoroughly optimised. While synthesis of most of the symmetrical TRSMs *via* multicomponent reactions are generally catalysed by an acid catalyst, the unsymmetrical counterparts are favoured in the presence of base catalysts.[35–37] Therefore, we carried out a model reaction involving an equimolar mixture of 4-hydroxycoumarin, 6-amino-1,3-dimethyluracil and *m*-nitrobenzaldehyde in the presence of a catalytic

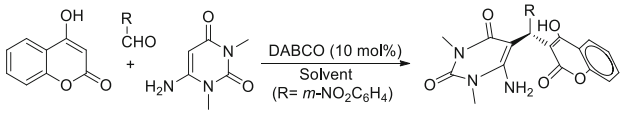
Table 1. Screening of catalyst[a].



Entry	Base catalyst	t/h	%Yield[b]
1	DABCO	1.5	90
2	DBU	5	71
3	DBN	5	62
4	4-DMAP	5	78
5	Et_3N	5	50
6	Hünig base	5	57
7	Amberlyst A21	5	80

[a]Reactions were carried out in 1 mmol scale.
[b]Isolated yield.

Table 2. Effect of solvents and temperature on the model reaction.



Entry	Solvent	Temperature	t/h	%Yield
1	EtOH	Reflux	1.5	90
2	MeOH	Reflux	2	65
3	MeCN	Reflux	12	78
4	CH_3Cl	Reflux	12	51
5	CH_2Cl_2	Reflux	12	52
6	H_2O	Reflux	3	85
7	THF	Reflux	12	35
8	DMSO	Reflux	12	28
9	Toluene	Reflux	12	55
10	**EtOH**	**RT; ultrasonication**	**1.5**	**90**
11	EtOH	50 °C; ultrasonication	1.5	93

[a]Reactions were carried out in 1 mmol scale.
[b]Isolated yield.

amount of various bases in 1 mL of ethanol at reflux conditions (Table 1). When the reaction was conducted with 10 mol% of DABCO, the reaction underwent complete conversion within 1.5 h to obviate 90% yield after recrystallization. As it can be seen from Table 1, other catalysts such as DBU, DBN, 4-DMAP, Et_3N, Hünig base and Amberlyst A21 gave comparatively low yields despite extending the reactions time.

The reaction conditions were then optimized and ethanol was found to be the solvent of choice (Table 2). Interestingly, the use of ultrasonic bath

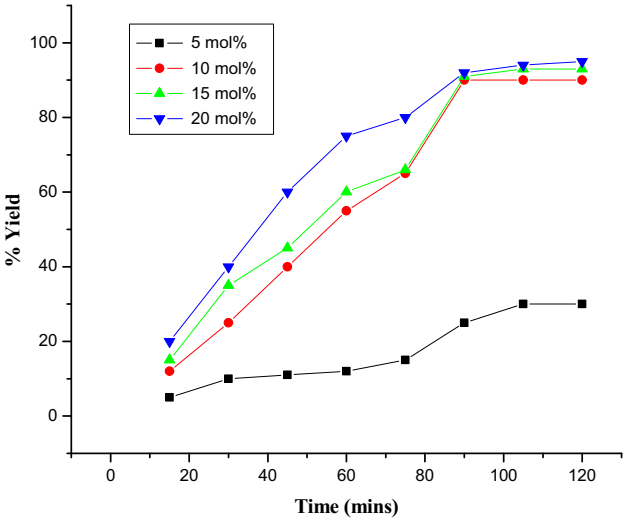


Figure 1. Optimization of catalyst loading.

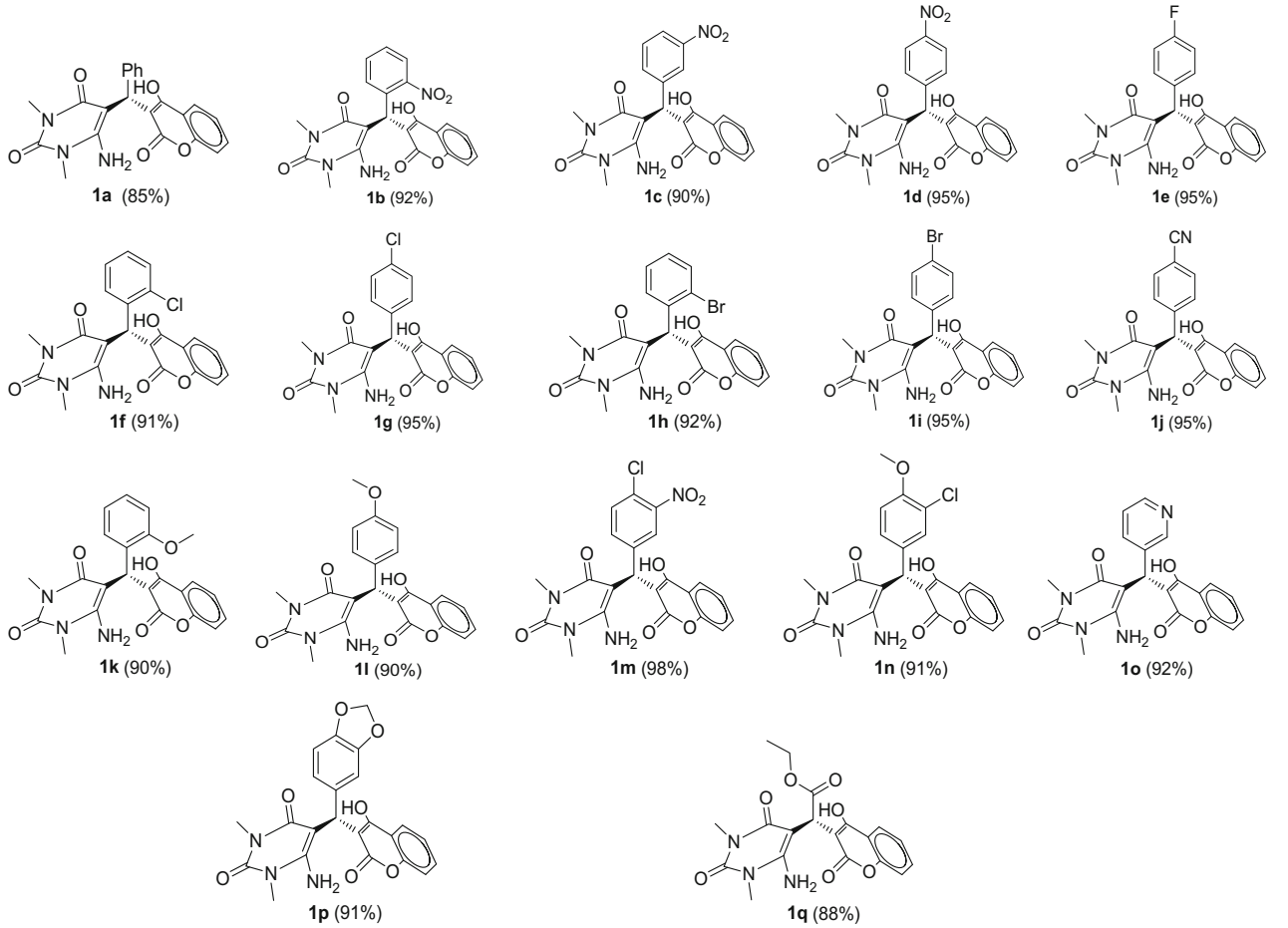
could accomplish the reaction at room temperature within the same time (1.5 h) to that of heating under reflux conditions to give 90% yield (Table 2, entry 10). However, the sonochemical effect was not pronounced enough upon increasing the reaction temperature to 50 °C for the said reaction. The catalyst loading was also optimized for sonochemical DABCO catalysed model reaction by carrying out the reaction at different catalyst loading, *viz.*, 5 mol%, 10 mol%, 15 mol%, and 20 mol% of aldehyde. The results (Figure 1) showed that the catalytic efficiency is optimum with 10 mol% catalyst loading with no visible benefit in an increase of catalyst loading till 20 mol%.

With the optimum condition in hand, a series of coumarin-based TRSMs (**1a–q**) were synthesized employing a variety of aldehydes including aromatic and heteroaromatic aldehydes (Table 3). Aromatic aldehydes having electron-withdrawing, as well as electron-donating functional groups, resulted in very good yield (entries **1a–1l**, **1p**). The reaction also worked well with heteroaromatic 3-pyridinecarbaldehyde (**1o**), disubstituted aldehydes (**1m**, **1n**) and ethyl glyoxalate (**1q**) giving the desired products in good to excellent yields. All the structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis data. The structure of one of the representative compounds (**1k**) was also confirmed single-crystal XRD experiment (CCDC 1828339) as shown in Figure 2.

3.2 *Pharmacology*

The synthesized compounds were then tested for their anthelmintic activity. A representative from each

Table 3. Synthesis of coumarin-based trisubstituted methanes (TRSMs) *via* Scheme 1[a,b].



[a]Reagents and conditions: aldehyde (1 mmol), 6-amino-1,3-dimethyluracil (1 mmol), 4-hydroxycoumarin (1 mmol), DABCO (10 mol%), ethanol (1 mL), ultrasonication, room temperature

[b]Isolated yield.

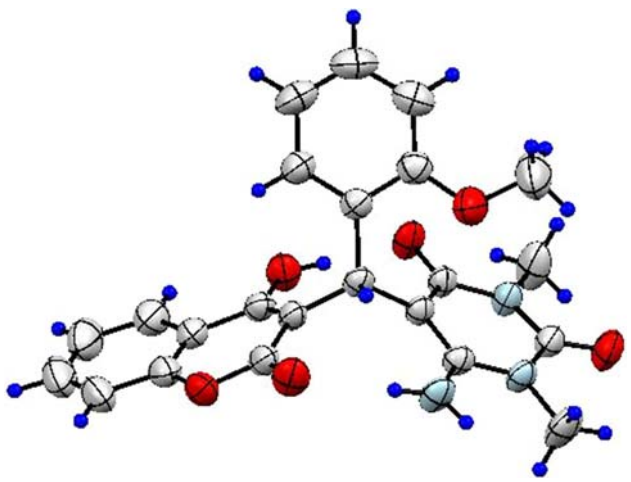


Figure 2. Ortep image of compound **1k** (CCDC 1828339).

phylum of helminth parasites (Platyhelminthes and Nemathelminthes) was used in this study. *Raillietina echinobothrida* (Platyhelminthes) was collected from the intestine of freshly slaughtered domestic fowl at local abattoirs in Shillong, India. *Syphacia obvelata* (Nemathelminthes) was obtained from the intestine of freshly necropsied Swiss mice, in which the infection was test maintained in the laboratory. All the compounds showed a dose-dependent activity against both the test parasites (Table 4).

A significant reduction ($P < 0.001$) was observed in paralysis and mortality time of parasites following exposure to a low (200 μg/mL) and a high (800 μg/mL) concentration of each compound. The results further indicated that the position of the substituents on the phenyl group in the molecule affect the

Table 4. *In vitro* efficacy of coumarin-based uTRSMs against nematode, *S. obvelata* and cestode, *R. echinobothrida*[a].

Entry	Compd.	Conc. (μg/mL)	*Syphacia obvelata*		*Raillietina echinobothrida*	
			Paralysis Time (h)	Mortality Time (h)	Paralysis Time (h)	Mortality Time (h)
1	**1a**	200	26.15 ± 0.70**	30.09 ± 0.50**	3.98 ± 0.12**	5.58 ± 0.13**
2		800	23.75 ± 0.20**	26.88 ± 0.20**	3.37 ± 0.08**	4.48 ± 0.12**
3	**1b**	200	30.77 ± 0.24*	34.92 ± 0.41*	5.02 ± 0.19**	6.32 ± 0.11**
4		800	27.90 ± 0.19*	32.76 ± 0.21*	3.33 ± 0.14**	5.38 ± 0.14**
5	**1c**	200	26.41 ± 0.39**	30.07 ± 0.44**	10.30 ± 0.10**	11.57 ± 0.05**
6		800	22.26 ± 0.16**	26.58 ± 0.20**	8.03 ± 0.10**	10.15 ± 0.14**
7	**1d**	200	14.09 ± 0.58**	15.84 ± 0.41**	2.23 ± 0.15**	3.03 ± 0.08**
8		800	11.64 ± 0.48**	13.71 ± 0.53**	0.92 ± 0.10**	2.19 ± 0.17**
9	**1e**	200	12.23 ± 0.19**	13.79 ± 0.11**	3.93 ± 0.11**	5.50 ± 0.22**
10		800	10.00 ± 0.71**	10.74 ± 0.44**	1.29 ± 0.05**	2.80 ± 0.08**
11	**1f**	200	29.05 ± 0.39*	31.83 ± 0.43**	7.25 ± 0.24**	8.88 ± 0.29**
12		800	26.22 ± 0.19*	29.91 ± 0.43**	4.71 ± 0.21**	5.90 ± 0.19**
13	**1g**	200	12.70 ± 0.26**	14.63 ± 0.24**	1.05 ± 0.10**	2.37 ± 0.08**
14		800	7.98 ± 0.23**	10.03 ± 0.18**	0.58 ± 0.05**	1.33 ± 0.14**
15	**1h**	200	33.76 ± 0.20	35.87 ± 0.07*	6.15 ± 0.11**	8.48 ± 0.20**
16		800	28.92 ± 1.40	31.97 ± 1.00*	5.10 ± 0.14**	7.43 ± 0.15 **
17	**1i**	200	11.43 ± 0.22**	13.96 ± 0.27**	1.67 ± 0.12**	2.84 ± 0.11**
18		800	8.28 ± 0.27**	11.38 ± 0.16**	0.96 ± 0.06**	1.52 ± 0.05**
19	**1j**	200	24.14 ± 0.60**	27.81 ± 0.80**	4.80 ± 0.10 **	6.39 ± 0.20**
20		800	21.73 ± 0.20**	23.73 ± 0.30**	1.94 ± 0.10**	4.19 ± 0.20**
21	**1k**	200	26.88 ± 0.18**	29.76 ± 0.25**	5.27 ± 0.16**	5.48 ± 0.14**
22		800	24.27 ± 0.30**	27.97 ± 0.41**	4.04 ± 0.10**	4.37 ± 0.06**
23	**1l**	200	17.42 ± 0.17*	18.91 ± 0.20**	3.84 ± 0.43**	5.36 ± 0.54**
24		800	16.31 ± 0.14*	17.67 ± 0.13**	0.85 ± 0.07**	1.82 ± 0.15**
25	**1m**	200	25.94 ± 0.63**	28.60 ± 0.46**	1.77 ± 0.38**	3.48 ± 0.42*
26		800	23.38 ± 0.76**	26.59 ± 0.70**	0.39 ± 0.06**	1.12 ± 0.09*
27	**1n**	200	24.02 ± 0.39**	28.06 ± 0.22**	7.32 ± 0.24**	8.60 ± 0.29**
28		800	23.48 ± 0.50**	26.57 ± 0.30**	2.79 ± 0.19**	4.71 ± 0.27**
29	**1o**	200	46.99 ± 0.32*	54.28 ± 0.86**	27.63 ± 0.39**	29.01 ± 0.40**
30		800	45.05 ± 0.35*	52.35 ± 1.06**	24.49 ± 0.52**	26.89 ± 0.72**
31	**1p**	200	23.82 ± 1.02**	28.92 ± 0.44**	6.55 ± 0.39**	7.83 ± 0.42**
32		800	21.74 ± 0.91**	27.33 ± 0.76**	5.59 ± 0.39**	6.94 ± 0.42**
33	**5**	800	17.56 ± 0.31**	20.10 ± 0.44**	0.44 ± 0.05**	1.15 ± 0.04**
34	**6**	800	25.62 ± 0.69**	30.69 ± 0.73**	9.41 ± 0.78*	11.42 ± 0.64**
35	**2**	800	27.30 ± 0.7*	30.84 ± 0.46**	1.52 ± 0.22**	4.11 ± 0.32**
36	**3**	800	32.38 ± 1.05	43.20 ± 0.8	22.66 ± 0.36**	27.35 ± 0.5**
37	Albendazole	800	21.92 ± 0.22**	25.17 ± 0.45**		
38	Praziquantel	800			0.55 ± 0.06**	1.35 ± 0.14**

[a]Data are expressed as mean ± standard error of the mean.

*$p < 0.05$ compared with control groups.

**$p < 0.001$ compared with control groups, one-way ANOVA, followed by Tukey's test.

anthelmintic efficacy of the compound. Interestingly, most of the compounds with substituents in the para position of the phenyl ring showed significantly higher anthelmintic activity than their ortho isomers. For example, at 800 μg/mL, compound **1f** showed a mortality of *R. echinobothrida* worms in 5.90 ± 0.19 h which was about 6 folds higher than the time taken by compound **1g**, which caused mortality of the same parasite only in 1.33 ± 0.14 h. In addition, some of the compounds also showed high selectivity towards the strain of parasites. While **1m** emerged out as the most active compound against *R. echinobothrida*, and caused the mortality of worms in 1.12 ± 0.09 h, at 800 μg/mL concentration, it was only moderately effective against the other test parasite, *S. obvelata*. On the other hand, compound **1g** and **1i** showed rather good efficacy against both the strains of parasites. This study also revealed that the compounds with halogen substituents at para-position (**1e**, **1g** and **1i**) of the phenyl ring demonstrate a high

activity against test parasites, while the halogen substituents at ortho- (**1f** and **1h**) and meta-position (**1n**) of the phenyl ring hardly had appreciable activity. Nevertheless, all the compounds carrying meta substituents (**1c**, **1m** and **1n**) including the one bearing chloro group at the para position showed reasonably low activity against *S. obvelata* suggesting the fact that the presence of a meta substituent may hinder binding of the compounds to the enzyme active site of the parasite. These findings also suggest that the mere presence of the halogens on the phenyl ring does not help, but the position and size of the substituents dictate the anthelmintic activity. Finally, the results of this study also suggest that efficacy of some compounds, such as **1e**, **1g**, and **1i** are higher in albendazole (ABZ) sensitive *Syphacia obvelata*, while the compounds **1g** and **1m** showed significant anthelmintic activity against Praziquantel (PZQ) sensitive *R. echinobothrida*. Due to its poor solubility in PBS, the anthelmintic activity of the compound **1q** could not be screened.

In order to study any synergistic effects on anthelmintic property of these compounds (**1a–p**), symmetrical TRSMs having bis(4-hydroxycoumarin), **5** and bis(6-amino-1, 3-dimethyluracil), **6** (Figure 3) were screened along with 4-hydroxycoumarin (**2**), 6-amino-1,3-dimethyluracil (**3**), and *p*-chlorobenzaldehyde against parasite strains *Syphacia obvelata* and *Raillietina echinobothrida* (Table 4). The results showed that the bis-compounds **5** and **6** (Entries 33-34) were significantly less effective than the corresponding TRMS **1g** against *Syphacia obvelata*, while compound **5** was found to be extremely effective against *Raillietina echinobothrida*. The compounds **2** (Entry 35) and **3** (Entry 36) showed poor effectiveness against both the species in comparison to Albendazole and Praziquantel standards.

3.3 *Molecular docking*

Molecular docking of anthelmintic drug with β-tubulin to study the activity by drug-tubulin interaction is already proven[38] because inhibition of β-tubulin of the helminths can severely affect their vital cellular functions such as mitosis, motility, and transport.[39–41] Therefore, in order to rationalize the anthelmintic activity of the synthesized compounds and understand their possible interactions, molecular docking simulation of all the compounds have been carried out against β-tubulin (pdb id: 1oj0).[38] The free energy bindings of all the compounds with the receptor are listed in Table 5. The molecular docking of the test

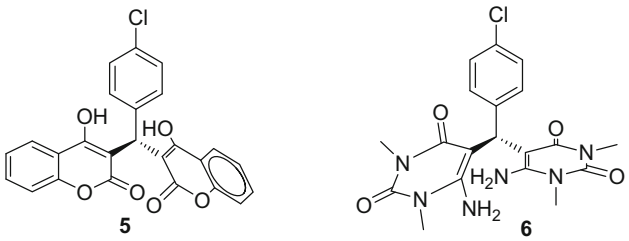


Figure 3. Some examples of biologically active coumarins and TRSMs.

Table 5. Molecular docking studies on anthelmintic activity of the compounds against the natomade, *Syphacia obvelata*.

Compd.	Docking score (kcal/mol)	*Syphacia obvelata* (Conc. 800 µg/mL)	
		Paralysis Time (h)	Mortality Time (h)
1a	− 2.79	23.75 ± 0.20**	26.88 ± 0.20**
1b	− 2.08	27.90 ± 0.19*	32.76 ± 0.21*
1c	− 1.92	22.26 ± 0.16**	26.58 ± 0.20**
1d	− 1.82	11.64 ± 0.48**	13.71 ± 0.53**
1e	− 4.65	10.00 ± 0.71**	10.74 ± 0.44**
1f	− 3.76	26.22 ± 0.19*	29.91 ± 0.43**
1g	− 4.06	7.98 ± 0.23**	10.03 ± 0.18**
1h	− 3.97	28.92 ± 1.40	31.97 ± 1.00*
1i	− 4.71	8.28 ± 0.27**	11.38 ± 0.16**
1j	− 3.69	21.73 ± 0.20**	23.73 ± 0.30**
1k	− 3.92	24.27 ± 0.30**	27.97 ± 0.41**
1l	− 3.09	16.31 ± 0.14*	17.67 ± 0.13**
1m	− 4.47	23.38 ± 0.76**	26.59 ± 0.70**
1n	− 3.57	23.48 ± 0.50**	26.57 ± 0.30**
1o	− 4.56	45.05 ± 0.35*	52.35 ± 1.06**
1p	− 3.57	21.74 ± 0.91**	27.33 ± 0.76**

compounds with the protein receptor revealed that among all the compounds, compound **1i** has shown the highest binding interaction at −4.71 kcal/mol against the protein receptor β-tubulin. Compounds **1e**, **1g** and **1o** are also found to exhibit good affinity toward the active site of β-tubulin with binding interaction of −4.65, −4.06 and −4.56 kcal/mol, respectively. The most important amino acid components involved in hydrophobic interaction with protein receptor are His227, Ser230, Ser234, Val 231, Phe242, Phe270, Val231, Gly235, Pro358, Arg318, Gln43, Phe20, Ile24, Val23 and Asp26. Binding mode of some of the active compounds, such as **1e** and **1g**, is shown in Figure 4. Figure 4(a) shows the binding interaction of the compound **1e** at the active site of the protein receptor. The compound is found to interact with the amino acid residue glutamic acid (Glu27) at a distance of about 1.81Å and serine (Ser234) at 2.08Å. Other

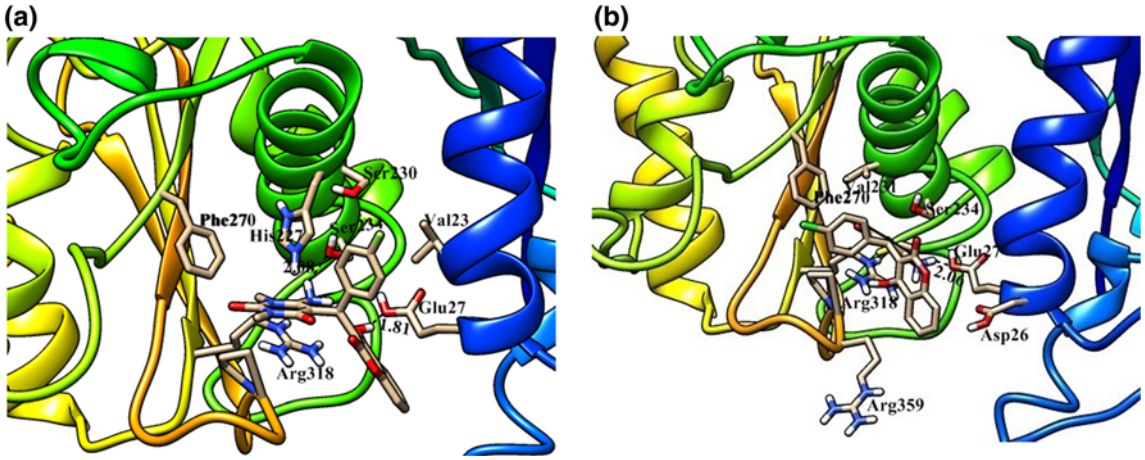


Figure 4. Best docking conformation of the two compounds (a) **1e** and (b) **1g** in the active site of β-tubulin. H-bonding interaction of the compounds with amino acid residue is shown. The remaining parts of the protein structure are not shown for clarity.

amino acid residue such as Ser230, Phe270, Pro358, Ser234 and Val23 also play a crucial rule in binding with the compound **1e** in the active site of β-tubulin. In Figure 4(b), the docking output of the compound **1g** with the tubulin receptor revealed that amino acid residue Gly235, Val231, Phe270, Pro358, Arg318, Ser234, Asp26 and Arg359 are involved in binding. A hydrogen-bonding interaction of the complex **1g** with amino acid residue Glu27 (2.06 Å) has been observed. From the observed binding energy values (Table 5) it can be considered that the compounds **1e**, **1g**, **1i** and **1o** are the good inhibitor of β-tubulin. The maximum binding energy of these compounds towards target protein can be attributed to their sterically unhindered structure and highly reactive functional groups like nitro, amino and hydroxyl groups which are involved in hydrogen bonding interaction. The docking results are in good agreement with the experimental study. The docking conformation of the compounds **1e** and **1g** in the active site of β-tubulin (Figure 4).

4. Conclusions

Selected coumarin-based TRSMs bearing 1,3-dimethyl 6-aminouracil scaffold were found to demonstrate a high level of anthelmintic activity *in vitro* against helminth parasites *Syphacia obvelata* (Nematoda) and *Raillietina echinobothrida* (Cestoda). The TRSMs were synthesized by developing a mild, environmentally benign and chromatography-free sonochemical multicomponent reaction in the presence of a catalytic amount of DABCO (10 mol%) at room temperature. Notably, most of the tested compounds with substituents in the para position of the phenyl ring showed comparatively better anthelmintic activity against both the cestode and nematode parasites as compared to ortho and meta substituted derivatives. The docking study revealed the binding interaction of all the optimized compounds with several amino acid residues in the active site of β-tubulin. The compounds showing good docking score with β-tubulin showed comparable anthelmintic activity experimentally as well.

Supplementary Information (SI)

The spectroscopic data, ^{1}H NMR and ^{13}C NMR spectra of the compounds are available at www.ias.ac.in/chemsci.

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