Preliminary Communication

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Antioxidant, α-glucosidase inhibitory and *in vitro* antitumor activities of coumarin-benzothiazole hybrids

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Abstract: Coumarin-benzothiazole hybrids are antitumor agents based on their antioxidant and α-glucosidase inhibitory activities. Compounds **5a–c** were selected by National Cancer Institute (NCI), USA, to be screened for antitumor activity at a single dose (10 μM) against a panel of 60 cancer cell lines. The most active compound **5c** was further screened at a five-dose level by NCI. Compound **5c** displays half maximal growth inhibition (GI_{50}) values of 0.24 and 0.33 μM against central nervous system (CNS) cancer (SNB-75) and ovarian cancer (OVCAR-4) cell lines, respectively. Compounds **5a–c** were also screened for their antioxidant and α-glucosidase inhibitory activities.

Keywords: antitumor activity; benzothiazoles; coumarins; hybridization; pharmacophore.

Glycosidases are a class of carbohydrate-hydrolase enzymes that catalyze hydrolysis of glycosidic bonds in oligosaccharides [1, 2]. The development of α-glycosidase inhibitors with potential therapeutic applications has received considerable attention recently [3, 4]. The emergence of drug resistance to cancer chemotherapeutic agents has directed significant research efforts toward development of new agents for cancer treatment utilizing molecular hybridization (MH) strategy of different pharmacophores with the aim of obtaining superior anticancer activity compared to the parent molecules [5–7]. Limited examples of lead compounds from natural sources display promising antitumor activity based on their potent glycosidase inhibition activity [8].

The imbalance between overproduction of reactive oxygen species (ROS) and cellular detoxification machinery in favor of ROS production, known as oxidative stress, leads to cellular damage and malfunction [9, 10]. Oxidative stress is directly associated with cancer progression, and there is a pressing need for development of potent antioxidant agents that can protect cellular organelles from ROS [11]. In this context, coumarin derivatives demonstrate intriguing antioxidant activity owing to scavenging of the initial radicals and propagating peroxyl radicals [12, 13]. Moreover, coumarin-based compounds show promising antitumor activity [14] and are potent inhibitors of α-glycosidase [15, 16]. Benzothiazole is a versatile synthetic scaffold with a wide spectrum of biological effects including potential antioxidant [17] and antitumor [18] activities. In addition, benzothiazole-containing agents show α-glycosidase inhibitory activity [19, 20].

Bromophenols (BPs), isolated from marine algae, demonstrate promising α-glycosidase inhibitory activity which has been attributed to the presence of bromo and hydroxy substituents [21, 22]. Therefore, BPs are promising lead compounds for the design of potential α-glycosidase inhibitors. Bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (BDDE, Figure 1) is a potent α-glycosidase inhibitor with a half maximal inhibitory concentration (IC_{50}) value of 0.098 μM [21] and a potential antitumor agent [23]. Investigation of the binding interactions between BDDE and α-glycosidase has identified a charged-hydrophobic-polar (C-H-P) binding pocket in α-glycosidase that fits BDDE [24]. The hydroxy groups of BDDE are involved in multiple hydrogen bonds with residues in the polar areas of the binding pocket, while the rest of the molecule is stabilized by hydrophobic interactions with nearby residues. The binding mode of BDDE to α-glycosidase is consistent with the structure-activity relationship established for hydroxycoumarin derivative (Figure 1) as potent α-glycosidase inhibitor with IC_{50} values in the nanomolar range [25]. It is proposed that hydrogen bonding and extensive hydrophobic interactions in a cooperative fashion are involved in the α-glycosidase inhibitory activity of the hydroxycoumarin derivatives. The basis of the cooperative hydrogen bonding and hydrophobic interactions has been derived from X-ray

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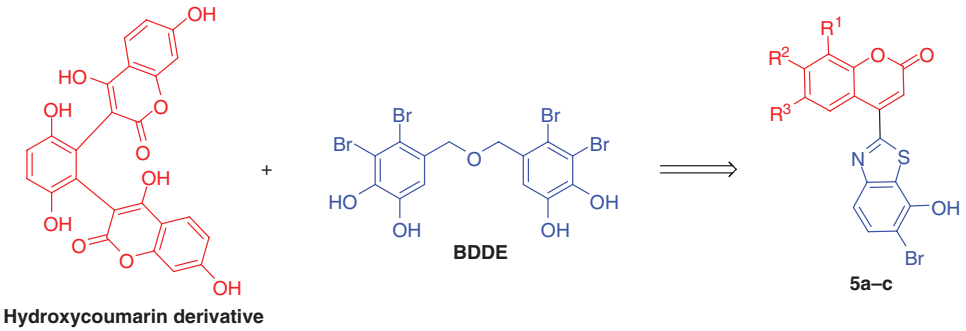


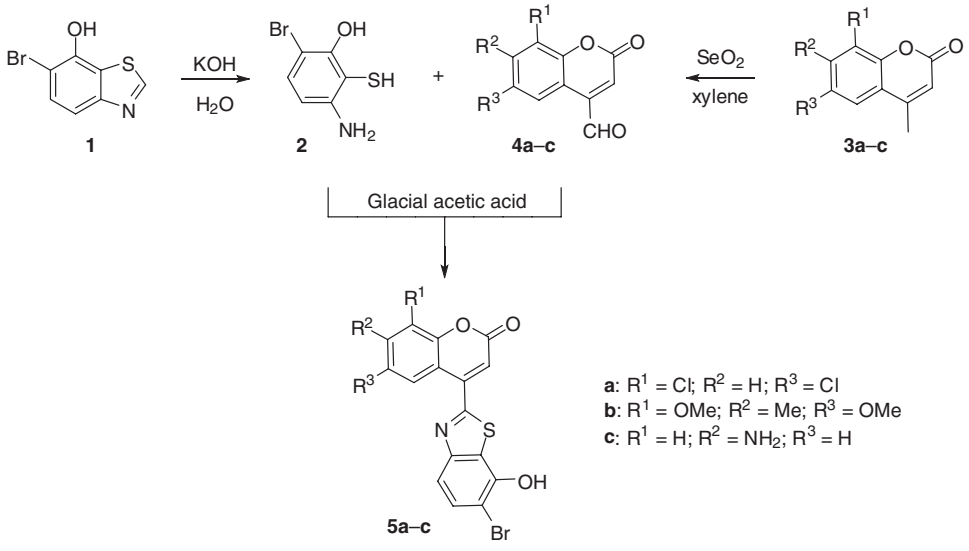
Figure 1 α-Glycosidase inhibitors reported in the literature and coumarin-benzothiazole hybrid compounds **5a–c**.

crystallographic analysis of maltose in a complex with *Thermotoga maritima* α-glucosidase AglA [26]. In the crystal structure, one of the glucose rings of maltose is bound by multiple hydrogen bonds to charged residues in the binding pocket, while the rest of the molecule is hydrophobically stacked to stabilize the interactions.

In this investigation, the design strategy of the coumarin-benzothiazole hybrids as α-glycosidase inhibitors (Figure 1) interrogates structural features of both the marine natural BDDE and coumarins. It was anticipated that the benzothiazole core with bromo and hydroxy substituents would be implicated in hydrophobic and hydrogen-bonding interactions with α-glycosidase similar to BPs. The coumarin moiety in the new hybrid compounds was speculated to be involved in additional hydrophobic and hydrogen bonding interactions in the hydrophobic and polar areas of the binding pocket. The synthesized coumarin-benzothiazole hybrids (Figure 1) were evaluated for their antitumor and antioxidant activities.

The target compounds **5a–c** of this study were synthesized according to the general approach outlined in Scheme 1. As can be seen, the starting aminothiophenol **2** was synthesized by hydrolysis of the benzothiazole **1** with aqueous potassium hydroxide. Treatment of methyl substituted coumarin derivatives **3a–c** with selenium dioxide in xylene proceeded smoothly to furnish formyl derivatives **4a–c** in serviceable yields. Subsequent condensation of **2** and **4a–c** in glacial acetic acid yielded coumarin-benzothiazole hybrids **5a–c** (Scheme 1).

Compounds **5a–c** were evaluated by the National Cancer Institute (NCI) *in vitro* for their antitumor activity [27]. A single dose (10 μM) of the tested compounds was used in the full NCI-60 cell lines panel assay. Compounds **5a,b** exhibited weak antitumor activity against all tested cell lines except for moderate activity of **5a** against central nervous system (CNS) and breast cancer cell lines. Compound **5c** displayed lethal effects (>100% inhibition) against non-small-cell lung cancer (HOP-62), CNS cancer



Scheme 1 Synthesis of compounds **5a–c**.

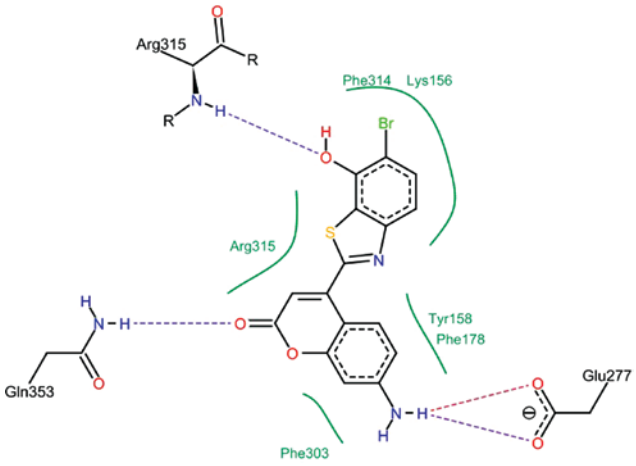


Figure 2 Interaction of compound **5c** with the binding site of the target enzyme. Dashed lines represent hydrogen bonds. Hydrophobic interactions are shown by green solid lines.

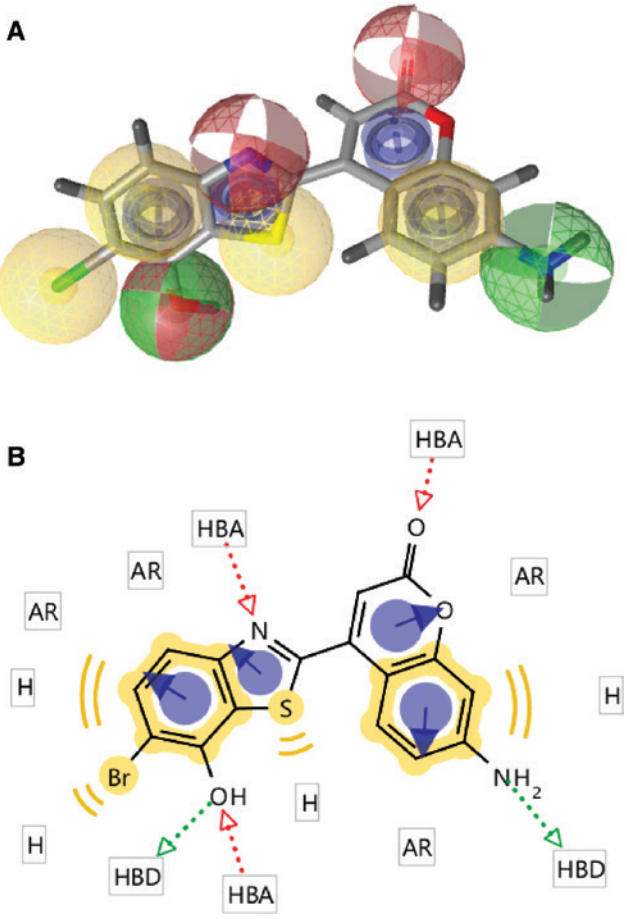


Figure 3 The 3D and 2D pharmacophoric maps of compound **5c**. (A) The 3D pharmacophoric map; the pharmacophore color coding is red for hydrogen acceptor, yellow for hydrophobic regions and green for hydrogen donors. (B) The 2D pharmacophoric map; HBA is hydrogen bond acceptor, H is hydrophobic center, HBD is hydrogen bond donor and AR is aryl.

(SNB-75) and ovarian cancer (SK-OV-3) cell lines. Subsequently, compound **5c** after passing this primary antitumor assay was carried over to the NCI five-dose screening. It can be suggested that the presence of a polar ionizable group on the coumarin moiety of these coumarin-benzothiazole hybrids is essential for antitumor activity. Potent antitumor activity of **5c** was evident against CNS cancer (SNB-75) and ovarian cancer (OVCAR-4) cell lines with half maximal growth inhibition values of 0.24 and 0.33 μM, respectively.

Compounds **5a–c** were also evaluated for their *in vitro* α-glucosidase inhibitory activity [28]. The results showed that compound **5c** exhibits promising inhibitory activity with an IC_{50} value of 6.32 ± 0.51 μM in comparison to miglitol as a reference compound (IC_{50} = 0.39 ± 0.02 μM). Compounds **5a,b** display moderate α-glucosidase inhibitory activity with IC_{50} values of 38.9 ± 1.43 and 21.47 ± 0.91 μM, respectively.

Antioxidant activities of compounds **5a–c** were determined using diphenylpicrylhydrazyl (DPPH) radical scavenging method [29]. In this test, compound **5c** displayed moderate antioxidant activity with an IC_{50} value of 35.17 ± 1.34 μM which is comparable to the activity of the standard reference ascorbic acid (IC_{50} = 22.8 ± 0.71 μM). Compounds **5a,b** displayed similar antioxidant capacity with IC_{50} values of 44.5 ± 2.66 and 41.36 ± 2.12 μM, respectively. The correlation between antitumor activity of **5c** to its α-glucosidase inhibitory and antioxidant activities in comparison to **5a,b** suggests that these activities represent the basis of its antitumor profile.

Saccharomyces cerevisiae isomaltase crystal structure (PDB ID: 3AJ7) shows high sequence similarity (72.4%) with α-glucosidase and was utilized in this investigation for molecular docking studies. Compound **5c** displays significantly preferential binding to the target enzyme with an estimated binding energy of –21.59 kcal mol^{-1}, which is in good agreement with the result of the *in vitro* α-glucosidase inhibition assay. The detailed analysis of the binding interaction is displayed in the two-dimensional (2D) binding mode of **5c** in Figure 2. As can be seen, the coumarin moiety of **5c** is stretched into a hydrophobic pocket of the target enzyme revealing hydrophobic interactions with Phe303, Phe178 and Tyr158. The carbonyl group of the coumarin scaffold interacts by hydrogen bonding with Gln353. It is noteworthy to mention that hydrogen bonding of the amino group in **5c** with Glu277 further stabilizes embedding of the coumarin moiety in the hydrophobic pocket, compared to **5a,b** that lack this interaction. The benzothiazole moiety of

5c is involved in π-π interactions with Phe314 and Lys156, as well as hydrogen bonding with Arg315 (Figure 2).

Three-dimensional (3D) and 2D pharmacophoric maps for the structural features of compound **5c** (the most active member of this study) were created by LigandScout software and are presented in Figure 3A and B, respectively. The investigated pharmacophoric features include hydrogen bond donors and acceptors as directed vectors, positive and negative ionizable regions as well as lipophilic areas that are represented by spheres. These pharmacophoric maps of **5c** may help design more potent antitumor coumarin-benzothiazole hybrids.

In conclusion, coumarin-benzothiazole hybrids **5a–c** were introduced in this investigation as a novel scaffold of potential antitumor agents. The substitution pattern of the coumarin moiety in the new hybrid molecules greatly affects their biological activity. Compound **5c** is the most active member of this study according to NCI's single- and five-dose assays. Intriguing antioxidant and α-glucosidase inhibitory activities of **5c** are in good agreement with the antitumor screening results. The preliminary results reported in this study may help design new coumarin-benzothiazole hybrids as antitumor agents based on the nature and number of polar substituents on the coumarin nucleus.

Experimental

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ on a Bruker spectrometer. Melting points were recorded using a capillary melting point apparatus and are uncorrected. HRMS were obtained in positive ion mode using ESI on a double-focusing magnetic sector mass spectrometer.

The antitumor screening of compounds **5a–c** [27], the α-glucosidase inhibition assay [28], the antioxidant assay [29] and molecular modeling [30] were conducted as previously described.

3-Amino-6-bromo-2-mercaptophenol A mixture of compound **1** (1.15 g, 5 mmol) and KOH (2.80 g, 50 mmol) in water (10 mL) was heated at reflux overnight, then cooled and neutralized with 1 N HCl. The resultant precipitate was subjected to column chromatography eluting with 5% methanol in dichloromethane to give **2** as a dark yellow solid; yield 59%; mp 115–117°C; ^{1}H NMR: δ 4.16 (bs, 1H), 4.95 (s, 2H), 6.59 (d, 1H, J = 7.5 Hz), 7.12 (d, 1H, J = 7.5 Hz), 8.85 (s, 1H); ^{13}C NMR: δ 114.3, 117.4, 118.2, 125.8, 140.1, 159.1. HRMS. Calcd for C_6H_7BrNOS, $[M+H]^+$: *m/z* 219.9431. Found: *m/z* 219.9439.

6,8-Dichloro-2-oxo-2*H*-chromene-4-carbaldehyde (4a) A mixture of compound **3a** (2.29 g, 10 mmol) and selenium dioxide (1.23 g, 11.1 mmol) in xylene (100 mL) was heated at reflux overnight. The solvent was removed under reduced pressure and the crude product was subjected to silica gel column chromatography eluting with 2% methanol in dichloromethane to give **4a** as a light yellow solid; yield 72%; mp 130–132°C; ^{1}H NMR: δ 6.81 (s, 1H), 7.18 (s, 1H), 7.75 (s, 1H), 9.94 (s, 1H); ^{13}C NMR: δ 120.6, 122.4, 125.1, 128.9, 132.3, 133.5, 139.4, 152.3, 158.6, 192.6.

6,8-Dimethoxy-7-methyl-2-oxo-2*H*-chromene-4-carbaldehyde (4b) Using the procedure for the preparation of **4a**, the reaction of **3b** (2.34 g, 10 mmol) and selenium dioxide (1.23 g, 11.1 mmol) gave **4b** as a yellow solid after purification by silica gel column chromatography using 2% methanol in dichloromethane as eluent; yield 87%; mp 137–139°C; ^{1}H NMR: δ 2.19 (s, 3H), 3.51 (s, 3H), 3.58 (s, 3H), 6.65 (s, 1H), 6.90 (s, 1H), 9.98 (s, 1H); ^{13}C NMR: δ 10.2, 59.5, 60.1, 118.1, 122.1, 123.5, 127.8, 130.2, 134.8, 141.9, 143.8, 155.4, 190.4.

7-Amino-2-oxo-2*H*-chromene-4-carbaldehyde (4c) Using the procedure given for the preparation of **4a**, the reaction of **3c** (1.75 g, 10 mmol) and selenium dioxide (1.23 g, 11.1 mmol) gave **4c** as a yellow solid after purification by silica gel column chromatography using 5% methanol in dichloromethane as eluent; yield 51%, mp 155–157°C; ^{1}H NMR: δ 6.62 (s, 2H), 6.81 (s, 1H), 6.95 (s, 1H), 7.38 (d, 1H, *J* = 8.0 Hz), 7.70 (d, 1H, *J* = 8.0 Hz), 10.09 (s, 1H). HRMS. Calcd for $C_{10}H_8NO_3$, $[M+H]^+$: *m/z* 190.0504. Found: *m/z* 190.0509.

4-(6-Bromo-7-hydroxybenzothiazol-2-yl)-6,8-dichloro-2*H*-chromen-2-one (5a) A mixture of compound **4a** (1.33 g, 5.5 mmol) and compound **2** (1.1 g, 5 mmol) was heated under reflux in glacial acetic acid (10 mL) for 6 h, then cooled and diluted with water (50 mL). The resultant precipitate was purified by column chromatography using 1% methanol in dichloromethane as eluent to give **5a** as a yellow solid; yield 69%; mp 188–190°C; ^{1}H NMR: δ 5.07 (s, 1H), 6.46 (s, 1H), 6.99 (s, 1H), 7.08 (s, 1H), 7.51 (d, 1H, *J* = 8.2 Hz), 7.83 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR: δ 118.7, 122.3, 124.8, 125.9, 126.9, 127.7, 129.8, 130.0, 132.4, 132.8, 133.1, 140.5, 145.1, 146.3, 155.1, 163.9. HRMS. Calcd for $C_{16}H_7BrCl_2NO_3S$, $[M+H]^+$: *m/z* 441.8707. Found: *m/z* 441.8709.

4-(6-Bromo-7-hydroxybenzothiazol-2-yl)-6,8-dimethoxy-7-methyl-2*H*-chromen-2-one (5b) Using the procedure for the preparation of **5a**, the reaction of **4b** (1.36 g, 5.5 mmol) and compound **2** (1.1 g, 5 mmol) gave **5b** as a yellow solid after purification by flash column chromatography using 2% methanol in dichloromethane as eluent; yield 66%, mp 188–190°C. ^{1}H NMR: δ 2.27 (s, 3H), 3.71 (s, 3H), 3.76 (s, 3H), 5.45 (s, 1H), 6.83 (s, 1H), 7.01 (s, 1H), 7.42 (d, 1H, *J* = 7.5 Hz), 7.69 (d, 1H, *J* = 7.5 Hz); ^{13}C NMR: δ 15.3, 57.4, 58.1, 119.4, 123.1, 125.2, 125.7, 125.9, 127.0, 128.9, 130.2, 131.5, 132.7, 135.8, 137.1, 143.1, 144.5, 157.9, 161.3. HRMS. Calcd for $C_{19}H_{15}BrNO_5S$ $[M+H]^+$, *m/z* 447.9854. Found: *m/z* 447.9853.

7-Amino-4-(6-bromo-7-hydroxybenzothiazol-2-yl)-2*H*-chromen-2-one (5c) Using the procedure given for the preparation of **5a**, the reaction of **4c** (1.04 g, 5.5 mmol) and compound **2** (1.1 g, 5 mmol) gave **5c** as a deep yellow solid after purification by flash column chromatography using 5% methanol in dichloromethane as eluent; yield 79%, mp 170–172°C; ^{1}H NMR: δ 5.53 (s, 1H), 6.21 (s, 1H), 6.51 (s, 2H), 6.92 (s, 1H), 7.01–7.09 (m, 2H), 7.55 (d, 1H, *J* = 7.9 Hz), 7.85 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR: δ 120.5, 122.3, 125.6, 125.8, 126.4, 127.0, 132.1, 132.3, 134.2, 134.7, 135.3, 139.6, 142.4, 144.6, 156.2, 162.9. HRMS. Calcd for $C_{16}H_{10}BrN_2O_3S$, $[M+H]^+$: *m/z* 388.9595. Found: *m/z* 388.9590.

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