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 (GI50) 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 chemotherapy 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 (IC50) 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 IC50 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
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
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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 IC50 value of 6.32 ± 0.51 μM in comparison to miglitol as a reference compound (IC50 = 0.39 ± 0.02 μM). Compounds 5a,b display moderate α-glucosidase inhibitory activity with IC50 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 exhibited moderate antioxidant activity with an IC50 value of 35.17 ± 1.34 μM which is comparable to the activity of the standard reference ascorbic acid (IC50 = 22.8 ± 0.71 μM). Compounds 5a,b displayed similar antioxidant capacity with IC50 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

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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 Ligand-Scout 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**

1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded in CDCl3 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; 1H NMR: δ 4.61 (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); 13C NMR: δ 114.3, 117.4, 118.2, 125.8, 140.1, 159.1. HRMS. Calcd for C6H7BrNOS, [M + H]+: m/z 219.9439. Found: m/z 219.9439.

**6,8-Dichloro-2-o xo-2H-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; 1H NMR: δ 6.81 (s, 1H), 7.18 (s, 1H), 7.75 (s, 1H), 9.94 (s, 1H); 13C 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-2H-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; 1H 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); 13C NMR: δ 10.2, 59.5, 60.1, 118.1, 122.1, 123.5, 127.8, 130.2, 134.8, 141.9, 163.8, 155.4, 190.4.

**7-Amino-2-oxo-2H-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; 1H NMR: δ 3.62 (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 C6H8NO4S, [M + H]+: m/z 219.0504. Found: m/z 219.0509.

**4-(6-Bromo-7-hydroxybenzothiazol-2-yl)-6,8-dichloro-2H-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; 1H 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); 13C 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, 163.3, 155.1, 163.9. HRMS. Calcd for C16H7BrClNO3S, [M + H]+: m/z 441.8707. Found: m/z 441.8709.

**4-(6-Bromo-7-hydroxybenzothiazol-2-yl)-6,8-dimethoxy-2H-chromene-4-one (5b)** Using the procedure given 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; 1H 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); 13C 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, 1579, 1613. HRMS. Calcd for C16H9BrNO3S, [M + H]+: m/z 447.9854. Found: m/z 447.9853.

**4-(6-Bromo-7-hydroxybenzothiazol-2-yl)-6,8-dimethoxy-7-methyl-2H-chromene-2-one (5c)** Using the procedure 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 yellow solid after purification by flash column chromatography using 2% methanol in dichloromethane as eluent; yield 79%, mp 170–172°C; 1H NMR: δ 5.53 (s, 1H), 6.21 (s, 1H), 6.51 (s, 2H), 6.92 (s, 1H), 7.01–7.09 (m, 2H), 7.25 (d, 1H, J = 7.9 Hz), 7.85 (d, 1H, J = 7.9 Hz); 13C NMR: δ 120.5, 122.3, 125.6, 128.5, 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 C16H9BrNO3S, [M + H]+: m/z 388.9595. Found: m/z 388.9590.

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tives and their in vitro anticancer effects and antioxidant activi-


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