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Bioorganic & Medicinal Chemistry 15 (2007) 2810–2814

Bioorganic & Medicinal Chemistry

Synthesis of xanthone derivatives with extended π-systems as α-glucosidase inhibitors: Insight into the probable binding mode

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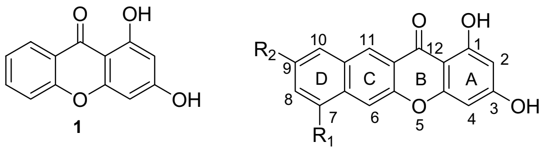
Received 12 January 2007; revised 13 February 2007; accepted 14 February 2007
Available online 16 February 2007

Abstract—A series of novel xanthone derivatives with extended π-systems, that is, benzoxanthones **2**–**4**, and their structurally perturbed analogs **5**–**9** have been designed and synthesized as α-glucosidase inhibitors. Their inhibitory activities toward yeast's α-glucosidase were evaluated with the aim to enrich the structure–activity relationship. The results indicated that benzoxanthones **2**–**4** were capable of inhibiting in vitro yeast's α-glucosidase 17- to 28-fold more strongly than xanthone derivative **1** that has smaller conjugated π-system. Benzoxanthone **8**, bearing angularly fused aromatic rings, and reduced benzoxanthone **5** showed decreased activities, strongly suggesting that linearly conjugated π-systems play a crucial role in the inhibition process. O-Methylation of 3-OH of benzoxanthone **2** and nitration at C4 position led to a large decrease in the activity. This indicates that 3-OH of benzoxanthone was crucial to the inhibitory activity, primarily as an H-bonding donor. The present results suggest that π–π stacking effect and H-bonding make substantial contributions to elicit the inhibitory activities of this general class of inhibitors.

1. Introduction

During the last few decades, there has been widespread interest in α-glucosidase (EC 3.2.1.20) because of its important role not only in carbohydrate digestion, but also in the processing of glucoproteins and glycolipids. The enzyme is also involved in a variety of metabolic disorder and other diseases such as diabetes, viral attachment, and cancer formation.[1–5] The biological and practical importance ensures the use of α-glucosidase inhibitors as a vital tool to understand the mechanisms of action of α-glucosidase and as therapeutic agents for some degenerative diseases. Therefore, considerable endeavors have been made to develop inhibitors that can probe the structure and function of α-glucosidase. To date, many α-glucosidase inhibitors[6–8] have been reported, such as acarbose and voglibose from microorganisms, and 1-deoxynojirimycin isolated from plants, however, they are largely confined to glycosidic derivatives. The needs for new diverse therapeutic agents necessitate the creation of new classes of inhibitors. These inhibitors may provide more insight into the interaction with α-glucosidase whose crystal structure remains presently unclear.

In these aspects, increasing attention has been paid to xanthones that are widely present as a class of secondary metabolites in some higher plants, fungi, and lichen families.[9–11] These naturally occurring compounds and their synthetic analogs have demonstrated multiple pharmacological properties, such as antioxidant,[12,13] anti-inflammatory,[14] and antimalarial[15] activities, inhibition of a variety of tumor cell lines' growth,[16–18] and modulation of PKC isoforms.[19] Recent studies by others[20–22] and us[23] have indicated that xanthone derivatives (e.g., compound **1**, Chart 1) are capable of inhibiting α-glucosidase with moderate to good activities. This preliminary work has highlighted the high potentials of xanthones as



2: $R_1 = R_2 = H$
3: $R_1 = H, R_2 = OH$
4: $R_1 = OH, R_2 = H$

Chart 1.

Keywords: Benzoxanthones; Synthesis; α-Glucosidase; Inhibitory activity.

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doi:10.1016/j.bmc.2007.02.030

a promising building motif for the development of a new class of potent α-glucosidase inhibitors.

However, little is known about the action mode of xanthones with α-glucosidases. In previous study we have shown that two or more phenolic hydroxyl groups are fundamental for the inhibitory activity,[23] however their roles are not clear. In addition, our attempt to enhance the activity by modifying the phenolic hydroxyl group with diverse substituents proved unsuccessful. This observation, together with the structural features inherent in xanthones, makes us aware that the conjugated π-systems in xanthones may contribute significantly to the inhibitory activity. With this thought in mind, we describe herein the synthesis of xanthone derivatives with extended π-systems, that is, benzoxanthones **2–4**, and a comparative study of their α-glucosidase inhibitory activities with those of structurally perturbed analogs **5–9** (Chart 1 and Scheme 1). The primary aim of this work was to gain some insight into the effect of each structural factor in benzoxanthones on the inhibitory activity as well as into the probable binding mode of these compounds toward α-glucosidase.

2. Results and discussion

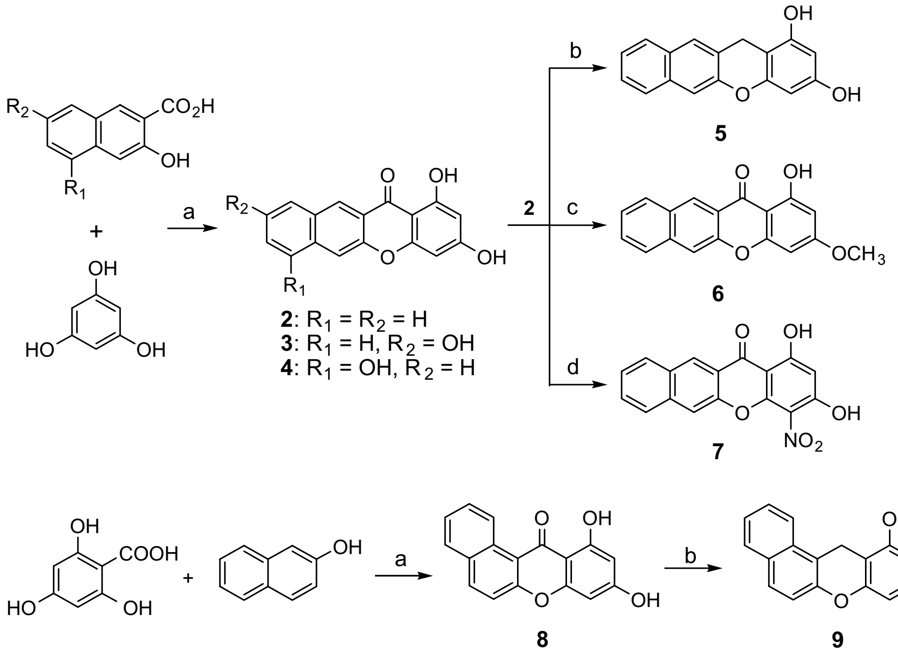
2.1. Chemistry

The synthetic route that was used to synthesize xanthone derivatives **2–9** is shown in Scheme 1. Compounds **2–4** and **8** were synthesized in 31–79% yields from the condensation of hydroxybenzoic acid or hydroxy-2-naphthoic acid with phloroglucinol or β-naphthol in the presence of anhydrous zinc chloride and phosphoryl chloride.[24,25] Compounds **5** and **9** were obtained in 29% and 17% yields, respectively, from the reduction of their corresponding hydroxylxanthones **2** and **8** by lithium aluminum hydride.[25,26] Selective O-methylation[27,28] of the 3-hydroxyl group of **2** with methyl iodide in acetone at room temperature afforded **6** in 83%. Nitration[6,28] of compound **2** with 70% HNO_3 in acetic acid gave compound **7**. All these xanthone derivatives were characterized by NMR, mass, IR, and elemental analyses (see Section 4).

2.2. α-Glucosidase inhibitory activity

The inhibitory activities of xanthone derivatives **2–9** toward yeast's α-glucosidase were evaluated by using methods similar to those described in the literature.[29–31] The obtained IC_{50} values, together with those of compound **1** and 1-deoxynojirimycin **10**, for comparison are shown in Table 1.

Under our assay condition, compound **1**, bearing three fused aromatic rings, showed very weak inhibitory activity. However, benzoxanthones **2–4** bearing four fused aromatic rings exhibited greatly enhanced inhibitory activities. For example, compound **2** having one more conjugated aromatic ring was at least 17-fold more active in inhibiting α-glucosidase than compound **1**. More significantly, benzoxanthones **2–4** were much more active than 1-deoxynojirimycin **10**, a classical α-glucosidase inhibitor. These results clearly indicate that benzoxanthones **2–4** are quite potent α-glucosidase inhibitors and the weak inhibitory activity of xanthones can be largely amplified by inserting one aromatic ring to extend the π-conjugated systems. In addition, benzoxanthone **8** bearing angularly fused aromatic rings was less favorable to the activity than compound **2** bearing linearly fused aromatic rings. Thus, it appears that yeast's α-glucosidase has apparent preference for linearly angular aromatic structures.



Scheme 1. Synthetic route for xanthone derivatives **2–9**. Reagents and conditions: (a) $ZnCl_2$, $POCl_3$, 70–80 °C; (b) $LiAlH_4$, THF, rt; (c) CH_3I, K_2CO_3/acetone, 0.5 h; (d) 70% HNO_3, AcOH, 60–70 °C, 1 h.

Table 1. α-Glucosidase inhibitory activities of compounds **1**–**10** (IC_{50}, μM)[a]

Compound	IC_{50}
1	160.8
2	9.3
3	5.8
4	8.0
5	27.8
6	31.3
7	20.1
8	39.9
9	34.9
10	26.4

[a] Determined against yeast's α-glucosidase in 50 mM phosphate buffer (pH 6.8) containing 5% v/v DMSO at 37 °C. The experiments were performed in triplicate and repeated at least three times, and the mean values were adopted.

To further clarify the role of π-system in the inhibitory activity, we reduced the carbonyl unit in benzoxanthones **2** and **8** to sp^3 hybridized methylene to afford compounds **5** and **9**, respectively. The reduction partially destroys the conjugated π-system, and in principle should weaken the ability of forming π-stacking interaction. As expected, compound **5** showed a decrease in the potency by three times. However, the reason why compound **9** showed comparable activities with **8** is not presently clear.[32]

The aforementioned results strongly suggest that the extended π-conjugated systems in **2**–**4** make substantial contributions to the remarkably enhanced inhibitory activities, most probably through π-stacking interaction with yeast's α-glucosidase.[33] To the best of our knowledge, this represents the first example that has demonstrated the crucial role of π-stacking effect in α-glucosidase inhibition. This result is in agreement with previous reports that π-stacking effect plays a significant role in biological activities,[34–37] and may be rationalized by taking into account the fact that there usually exist many aromatic amino acid residues in an enzyme that enables the formation of stable π-stacking interaction.

It is noteworthy that compounds **2**–**4** showed comparable inhibitory activities, and thus the additional 9-OH (in **3**) or 7-OH (in **4**) was not crucial to the activity, however this is not the case for 3-OH. As shown in our data, O-methylation of 3-OH led to a large decrease in the activity. This indicates that 3-OH in compound **2** was crucial, primarily as an H-bonding donor to interact with α-glucosidase since hydroxyl group is an H-bonding donor/acceptor, while methoxy group can only act as an H-bonding acceptor. This was further supported by the decreased activity observed with compound **7** in which 3-OH as an H-bonding donor forms strong intramolecular H-bond with nitro group at C4 position.

3. Conclusions

In summary, a series of novel benzoxanthones and their structurally perturbed analogs have been synthesized and fully characterized by NMR, IR, MS, and elemental analyses. The results indicate that bezoxanthones exhibited quite high inhibitory activities, and thus are exploitable as potent α-glucosidase inhibitors. Structural variation on benzoxanthones has provided some insight into their potencies that may be as a result of cooperative π-stacking and H-bonding interactions with α-glucosidase. Further efforts aiming at clarifying the mechanisms underlying the α-glucosidase inhibitory activities of xanthones are continuing in our laboratories, which will be reported in due course.

4. Experimental

NMR spectra were recorded on a Varian INOVA 300 MB NMR spectrometer in either $CDCl_3$ or d_6-acetone or d_6-DMSO and tetramethylsilane was used as an internal standard. Mass spectra were measured on a DSQ low resolution mass spectrometer or on a Shimadzu LCMS-2010A liquid chromatography mass spectrometer. IR spectra were obtained on a Bruker EQUINOX55 Fourier transformation infrared spectrometer. Elemental analyses were carried out on an Elementar Vario EL series elemental analyzer. Melting points were determined on a WRS-1B digital melting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-3150 scanning spectrophotometer.

Compound **1** was prepared according to the reported method.[23] 1-Deoxynojirimycin, *p*-nitrophenyl (PNP) glycoside, and α-glucosidase (from baker's yeast) used in this study were purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical quality and used upon received.

4.1. Synthesis of compounds 2–4 and 8

General procedures: To a mixture of hydroxybenzoic acid or hydroxy-2-naphthoic acid (0.01 mol), phloroglucinol or β-naphthol (0.01 mol), and freshly fused zinc chloride (5 g), phosphoryl chloride (30–50 mL) was added. The reaction mixture was stirred at 70 °C for 1.5–4 h, poured into crushed ice, and allowed to stand overnight. The solid was collected by filtration, washed with saturated aqueous sodium bicarbonate and water, and dried. The crude products were purified by flash column chromatography to afford **2**–**4** and **8** as yellow solids.

4.1.1. 1,3-Dihydroxy-12*H*-benzo[*b*]xanthen-12-one (2). Yield 79% from 3-hydroxy-2-naphthoic acid and phloroglucinol. Mp 283–285 °C (278–279 °C[24]); ^1H NMR (300 MHz, d_6-acetone) δ: 12.97 (s, 1H), 9.91 (s, 1H), 8.86 (s, 1H), 8.22 (d, $J = 8.4$ Hz, 1H), 8.06 (d, $J = 8.4$ Hz, 1H), 8.00 (s, 1H), 7.74–7.68 (m, 1H), 7.62–7.56 (m, 1H), 6.46 (d, $J = 2.4$ Hz, 1H), 6.28 (d, $J = 2.4$ Hz, 1H).

4.1.2. 1,3,9-Trihydroxy-12*H*-benzo[*b*]xanthen-12-one (3). Yield 42% from 3,7-dihydroxy-2-naphthoic acid and phloroglucinol. Mp >300 °C; IR (KBr): 3458, 3267, 2925, 1647, 1611, 1520, 1469, 1402, 1313, 1267, 1167, 1078, 832, 750, 710 cm^{-1}; ^{1}H NMR (300 MHz, d_6-acetone) δ: 13.03 (s, 1H), 9.89 (s, 1H), 8.98 (s, 1H), 8.62 (s, 1H), 7.96 (d, $J = 9.0$ Hz, 1H), 7.92 (s, 1H), 7.47 (d, $J = 2.7$ Hz, 1H), 7.38 (dd, $J = 9.0, 2.7$ Hz, 1H), 6.44 (d, $J = 2.7$ Hz, 1H), 6.26 (d, $J = 2.7$ Hz, 1H); ESI-MS *m/z*: 293 ([M−H]$^-$); Anal. Calcd for $C_{17}H_{10}O_5$: C, 69.39; H, 3.43. Found: C, 69.45; H, 3.51.

4.1.3. 1,3,7-Trihydroxy-12*H*-benzo[*b*]xanthen-12-one (4). Yield 35% from 3,5-dihydroxy-2-naphthoic acid and phloroglucinol. Mp >300 °C; IR (KBr): 3400, 2927, 1636, 1607, 1501, 1475, 1427, 1301, 1280, 1164, 1079, 1034, 1007, 810, 740, 630, 600 cm^{-1}; ^{1}H NMR (300 MHz, d_6-acetone) δ: 12.98 (s, 1H), 9.91 (br, 1H), 9.40 (br, 1H), 8.77 (s, 1H), 8.19 (s, 1H) 7.69 (d, $J = 8.1$ Hz, 1H), 7.39 (t, $J = 8.1$ Hz, 1H), 7.09 (d, $J = 8.1$ Hz, 1H), 6.46 (d, $J = 2.7$ Hz, 1H), 6.26 (d, $J = 2.7$ Hz, 1H); ESI-MS *m/z*: 293 ([M−H]$^-$); Anal. Calcd for $C_{17}H_{10}O_5$: C 69.39, H 3.43. Found: C, 69.10; H, 3.67.

4.1.4. 9,11-Dihydroxy-12*H*-benzo[*a*]xanthen-12-one (8).[38] Yield 31% from 2,4,6-trihydroxybenzoic acid and β-naphthol. ^{1}H NMR (300 MHz, d_6-acetone) δ: 13.42 (s, 1H), 9.95 (d, $J = 9.0$ Hz, 1H), 9.78 (br, 1H), 8.34 (d, $J = 9.0$ Hz, 1H), 8.06 (d, $J = 9.0$ Hz, 1H), 7.82–7.76 (m, 1H), 7.68–7.63 (m, 1H), 7.61 (d, $J = 9.0$ Hz, 1H), 6.51 (d, $J = 2.1$ Hz, 1H), 6.33 (d, $J = 2.1$ Hz, 1H); ESI-MS *m/z*: 277 ([M−H]$^-$).

4.2. Synthesis of compounds 5 and 9

General procedures: To a dry flask flushed with nitrogen and equipped with a reflux condenser was added $LiAlH_4$ (50–100 mg, excess) in anhydrous THF (30 mL). The benzoxanthone analogs (1.0 mmol) in THF (5 mL) were added dropwise at room temperature, and the mixture was then refluxed for 6–10 h. Excess $LiAlH_4$ was destroyed by the addition of ethyl acetate. The reaction mixture was poured into ice-water and then extracted with ethyl acetate. The crude products obtained after removal of the solvent were purified by flash column chromatography to afford **5** and **9** as yellow solids.

4.2.1. 1,3-Dihydroxy-12*H*-benzo[*b*]xanthene (5). Yield 29% from compound **2**. Mp 236 °C (dec); IR (KBr): 3426, 3055, 1628, 1509, 1459, 1272, 1241, 1164, 1043, 1003, 859, 810, 735, 622 cm^{-1}; ^{1}H NMR (300 MHz, d_6-acetone) δ: 8.60 (s, 1H), 8.26 (s, 1H), 7.82–7.77 (m, 3H), 7.43 (s, 1H), 7.41–7.32 (m, 2H), 6.20 (d, $J = 2.1$ Hz, 1H), 6.13 (d, $J = 2.1$ Hz, 1H), 4.06 (s, 2H); ESI-MS *m/z*: 263 ([M−H]$^-$); Anal. Calcd for $C_{17}H_{12}O_3$: C, 77.26; H, 4.58. Found: C, 77.11; H, 4.33.

4.2.2. 9,11-Dihydroxy-12*H*-benzo[*a*]xanthene (9). Yield 17% from compound **8**. Mp 205–207 °C; IR (KBr): 3433, 3050, 1625, 1519, 1450, 1262, 1241, 1163, 1048, 1008, 854, 811, 737, 626 cm^{-1}; ^{1}H NMR (300 MHz, d_6-acetone) δ: 9.91 (d, $J = 8.7$ Hz, 1H), 9.18 (s, 1H), 8.58 (s,1H), 8.31 (d, $J = 8.7$ Hz, 1H), 8.16 (d, $J = 8.7$ Hz, 1H), 7.81–7.74 (m, 1H), 7.65–7.60 (m, 1H), 7.60 (d, $J = 8.7$ Hz, 1H), 6.21 (d, $J = 2.1$ Hz, 1H), 6.13 (d, $J = 2.1$ Hz, 1H), 4.25 (s, 2H); ESI-MS *m/z*: 263 ([M−H]$^-$); Anal. Calcd for $C_{17}H_{12}O_3$: C, 77.26; H, 4.58. Found: C, 77.53; H, 4.39.

4.3. 1-Hydroxy-3-methoxy-12*H*-benzo[*b*]xanthen-12-one (6)

To a solution of xanthone **2** (139 mg, 0.5 mmol) in dry acetone (30 mL) and potassium carbonate (276 mg, 2.0 mmol) was added 10-fold excess iodomethane. The mixture was stirred at room temperature for 0.5 h. The excess iodomethane and acetone were removed under reduced pressure. Then the reaction mixture was partitioned between dilute hydrochloric acid (50 mL, 5%) and $CHCl_3$. The crude product obtained after removal of the solvent was purified by flash column chromatography (petroleum ether/CH_2Cl_2, 3:1) to afford **6** (121 mg, 83%) as a yellow solid. Mp 208–210 °C; IR (KBr): 3427, 2940, 1652, 1589, 1510, 1454, 1348, 1160, 1029, 824, 756 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.89 (s, 1H), 8.79 (s, 1H), 8.02 (d, $J = 8.4$ Hz, 1H), 7.87 (d, $J = 8.4$ Hz, 1H), 7.74 (s, 1H), 7.63–7.58 (m, 1H), 7.51–7.46 (m, 1H), 6.42 (d, $J = 2.1$ Hz, 1H), 6.32 (d, $J = 2.1$ Hz, 1H), 3.91 (s, 3H); EI-MS *m/z* (%): 292 ([M]$^+$ 100); Anal. Calcd for $C_{18}H_{12}O_4$: C, 73.97; H, 4.14. Found: C, 73.91; H, 4.09.

4.4. 1,3-Dihydroxy-4-nitro-12*H*-benzo[*b*]xanthen-12-one (7)

Nitric acid (70%, 0.5 mL) in acetic acid (5 mL) was slowly added to a solution of compound **2** (2 mmol) in acetic acid (20 mL). The mixture was stirred at 60 °C for 1 h and then poured into ice-cooled water (200 mL). The formed precipitates were filtered, washed with water, and recrystallized from ethanol to give **7** (342 mg, 53%) as a yellow solid. Mp 272–274 °C; IR (KBr): 3420, 3044, 2923, 2853, 1641, 1593, 1497, 1421, 1351, 1307, 1184, 876, 744, 624 cm^{-1}; ^{1}H NMR (300 MHz, d_6-DMSO) δ: 13.07 (s, 1H), 8.86 (s, 1H), 8.25 (d, $J = 8.1$ Hz, 1H), 8.13 (s, 1H), 8.07 (d, $J = 8.1$ Hz, 1H), 7.71 (t, $J = 8.1$ Hz, 1H), 7.59 (t, $J = 8.1$ Hz, 1H), 6.31 (s, 1H); EI-MS *m/z* (%): 323 ([M]$^+$ 100); Anal. Calcd for $C_{17}H_9NO_6$: C, 63.16; H, 2.81; N, 4.33. Found: C, 63.25; H, 2.90; N, 4.49.

4.5. Enzyme assays

The inhibitory activities of all the xanthone derivatives were measured by using the methods similar to those described previously.[29–31] Typically, α-glucosidase activity was assayed in 50 mM phosphate buffer (pH 6.8) containing 5% v/v dimethylsulfoxide and the PNP glycoside was used as a substrate. The inhibitors were pre-incubated with the enzyme at 37 °C for 0.5 h. The substrate was then added and the enzymatic reaction was carried out at 37 °C for 60 min. The reaction was monitored spectrophotometrically by measuring the absorbance at 400 nm. The assay was performed in triplicate with five different concentrations around the IC_{50} values that were roughly estimated in the first round of experiments, and the mean values were adopted.

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32. We have shown in previous study that inhibitory activities of xanthones show a significant dependence on the number of phenolic hydroxyl groups.[23] Thus, one possibility for the comparable activities shown by **8** and **9** is that the reduction destroys the intramolecular H-bonding between the carbonyl moiety and 1-OH, and thus releases one phenolic hydroxyl group that may contribute to the activity. That is, in this case, phenolic hydroxyl group may be more important to enhance the inhibitory activity than conjugated π-system.
33. One other possibility for the enhanced activities observed with **2**–**4** is an increase in the hydrophobicity of the molecules due to the insertion of one more aromatic ring. However, this may be ruled out by the fact that compounds **2**–**4** show comparable inhibitory activities and by our previous observation that introducing hydrophobic groups onto xanthones does not enhance the inhibitory activities.[23]
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