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Synthesis and biological evaluation of novel benzo[*b*]xanthone derivatives as potential antitumor agents

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Abstract: Nine novel aminoalkoxy substituted benzo[*b*]xanthones (**3a**–**i**) were synthesized. Their antitumor activities were evaluated in five human solid tumor cell lines, including Hep-G2, BEL-7402, HeLa, MGC-803 and CNE, by the MTT (2-(4,5-dimethyl-thiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) method. The results showed that most of the compounds displayed moderate to good inhibitory activities on the tested cancer cell lines *in vitro*, among them compounds **3a** and **3h** showed higher antitumor activity than the other tested compounds against most cell lines. The influences of two kinds of structural factors, *i.e.*, the terminal amino group and length of the carbon spacers, on the anticancer activities were explored to discuss preliminary structure–activity relationships.

Keywords: xanthone; benzo[*b*]xanthone; *in vitro* anticancer activity.

INTRODUCTION

Xanthones, a class of oxygen-containing heterocyclic compounds widely distributed in nature,[1] have diverse pharmacological activities, such as antioxidant activity,[2,3] topoisomerase II inhibitory activity,[4] antitumor activity,[5] α-glucosidase inhibitory activity,[6–9] cholesterol acyltransferase (ACAT) inhibitory activity,[10] antibacterial and antifungal activity,[11] based on their diverse structures. Their interesting structural scaffolds and pharmacological importance have attracted many scientists to isolate or synthesize these compounds as novel drug candidates. Especially, the effective inhibitory activity against human cancer cell lines has attracted considerable attention and many xanthone derivatives with excellent anticancer activity have been obtained.[12–15] For example, Shen *et al.* reported xanthone derivatives substituted by six different groups, of which the

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piperidinylethoxy-substituted xanthone could efficiently prohibit the growth of cancer cells.[16] Although preliminary work has highlighted the high class of the potent anticancer potentials of xanthone as a promising building motif for the development of new drugs, more efforts on structural modification of xanthone as well as on their pharmacological activity should be realized.

In the past few decades, medicinal chemistry researchers have mainly focused on the introduction of various side chains onto the aromatic ring core of xanthone in attempts to improve its bioactivity; however, little attention was paid to the structural modifications and anticancer activities of benzoxanthone, which possesses an extended π-system. Considering that a pharmacophore might improve its physicochemical and biological activities by the introduction of a nitrogen-containing side chain[17] and the structure of 1,3-dihydroxybenzoxanthone, in the herein reported study, a series of novel benzo[*b*]xanthone derivatives with terminal amines linked by different carbon spacers were prepared and their effect on the growth of five human cancer cell lines, *i.e.*, Hep-G2, BEL-7402, HeLa, MGC-803 and CNE, evaluated.

The primary aim of this work was to gain some insight into the effect of various terminal amines and different carbon lengths on benzo[*b*]xanthones on antitumor activities and an interesting structure–activity relationship. The obtained results revealed that aminoalkoxy-substituted benzo[*b*]xanthones provided interesting scaffolds in the search for potential anticancer drugs. The chemical structures of xanthone and benzo[*b*]xanthone are shown in Fig. 1.

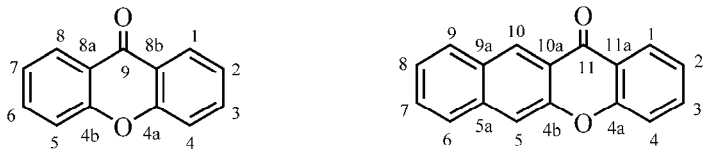


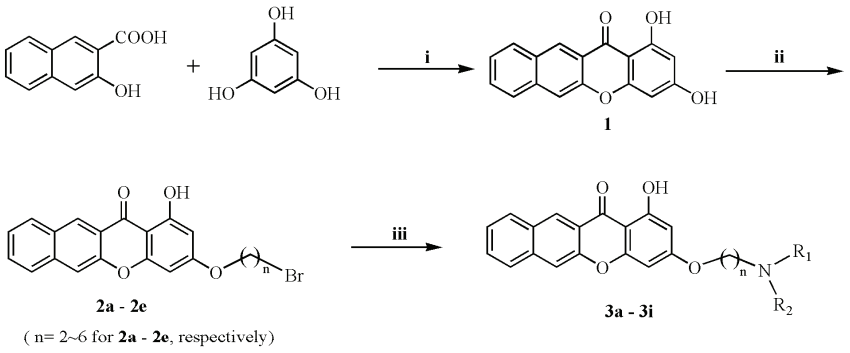
Fig. 1. Chemical structures of xanthone and 12*H*-benzo[*b*]xanthen-12-one.

RESULTS AND DISCUSSION

Chemistry

The synthetic route of benzoxanthone derivatives **3a**–**i** is shown in Scheme 1. Compound **1** was synthesized in 35 % yield from the condensation of 3-hydroxy-2-naphthoic acid with phloroglucinol (1,3,5-trihydroxybenzene) in the presence of anhydrous zinc chloride and phosphorus oxychloride as a condensing agent.[7] Compounds **2a**–**e**, which could be prepared from 1,3-dihydroxy-12*H*--benzo[*b*]xanthen-12-one with α,ω-dibromoalkane using K_2CO_3 as the base in anhydrous acetone.[5,8,9] were the key intermediates for the synthesis of the investigated compounds, Finally, amination of compounds **2a**–**e** with appropriate

amines in ethanol afforded the novel benzo[*b*]xanthone derivatives **3a**–**i**,[3,18] which could be purified by chromatography on a silica gel column, as yellow solids. The synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.



Compound	3a	3b	3c	3d	3e	3f	3g	3h	3i
n	4	4	4	4	4	2	3	5	6
$-NR^1R^2$									

Regents and conditions: (i) $ZnCl_2$, $POCl_3$, 70℃, 2-3 h; (ii) $Br(CH_2)_nBr$, K_2CO_3, acetone, 55℃, 24 h; (iii) R^1R^2NH, MeOH, 78℃, 6 h

Scheme 1. Synthetic route to the benzo[*b*]xanthone derivatives.

Antiproliferative activities

In the present study, the antiproliferative activity of the synthesized compounds was tested *in vitro* on five human tumor cell lines, *i.e.*, human hepatocarcinoma (Hep-G2 and BEL-7402), human cervix tumor (HeLa), human gastric cancer (MGC-803) and human nasopharyngeal carcinoma (CNE). 5-Flurouracil (5-Fu) was used as a positive control. The typical MTT (2-(4,5-dimethyl-thiazol--2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) assay procedure was applied for this assessment. The inhibitory activities of the compounds are given in Table I.

The results depicted in Table I indicated that most of the prepared compounds exhibited moderate to good inhibitory activities with the IC_{50} values at micromole level concentration against all of the cancer cell lines and all of the compounds demonstrated selective cytotoxicity against MGC-803 cells, except for compound **3e**. To optimize the structure favorable for anticancer activities, two kinds of structural factors, *i.e.*, the terminal amino substituent and the length of

TABLE I. IC_{50} values, determined by the MTT assay, for the benzoxanthone derivatives towards Hep-G2, BEL-7402, HeLa, MGC-803 and CNE cells

Compd./cell	IC_{50}[a] / μM				
	Hep-G2	BEL-7402	HeLa	MGC-803	CNE
5-Fu	21.30±2.53	20.95±1.90	51.88±2.53	7.76±1.09	51.00±2.82
3a	3.51±0.72	1.64±0.33	1.59±0.14	0.85±0.15	5.47±2.10
3b	7.56±1.09	5.74±1.37	6.22±0.56	0.76±0.09	12.39±0.88
3c	4.08±0.50	1.41±0.70	3.72±0.57	0.49±0.05	6.51±0.44
3d	5.70±0.39	5.53±0.58	8.45±1.97	0.39±0.06	20.29±1.14
3e	>100	>100	>100	>100	>100
3f	9.86±0.20	16.73±3.36	19.24±1.49	1.92±0.21	>100
3g	5.49±0.54	8.18±0.90	8.93±0.88	0.70±0.08	5.11±0.44
3h	1.72±0.67	10.08±1.46	3.41±0.17	0.86±0.14	4.41±0.31
3i	2.35±0.69	6.81±1.11	4.26±0.95	1.48±0.12	9.61±0.86

[a]The drug concentration required to inhibit tumor cell proliferation by 50 % after continuous exposure of 48 h; each value represents the mean ± *SD* from three independent experiments

the carbon spacer, were considered. First, compounds **3a**–**e**, in which the benzoxanthone ring system was linked to different terminal amino substituents by a 4-carbon spacer at the C-3 position, were investigated to determine the influence of the different amino substituents on the antiproliferative activity. It was shown that the dimethylamine-substituted compound **3a** holds the best inhibitory activity on the cancer cell lines in most cases. Compound **3a** has best activities against the cancer cell lines of Hep-G2, HeLa and CNE in this series, with IC_{50} values of 3.51, 1.59 and 5.47 μM, respectively. However, for the cell lines BEL-7402 and MGC-803, compounds **3c** and **3d**, which have *N*-heterocyclic rings at the C-3 position of 1,3-dihydroxy-12*H*-benzo[*b*]xanthen-12-one, were more active than compound **3a**. Interestingly, no notable activities were observed for the morpholino-substituted compound **3e** at a concentration of 100 μM against any of the tested cancer cell line. Compound **3b** also exhibited weaker inhibitory activities than compound **3a**, except for the cell line MGC-803. From the above comparisons, it is obvious that compound **3a** was the most potent compound of the derivatives **3a**–**e**, which indicated that the terminal dimethylamino group was favorable for the growth inhibitory activity in this series. Next, compounds **3f**–**i** as well as **3a**, in which the benzoxanthone ring system was linked to dimethylamino group by different carbon spacers, were investigated to explore the influence of the different length of carbon spacers on the anticancer activity. In this series, compounds **3a** and **3h**, which contained a four or five carbon spacer at position C-3 position respectively, showed higher inhibitory activities than the other compounds. Meanwhile, compound **3f**, which had two carbon spacers in between benzoxanthone and the terminal dimethylamino group, displayed the lowest activities. Comparing the IC_{50} values of compounds **3a** and **3h**, compound **3a** showed greater anticancer activities against the cancer cell line BEL-7402 and

HeLa, but lower activities against the Hep-G2/ and CNE cell lines, and equivalent activities for the MGC-803 cell line; thus they had almost equivalent anticancer activities. Although more studies are required to establish a structure–activity relationship, these preliminary results suggest that a four or five carbon spacer and a terminal dimethylamino group at position C-3 of the benzoxanthone scaffold (compounds **3a** and **3h**) are favorable for growth inhibitory activity against most cancer cell lines.

EXPERIMENTAL

All the solvents and reactants were of analytical grade and were used without further purification unless stated. Melting points were determined on a WRS-IA apparatus without correction. The elemental analyses were realized using an Elementar Vario EL CHNS elemental analyzer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Bruker Avance AV500 spectrometer using TMS as an internal standard. The IR spectra were recorded on a Nicolet ESP 360 FTIR instrument. The mass spectra were obtained on a Bruker Esquire HCT spectrometer. Analytic and spectroscopic data for compounds **1** and **2a**–**e** are given in Supplementary material to this paper.

*Synthesis of 1,3-dihydroxy-12*H*-benzo[*b*]xanthen-12-one (**1**)*

Compound **1** was synthesized by an improved literature method.[7] Thus, to a mixture of 3-hydroxy-2-naphthoic acid (9.4 g, 0.050 mol), phloroglucinol (6.3 g, 0.050 mol) and freshly fused zinc chloride (20 g) was added phosphoryl chloride (50–60 mL). The reaction mixture was stirred at 70 °C for 2–3 h until the starting material had disappeared as controlled by TLC. On completion of the reaction, the mixture was cooled to room temperature, poured onto crushed ice and allowed to stand overnight. The solid was collected by filtration, washed with saturated aqueous sodium bicarbonate and water, and dried to give a red solid. The crude products were purified by silica gel column chromatography eluting with petroleum ether (PE):EtOAc = 3:1 (v/v) to afford compound **1** as a yellow solid.

*General procedure for the synthesis of ω-bromoalkoxy-substituted benzo[*b*]xanthones (**2a**–**e**)*

With improved methods based on literatures procedures,[5,8,9] to a mixture of compound **1** (1.112 g, 4 mmol) and anhydrous K_2CO_3 (1.656 g, 12.0 mmol) in anhydrous acetone (30 mL) was added 1,2-dibromoethane or 1,3-dibromopropane or 1,4-dibromobutane or 1,5-dibromopentane or 1,6-dibromohexane (20 mmol) with a syringe. The reaction mixture was stirred overnight at 55 °C. The mixture was filtered, washed with water, then the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography eluting with PE:EtOAc = 25:1 (v/v) to give compounds **2a**–**e** as yellow solid, respectively.

*Synthesis of aminoalkoxy substituted benzo[*b*]xanthones (**3a**–**i**)*

General procedure. To a solution of **2a**–**e** in 15 mL of anhydrous ethanol was added dimethylamine, diethylamine, pyrrolidine, piperidine or morpholine, followed by heating at 78 °C for 6 h until the starting material had disappeared as evidenced by TLC and then cooled to room temperature. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The crude products were purified by column chromatography.

*Compound **3a***. Reactants: **2c** (0.06 g, 0.15 mmol) and an aqueous solution of dimethylamine (30 %, 2 mL, 9.97 mmol); elution solvent for column chromatography, chloroform:methanol, 100:8 (v/v).

*Compound **3b***. Reactants: **2c** (0.060 g, 0.15 mmol) and diethylamine (2 mL, 19.4 mmol); elution solvent for column chromatography, chloroform:methanol, 100:4 (v/v).

*Compound **3c***: Reactants: **2c** (0.060 g, 0.15 mmol) and pyrrolidine (0.25 mL, 3 mmol); elution solvent for column chromatography, chloroform:methanol, 100:6 (v/v).

*Compound **3d***. Reactants: **2c** (0.060 g, 0.15 mmol) and piperidine (0.25 mL, 2.52 mmol); elution solvent for column chromatography, chloroform:methanol, 100:1 (v/v).

*Compound **3e***. Reactants: **2c** (0.060 g, 0.15 mmol) and morpholine (0.25 mL, 2.88 mmol); elution solvent for column chromatography, chloroform:methanol, 100:1 (v/v).

*Compound **3f***: Reactants: **2a** (0.060 g, 0.16 mmol) and an aqueous solution of dimethylamine (30 %, 2 mL, 9.97 mmol); elution solvent for column chromatography, chloroform:methanol, 100:3 (v/v).

*Compound **3g***. Reactants: **2b** (0.060 g, 0.15 mmol) and dimethylamine aqueous solution (2 mL, 30%, 9.97 mmol); elution solvent for column chromatography, chloroform:methanol, 100:8 (v/v).

*Compound **3h***. Reactants: **2d** (0.060 g, 0.14 mmol) and dimethylamine aqueous solution (30%, 2 mL, 9.97 mmol); elution solvent for column chromatography, chloroform:methanol, 100:8 (v/v).

*Compound **3i***. Reactants: **2e** (0.060 g, 0.14 mmol) and dimethylamine aqueous solution (30%, 2 mL, 9.97 mmol); elution solvent for column chromatography, chloroform:methanol, 100:8 (v/v).

Growth of cell and chemicals

The human hepatocarcinoma cell lines (Hep-G2 and BEL-7402), human cervix tumor cell line (HeLa), human gastric cancer cell line (MGC-803) and human nasopharyngeal carcinoma cell line (CNE) were obtained from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). All cell lines were maintained in RPMI 1640 medium supplemented with 10 % fetal bovine serum and 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. The cells were kept at 37 °C in a humidified atmosphere containing 5 % CO_2. The nine benzo[*b*]xanthone derivatives **3a**–**i** were applied in DMSO to 10 mM and stored at −80 °C. MTT (2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) was obtained from Amresco Chemical Corp (USA) and was dissolved in 0.01 M PBS to 5 mg mL^{-1}. Deionized water was used in all experiments.

Cell proliferation assay (MTT assay)

Inhibition of cell proliferation by the benzo[*b*]xanthone derivatives was measured using the MTT assay. Briefly, cells were plated in 96-well culture plates at the density of 5000 cells per well in RPMI 1640 medium in 200 μL aliquots. After 24 h incubation, the cells were treated with the benzo[*b*]xanthone derivatives (0.3, 1, 3, 10 and 30 μM, four wells per concentration) for 48 h. Then 20 μL of a 5 mg mL^{-1} MTT solution was added to each well and the cells were further incubated at 37 °C for another 4 h. The supernatant was discarded, 150 μL DMSO per well was added and the absorbance (*A*) was measured at 490 nm. The cell inhibition (*IR*) was calculated using the following equation:

$$IR\ (\%) = \left[\frac{(A_c - A_t)}{A_c}\right] \times 100$$

where A_c and A_t are the absorption of the control and treated samples, respectively.

The IC_{50}, values, calculated by the logit method,[19] were taken as the concentrations of the benzo[*b*]xanthone derivatives causing 50 % inhibition of cell viabilities.

CONCLUSIONS

In this work, nine novel aminoalkoxy substituted benzo[*b*]xanthone were synthesized and their *in vitro* antitumor activities evaluated. The results suggested that a four or five carbon spacer and a terminal dimethylamino group at position C-3 of benzo[*b*]xanthone scaffold were favorable for the growth inhibitory activity. Thus, compounds **3a** and **3h** were the most potent compounds, which possess potential as future antitumor agents. The molecular mechanisms of their antitumor action will be reported in due course.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically at http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И ИСПИТИВАЊЕ БИОЛОШКЕ АКТИВНОСТИ НОВИХ ДЕРИВАТА БЕНЗОКСАНТОНА КАО ПОТЕНЦИЈАЛНИХ АНТИТУМОРСКИХ АГЕНАСА

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Описана је синтеза и карактеризација девет нових супституисаних деривата бензо[*b*]ксантона (**3a–i**). Испитивана је њихова антитуморска активност према пет типова ћелијских линија хуманог тумора, Hep-G2, BEL-7402, HeLa, MGC-803 и CNE, MTT методом. Резултати су показали да већина деривата показује умерену до добру *in vitro* инхибиторну активност према ћелијским линијама канцера, од којих једињења **3a** и **3h** показују највећу активност. Испитиван је утицај терминалне амино групе и дужине низа на антиканцерску активност и дискутована је прелиминарна веза између структуре и активности.

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