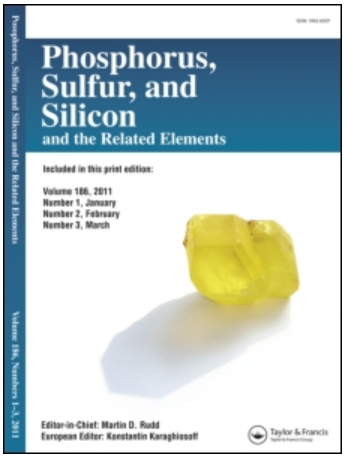

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Tianzhi Yu[a]; Peng Zhang[ab]; Yuling Zhao[b]; Hui Zhang[a]; Duowang Fan[a]; Wenkui Dong[b]; Lan Ding[c]
[a] Ministry of Education, Key Laboratory of Opto-Electronic Technology and Intelligent Control (Lanzhou Jiaotong University), Lanzhou, China [b] School of Chemical and Biological Engineering, Lanzhou Jiaotong University, Lanzhou, China [c] College of Life Science, Northwest Normal University, Lanzhou, China

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Synthesis and Cytotoxic Activity of Coumarin Derivatives Containing Benzotriazole Moieties

Tianzhi Yu,[1] Peng Zhang,[1,2] Yuling Zhao,[2] Hui Zhang,[1] Duowang Fan,[1] Wenkui Dong,[2] and Lan Ding[3]
[1]Key Laboratory of Opto-Electronic Technology and Intelligent Control (Lanzhou Jiaotong University), Ministry of Education, Lanzhou, China
[2]School of Chemical and Biological Engineering, Lanzhou Jiaotong University, Lanzhou, China
[3]College of Life Science, Northwest Normal University, Lanzhou, China

Three coumarin derivatives containing benzotriazole moieties, 3-(1-benzotriazolyl)-4-methyl-coumarin (BMC), 3-(1-benzotriazolyl)-4-methyl-benzo[7,8]coumarin (BMBC), and 3-(4-methyl-1-benzotriazolyl)-4-methyl-benzo[7,8]coumarin (MBMBC), were synthesized. Among them, 3-(4-methyl-1-benzotriazolyl)-4-methyl-benzo[7,8]coumarin has been structurally characterized by X-ray crystallography. The cytotoxic effect by Sulforhodamine B (SRB) assay with these compounds against the human hepatocellular carcinoma cell lines (HepG-2) was carried out in vitro. The preliminary results showed that all compounds inhibit HepG-2 growth effectively.

Keywords Benzotriazole; coumarin derivatives; cytotoxic activity; synthesis

INTRODUCTION

Coumarin and its derivatives are an important class of naturally occurring benzopyrone derivatives with useful pharmacological activity,[1–3] which are widely distributed in natural plants. Some of the coumarin derivatives are also reported as antibacterial agents,[4] and possess anti-HIV[5] and anticancer activities.[1,5,6] The pharmacological and biochemical properties, and therapeutic applications of coumarins depend upon the types of substituents in their basic structure. It was suggested

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Address correspondence to Tianzhi Yu, Key Laboratory of Opto-Electronic Technology and Intelligent Control (Lanzhou Jiaotong University), Ministry of Education, Lanzhou 730070, China. E-mail: ytz823@hotmail.com

that alterations in the chemical structure of coumarins could influence their biological properties. In addition, benzotriazole derivatives are of biological, chemical, and industrial importance. The 1*H*-1,2,3-Benzotriazole moiety has a documented prominence in a number of antitumor aromatase inhibitors.[7] Benzotriazole derivatives exhibit good pharmacological activities such as analgesic, anti-inflammatory, antifungal, antineoplastic, antiviral, and antihypertensive activities.[8]

In view of the biological importance of the coumarin and 1*H*-1,2,3-benzotriazole moieties, it prompted us to synthesize new heteroaromatic derivatives containing both these systems. Recently, we synthesized three coumarin derivatives containing benzotriazole moieties (see Figure 1) and tested them in vitro against the human hepatocellular carcinoma cell lines (HepG-2).

RESULTS AND DISCUSSION

Synthesis

The coumarin derivatives 3-(4-methyl-1-benzotriazolyl)-4-methyl-benzo[7,8]coumarin (MBMBC), 3-(1-benzotriazolyl)-4-methyl-benzo [7,8]coumarin (BMBC), and 3-(1-benzotriazolyl)-4-methyl-coumarin (BMC) were obtained from the starting substances 4-methyl-1*H*-benzotriazole, 1*H*-benzotriazole, 2-acetyl-1-naphthol, and 2-acetyl-1-phenol. The compounds were synthesized in three steps. The first step was alkylation of benzotriazole derivatives with chloroacetic acid in dry toluene. The second step was esterification of (4-methyl-1-benzotriazolyl-) acetic acid or (1-benzotriazolyl-) acetic acid with 2-acetyl-1-naphthol and 2-acetyl-1-phenol, respectively, in the presence of phosphorus oxychloride in dry pyridine. The final step was cyclodehydration of the intermediates using potassium hydroxide as catalyst in dry pyridine. Noteworthy, in the same experimental conditions, Liu et al. claimed that the esters took place in a rearrangement in third reaction step and yielded 1, 3-diketones.[11] However, due to the acidic character of the methylene group of the intermediate activated by strong electron-withdrawing benzotriazolyl moiety, we suggest that the intermediates were cyclodehydrated to form coumarin derivatives. When treated with a base, the methylene group close to the benzotriazole is easier to deprotonate, therefore, a stable carbanion forms. The carbanion connects to the carbonyl group, and the oxy anion attacks the hydrogen intramolecularly. An $H\text{-}O^-$ is then eliminated, and a new C=C bond is formed. The crystal structures of the compounds corroborate our deductions.

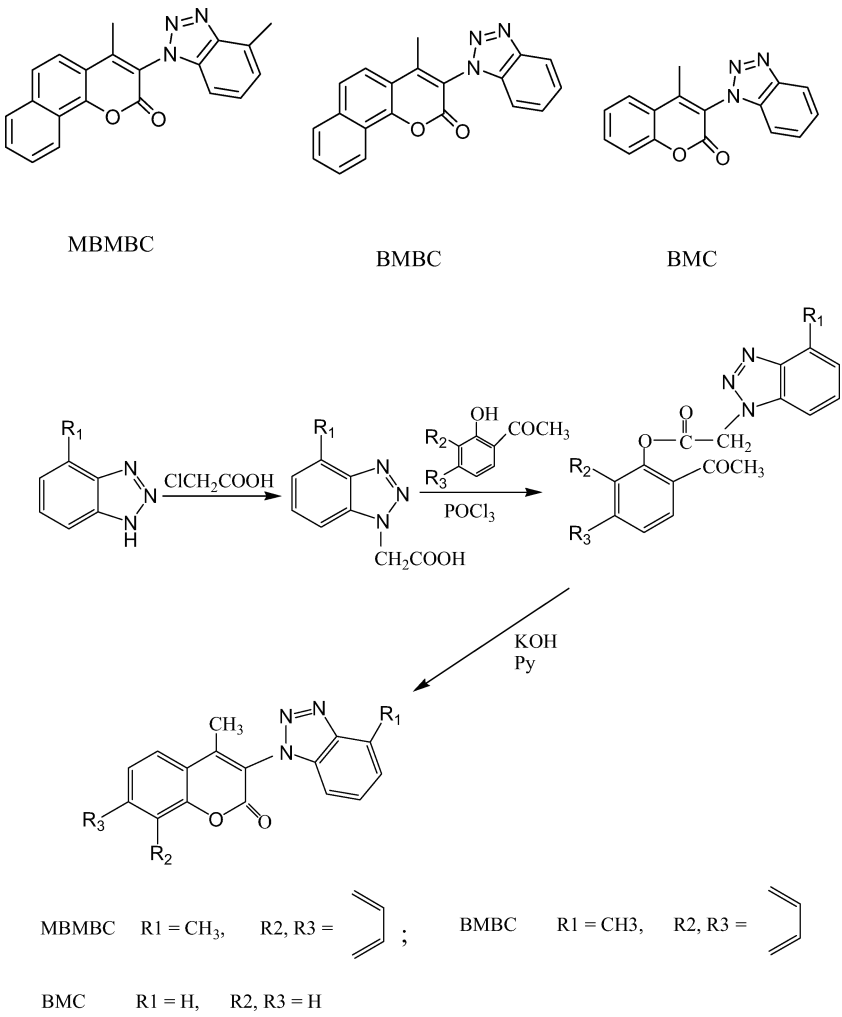

FIGURE 1 Structures and the synthetic route of the coumarin derivatives.

X-Ray Crystallographic Analysis

The crystal structure and packing diagram of MBMBC are illustrated in Figures 2 and 3, respectively. An acceptable crystal of MBMBC suitable for X-ray analysis was obtained by slow evaporation of an ethanol solution, and the structure was determined by X-ray crystallography. Crystal data: $C_{21}H_{15}N_3O_2$, M = 341.36, monoclinic, crystal dimensions 0.21 × 0.20 × 0.12 mm, space group $P2_1/n$, a = 7.3632(9) Å, b =

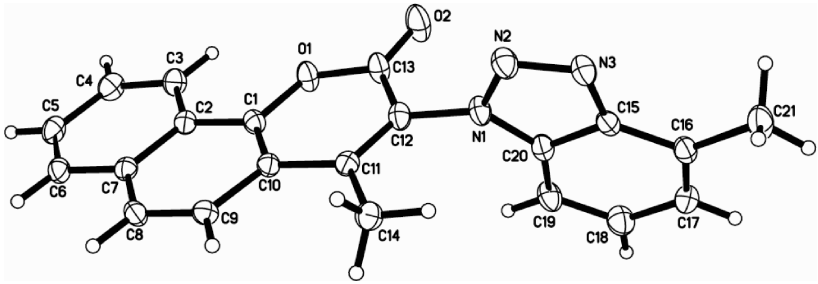

FIGURE 2 Crystal structure of MBMBC.

14.0875(18) Å, c = 16.004(2) Å, $\alpha = 90^\circ$, $\beta = 93.699(2)^\circ$, $\gamma = 90^\circ$, λ = 0.71073 Å, T = 187(2) K, U = 1656.6(4) Å^3, Z = 4, Dc = 1.369 g cm^{-3}, μ = 0.090 mm^{-1}, F(000) = 712; 8679 reflections measured, 3032 unique (R_{int} = 0.0272), 237 refined parameters, transmission factors 0.9890 and 0.9815. The final discrepancy factors were R_1 = 0.0523, wR_2 = 0.1329, goodness-of-fit S = 1.024 on F^2, largest difference peak and hole 0.510 and –0.237 eÅ^{-3}. CCDC reference number 673252.

Because of the steric interaction between 4-positioned methyl group and 3-positioned carbonyl oxygen in the coumarin ring and benzotriazole group, the benzotriazole skeleton is not coplanar with the benzocoumarin ring and the dihedral angle is 80.54° (Figure 2). The dihedral angle of MBMBC is larger than that of BMBC (60.4°)[10] and BMC (72°).[9] It is shown that the mutual repulsion of the two electron-rich atoms, the carbonyl oxygen of coumarin and the 2-positioned benzotriazolyl nitrogen, is larger than that of other two compounds owing to the 4-positioned electron-releasing CH_3 of benzotriazole ring in MBMBC.

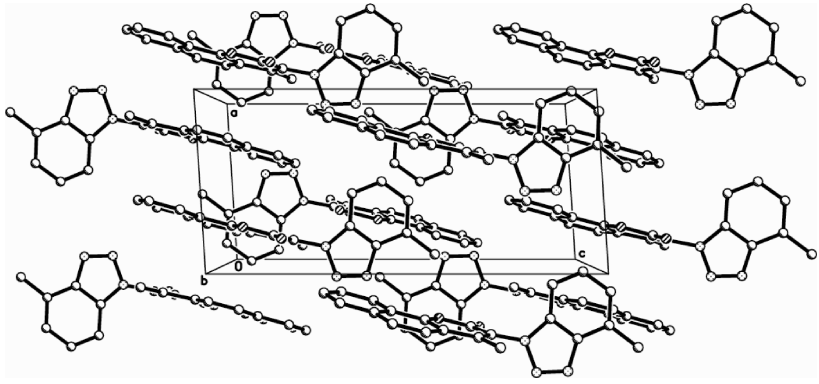

FIGURE 3 Packing diagram along a-axis. H atoms are omitted for clarity.

These results show that the benzotriazole and coumarin systems are not coplanar, and not conjugated.

From Figure 3, the interplanar distance of coumarin rings between two adjacent molecules along *a*-axis is approximately 6.911 Å, which is far from the value of the intermolecular π-π stacking interaction. It is means that there are not intermolecular π-π stacking interactions between the MBMBC molecules in the crystal lattice. Weak intermolecular π-π stacking interactions are noted in the BMBC molecules and in the BMC molecules; the interplanar distance of the coumarin rings is 3.59 Å for BMBC and 3.93 Å for BMC.

Cytotoxic Activity

In the present study, we performed a comparative evaluation of the cytotoxic effects of the three compounds against the human hepatocellular carcinoma cell lines (HepG-2) using the standard Sulforhodamine B (SRB) assay. The in vitro cytotoxicity assay was carried out according to procedures described by Rubinstein et al.[12] The values of IC_{50} (the concentration of test compounds required to reduce the cell survival fraction to 50% of the control) were calculated from dose response curves and used as a measure of cellular sensitivity to a given treatment. HepG-2 was incubated with RPMI-1640 medium containing 5% fetal bovine serum and grown at 37°C in a humidified atmosphere with 5% carbon dioxide. In order to maintain the cells in log-phase cellular suspension, aliquots were re-fed with fresh RPMI-1640 medium two or three times per week. The stock solutions of the tested compounds were freshly prepared in dimethyl sulfoxide (DMSO) and consequently diluted in RPMI-1640, and the final concentration never exceeded 0.5%.

The 48-h exposure of HepG-2 cells with the tested compounds resulted in a concentration-dependent reduction of cell viability. Figure 4 shows the concentration-response curves of incubation of HepG-2 cells in the presence of various concentrations of the compounds after 48 h. The corresponding IC_{50} values are listed in Table I, which can allow a quantitative merit for assessment of the relative potencies of the compounds under investigation. The results indicated that these compounds possess certain cytotoxicity at μg/mL scale.

From the results above, the in vitro studies indicated that all the tested compounds exhibit higher cytotoxic activity against HepG-2 cells. In terms of the relative potency of the compounds, compound BMC (IC_{50} value of 26.41 μg/mL) proved to be superior to the other two compounds yielding the cytotoxic sequence BMC > MBMBC > BMBC. The results clearly show that the conjugation of the coumarin

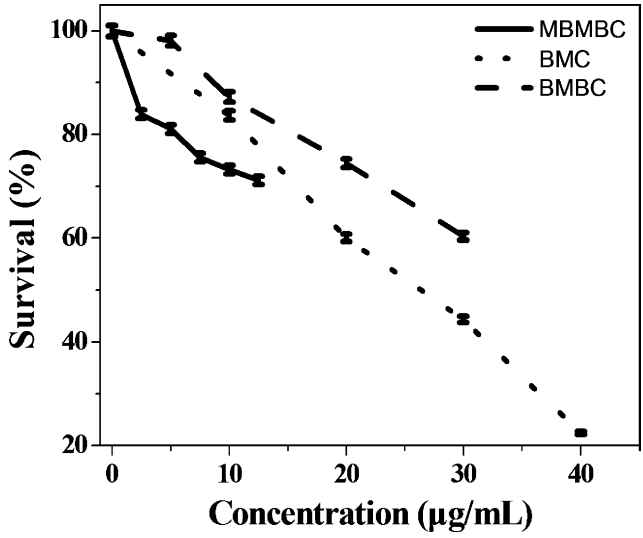

FIGURE 4 Cyctotoxic effects of MBMBC, BMBC, and BMC against HepG-2 cells after 48 h exposure as assessed by the standard Sulforhodamine B assay. Each data point represents the arithmetic mean of at least six independent experiments.

ring and the substitution on the benzotriazole ring substantially affect the cell-killing ability of the molecules. The cell-killing ability of the molecules decreased with increasing conjugation of the coumarin ring of the compounds. Compared with BMBC, MBMBC has a 4-positioned electron-releasing CH_3 in the benzotriazole ring; the electron density of triazole ring is higher, so the cell-killing ability of MBMBC is higher than that of BMBC.

CONCLUSIONS

We have synthesized three coumarin derivatives containing benzotriazole moieties, and their cytotoxic effect against the human

TABLE I Cytotoxic Activity of the Tested Compounds

Compounds	IC_{50} (μg/mL)
MBMBC	27.78 ± 2.37
BMBC	36.84 ± 3.55
BMC	26.41 ± 2.28

hepatocellular carcinoma cell lines (HepG-2) was carried out in vitro. It is clear from the results that these compounds are likely to have much higher HepG-2 inhibitory activity. The relationship between the structure and the cytotoxic property of the compounds was examined. The results show that the conjugate degree of the coumarin ring and the substitution on the benzotriazole ring can drastically alter the cytotoxic property of the compounds. Such results would lead us to modify our new target molecules by introducing more potential groups on the coumarin ring and the benzotriazole ring.

EXPERIMENTAL

Reagents and Chemicals

2-Acetyl-1-naphthol and 2-acetyl-1-phenol from Acros Organics and 4-methyl-1*H*-benzotriazole and 1*H*-benzotriazole from Aldrich were used without further purification. Chloroacetic acid and phosphorus oxychloride were analytical grade reagents from Tanjin Fuchen Chemical Reagent Factory. Phosphorus oxychloride was dried and redistilled before using. MBMBC, BMBC, and BMC were obtained by similar procedures. The synthesis of BMBC and BMC were described in earlier articles.[9,10]

Apparatus

IR spectra (400–4000 cm^{-1}) were measured on a Shimadzu IR Prestige-21 FT-IR spectrophotometer. ^{1}H-NMR spectra were obtained on Unity Varian-500 MHz. Melting points were measured by using an X-4 microscopic melting point apparatus made by Beijing Taike Instrument Limited Company, and the thermometer was uncorrected.

Procedure

Synthesis of (4-Methyl-1-benzotriazolyl-) Acetic Acid

50.00 g (0.376 mol) of 4-methyl-1*H*-benzotriazole and 32.12 g (0.342 mol) of chloroacetic acid were dissolved in dry toluene (300 mL), and then the solution was heated to 120°C and refluxed for 18 h with magnetic stirring. After cooling down to room temperature, it was poured into a concentrated aqueous solution of $NaHCO_3$. The water phase was separated and extracted with dichloromethane three times. The aqueous solution was adjusted to a pH of 4 by adding 2N HCl and allowed to sit overnight. The precipitate was filtered, then washed successively with distilled water and methanol. The product was dried under

reduced pressure and purified with recrystallization from 90% ethanol to yield 29.15 g (44.6%) of white crystalline solid; mp 201–203°C. IR (KBr pellet, cm^{-1}): 3431 ($\nu_{H\text{-}O\text{-}C=O}$), 2862, 2918 ($\nu_{CH2,}$ ν_{CH3}), 1730 ($\nu_{C=O}$). 1H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.50 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 7.37–7.94 (m, 3H, Ar-H).

Synthesis of (2-Acetyl) Naphthol (4-Methyl-1-benzotriazolyl-) Acetate

2.00 g (0.0107 mol) of 2-acetyl-1-naphthol and 2.21 g (0.0115 mol) of (4-methyl-1-benzotriazolyl-) acetic acid were dissolved in dry pyridine (20 mL). Then phosphorus oxychloride (1 mL) was added dropwise with magnetic stirring at 5–10°C, and the mixture was stirred for 10 h at room temperature. After the reaction was complete, the mixture was poured into an aqueous solution of HCl containing broken ice with vigorous stirring, and a white precipitate was produced. The precipitate was filtered, and washed successively with a diluted aqueous solution of $NaHCO_3$ (10%) and distilled water, respectively. After ethanol recrystallization, filtration, and drying in vacuo, 1.82 g (47.1%) of white flocculent crystals was obtained. mp 168–170°C. IR (KBr pellet, cm^{-1}): 1768 (ester C=O), 1681 (ketone C=O), 3057, 2985, 2953, 1465, 1357, 1180. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.52 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 5.92 (s, 2H, CH_2), 7.38–7.97 (m, 9H, Ar-H).

Synthesis of 3-(4-Methyl-1-benzotriazolyl)-4-methyl-benzo [7,8]coumarin (MBMBC)

Into a one-neck, 100 mL round-bottomed flask, (2-acetyl) naphthol (4-methyl-1-benzotriazolyl-) acetate (1.30 g (0.0036 mol)) and dry pyridine (11 mL) were placed, then potassium hydroxide (0.30 g (0.0053 mol)) was added stepwise, and the reaction mixture was stirred vigorously for 5 h at room temperature. The mixture was poured into an aqueous solution of HCl containing broken ice with vigorous stirring, and a primrose yellow precipitate was formed. Then the crude product was filtered and washed with distilled water, and purified with recrystallization from chloroform:ethanol mixed solvent (5:1) to yield 0.81 g (65.9%) of a primrose yellow crystalline solid. mp > 250°C. IR (KBr pellet, cm^{-1}): 1728 ($\nu_{C=O}$), 3056, 2924, 1637, 1395, 1284, 1116. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 2.42 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.33–8.66 (m, 9H, Ar-H).

Crystallography

A suitable single crystal of MBMBC was obtained by evaporation of an ethanol solution. The diffraction data were collected with a Bruker

Smart Apex CCD area detector using a graphite monochromated MoKα radiation (λ = 0.71073 Å) at 187(2) K.[13] The structure was solved by using the program SHELXL and Fourier difference techniques, and refined by the full-matrix least-squares method on F^2. All hydrogen atoms were added theoretically.[14]

SUPPORTING INFORMATION

The crystallographic data (excluding structure factors) of MBMBC has been deposited with the Cambridge Crystallographic Center as supplementary publication no. CCDC-673252.

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