Med Chem Res (2010) 19:970–983
DOI 10.1007/s00044-009-9243-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, structure characterization, and biological evaluation of some new 1,2,3-benzotriazole derivatives

Jun Wan · Xia Yan · Cuiping Ma · Sai Bi · Hai-Liang Zhu

Received: 17 February 2009 / Accepted: 25 August 2009 / Published online: 30 September 2009

Abstract Ten novel benzotriazole compounds were synthesized. Their chemical structures were confirmed by ^{1}H NMR, IR, and elemental analyses, coupled with three selected single-crystal structures (compounds **A2**, **B3**, and **B5**). Their antimycotic and antitumor activities were also investigated. The title compounds showed some antitumor activities, especially in the case of **A3** and **A4**, which showed the most potent activity of propagation inhibition in liver and galactophore cancer cells.

Keywords Benzotriazole compounds · Antimycotic activities · Antitumor activities · Crystal structure

Introduction

Heterocyclic compounds containing nitrogen are considered to be the most effective anticancer drugs used either as single agents or in combination for cancer therapy (Lin *et al.*, 2005; Fang *et al.*, 2006; Wang *et al.*, 2006). Some azole compounds, especially triazole or benzimidazole derivatives, have been reported to have cytotoxic potency on human cancer cells, e.g., breast carcinoma and liver cancer

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(Saüczewski *et al.*, 2004; Pagliai *et al.*, 2006; Leonetti *et al.*, 2004; Saberi *et al.*, 2006). During recent years studies have shown that several benzotriazole compounds can act to diminish inflammation, as antivirus, antifungal, and antimumor agents, and as selective inhibitors of PTP1B and antidepressants, resulting from the potent bioactivity of benzotriazole (Xu *et al.*, 2003; Emami *et al.*, 2004; Biagi *et al.*, 2001; Scapin *et al.*, 2003; Antonio *et al.*, 2004). Moreover, Touami *et al.* (1997) also reported that the novel conjugates of benzotriazole photonucleases and DNA minor groove binders exhibit enhanced cleavage efficiency and unique cleavage selectivity (Touami *et al.*, 1997). Al-Soud *et al.* (2003) reported that some triazole derivations showed remarkable activity against leukemia, ovarian, renal, and lung cancers McClure *et al.* (2005) also reported that benzotriazoles are significantly more potent inhibitors applied to p38 MAP kinase experimentally than benzoimidazolones. The X-ray crystal structure of benzotriazole derivatives also shows that the triazole group as the H-bond acceptor, but unexpectedly as a dual acceptor, inducing movement of the crossover connection of p38R, was the most useful in ranking potency (McClure *et al.*, 2005). And niacin has long been used for the treatment of lipid disorders and for the prevention of atherosclerosis, as a result of its ability to raise high-density lipoprotein (HDL) levels (Semple *et al.*, 2006). The design and synthesis of new benzotriazole compounds with higher physiological and biological properties have been the focus of recent studies in our laboratories (Wan *et al.*, 2006; Zhang *et al.*, 2007a).

Exploiting the concept of bioisosterism (Cui and Nan, 2006), a series of novel title compounds including benzotriazole was synthesized. Single crystals suitable for X-ray measurements of three compounds were also obtained by slow evaporation of mixed solvents at room temperature. Furthermore, their biological and antitumor activities were investigated. The results show that the compounds 3-(1H-benzo[*d*][1,2,3]triazol-1-yl)-1-oxo-1-*p*-tolylpropan-2-yl nicotinate and 3-(1H-benzo[*d*][1,2,3] triazol-1-yl)-1-(4-chlorophenyl)-1-oxopropan-2-yl nicotinate possess potent propagation inhibition activity in liver and galactophore cancer cells, respectively, and the toxicity of the compounds has a time-quantity efficiency relationship.

Experimental

General comments

All reagents were obtained from commercial suppliers and were used without further purification. Solvents for reactions, extraction, and chromatography were of analytical grade. Intermediates I and II were readily prepared according to the reported methods (Wan *et al.*, 2006; Abonia *et al.*, 2004). To a 250-m flask were added 0.02 mol of intermediate II in 50 ml of acetic and 0.05 mol of sodium acetate. Then bromine, 0.02 mol (3.2 g), was added dropwise, with stirring, at room temperature. The reaction was maintained until the mixture became colorless for about 4 h. Then 50 ml of water and 20 ml of chloroform were added. The organic layer was successively washed with saturated sodium bicarbonate solution and

brine, then dried over anhydrous magnesium sulfate to get III. Melting points were measured on a Yanaco MP-500 melting-point apparatus and were uncorrected. Elemental analyses were measured with a Vario EL III analyzer. IR spectra (4000–400 cm^{-1}), as KBr pellets, were recorded on a Nicolet FT-IR 510P spectrophotometer. ^1H NMR was recorded on a 500-MHz JEOL FX-90Q NMR spectrometer in $CDCl_3$ as the solvent and with TMS as an internal standard. Column chromatography was carried out on silica gel (unactivated, neutral, 100–200 mesh).

Preparation of **A1–A6**, **B1**, and **B3–B5**

Triethylamine (2.8 ml, 0.02 mol) was added dropwise, with stirring, to a solution of intermediate III (0.02 mol) and nicotinic acid or isonicotinic acid (0.02 mol) in acetone at 0°C. The mixture was stirred for another 5 h in an ice-water bath, then filtered, and the filtrate was concentrated to give a crude product which was poured into a saturated sodium chloride solution, followed by extraction with chloroform. The combined organic extracts were washed with water, dried with $MgSO_4$, and filtered. Removal of the solvent gave the residue, which was chromatographed on silica or recrystallized to get the desired products of **A1**–**A6**, **B1**, and **B3**–**B5** in pure form. Similarly, the single crystals were obtained by slow evaporation of mixed solvents at room temperature over a period of several days.

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-oxo-1-phenylpropan-2-yl nicotinate (**A1**)*

Purified by recrystallization from petroleum ether/DMF (5:1, v/v). Yield, 37.8%; yellow solid; m.p., 145.8–147.0°C. Anal. Calcd. for $C_{21}H_{16}N_4O_3$ (%): C 67.73, H 4.33, N 15.05. Found (%): C 67.53, H 4.43, N 15.34. ν_{max}/cm^{-1} (KBr): 3133 (=C–H), 1750, 1710 (s, C=O), 1585 (s, C=N), 1102, 1227 (s, C–O). ^1H NMR ($CDCl_3$): 5.18 and 5.31(2H, CH_2), 6.70 (1H, CH), 7.26–9.07 (13H, phenyl H).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-(4-methoxyphenyl)-1-oxopropan-2-yl nicotinate (**A2**)*

Purified by column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as the eluent; the single crystal suitable for X-ray measurements was obtained by slow evaporation of petroleum ether and alcohol (1:2, v/v) at room temperature over a period of several days. Yield, 35.1%; yellow solid; m.p., 160.1–161.8°C. Anal. Calcd. for $C_{22}H_{18}N_4O_4$ (%): C 65.66, H 4.51, N 13.92. Found (%): C 65.86, H 4.35, N 13.88., ν_{max}/cm^{-1} (KBr): 3130(=C–H), 1750, 1710 (s, C=O), 1585 (s, C=N), 1102, 1227 (s, C–O)., ^1H NMR ($CDCl_3$): 3.90 (3H, methoxyl, CH_3), 5.18 and 5.31 (2H, CH_2), 6.68 (1H, CH), 6.99–9.08 (12H, phenyl H). ESI–MS *m/z* 403.1 (MH^+).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-oxo-1-p-tolylpropan-2-yl nicotinate (**A3**)*

Purified by recrystallization from ethanol; the single crystal suitable for X-ray measurements was obtained by slow evaporation of ethyl acetate and petroleum ether (1:1, v/v) at room temperature over 1 week. Yield, 33.5%; yellow solid; m.p.,

139.5–141.2°C. Anal. Calcd. for $C_{22}H_{18}N_4O_3$ (%): C 68.38, H 4.70, N 14.50. Found (%): C 68.62, H 4.66, N 14.38. ν_{max}/cm^{-1} (KBr): 3060 (=C–H), 1729, 1690 (s, C=O), 1605 (s, C=N), 1281, 1117 (s, C–O). ^1H NMR ($CDCl_3$): 2.45(3H, methyl), 5.53 (2H, CH_2), 6.86 (1H, CH), 7.41–9.04 (12H, phenyl H).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-(4-chlorophenyl)-1-oxopropan-2-yl nicotinate (**A4**)*

Purified by column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as the eluent. Yield, 44.3%; yellow solid; m.p., 137.8–138.5°C. Anal. Calcd. for $C_{21}H_{15}ClN_4O_3$ (%): C 62.00, H 3.72, N 13.77. Found (%): C 61.89, H 3.88, N 13.76. ν_{max}/cm^{-1} (KBr): 3072 (=C–H), 1730, 1692 (s, C=O), 1588 (s, C=N), 1260, 1098 (s, C–O). ^1H NMR ($CDCl_3$): 5.56 (2H, CH_2), 6.87 (1H, CH), 7.41–8.20 (12H, phenyl H).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-(2,4-dichlorophenyl)-1-oxopropan-2-yl nicotinate (**A5**)*

Purified by recrystallization from H_2O/acetone (3:1, v/v). Yield, 36.4%; yellow solid; m.p., 92.8–94.3°C. Anal. Calcd. for $C_{21}H_{14}Cl_2N_4O_3$ (%): C 57.16, H 3.20, N 12.70. Found (%): C 57.23, H 3.41, N 12.89. ν_{max}/cm^{-1} (KBr): 3081 (=C–H), 1731, 1714 (s, C=O), 1582 (s, C=N), 1113, 1287 (s, C–O). ^1H NMR ($CDCl_3$): 5.29 (2H, CH_2), 6.53 (1H, CH), 7.27–8.74 (11H, phenyl H).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-(2,5-dichlorophenyl)-1-oxopropan-2-yl nicotinate (**A6**)*

Purified by column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as the eluent. Yield, 38.5%; yellow solid; m.p., 165.4–166.5°C. Anal. Calcd. for $C_{21}H_{14}Cl_2N_4O_3$ (%): C 57.16, H 3.20, N 12.70. Found (%): C 57.52, H 3.17, N 13.04., ν_{max}/cm^{-1} (KBr): 3089 (=C–H), 1730, 1635 (s, C=O), 1591 (s, C=N), 1272, 1104 (s, C–O). ^1H NMR ($CDCl_3$): 5.74 (2H, CH_2), 6.88 (1H, CH), 7.29–9.03 (11H, phenyl H).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-oxo-1-phenylpropan-2-yl isonicotinate (**B1**)*

Purified by column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as the eluent. Yield, 40.2%; yellow solid; m.p., 166.2–167.5°C. Anal. Calcd. for $C_{21}H_{16}N_4O_3$ (%): C 67.73, H 4.33, N 15.05, Found (%): C 67.85, H 4.37, N 15.67, ν_{max}/cm^{-1} (KBr): 3063 (=C–H), 1733, 1687 (s, C=O), 1592 (s, C=N), 1283, 1124 (s, C–O), ^1H NMR ($CDCl_3$): 5.17 and 5.33 (2H, CH_2), 6.68 (1H, CH), 7.26–8.73 (13H, phenyl H).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-oxo-1-p-tolylpropan-2-yl isonicotinate (**B3**)*

Purified by recrystallization from petroleum ether/acetone (2:1, v/v); single crystals suitable for X-ray measurements were obtained by slow evaporation of acetone and

alcohol (1:2, v/v) at room temperature over a period of 5 days. Yield, 39.5%; yellow solid; m.p., 174.2–175.0°C. Anal. Calcd. for $C_{22}H_{18}N_4O_3$ (%): C 68.38, H 4.70, N 14.50. Found (%): C 68.71, H 4.58, N 14.82., ν_{max}/cm^{-1} (KBr): 3053 (=C–H), 1737, 1684 (s, C=O), 1605 (s, C=N), 1286, 1125 (s, C–O)., ^1H NMR ($CDCl_3$): 2.46 (3H, methyl, CH_3), 5.38 (2H, CH_2), 6.86 (1H, CH), 7.44–8.78 (12H, phenyl H).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-(4-chlorophenyl)-1-oxopropan-2-yl isonicotinate (**B4**)*

Purified by column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as the eluent. Yield, 44.8%; yellow solid; m.p., 151.2–152.5°C. Anal. Calcd. for $C_{21}H_{15}ClN_4O_3$ (%): C 62.00, H 3.72, N 13.77. Found (%): C 62.13, H 4.02, N 13.96., ν_{max}/cm^{-1} (KBr): 3045 (=C–H), 1739, 1688 (s, C=O), 1586 (s, C=N), 1268, 1124 (s, C–O). ^1H NMR ($CDCl_3$): 5.57 (2H, CH_2), 6.88 (1H, CH), 7.42–8.78 (12H, phenyl H). ESI–MS *m/z* 407.1 (MH^+).

*3-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-(2,4-dichlorophenyl)-1-oxopropan-2-yl isonicotinate (**B5**)*

Purified by column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as the eluent; the single crystal suitable for X-ray measurements was obtained by slow evaporation of acetone and alcohol (1:1, v/v) at room temperature over a period of several days. Yield, 43.0%; yellow solid; m.p., 115.0–117.2°C. Anal. Calcd. for $C_{21}H_{14}Cl_2N_4O_3$ (%): C 57.16, H 3.20, N 12.70. Found (%): C 57.43, H 3.46, N 13.01. ν_{max}/cm^{-1} (KBr): 3087 (=C–H), 1736, 1713 (s, C=O, 1581 (s, C=N), 1287, 1118 (s, C–O)., ^1H NMR ($CDCl_3$): 5.57 (2H), 6.88 (1H), 7.42–8.78 (11H, phenyl H).

Crystallographic data and structure determination

A colorless crystal, **A2** (0.13 × 0.11 × 0.08), a colorless crystal, **B3** (0.37 × 0.18 × 0.07), and a colorless crystal, **B5** (0.33 × 0.25 × 0.11), were mounted on a Bruker SMART 1000 CCD area diffractometer for data collection using ω-scan mode. A total of 33,084, 5391, and 11,343 reflections were collected in the range of $1.43 \leq \theta \leq 26.05$, $2.08 \leq \theta \leq 26.01$, and $1.81 \leq \theta \leq 26.08$ using a MoKα radiation device ($\lambda = 0.71073$ Å) equipped with a graphite monochromator at 293(2) K (Siemens, 1996), of which 11,926, 3668, and 4066 were independent ($R_{int} = 0.070$) and 5006, 2432, and 3235 were observed at $I > 2\sigma(I)$. Empirical absorption corrections were carried out using the SADABS (Sheldrick, 1996) program.

The structures of **A2**, **B3**, and **B5** were solved by direct methods and refined by full-matrix least-squares on F^2 with the SHELXTL (Sheldrick, 1997) software package. All non-H atoms were anisotropically refined. All H atoms were located on difference Fourier maps and refined isotropically. The water hydrogen atoms of **B5** were located by means of difference synthesis and refined isotropically. For **A2** the final $R = 0.1227$ and $\omega R = 0.2897$ ($\omega = 1/[\sigma^2(F_o^2) + (0.1000P)^2 + 0.0000P]$, where $P = (F_o^2 + 2F_c^2)/3)$ with $I > 2\sigma(I)$. $S = 1.441$, $(\Delta/\sigma)_{max} = 0.001$,

$(\Delta\rho)_{max} = 0.793$, and $(\Delta\rho)_{min} = -0.630$ e/Å^3. For **B3** the final $R = 0.0568$ and $\omega R = 0.1201$ ($\omega = 1/[\sigma^2(F_o^2) + (0.0532P)^2 + 0.1631P]$, where $P = (F_o^2 + 2F_c^2)/3$) with $I > 2\sigma(I)$. $S = 1.035$, $(\Delta/\sigma)_{max} = 0.001$, $(\Delta\rho)_{max} = 0.186$, and $(\Delta\rho)_{min} = -0.197$ e/Å^3. For **B5** the final $R = 0.0390$ and $\omega R = 0.0962$ ($\omega = 1/[\sigma^2(F_o^2) + (0.0530P)^2 + 0.3967P]$, where $P = (F_o^2 + 2F_c^2)/3$) with $I > 2\sigma(I)$. $S = 1.026$, $(\Delta/\sigma)_{max} = 0.001$, $(\Delta\rho)_{max} = 0.225$, and $(\Delta\rho)_{min} = -0.220$ e/Å^3. Atomic scattering factors and anomalous dispersion corrections were taken from *International Tables for X-Ray Crystallography* (Wilson, 1992). Software packages used to prepare material for publication were SHELXTL, PARST (Nardelli, 1995), and PLATON (Spek, 1990).

Biological activities

Testing of primary biological activities was performed in an isolated culture (Zhang *et al.*, 2007b). Under sterile conditions, 1 ml of sample was added to culture plates, followed by 9 ml of culture medium. The final concentration was 50 μg/ml. The blank assay was performed with 1 ml of sterile water. A circle of mycelium with a diameter of 4 mm was cut using a drill. Culture plates were cultivated at 24 ± 1°C. The extended diameters of the circle mycelium were measured after 72 h. The relative inhibition rate of the circle mycelium was compared with a blank assay.

Short-term bioassay of antitumor activity

Cell viability was measured by the MTT (3-[4,5-dimethyl-2-yl]-2,5-diphenyltetrazolium bromide) method (Hansen *et al.*, 1989). Cells in DMEM were seeded at a density of 9 × 103 cells per well in 96-well plates for 24 h. After exposure to different concentrations of compounds dissolved in fresh DMEM for various times, 20 μl of MTT (50 mg/ml) dissolved in DMEM was added. Cells were incubated at 37°C in the dark for 4 h, MTT was removed, and 100 μl of a lysing buffer (10%, w/v, sodium dodecyl sulfate [SDS] dissolved in a solution of 50% each *N*,*N*-dimethyl formamide and deionized water, pH 4.7) was added. Absorbance at 570 nm was determined using a microplate reader (SpectraMax190; Molecular Devices) after shaking in the dark for 15 min. Cell viability was expressed as a percentage of the cell survival rate.

Results and discussion

Synthesis

The synthesis of compounds **A1**–**A6**, **B1**, **and B3**–**B5** is illustrated in Scheme 1. Benzotriazolylpropiophenones (II) were prepared according to reported methods (Wan *et al.*, 2006; Abonia, *et al.*, 2004). Bromine-substituted benzotriazolylpropiophenones were obtained in the presence of sodium acetate and acetic acid instead of $AlCl_3$ (Kasiotis and Haroutounian, 2006); when the ratio of II and sodium acetate

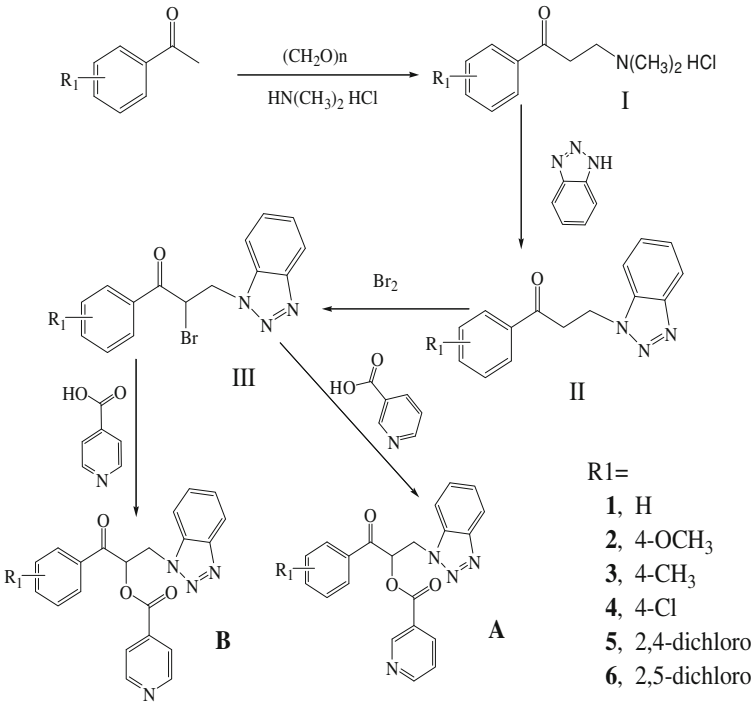
was 1:2, the reaction temperature was decreased to 30°C to obtain the monobromide. Using triethylamine as the binding acid reagent, nicotinates **A1**–**A6** and isonicotinates **B1** and **B3**–**B5** were obtained at room temperature in good yields (Scheme 1).

Compounds **A1**–**A6**, **B1**, and **B3**–**B5** were characterized by elemental, IR, and ^{1}H NMR spectroscopic analysis. Single crystals suitable for X-ray measurements of compounds **A2**, **B3**, and **B5** were obtained by slow evaporation of petroleum ether and alcohol (1:2, v/v), acetone and alcohol (1:2, v/v), and acetone and alcohol (1:1, v/v), respectively, at room temperature over a period of days.

Crystal structure of compounds **A2**, **B3**, and **B5**

Crystal data and structure refinement for compounds **A2**, **B3**, and **B5** are listed in Table 1. Figures 1, 3, and 5 show the molecular structure of **A2**, **B3**, and **B5**, and their packing diagrams are shown in Figs. 2, 4, and 6, respectively.

In the crystal structure of **A2**, three independent molecules exist in each unit cell. The bond lengths of C–N in **A2** are in the range of 1.290–1.400 Å, shorter than the single-bond length of 1.48 Å and longer than the typical C=N distance of 1.28 Å, indicating a partial double character. This can be interpreted in terms of conjugation in the benzotriazole ring system. The benzotriazole moieties are nearly coplanar,



Scheme 1 Synthesis of title compounds

Table 1 Crystal data and structure refinement for **A2**, **B3**, and **B5**

	A2	**B3**	**B5**
Chem. formula	$C_{22}H_{18}N_4O_4$	$C_{22}H_{18}N_4O_3$	$C_{21}H_{14}Cl_2N_4O_3{\cdot}H_2O$
Fw	402.40	386.40	459.28
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	P21/c	*P*-1	P21/c
a (Å)	23.059(4)	9.840(2)	11.2644(11)
b (Å)	9.3484(17)	10.050(2)	12.8260(13)
c (Å)	29.986(5)	10.994(2)	14.2814(14)
α (°)	90	114.635(3)	90
β (°)	110.433(3)	97.113(4)	92.659(2)
γ (°)	90	98.384(4)	90
V (Å^3)	6057.2(18)	956.9(3)	2061.1(4)
Z	12	2	4
ρ (g/cm^3)	1.324	1.341	1.480
μ (mm^{-1})	0.116	0.092	0.352
F (000)	2520	404	944
Abs. correct	SABABS	SABABS	SABABS
Cryst. color	Colorless	Colorless	Colorless
Cryst.l size (mm^3)	0.13 × 0.11 × 0.08	0.37 × 0.18 × 0.07	0.33 × 0.25 × 0.11
θ	1.43–26.05	2.08–26.01	1.81–26.08
No. reflects meads	33,084	5,391	11,343
No. unique reflects	11,926	3,668	4,066
No. params	361	262	288
R	0.1227	0.0568	0.0390
ωR	0.3327	0.1394	0.1039
S	1.44	1.03	1.03

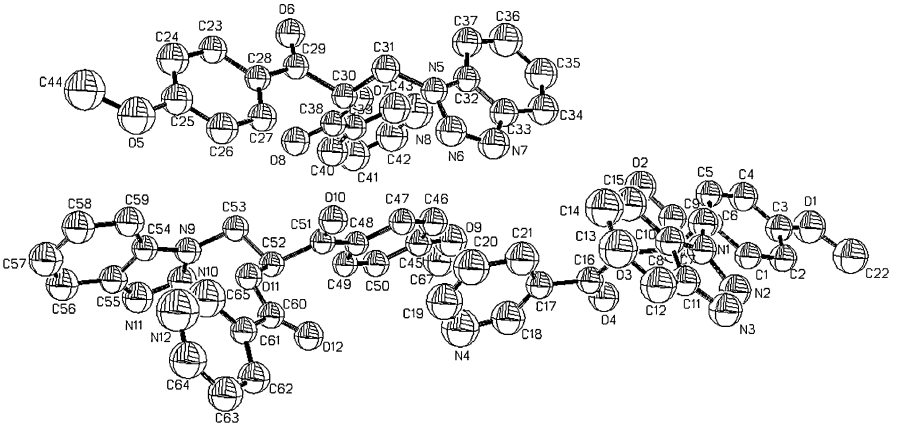


Fig. 1 Molecular structure of **A2**

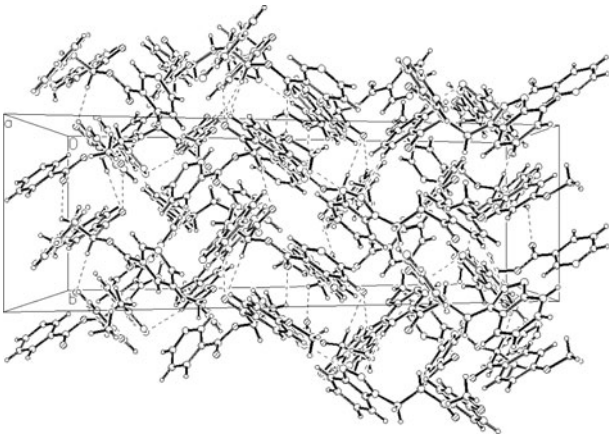
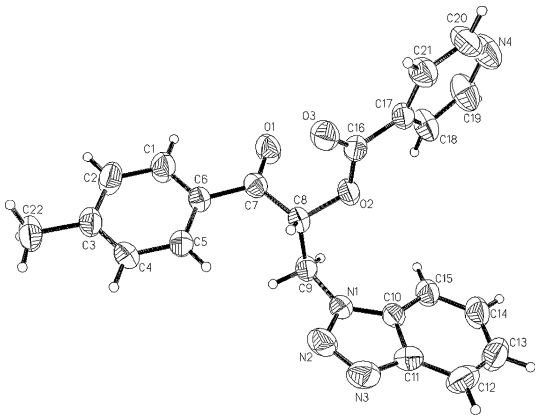


Fig. 2 Packing diagram of **A2** along the *c* axis

Fig. 3 Molecular structure of **B3**



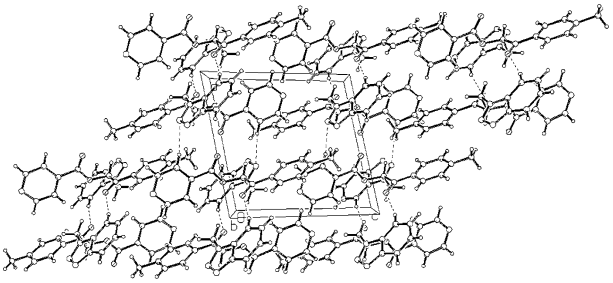
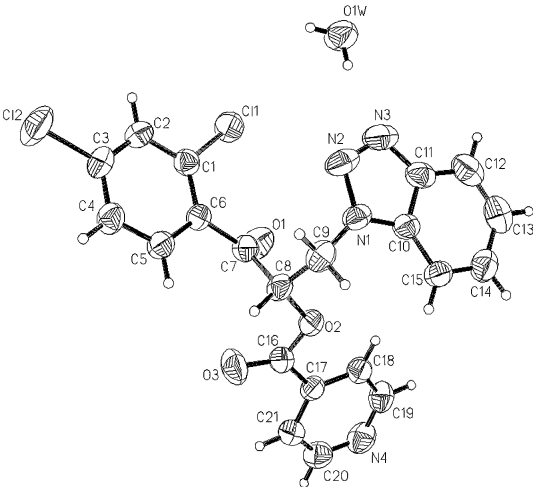


Fig. 4 Packing diagram of **B3** along the *b* axis

with dihedral angles of 0.8(3)°, 1.8(3)°, and 2.1(3)° between the phenyl plane and its fused triazole plane. The C7–C8–C9, C29–C30–C31, and C51–C52–C53 angles are 109.5(1)°, 109.6(1)°, and 111.2(1)°, respectively, determined by the sp^3



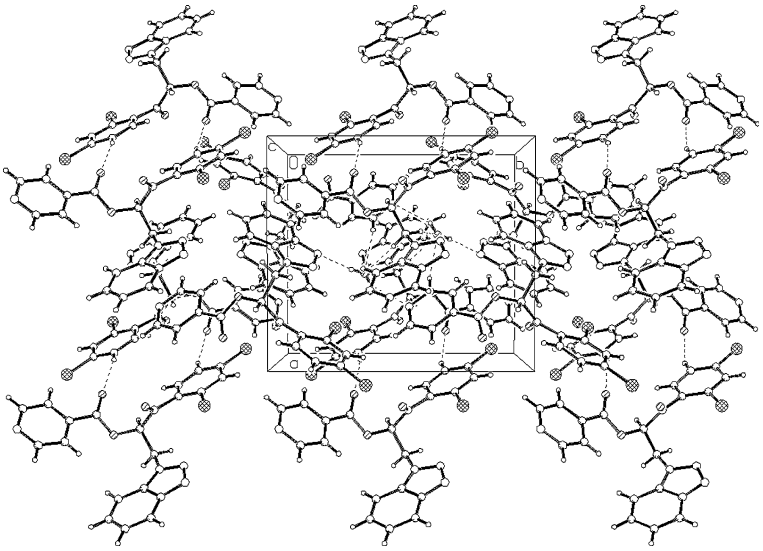


Fig. 5 Molecular structure of **B5**

Fig. 6 Packing diagram of **B5** along the *c* axis

hybridization state of C8, C30, and C52, respectively. In a unit cell, three independent molecules are connected by C–H···O and C–H···N hydrogen bonding interactions.

For **B3**, all bond lengths and angles are within normal ranges. The angle between the C10–C15 phenyl plane and its fused triazole plane is 0.86(1)°, which indicates that the two planes are essentially coplanar, but the benzotriazole ring system makes a dihedral angle of 87.41(1)° and 18.63(1)° with the pyridine ring and the other phenyl plane, respectively, while the dihedral angle between the latter aromatic

rings is 88.84(1)°. As a result, the whole compound is not a planar molecule. In the crystal structure, two molecules are linked by two mutual intermolecular hydrogen bonds, C15–H15A···O1, resulting in a centrosymmetric dimer. Furthermore, the dimers are linked by another intermolecular hydrogen bond, C21–H21A···N2, leading to the three-dimensional network structure.

In the structure of **B5**, the asymmetric unit consists of a monomeric compound and one water molecule, which are incorporated during recystallization. The bond lengths and angles show normal values, which are comparable to those in **A2** and **B3**. Within the asymmetric unit, the water acts as both hydrogen bond donor and hydrogen bond acceptor. In the crystal structure, molecules are linked into dimers by intermolecular hydrogen bonds, C14–H4B···O3. Furthermore, the crystal packing is stabilized by π–π stacking interactions with a distance of Cg1···Cg2 (1–x, $-1/2 + y$, 1/2–z) of 3.671 Å, where Cg1 and Cg2 denote the centroids of rings N1–N3/C10/C11 and N4/C17–C21, respectively.

Biological activities

The biological activities of compounds **A1**–**A5** and **B1** were tested, including their fungicidal inhibiting activities at 50 μg/ml. Experimental results on the fungicidal inhibiting activities of these compounds are reported in Table 2. On the whole, they exhibit less efficiency on tomato early blight, peanut *Cercosporium arachidicola*, and asparagus stem wilt and better efficiency on apple ringspot.

Antitumor biological activities

Antitumor biological activities of compounds were also tested by the method of MTT. Experimental results show that compounds **A3** and **A4** have antitumor activity for hepatoma 7402 cells and breast cancer 4T-1 cells. Results of short-term bioassay of antitumor activity of compounds **A3** and **A4** are presented in Figs. 7 and 8. They show that compound **A3** affects liver cancer 7402 cell viability, and the liver cancer 7402 cell was inhibited. The results also show that the toxicity of compound **A3** has a time-quantity efficiency relationship (Fig. 7).

MTT assay showed that application of **A4** induced dose-dependent cell damage (Fig. 8). While **A4** at a concentration of 0.2 mM was used to estimate a

Table 2 Fungicidal activities of compounds **A1**, **A3**–**A5**, and **B1**

Compound	Fungicidal activity (c = 0.005% inhibition)			
	Tomato early blight	Peanut *Cercosporium arachidicola*	Apple ringspot	Stem wilt of asparagus
A1	8.3	15.0	56.8	14.3
A3	10.0	0	24.4	21.4
A4	15.0	18.8	19.5	28.6
A5	35.0	31.3	31.7	21.4
B1	7.4	13.0	36.7	0

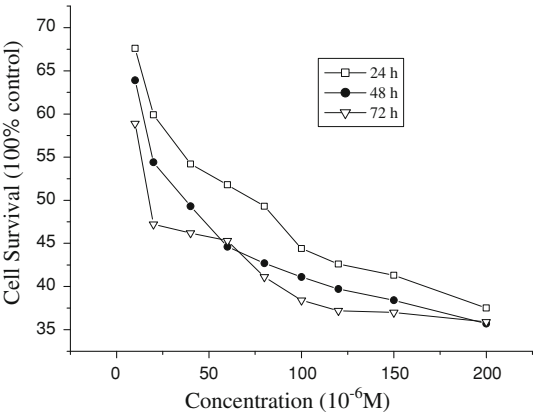


Fig. 7 Effects of different concentrations of compound **A3** on survival rates of hepatoma 7402 cells

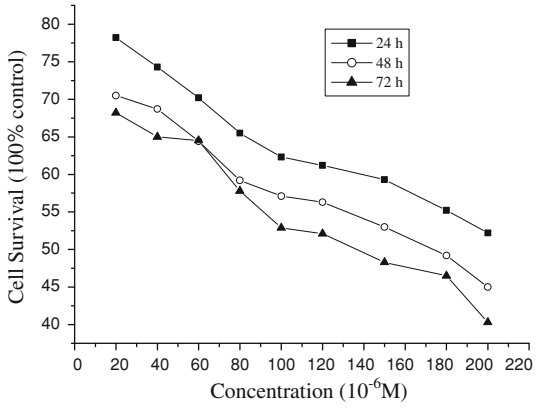


Fig. 8 Effects of different concentrations of compound **A4** on survival rates of breast cancer 4T-1 cells

time-dependent viability loss, cell viability after incubation with A4 at concentrations of 0.2 mM for 24, 48, and 72 h of treatment was 52.2, 45.0, and 40.3% of the control value, respectively. Results of the MTT assay showed that **A4** treatment significantly reduced the viability of the breast cancer cell 4T-1, and the toxicity of compound **A4** has a time-quantity efficiency relationship (Fig. 8).

Studies on the mechanism of antitumor activity are ongoing.

Acknowledgments This work was supported by the Natural Science Foundation of Shandong Province (Grants Y2007B50 and Y2006B10), Outstanding Adult-Young Scientific Research Encouraging Foundation of Shandong Province (2008BS09017), and Qingdao University of Science and Technology Dr. Seed funding.

References

Abonia R, Insuastya B, Quirogaa J, Noguerasb M, Meierc H (2004) Use of β-(N,N-dialkylamino) propiophenones in the synthesis of nitrogenated heterocyclic compounds. Mini Rev Org Chem 1:387–402

Al-Soud YA, Al-Masoudi NA, Ferwanah A (2003) Synthesis and properties of new substituted 1,2,4-triazoles: potential antitumor agents. Bioorg Med Chem 11(8):1701–1708

Antonio C, Michele P, Gianpiero B, Bernardetta B, Marta M, Roberta L (2004) Synthesis and antiproliferative activity of 3-aryl-2-[1*H*(2*H*)-benzotriazol-1(2)-yl]acrylonitriles variously substituted: Part 4. Il Farmaco 59:637–644

Biagi G, Giorgi I, Livi O, Scartoni V, Barili PL, Calderone V (2001) New 5-substituted-1-(2-hydroxybenzoyl)-benzotriazoles, potassium channel activators IV. Il Farmaco 56:827–834

Cui YM, Nan FJ (2006) Biological electron emission principle of lead compounds in drug optimization. Chin Bull Life Sci 18:161

Emami S, Falahati M, Banifatemi A, Shafiee A (2004) Stereoselective synthesis and antifungal activity of (*Z*)-*trans*-3-azolyl-2-methylchromanone oxime ethers. Bioorg Med Chem 12:5881–5889

Fang L-Y, Zhang G-S, Li C-L, Zheng X-C, Zhu L-Z, Xiao J-J, Szakacs G, Nadas J, Chan K-K, Wang P-G, Sun D-X (2006) Discovery of a daunorubicin analogue that exhibits potent antitumor activity and overcomes P-gp-mediated drug resistance. J Med Chem 49:932–941

Hansen MB, Nielsen SE, Berg K (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods 119:203–210

Kasiotis KM, Haroutounian SA (2006) 2-Pyridin-2-yl-1*H*-indole derivatives: sSynthesis, estrogen receptor binding affinity, and photophysical properties. Bioorg Chem 34:1–14

Leonetti F, Favia A, Rao A, Aliano R, Paluszcak A, Hartmann RW, Carotti A (2004) Design, synthesis, and 3D QSAR of novel potent and selective aromatase inhibitors. J Med Chem 47:6792–6803

Lin R, Connolly PJ, Huang SL, Wetter SK, Lu YH, Murray WV, Emanuel SL, Gruninger RH, Fuentes-Pesquera AR, Rugg CA, Middleton SA, Jolliffe LK (2005) 1-Acyl-1H-[1,2,4]triazole-3,5-diamine analogues as novel and potent anticancer cyclin-dependent kinase inhibitors: synthesis and evaluation of biological activities. J Med Chem 48:4208–4211

McClure KF, Abramov YA, Laird ER, Barberia JT, Cai W, Carty TJ, Cortina SR, Danley DE, Dipesa AJ, Donahue KM, Dombroski MA, Elliott NC, Gabel CA, Han S, Hynes TR, LeMotte PK, Mansour MN, Marr ES, Letavic MA, Pandit J, Ripin DB, Sweeney FJ, Tan D, Tao Y (2005) Theoretical and experimental design of a typical kinase inhibitor: application to p38 MAP kinase. J Med Chem 48:5728–5737

Nardelli NJ (1995) *PARST*95—an update to *PARST*: a system of Fortran routines for calculating molecular structure parameters from the results of crystal structure analyses. Appl Crystallogr 28:659

Pagliai F, Pirali T, Grosso ED, Brisco RD, Tron GC, Sorba G, Genazzani AA (2006) Rapid synthesis of triazole-modified resveratrol analogues via click chemistry. J Med Chem 49:467–470

Saberi MR, Vinh TK, Yee SW, Griffiths BJN, Evans PJ, Simons C (2006) Potent CYP19 (aromatase) 1-[(benzofuran-2-yl)(phenylmethyl)pyridine, -imidazole, and -triazole inhibitors: synthesis and biological evaluation. J Med Chem 49:1016–1022

Saüczewski F, Reszka P, Gdaniec M, Grünert R, Bednarski PJ (2004) Synthesis, X-ray crystal structures, stabilities, and in vitro cytotoxic activities of new heteroarylacrylonitriles. J Med Chem 47:3438–3439

Scapin G, Patel SB, Becker JW, Wang Q, Desponts C, Waddleton D, Skorey K, Cromlish W, Bayly C, Therien M, Gauthier JY, Li CS, Lau CK, Ramachandran C, Kennedy BP, Asante-Appiah E (2003) The structural basis for the selectivity of benzotriazole inhibitors of PTP1B. Biochemistry 42:11451–11459

Semple G, Skinner PJ, Cherrier MC, Webb PJ, Sage CR, Tamura SY, Chen R, Richman JG, Connolly DT (2006) 1-Alkyl-benzotriazole-5-carboxylic acids are highly selective agonists of the human orphan G-protein-coupled receptor GPR109b. J Med Chem 49:1227–1230

Sheldrick GM (1996) SADABS program for empirical absorption correction of area detector data. University of Göttingen, Göttingen

Sheldrick GM (1997) SHELXTL V51 software reference manual. Bruker AXS, Madison, WI

Siemens (1996) SMART and SAINT area detector control and integration software. Siemens Analytical X-Ray Systems, Madison, WI

Spek AL (1990) PLATON, an integrated tool for the analysis of the results of a single crystal structure determination. Acta Crystallogr A 46:C34

Touami SM, Poon CC, Wender PA (1997) Sequence specific DNA cleavage by conjugates of benzotriazoles and minor groove binders. J Am Chem Soc 119:7611–7612

Wan J, Li CL, Li XM, Zhang SS (2006) Synthesis, structure and biological activities of 2-(1H–1,2,3-benzotriazol-1-yl)-1-(4-methylphenyl)-2-(1H–1, 2, 4-triazol-1-yl)-1-ethanone. Chem Res Chin Univ 22:465–467

Wang YQ, Li LF, Tian ZM, Jiang W, Larrick JW (2006) Synthesis and antitumor activity of CBI-bearing ester and carbamate prodrugs of CC-1065 analogue. Bioorg Med Chem 14:7854–7861

Wilson AJ (1992) International tables for X-ray crystallography. Vol C. Kluwer Academic, Dordrecht, Netherlands

Xu LZ, Jian FF, Gao HR, Chong CY (2003) Synthesis, structure and biological activities of S-[α-(4-methoxyphenylcarbonyl)-2-(1,2,4-triazole-1-yl)] ethyl-N,N-dimethyldithiocarbamate. Chin J Chem 21:1615–1617

Zhang SS, Wan J, Li CL, Li M, Qu B (2007a) Syntheses and biological activities of novel triazole compounds. J Heterocycl Chem 44:75–78

Zhang SS, Wan J, Li XM, Li CL, Xu LZ (2007b) Syntheses, structure and biological activities of 2-(1H-benzo[1,2,3]triazole-1–y1)-1-(3-methyl-4-bromobipheny1)-2-(1H–1,2,4-triazole-1–y1)ethanon. Chem Res Chin Univ 23:120–122