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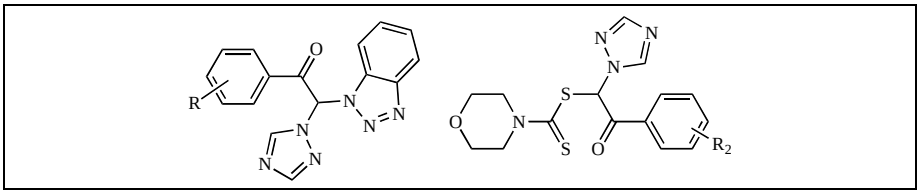

Fifteen novel compounds bearing the triazole moiety were synthesized and structurally characterized. The title compounds showed some antimycotic and plant growth regulating activities, especially in the case of **4d** and **4c**. The latter compound affected also the liver cancer 7402 cell viability.

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INTRODUCTION

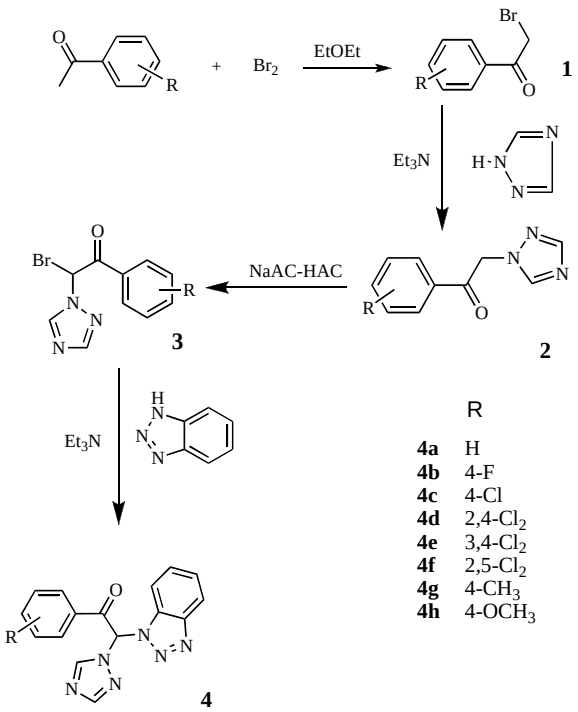
1,2,4-Triazole derivatives represent an interesting class of heterocycles [1] and have become one of the most rapidly expanding groups of antifungal compounds with the advantage of toxicity, high oral bioavailability and broad-spectrum activity against most yeasts and filamentous fungi [2-6]. The triazole fungicides are agrochemicals used world wide in the agricultural industry due to their wide spectrum of action [7]. Some of the new triazole antifungals with higher activity and lower toxicity can inhibit systemic fungal infections in patients suffering with tumors or immunodeficiency [8]. Some triazoles are also efficient stimulators of heart function [9-11], inhibitors of histidine biosynthesis [12], and fungicides of the second generation [13,14].

At present, the studies on triazole derivates are mainly concentrated on compounds with triazole as the only active group [15]. Reports of triazole compounds that contain both triazole group and other active group in a single molecule has rarely been found. A variety of benzotriazoles exhibit growth inhibiting activities against some microorganisms and other derivatives endowed with anti-inflammatory properties [16]. Morpholine derivatives, as an important type of fungicides, have attracted much interest because of their inward absorbent and broad-spectrum activities, and dialkyl-substituted dithiocarbamate salts have shown interesting biological effects as broad-range fungicides, too [17]. Taking advantage of the concept of bioisosterism, fifteen novel compounds containing benzotrizole and morpholine-carbodithioate were synthesized in order to search for new triazole compounds with higher bioactivity. In this paper, we reported the synthesis, structure characterization and biological activities of fifteen novel triazole compounds.

RESULTS AND DISCUSSION

Triazolylethanones and bromine substituted triazolylethanones were prepared according to the reported methods [16,17]. Triazole derivatives **4** and **8** were obtained in good yields by reacting in the presence of triethylamine at r.t. intermediates **3** and **7** with triazole or morpholine carbodithioate, respectively, as reported in the Schemes 1 and 2.

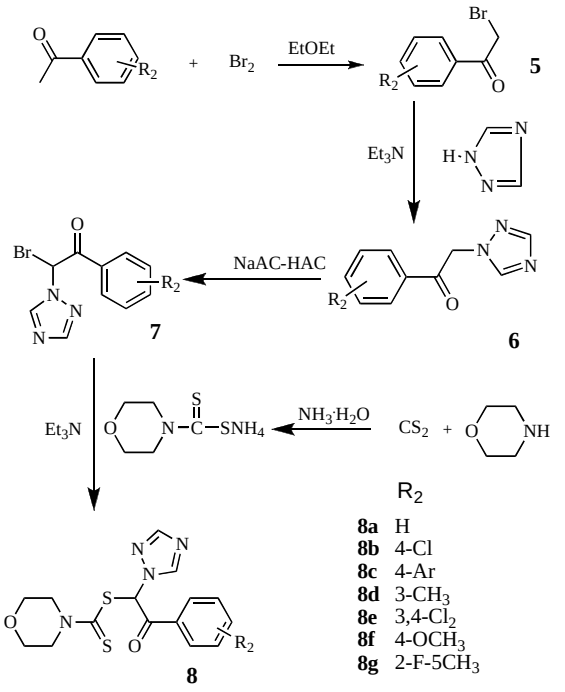
Scheme 1. Synthesis of **4**.



In ice-water bath, to a solution of intermediate **3** (0.02 mol) in chloroform (20 mL) was added benzotriazole (2.4

g, 0.02 mol) and triethylamine (2.8 mL, 0.02 mol) which was used as the binding acid reagent in acetone (10 mL) under stirring, and the mixture was stirred at room temperature for about 2 h. The solution was filtered, concentrated and purified by flash column chromatography (silica gel, using petroleum ether and ethyl acetate, 3/1~5 (v/v) as eluent) to afford the compounds **4a**-**4h**.

Scheme 2. Synthesis of **8**.



To a solution of morpholine carbodithioate (3.6 g, 0.02 mol) in acetone (10 mL) was added dropwise **7** (0.02 mol) in chloroform (20 mL), and the mixture was stirred at room temperature for about 3 h. The solution was filtered, concentrated and purified by flash column chromatography (silica gel, using petroleum and ethyl acetate, 3:1~3:5(v/v) as eluent) to afford compounds **8a**-**8g**.

Fungicidal and Plant Growth Regulating Activities. The fungicidal activities at 50 mg/mL (*N*,*N*-dimethyl formamide and deionized water, 1:1 v/v) and plant growth regulating activities of compounds **4a**-**4g**, **8b**, **8c**, **8e** and **8g** have been tested, and fungicidal-inhibiting activities at 50 mg/mL and plant growth regulating activities at 10 mg/mL. The experimental results with inhibiting fungicidal and plant growth regulating activities of these compounds are shown in Table 1.

On the whole, they exhibit better efficiency on apple ringspot and rooting of cucumber cotyledon. As far as substitution is concerned, morpholine-carbodithioate substitution derivatives have better fungus-inhibiting activities on apple ringspot and tomato early blight, but lower plant growth regulating activities on rooting of cucumber cotyledon than benzotriazole substitution derivatives.

Of all the fifteen compounds, compound **4d** (2-benzotriazol-1-yl-1-(2,4-dichlorophenyl)-2-[1,2,4]triazole-1-yl-ethanone) has the highest plant growth regulating activities on rooting of cucumber cotyledon, its regulating rate reaching 255.0% at 10 mg/mL. And **4c** (2-benzotriazol-1-yl-1-(4-chloro-phenyl)-2-[1,2,4]triazole-1-yl-ethanone) also has higher plant growth regulating activities on rooting of cucumber cotyledon, and its regulating rate reaches 155.0%.

Short-Term Bioassay of Antitumor Activity. Cell viability was measured by the MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) method [18]. Cells in DMEM were seeded at a density of 9×103 cells per

Table 1
The Biological Activities of the Compounds

Comp.	Fungicidal activities (c=0.005%, inhibition)					Plant growth regulating activities(c=0.001%)
	Wheat gibberellin	Tomato early blight	Peanut cercosporiumar achidicola	Apple ringspot	Stem wilt of asparagus	Rooting of cumcumber cotyledon
4a	38.5	41.3	31.4	50.6	19.2	125.0
4b	40.4	36.9	34.3	37.7	30.8	100.0
4c	30.8	19.6	14.3	44.2	19.2	155.0
4d	30.8	34.8	28.6	35.1	30.8	255.0
4e	42.3	41.3	37.1	49.4	42.3	85.0
4f	30.8	32.6	0	54.5	11.5	100.0
4g	42.3	36.9	28.6	44.2	19.2	70.0
8b	57.7	58.7	54.3	59.7	46.2	35.0
8c	42.3	45.7	22.9	44.2	11.5	120.0
8e	42.3	41.3	37.1	49.4	42.3	35.0
8g	42.3	56.5	14.3	48.1	30.8	15.0

well in 96-well plates for 24 h. After exposure to different concentrations of compound **4c** 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 and then MTT was removed, 100 μL of a lysing buffer (10% w/v of sodium dodecyl sulfate (SDS) dissolved in a solution of 50% each of *N*,*N*-dimethyl formamide and deionized water, pH 4.7) were 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 optical density (OD).

Table 2

The Short-term Bioassay of Cytotoxicity of Compound **4c**

Compound	concentration	24 hour			48 hour		
	mmol/L	OD_{570}	GI/%	GT/%	OD_{570}	GI/%	GT/%
comparision	—	1.03±0.06	100	0	1.14±0.15	100	0
5-fluorouracil	0.100	0.76±0.14	73.8	26.2	0.75±0.05	66.1	33.8
4c	0.295	0.99±0.10	96.1	3.8	0.84±0.05	73.6	26.3
	1.480	0.98±0.09	95.1	4.8	0.83±0.06	72.8	28.1
	2.950	0.95±0.11	92.2	7.7	0.81±0.03	70.1	28.9
	14.80	0.90±0.15	87.3	12.6	0.80±0.08	70.2	29.8
	29.50	0.83±0.13	80.5	19.4	0.77±0.05	67.5	32.5

Inhibitor rate (GT) = (the value of negative comparision OD–the value of druggery OD) /the value of negative comparision OD; growth index (GI) = the value of druggery OD/the value of negative comparision OD

The antitumor biological activities of compound **4d**, **4c** and **4h** have also been tested by the method of MTT. The experimental results show that the compound **4c** has the antitumor activity, while compound **4d** and **4h** have lower inhibitor rate. The results of short-term bioassay of antitumor activity of compound **4c** are presented in Table 2.

The result shows that compound **4c** affects liver cancer 7402 cell viability, and the liver cancer 7402 cell were inhibitor. The result also shows that the toxicity of compound **4c** has the time quantity effective relationship.

Synthesis of the triazole derivatives containing benzotriazole or morpholine-carbodithioate was obtained. Of particular significance for biological activities were the higher plant growth regulating activities of triazole derivatives containing benzotriazole compounds, especially for **4d** (2-benzotriazol-1-yl-1-(2,4-dichloro-phenyl)-2-[1,2,4]triazole-1-yl-ethanone) and **4c** (2-benzo-triazol-1-yl-1-(4-chloro-phenyl)-2-[1,2,4]triazole-1-yl-ethanone). They can be utilized as a plant growth regulation in agriculture. Compound **4c** could act as tumor cell inhibitors, while studies on the mechanism of antitumor activity are ongoing.

EXPERIMENTAL

Genernal Methods. All the chemicals were obtained from commercial sources and used directly without further purification. Triazolone and bromine substituted triazolone were prepared according to the reported methods [19]. 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 spectra were measured with a JNM-ECP600 nuclear magnetic resonance spectrometer ($CDCl_3$ as solvent, TMS as internal standard). The melting points were determined on Yanaco MP-500 melting point apparatus.

2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-phenyl-2-(1*H*-1,2,4-triazole-1-yl)ethanone (4a). Yield 45.5%, mp 82.9-84.9 °C, *Anal.* $C_{16}H_{12}N_6O$, Found: C 63.61, H 4.05, N 27.62, M^+ 304. calcd: C 63.15, H 3.97, N 27.62 M^+ 304). ν_{max}/cm^{-1} (KBr) 1714 (s, C=O), 1509 (s, C=N), δ_H ($CDCl_3$) 7.48-8.01 (9H, Ar-H), 7.48 (1H, C-H), 8.91 (1H, Tr-H), 9.37 (1H, Tr-H).

2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-(4-fluorophenyl)-2-(1*H*-1,2,4-triazole-1-yl)ethanone (4b). Yield 42.0%, mp 140.2-142.9 °C, *Anal.* $C_{16}H_{11}FN_6O$, Found: C 59.73, H 3.65, N 26.55. calcd: C 59.63, H 3.44, N 26.08. ν_{max}/cm^{-1} (KBr) 1711 (s, C=O), 1508 (s, C=N), δ_H ($CDCl_3$) 7.11-8.11 (8H, Ar-H), 7.75 (1H, C-H), 8.56 (1H, Tr-H), 8.63 (1H, Tr-H).

2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(4-chlorophenyl)-2-(1*H*-1,2,4-triazole-1-yl)ethanone (4c). Yield 44.3%, mp 166.9-167.3 °C, *Anal.* $C_{16}H_{11}ClN_6O$, Found: C 57.02, H 3.62, N 24.55. calcd: C 56.72, H 3.27, N 24.79. ν_{max}/cm^{-1} (KBr) 1711 (s, C=O), 1506 (s, C=N), δ_H ($CDCl_3$) 7.40-8.11 (8H, Ar-H), 7.75 (1H, C-H), 8.55 (1H, Tr-H), 8.60 (1H, Tr-H).

2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazole-1-yl)ethanone (4d). Yield 39.5%, mp 180.1-181.8 °C, *Anal.* $C_{16}H_{10}Cl_2N_6O$, Found: C 51.80, H 2.80, N 23.01. calcd: C 51.49, H 2.70, N 22.52. ν_{max}/cm^{-1} (KBr) 1725 (s, C=O), 1505 (s, C=N), δ_H ($CDCl_3$) 7.34-8.08 (7H, Ar-H), 7.34 (1H, C-H), 8.49 (1H, Tr-H), 8.79 (1H, Tr-H).

2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-(3,4-dichlorophenyl)-2-(1*H*-1,2,4-triazole-1-yl) ethanone (4e). Yield 40.2%, mp 188.6-190.9 °C, *Anal.* $C_{16}H_{10}Cl_2N_6O$, Found: C 51.73, H 2.93, N 22.83. calcd: C 51.49, H 2.70, N 22.52. ν_{max}/cm^{-1} (KBr) 1708 (s, C=O), 1505 (s, C=N), δ_H ($CDCl_3$) 7.48-8.10 (7H, Ar-H), 7.48 (1H, C-H), 8.55 (1H, Tr-H), 8.59 (1H, Tr-H).

2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-(2,5-dichlorophenyl)-2-(1*H*-1,2,4-triazole-1-yl) ethanone (4f). Yield 41.4%, mp 206.5-207.2 °C, *Anal.* $C_{16}H_{10}Cl_2N_6O$, Found: C 51.80, H 2.93, N 23.00. calcd: C 51.49, H 2.70, N 22.52. ν_{max}/cm^{-1} (KBr) 1737 (s, C=O), 1507 (s, C=N), δ_H ($CDCl_3$) 7.47-8.09 (7H, Ar-H), 7.56 (1H, C-H), 8.97 (1H, Tr-H), 9.32 (1H, Tr-H).

2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-*p*-tolyl-2-(1*H*-1,2,4-triazole-1-yl)ethanone (4g). Yield 44.3%, mp 134.0-136.0 °C, *Anal.* $C_{17}H_{14}N_6O$, Found: C 64.59, H 4.65, N 26.62, M^+ 318. calcd: C 64.14, H 4.43, N 26.40 M^+ 318. ν_{max}/cm^{-1} (KBr) 1711 (s, C=O), 1508 (s, C=N), δ_H ($CDCl_3$) 2.33 (3H, CH_3-H), 7.30-8.01 (8H, Ar-H), 7.28 (1H, C-H), 8.90 (1H, Tr-H), 9.33 (1H, Tr-H).

2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-(4-methoxyphenyl)-2-(1*H*-1,2,4-triazole-1-yl) ethanone (4h). Yield 47.3%, mp 167.0-169.0 °C, *Anal.* $C_{17}H_{14}N_6O_2$, Found: C 61.61, H 4.25, N 25.60, M^+ 334. calcd: C 61.07, H 4.22, N 25.14, M^+ 334. ν_{max}/cm^{-1} (KBr) 1696 (s, C=O), 1509 (s, C=N), δ_H ($CDCl_3$) 5.77 (3H, OCH_3-H), 7.37-7.94 (8H, Ar-H), 7.28 (1H, C-H), 7.95 (1H, Tr-H), 8.02 (1H, Tr-H).

2-Oxo-2-phenyl-1-(1*H*-1,2,4-triazol-1-yl)ethylmorpholine-4-carbodithioate (8a). Yield 52.3%, mp 120.4-122.2, *Anal.* $C_{15}H_{16}N_4O_2S_2$, Found: C 51.92, H 4.75, N 15.96, S 18.52, M^+ 348. calcd: C 51.70, H 4.63, N 16.08, S 18.40, M^+ 348. ν_{max}/cm^{-1} (KBr) 1690 (s, C=O), 1500 (s, C=N), 1235 (m, C=S), 652 (m, C–S).

2-(4-Chlorophenyl)-2-oxo-1-(1*H*-1,2,4-triazol-1-yl)ethyl morpholine-4-carbodithioate (8b). Yield 53.4%, mp 152.2-152.6 °C, *Anal.* $C_{15}H_{15}ClN_4O_2S_2$, Found: C 47.35, H 4.05, N 14.38, S 16.90, M^+ 382. calcd C 47.05, H 3.95, N 14.63, S 16.75, M^+ 382. ν_{max}/cm^{-1} (KBr) 1692 (s, C=O), 1500 (s, C=N), 1236 (m, C=S), 640 (m, C–S), δ_H ($CDCl_3$) 3.73-4.38 (8H, Maph-H), 7.48-7.99 (4H, Ar-H), 7.48 (1H, C-H), 8.54 (1H, Tr-H), 8.66 (1H, Tr-H).

Morpholine-4-carbodithioic acid 2-biphenyl-4-yl-2-oxo-1-[1,2,4]triazol-1-yl-ethyl ester (8c). Yield 45.0%, mp 160.0-161.2 °C, *Anal.* $C_{21}H_{20}N_4O_2S_2$, Found: C 59.50, H 4.75, N 13.15, S 15.32, M^+ 424. calcd: C 59.41, H 4.75, N 13.20, S 15.11, M^+ 424. ν_{max}/cm^{-1} (KBr) 1711 (s, C=O), 1500 (s, C=N), 1269 (m, C=S), 659 (m, C–S), δ_H ($CDCl_3$) 3.73-4.32 (8H, Maph-H), 7.52-8.14 (9H, Ar-H), 7.45 (1H, C-H), 8.71 (1H, Tr-H), 8.80 (1H, Tr-H).

2-Oxo-2-*m*-tolyl-1-(1*H*-1,2,4-triazol-1-yl)ethylmorpholine-4-carbodithioate (8d). Yield 39.5%, mp 128.8-129.3 °C, *Anal.* $C_{16}H_{18}N_4O_2S_2$, Found: C 53.92, H 4.95, N 15.26, S 17.38, M^+ 362. calcd: C 53.02, H 5.01, N 15.46, S 17.69, M^+ 362. ν_{max}/cm^{-1} (KBr) 1692 (s, C=O), 1499 (s, C=N), 1237 (m, C=S), 664 (m, C–S), δ_H ($CDCl_3$) 2.39 (3H, CH_3-H), 3.77-4.31 (8H, Maph-H), 7.51-7.93 (4H, Ar-H), 7.45 (1H, C-H), 8.65 (1H, Tr-H), 8.77 (1H, Tr-H).

2-(3,4-Dichlorophenyl)-2-oxo-1-(1*H*-1,2,4-triazol-1-yl)ethyl morpholine-4-carbodithioate (8e). Yield 48.6%, mp 142.6-143.1 °C, *Anal.* $C_{15}H_{14}Cl_2N_4O_2S_2$, Found: C 43.38, H 3.45, N 13.26, S 15.54. calcd: C 43.17, H 3.38, N 13.42, S 15.37. ν_{max}/cm^{-1} (KBr) 1694 (s, C=O), 1501 (s, C=N), 1233(m, C=S), 675 (m, C–S), δ_H ($CDCl_3$) 3.74-4.38 (8H, Maph-H), 7.86-8.14 (3H, Ar-H), 7.58 (1H, C-H), 8.52 (1H, Tr-H), 8.65 (1H, Tr-H).

2-(4-Methoxyphenyl)-2-oxo-1-(1*H*-1,2,4-triazol-1-yl)ethyl morpholine-4-carbodithioate (8f). Yield 45.4%, Anal. $C_{16}H_{18}N_4O_3S_2$, Found: C 51.02, H 4.75, N 14.67, S 17.13, M^+ 378. calcd: C 50.78, H 4.79, N 14.80, S 16.94, M^+ 378. ν_{max}/cm^{-1} (KBr) 1692 (s, C=O), 1500 (s, C=N), 1234 (m, C=S), 631 (m, C–S).

2-(2-Fluoro-5-methylphenyl)-2-oxo-1-(1*H*-1,2,4-triazol-1-yl)ethyl morpholine-4-carbodithioate (8g). Yield 47.3%, mp 171.3-171.8 °C, *Anal.* $C_{16}H_{17}FN_4O_2S_2$, Found: C 50.61, H 4.62, N 14.69, S 16.90. calcd: C 50.51, H 4.50, N 14.73, S 16.86. ν_{max}/cm^{-1} (KBr) 1689 (s, C=O), 1496 (s, C=N), 1210(m, C=S), 631 (m, C–S), δ_H ($CDCl_3$) 2.34 (3H, CH_3-H), 3.79-4.32 (8H, Maph-H), 7.27-7.91 (3H, Ar-H), 6.98 (1H, C-H), 8.56 (1H, Tr-H), 8.62 (1H, Tr-H).

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