Synthesis, Crystal Structure and Antifungal Activity of 1-(3,3 -Dimethyl-2-oxobutyl)-N-phenyl-1*H*-1,2,4-triazole-3-carboxamide

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Summary: Fungicides play a vital role in protecting crops from fungal damage.However, fungicide resistance is one of the most important issues in modern agriculture. Hence, it is very necessary to develop new fungicides constinuously.Triazole compounds have received considerable interest in agricultural chemistry due to a novel action mode, extremely high activity against phytopathogenic fungi, low acute toxicity to mammals, and environmentally benign characteristics. The title compound 1-(3,3-dimethyl -2-oxobutyl)-N-phenyl-1*H*-1,2,4-triazole-3-carboxamide 5, synthesized using methyl 1*H*-1,2,4 -triazole-3-carboxylate 1 as the start material, was successfully obtained via multiple synthesis route and finally characterized by ^{1}H NMR,^{13}C NMR, HRMS and single-crystal X-ray diffraction. Compound 5 ($C_{15}H_{18}N_4O_2$, *Mr* = 287.1500) belongs to the orthorhombic system, space group *P*n2$_1$a, with *a* = 14.49451(11)Å, *b* = 20.34686(18)Å, *c* = 10.17021(9)Å, *V* =2999.38(4) Å^3, *Z* = 8, *Dc*= 1.268 g/cm^3, *T* = 293.55(14) K, μ (Cu$K\alpha$) = 0.710 mm^{-1}, F (000) =1216.0, the final *R*=0.0448, and *wR* = 0.1260 with $I>=2\sigma(I)$. Furthermore, the results from biological assays indicated that the title compound exihibited a similar antifungal activity（EC_{50} =8.98 mg. L^{-1}）compared to triadimefon (EC_{50}=5.6 mg.L^{-1})against *G. cingulatal*. And had different degrees of weak activity against other phytopathogenic fungi, including *A. solani*, *S. sclerotiorum*, *G. saubinetii* and *T. cucumeris*. Potentially, the result lay the foundation for the development of novel fungicides.

Keywords: Synthesis; Crystal structure; Triazole; Amide; Antifungal activity

Introduction

Fungicides, a major part of crop protection products, widely applied to major crops such as rice, corn and wheat, are playing an essential role by increasing both crop quality and yield [1]. Triazole fungicides belong to the sterol demethylation inhibitor (DMI) group, which is characterized by the inhibition of ergosterol biosynthesis, an important component of the fungal cell membrane [2], have been extensively and widely used in wheat, rice and corn fields for managing fungal diseases, including leaf rust, powdery mildew, leaf spots gibberellic diseases and sheath blight [3-4]. However, in recent years the developments of resistance to currently available triazole-antifungicide have been reported around the world [5-7]. Fungal diseases management is still a challenge for researchers, and continuous innovation is essential to maintain the effectiveness of pathogen management.

1,2,4-triazole derivatives are often used in the medicinal and agricultural areas. Some of them had been developed as commercial pesticides, such as Diniconazole [8], Triadimefon [9], Triadimenol [10], Flusilazole [11], Epoxiconazole [12], Prothioconazole [13] and Tebuconazole [14]. Also, tert-butyl ketone structural element displayed outstanding activity in agrochemical, such as fungicidal activity [15-16], phytohormone activity [17-18]. Meanwhile, Amide fungicides are widely used for the highly effective fungicidal activity, and performed outstanding effects on downy mildew on soybean, cucumber, millet, potato and eggplant caused by Phytophthora [19]. Unfortunately, after using for a period of time, the fungicides will be gradually out of severce [20-22]. Therefore, it is urgent to develop some innovative compounds displaying excellent fungicidal activity.

In view of these facts mentioned above, the title compounds were designed by introducing tert-butyl ketone and amide pharmacophore into 1,2,4-triazole scaffold. A new 1,2,4-triazole derivative was synthesized and characterized by ^{1} H NMR, ^{13} C NMR and HRMS. The single-crystal structure of the title compound was determined by X-ray diffraction. The antifungal activity of the title compound was investigated.

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EXPERIMENTAL

General Techniques

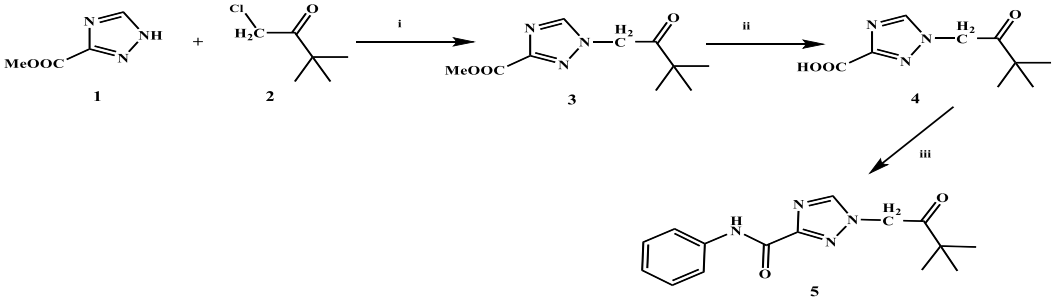
1*H*-1,2,4-triazole-3-carboxylate,1-chloro-3,3-dimethyl-butan-2-one, aniline and 2-(7- Azaben zotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate(HATU), Potassium carbonate (K_2CO_3) and polyethylene glycol-600 (PEG-600) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. All reactions were monitored by TLC with GF254 silica gel plates. Flash column chromatography was carried out using 200-300 mesh silica gel by using certain ratio of eluent with petroleum ether/ethyl acetate/methanol. Single crystal of compound **5** was cultured in a system of dichloromethane and petroleum ether. The melting point was measured on a Hanon MP100 automatic melting point apparatus (Jinan Hanon Instruments Co., Ltd., Jinan, Shandong, China), using an open capillary tube. The NMR spectra was recorded at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) on a Bruker AV-400 spectrometer with TMS as an internal standard. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. High-resolution mass spectral analysis was carried out on an FTICR-MS Varian 7.0 T FTICR-MS instrument (Varian IonSpec, Lake Forest, CA, USA). Single-crystal X-ray structure was measured on a Bruker SMART APEX II X-ray single crystal diffractometer (Bruker AXS, Karlsruhe, BW, Germany).

Synthetic Precedure

The synthetic route of Compound **5** is outlined in Scheme 1. The intermediates **3** and **4** were synthesized according to the reference [23]. A mixture of 1-(3,3-dimethyl-2-oxobutyl)-1H-1,2,4-triazole-3-carboxylic acid **4** (506.4 mg, 2.4 mmol), triethylamine (600 μL) and HATU(1 eq) in dichloromethane (50 mL) was stirred at r.t for 1 h. Afterwards, aniline (232.8 mg, 2.4 mmol) was added and stirring was continued for 40 min at same temperature. The reaction mixture was washed with water(310 mL) and saturated salt water (30 mL), dried over anhydrous $MgSO_4$, filtered through a Celite pad (i.d. = 8 cm; height = 1.5 cm) and concentrated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether / ethyl acetate/ methanol 3:2:0.5) to provide compound **5** (420 mg, 61%) as white powder, m.p. 152-154°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.22 (s, 1H), 7.71 (d, J = 7.7Hz, 2H), 7.37 (t, J = 7.9 Hz, 2H), 7.16 (d, J = 7.4 Hz, 1H), 5.32 (s, 2H), 1.27 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ205.76, 156.89, 156.57, 145.63, 137.33, 129.12, 124.67, 119.90, 54.08, 43.58, 26.14. HR-ESI-MS for (ESI^+) for $C_{15}H_{18}N_4O_2$ (m/z): calcd. 287.1463, found: 287.1500$[M+H]^{+.}$

Structure Determination

Single crystals of Compound **5** were obtained by recrystallization from a system of dichloromethane and petroleum ether at room temperature. The crystal dimensions were 0.20 × 0.05 × 0.05mm ^{3}. The reflection data of Compound **5** was collected by using X-radiation (λ = 1.54184 Å) at 293(14) K via a Bruker SMART APEX II X-ray single crystal diffractometer (Bruker AXS, Karlsruhe, BW, Germany). A total of 30970 reflections were collected, 5813 of which were independent with R_{int} = 0.0239. The structure of Compound **5** was solved via a direct method using SHELXS-97 (University of Gottingen, Gottingen, NI, Germany). The solutions were refined by full-matrix least squares techniques on F^2 by SHELXL-2015 program [24]. The final cycle of refinement gave R = 0.0461 and wR = 0.1282. Selected crystallographic data of the Compound **5** is provided in Table 1.



Scheme-1: Synthesis route of compound **5**. Regent and condition: (i) AcOEt, K_2CO_3-PEG (600), 56 ℃, 78 %; (ii)NaOH (10 %), MeOH: H_2O (V/V) =1:1, 30 min, rt, ≧99%; (iii) Aniline, DMC, HATU, TEA, r.t, 61 %.

Table-1: Selected crystallographic data of Compound **5**.

Parameter	Result	Parameter	Result
CCDC No.	2088260	α/°	90
Empirical formula	$C_{15}H_{18}N_4O_2$	β/°	90
Formula weight	286.33	γ/°	90
Temperature/K	293.55(14)	Volume/Å^3	2999.38(4)
Crystal system	orthorhombic	Z	8
Space group	$Pn2_1a$	ρ_{calc}g/cm^3	1.268
a/Å	14.49451(11)	Reflections collected	30970
b/Å	20.34686(18)	Independent reflections	[R_{int} = 0.0239, R_{sigma} = 0.0148]
c/Å	10.17021(9)	Data/restraints/parameters	5813/1/385
μ/mm^{-1}	0.087	Goodness-of-fit on F^2	1.039
F(000)	1216.0	Final R indexes [I>=2σ (I)]	R_1 = 0.0448, wR_2 = 0.1260
Crystal size/mm^3	0.2× 0.05 × 0.05	Final R indexes [all data]	R_1 = 0.0461, wR_2 = 0.1282
Radiation	CuKα (λ =1.54184)	Largest diff. peak/hole / e Å^{-3}	0.38/-0.19
2θ/°	8.692 to 143.53		
Index ranges	-17≤ h ≤ 12, -25≤ k ≤ 24, -12 ≤ l ≤ 12		

Antifungal Activity

The antifungal activities of Compound **5**, Triadimefon were tested in vitro against *Alternaria solani*, *Scherotinia sclerotiorum*, *Gibberella saubinetii*, *Glonemancingulatal* and *Thanatephorus cucumeris* The five kinds of test fungi were were purchased from National Microbial Resource Center (Beijing, China). After retrieval from the storage tube, the strains were incubated on potato dextrose agar (PDA) at 27 °C for 4 days to get new mycelia for the antifungal tests. The fungicidal activity of the target compound was tested in vitro against the five plant pathogenic fungi using the mycelia growth inhibition method [25]. The tested compound were dissolved in DMSO to prepare a 1000 mg.L^{-1} stock solution before mixing with PDA. The media containing compounds at a concentration of 50mg.L^{-1}were then poured into sterilized Petri dishes for initial screening. After two days at 27°C, the colony diameter of each strain was measured. Percentage inhibition rate was calculated as (1−a/b)×100%, where a represents the colony diameter in the Petridishes with tested compounds and b is the mean colony diameter in control Petri dishes. Each test was repeated three times. The 1000 mg.L^{-1} solution was diluted to 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg.L^{-1} and the above experiments were repeated, the inhibition rates were calculated separately. The EC_{50} values were calculated using SPSS Statistics v 22.0.

Result and Discussion

Description of the Structure

Compound **5** crystallized in the orthorhombic system. The $Pn2_1a$ space group and the molecular structure of Compound **5** are depicted in Figure 1. Selected molecular struc ture parameters (bond lengths and bond angles) for Compound **5** can be found summa rized in Table 1. The packing arrangement is shown in Figure 2.

The crystal data for Compound **5** was deposited at the Cambridge Crystallographic Data Centre (12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) as supplementary publication No. CCDC-2088260. Crystallographic data for this crystal is available free of charge at the following website: http: www.ccdc.cam.ac.uk/data_request/cif or from the Cambridge Crystallographic Data Centre.

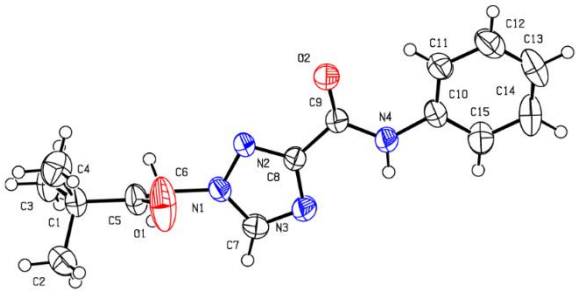


Fig. 1: Crystal structure of Compound **5**.

The bond lengths and bond angles of the aromatic rings (phenyl and triazolyl) in this crystal structure are in accordance with the general normal ranges [26-27]. The phenyl ring and triazolyl ring were both connected by an amide group, and the C(9)=O(2) bond length in this amide group was 1.219(3) Å. Another carbonyl(C(1)=O(5)) bond length was 1.202(4). The both is shorter to the general C=O double-bond length reported in the literature [28-29]. Compared to the normal C–N bond (1.47~1.50 Å), the shorter C(7)-N(1) (1.331(2) Å)、C(7)-N(3) (1.322(4) Å) and C(8)-N(2) (1.320(3) Å) bonds in the triazole ring, C(9)-N(4) (1.351(4) Å) of amide and C(10)-N(4)(1.421(4) Å) may be due to the effect of conjugation. The bond angles of triazole ring (N (1)/N (2)/C (7)/N (3)/C (8)) varied from 102.0(2)˚to 115.3(2)˚ with the average of 108˚, and benzene ring (C (10)/C (11)/C (12)/C (13)/C (14)/C (15) varied from 119.6(3) ˚to 120.2(3)˚with the average of 120

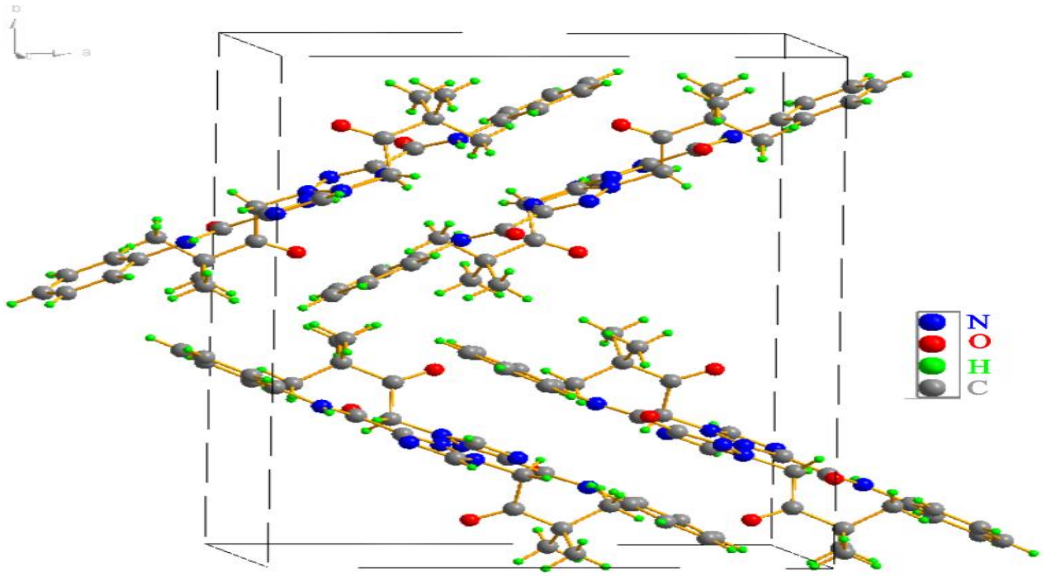


Fig. 2: Packing arrangement of Compound 5 in the unit cell.

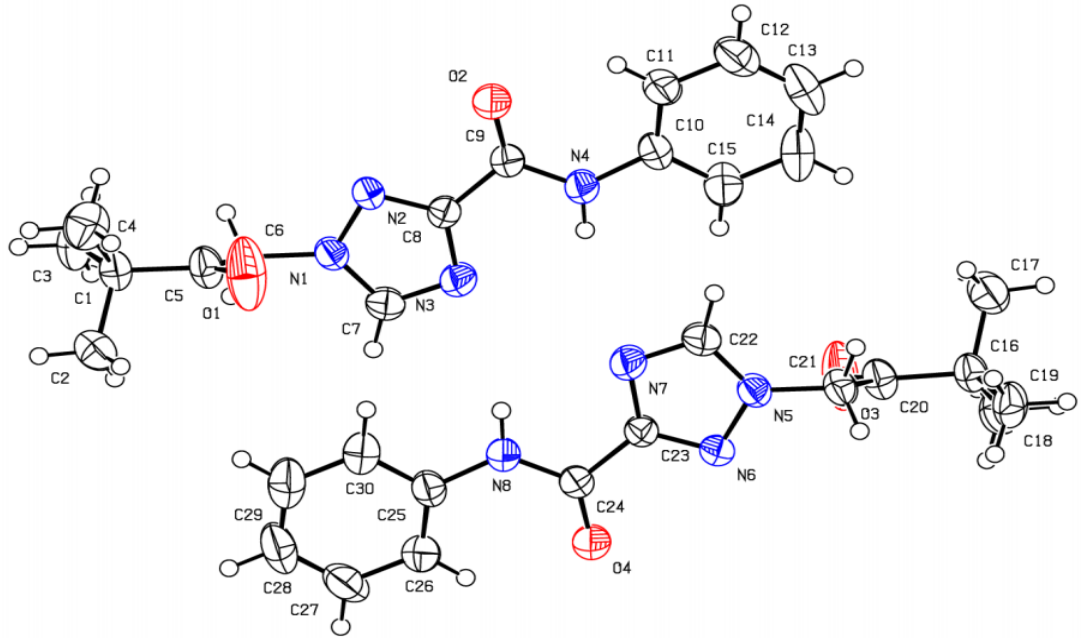


Fig. 3: Crystal unit stacking of compound **5.**

Table-2. EC_{50} values of compound **5**, triadimefon against *Alternaria solani*, *Scherotinia sclerotiorum*, *Gibberella saubinetii*, *Gloneman cingulatal* and *Thanatephorus cucumeris* [1].

	EC_{50} (± SD) $mg.L^{-1}$				
	A. solani	***S. sclerotiorum***	***G. saubinetii***	***G. cingulatal***	***T. cucumeris***
Compound 5	**125.24±1.786**	**78.22±1.334**	**59.98±2.311**	**8.98±0.484**	**66.20±1.681**
Triadimefon	**1.05±0.922**	**8.45±0.264**	**19.35 ±0.459**	**5.6±0.504**	**16.63±0.162**

The crystal packing characteristics of Compound **5** in the unit cell are described in Figure 2. Two adjacent molecules with a head to tail arrangement (Figure 3) are found in the crystal packing. In the intermolecular, it is worth noting that face-to-face π-π stacking pattern of the title compound, which would be proved by the relative positions between triazole ring and phenyl ring of the two molecules: the centroid separation of them is 3.5696 Å, and their angle is 6.9547°. These interactions are estimated to play a role in stabilizing the crystal structure.

Spectroscopic Properties

The structure of compound **5** was confirmed via melting point, ^{1}H NMR, ^{13}C NMR, and HRMS analysis. Signals corresponding to the C-H proton in the triazole ring and N-H proton in the amide group were observed at δ 8.22 and δ 8.96, respetively. The signals corresponding to the protons on the benzene ring were observed at δ 7.16-7.71. The HRMS data of compound **5** was in good agreement with the theoretical data that was calculated on the basis of the molecular formula.

Evalution of Antifungal Activity

The *in vivo* fungicidal activities of the compound 5 against *Alternaria solani, Scherotinia sclerotiorum, Gibberella saubinetii, Gloneman cingulatal,* and *Thanatephorus cucumeris* were evaluated, and triadimefon was used as controls(Table-2). The primary bioassay showed the title compound exhibits weak inhibiting activity towards*Alternaria solani, Scherotinia sclerotiorum, Gibberella saubinetii,* and *Thanatephorus cucumeris* with EC_{50} of 125.24 mg. L^{-1}, 78.22 mg. L^{-1}, 59.98 mg. L^{-1}, and 66.20 mg.L^{-1}, respectively. Interestingly, the inhibitory activity of title compound against *Gloneman cingulatal* was close that of triadimefon. These results indicated that compound **5** could be further used as a lead compound to develop novel fungicides.

[1]*A. solani*: *Alternaria solani*; *S. sclerotiorum*: *Scherotinia sclerotiorum*; *G. saubinetii*: *Gibberella saubinetii*; *G. cingulatal*: *Gloneman cingulatal* and *T. cucumeris*: *Thanatephorus cucumeris*. The experiment was carried out in three triplicates. The data of the fungicidal activities were statistically analyzed using the SPSS 22.0 software package to obtain EC_{50} values. The latter represent the mean ± standard deviation (SD) of triplicate experiments.

Conclusions

In summary, the compound 1-(3,3-dimethyl-2-oxobutyl)-N-phenyl-1*H*-1,2,4 -triazole-3-carboxamide **5** was synthesized and characterized by ^{1}H NMR, ^{13}C NMR, HRMS, and X-ray diffraction. The synthesis followed a strategy of inserting an amide group between a benzene ring and a triazole ring. The biological assay results indicated compound **5** showed a similar antifungal activity compared to triadimefon against *Gloneman cingulatal*. Potentially, the results obtained will lay the foundation for the design and development of novel fungicides.

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