Synthesis, Crystal Structure and Antitumor Activity of 4-*tert*-Butyl-*N*-(2-fluorophenyl)-5-(1*H*-1,2,4-triazol-1-yl)-thiazol-2-amine①

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ABSTRACT The title compound has been synthesized by the reaction of 1-bromo-3,3-dimethyl-1- (1*H*-1,2,4-triazol-1-yl)butan-2-one with 1-(2-fluorophenyl)thiourea, and its crystal structure was determined by single-crystal X-ray diffraction. The crystal belongs to the orthorhombic system, space group *Pbca* with a = 15.2568(6), b = 12.1533(5), c = 16.7307(7) Å, Z = 8, V = 3102.2(2) Å^3, M_r = 317.39, D_c = 1.359 g/cm^3, S = 1.05, μ = 0.223 mm^{-1}, F(000) = 1328, the final R = 0.034 and wR = 0.097 for 2590 observed reflections ($I > 2\sigma(I)$). X-ray crystal structure presents the intramolecular N–H···N hydrogen bond, which plays an important role in stabilizing the crystal structure. In addition, the preliminary biological test on the title compound shows good antitumor activity, with IC_{50} of 0.122 μmol/mL against the *Hela* cell line.

Keywords: 4-*tert*-butyl-*N*-(2-fluorophenyl)-5-(1*H*-1,2,4-triazol-1-yl)thiazol-2-amine, synthesis, crystal structure, antitumor activity

1 INTRODUCTION

As an important class of nitrogen heterocycles, thiazoles have been used as insecticides, fungicides, herbicides and so on for their excellent biological activity, low toxicity and environmental friendly[1]. Recently, many researches have reported that thiazole derivatives also have antitumor[2], antiviral[3], antioxidant[4], weeding[5] and analgesic[6] activities. On the other hand, 1,2,4-triazoles play an important role in many biological processes[7], which contributed to their popularity as antimycotic agents, agricultural fungicides and plant growth regulators[8-10]. Holla[11] synthesized 2-arylamino-4-(2,4-dichloro-5-fluorophenyl)thiazoles and evaluated their antibacterial and anti-inflammatory activities. Narayana[12] prepared 4-(2-arylamino-1,3-thiazol-4-yl)benzene-1,2-diols and some of them were found to possess excellent antibacterial and antifungal activities. Alejandra[13] reported the synthesis and biological activity of 5-(3,4-dimethoxyphenyl)-2-arylamino thiazole. Luo[14] reported the synthesis and insecticidal activity of 4-(7-methoxy-2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)-*N*-arylthiazol-2-amine (**1**), which demonstrated the most significant insecticidal activity. Furthermore, the chiral crystal structures of 4-(7-methoxy-2,2-dimethyl-2,3-dihydro-benzofuran-5-yl)-*N*-(pyridin-2-yl)thiazol-2-amine and 4-(7-methoxy-2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)-2-(3,4-xyleneamino)thiazole were obtained from their non-chiral molecules[15-16].

In this paper, we designed and synthesized a new thiazole derivative by incorporating the *tert*-butyl and 1,2,4-triazole groups into the title compound **2**

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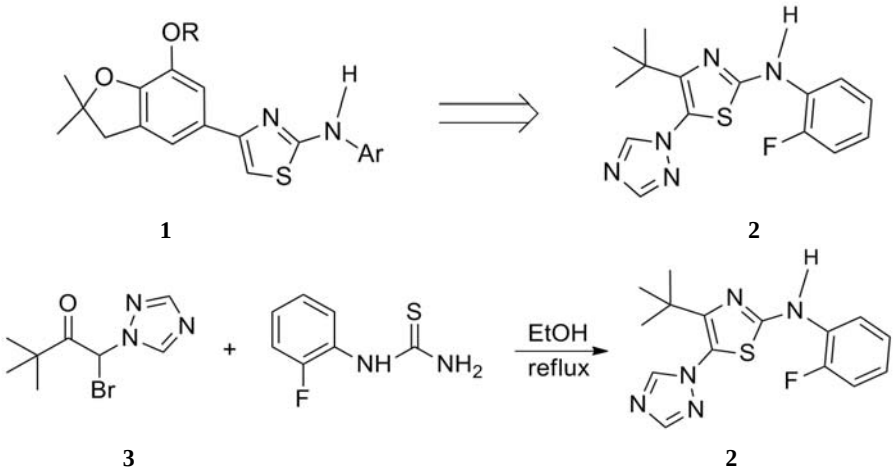
based on **1** (Scheme 1), and such molecule is expected to exhibit improving antitumor activity. Herein, we report the synthesis, characterization and bioactivity of the new compound 4-*tert*-butyl-*N*-(2-fluorophenyl)-5-(1*H*-1,2,4-triazol-1-yl)thiazol-2-amine (**2**). The synthesis route is depicted in Scheme 1.



Scheme 1. Design and synthetic route of the title compound (2)

2 EXPERIMENTAL

2. 1 Instruments and general methods

All solvents were of reagent grade. All chemicals were analytical reagents and used directly without further purification. Melting point was measured on an X-4 electrothermal digital melting point apparatus and uncorrected. ^{1}H NMR spectra were recorded on a Bruker advanced instrument with TMS as internal standard at 400 MHz with chemical shifts (δ) expressed in ppm.

2. 2 Synthesis of the title compound

1-Bromo-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one (**3**, 0.01 mol) and 1-(2-fluorophenyl)thiourea (0.01 mol) were dissolved in ethanol and refluxed with stirring for 5 h, then the reaction solution was adjusted to neutral by adding ammonia and extracted with ethyl acetate. After the excess solvent was evaporated, the residue was recrystallized from ethanol and dried to give **2**. Yield: 15.8%, m.p.: 193–195 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.10(s, 9H, 3×CH_3), 7.05(t, J = 6.4 Hz, 1H, C_6H_4 4-H), 7.20(d, J = 8.0 Hz, 1H, C_6H_4 6-H), 7.28(d, J = 8.0 Hz, 1H, C_6H_4 3-H), 8.42(t, J = 8.0 Hz, 1H, C_6H_4 5-H), 8.22(s, 1H, $C_2H_2N_3$ 3-H), 8.93(s, 1H, $C_2H_2N_3$ 5-H), 10.17(brs, 1H, NH).

2. 3 X-ray structure determination

The crystals of the title compound suitable for X-ray structure determination were obtained by slowly evaporating an ethanol solution for about 2 days at room temperature. A colorless block crystal with dimensions of 0.44mm × 0.42mm × 0.37mm was selected and mounted in air onto thin glass fibers. X-ray intensity data were measured at 173(2) K on a Bruker AXS SMART 1000 CCD diffractometer equipped with a graphite-monochromatic Mo$K\alpha$ (λ = 0.71073 Å) radiation. Corrections for incident and diffracted beam absorption effects were applied using SADABS[17]. The structure was solved by direct methods with SHELXS-97[18] and expanded by difference Fourier techniques. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were added according to theoretical models. The structure was refined by full-matrix least-squares techniques on F^2 with SHELXL-97[19]. The final refinement gave R = 0.034, wR = 0.097 (w = $1/[\sigma^2(F_o^2) + (0.0471P)^2 + 1.7514P]$, where P = $(F_o^2 + 2F_c^2)/3$), $(\Delta/\sigma)_{max}$ = 0.001, S = 1.05, $(\Delta\rho)_{max}$ = 0.32 and $(\Delta\rho)_{min}$ = –0.32 e/Å^3 for 2590 observed reflections with $I > 2\sigma(I)$. The selected bond lengths and bond angles are given in Table 1.

Table 1. Selected Bond Lengths (Å) and Bond Angles (°)

Bond	Dist.	Bond	Dist.	Bond	Dist.
S(1)−C(5)	1.7395(16)	C(4)−N(4)	1.386(2)	C(7)−C(8)	1.369(2)
S(1)−C(3)	1.7490(16)	C(4)−C(12)	1.519(2)	C(8)−C(9)	1.382(3)
C(1)−N(3)	1.315(2)	C(5)−N(4)	1.300(2)	C(9)−C(10)	1.382(3)
C(1)−N(1)	1.339(2)	C(5)−N(5)	1.368(2)	C(10)−C(11)	1.387(2)
C(2)−N(2)	1.312(2)	C(6)−C(7)	1.390(2)	C(12)−C(13)	1.523(3)
C(2)−N(3)	1.362(2)	C(6)−C(11)	1.392(2)	C(12)−C(14)	1.528(2)
C(3)−C(4)	1.356(2)	C(6)−N(5)	1.408(2)	C(12)−C(15)	1.530(3)
C(3)−N(1)	1.411(2)	C(7)−F(1)	1.3628(19)	N(1)−N(2)	1.3725(19)
Angle	(°)	Angle	(°)	Angle	(°)
C(5)−S(1)−C(3)	87.33(7)	N(4)−C(5)−S(1)	115.57(12)	C(4)−C(12)−C(14)	108.37(14)
N(3)−C(1)−N(1)	110.81(15)	N(5)−C(5)−S(1)	118.97(12)	C(13)−C(12)−C(14)	108.48(18)
N(3)−C(1)−H(1)	124.6	C(7)−C(6)−C(11)	117.37(15)	C(4)−C(12)−C(15)	107.53(14)
N(1)−C(1)−H(1)	124.6	C(7)−C(6)−N(5)	117.96(15)	C(13)−C(12)−C(15)	110.5(2)
N(2)−C(2)−N(3)	115.63(15)	C(11)−C(6)−N(5)	124.64(15)	C(14)−C(12)−C(15)	108.03(17)
C(4)−C(3)−N(1)	130.23(15)	F(1)−C(7)−C(8)	119.04(15)	C(1)−N(1)−N(2)	109.41(13)
C(4)−C(3)−S(1)	111.82(12)	F(1)−C(7)−C(6)	117.49(14)	C(1)−N(1)−C(3)	128.75(14)
N(1)−C(3)−S(1)	117.90(11)	C(8)−C(7)−C(6)	123.47(16)	N(2)−N(1)−C(3)	121.80(13)
C(3)−C(4)−N(4)	113.49(14)	C(7)−C(8)−C(9)	118.32(16)	C(2)−N(2)−N(1)	101.82(13)
C(3)−C(4)−C(12)	130.10(15)	C(9)−C(10)−C(11)	121.02(17)	C(1)−N(3)−C(2)	102.34(14)
N(4)−C(4)−C(12)	116.38(14)	C(10)−C(11)−C(6)	119.86(16)	C(5)−N(4)−C(4)	111.78(13)
N(4)−C(5)−N(5)	125.46(15)	C(4)−C(12)−C(13)	113.74(15)	C(5)−N(5)−C(6)	124.46(14)

2. 4 Bioassay of the antitumor activities

Antitumor activity of the title compound was tested by the typical MTT method according to the literature method[20].

3 RESULTS AND DISCUSSION

The ^{1}H NMR data for the product are in good agreement with the structure of **2**. X-ray analysis reveals that crystals of the title compound are made up of monoclinic unit cells, each containing eight molecules. The structure of the title compound with atomic numbering scheme is shown in Fig. 1.

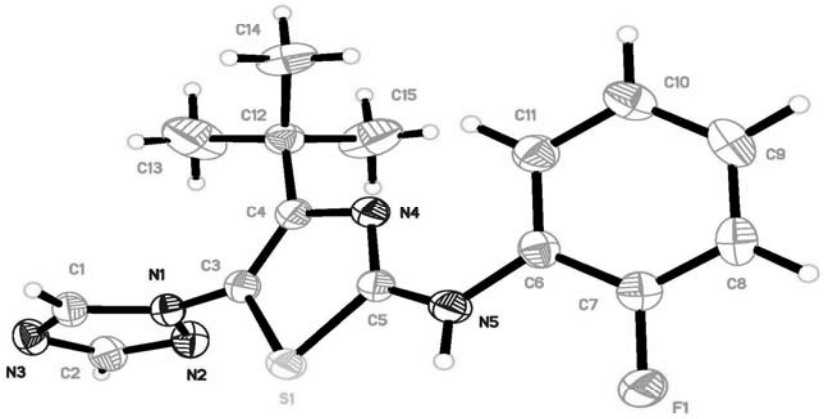


Fig. 1. X-ray crystal structure of the title compound with atom labels and 50% probability displacement ellipsoids

In the crystal structure of the title compound, the phenyl ring (Plane 1), thiazole ring (Plane 2), and triazolyl ring (Plane 3) form the three planes of the molecule. The dihedral angles between planes 1 and 2 and between planes 2 and 3 are 26.835(5) and 85.813(5)°, respectively, with the latter to be almost perpendicular. The angles of C(5)–S(1)–C(3), C(4)–C(3)–S(1) and C(1)–N(1)–C(3) are 87.33(7), 111.82(1) and 128.75(1)°, respectively, which are in accordance with the literature[20-21].

As outlined in Fig. 1 and Table 1, the bond lengths of C(2)–N(2) and C(1)–N(3) are 1.312(2) and 1.315(2) Å, belonging to the typical C=N bond[22]. Compared to the normal C–N bond distance of 1.47–1.50 Å, the shorter C(2)–N(3) bond distance of 1.362 Å may be due to the effect of conjugation[23]. The S(1)–C(5) and S(1)–C(3) bond lengths of 1.739 and 1.749 Å are also shorter than the typical S–C bond distance of 1.82 Å.

As shown in Fig. 2, an intramolecular hydrogen bond N(5)–H···N(3) can be seen between the amino and triazole groups, the distance and angle of which are 3.039(2) Å and 148.5°, respectively, belonging to the typical V-type hydrogen bond. The intramolecular hydrogen bonds play an important role in stabilizing the crystal structure.

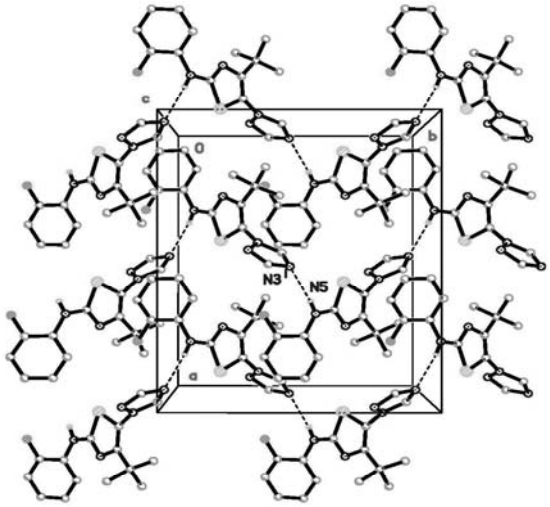


Fig. 2. Hydrogen bonding diagram. H atoms have been omitted for clarity

The compound was tested at five different concentrations in vitro and the experiments were performed in triplicate and the average inhibition is shown in Table 2. The IC_{50} was calculated by SPSS.

The antitumor activity test in vitro showed that the title compound exhibits good antitumor activity with the IC_{50} of 0.122 μmol/mL against *Hela*. The result of preliminary bioassay indicated that further researches on the title compound are of great significance.

Table 2. Antitumor Activity of the Title Compound against *Hela*

Concentration/μmol·L^{-1}	0.500	0.250	0.100	0.050	0.025
inhibition/ %	0.909	0.640	0.420	0.245	0.149

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