*Original research paper

Synthesis, spectroscopic characterization, X-ray crystallographic studies and antibacterial screening of 1-(5-(4-chlorophenyl)thiazol-2-yl)hydrazine hydrobromide

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ABSTRACT

The synthesis of 1-(5-(4-chlorophenyl)thiazol-2-yl)hydrazine hydrobromide was achieved in a single step reaction by condensation of 2-bromo-1-(4-chlorophenyl)ethanone with thiosemicarbazide in absolute ethanol. The structure of the target compound was deduced by modern spectroscopic techniques like FTIR, ^{1}H and ^{13}C NMR spectroscopy and unequivocally confirmed by crystallographic data. The title compound crystallized in the orthorhombic space group P 2_1 2 2_1 with unit cell parameters a = 6.6861(6) Å, b = 8.9683(12) Å, c = 20.4035(5) Å, $\alpha = \beta = \gamma = 90°$, V = 1223.5(2) Å^3, Z = 4, Dc = 1.751 mg/m^3, F(000) = 640 and μ = 8.058 mm^{-1}. The synthesized compound was also evaluated for antibacterial activity.

Keywords: Condensation; thiazole; crystal structure; antibacterial activity.

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1. INTRODUCTION

Thiazole is a diverse scaffold in heterocyclic chemistry and is found in various natural products (e.g., epothilone) and pharmacologically essential compounds including anticancer, antiviral and antidiabetic drugs [1-4]. These are ubiquitous building blocks in medicinal chemistry and found to exhibit broad spectrum of biological activities including antibacterial and antifungal activities. Thiazoles and their derivatives are reported as herbicidal, fungicidal, antiallergic, anti-inflammatory, antitubercular, antiarthritic, anti-HIV, analgesic and psychotropic agents [5-10]. The recent literature [11-15] has been investigated for their considerable antimicrobial activity against a variety of clinically vital fungal strains. In particular, these studies confirmed that thiazole derivatives are excellent pharmacophores for the design of bioactive molecules [16,17].

Infectious diseases remain serious and growing threatens to human health worldwide during the past few decades [18,19]. The decrease of susceptibility to antimicrobial agents in current use has also been increasing for a great variety of pathogens and the resistance to multiple drugs is more and more rampant for several microorganisms. Therefore the urgent need for innovation or optimization of antimicrobial agents active against these defiant strains is of vital significance [20,21].

In corollary of the fascinating biological and pharmaceutical properties and synthetic efficacy, we report herein the synthesis of a novel thiazole derivative combined with crystallographic studies.

2. MATERIAL AND METHODS

2.1. General

Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AV-300 spectrometer at 300 and 75 MHz respectively. Spectra were measured in DMSO-d_6 solution using residual solvent peak as the reference and coupling constants were measured in Hertz. Infrared spectrum was recorded on Bruker Optics Alpha FTIR Spectrophotometer. Melting point was determined on a Sanyo Gallenkamp melting point apparatus in open capillary tube and remains uncorrected. Analytical thin-layer chromatography was carried out with Merck silica gel 60 F254 aluminum backed sheets.

2.2. Synthesis

2.2.1. Synthesis of 1-(5-(4-chlorophenyl)thiazol-2-yl)hydrazine hydrobromide (3)

4-Chlorophenacyl bromide **(1)** (1 mmol) and thiosemicarbazide **(2)** (1 mmol) in ethanol were refluxed for 30 min. The excess solvent was removed under pressure on rotary evaporator. The crude solid obtained was recrystallized from dry ethanol to afford the title compound [22].

Yield: 88 %; m.p 181-182 ^{o}C; IR (neat, cm^{-1}): 3447-3289 (N-H), 3054 (C_{sp2}-H), 1653 (C=N), 1585, 1499 ($C=C_{Ar}$), 1187 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.05 (s, 1H, N-H), 7.95 (d, 2H, J = 8.7 Hz, Ar-H), 7.62 (s, 1H, $C\text{-}H_{thiazole}$), 7.50 (d, 2H, J = 8.4 Hz, Ar-H), 5.62 (s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.8, 148.4, 133.0, 132.9, 129.2, 128.0, 107.9.

2.3. X-ray Structure Determination

A suitable single crystal of the target compound having dimensions 0.4048 × 0.2270 × 0.2195 mm was selected. All the reflection data for the title compound were collected on an Oxford SuperNova CCD diffractometer using Cu-Kα (l = 1.54184 Å) X-radiation at 130 K. A total of 2813 reflections were collected, of which 1883 (-7<=h<=7, -6<=k<=10, -24<=l<=23) were

71 treated as observed. The structure was solved by direct methods and refined by full-matrix least
72 squares using SHELX-97 [23]. With the exception of those hydrogen atoms bonded to nitrogen,
73 all other hydrogen atoms were refined in idealised positions.
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Table 1 Crystal data and structure refinement for title compound 3

Empirical formula	$(C_9H_8ClN_3S).HBr.H_2O$
Formula weight	324.63
Temperature	130.0(1) K
Wavelength	1.5418 Å
Crystal system	Orthorhombic
Space group	P 2_1 2 2_1
Unit cell dimensions	a = 6.6861(6) Å
	b = 8.9683(12) Å
	c = 20.4035(17) Å
	$\alpha = \beta = \gamma = 90°$
Volume	1223.5(2) $Å^3$
Z	4
Density (calculated)	1.762 mg/m^3
Absorption coefficient	8.059 mm^{-1}
F(000)	648
Crystal size	0.4048 × 0.2270 × 0.2195 mm^3
Theta range for data collection	4.33 to 67.50°
Index ranges	-7<=h<=7, -6<=k<=10, -24<=l<=23
Reflections collected	2813
Independent reflections	1883 [R(int) = 0.0124]
Completeness to theta = 67.50∞	99.7 %
Absorption correction	Gaussian
Max. and min. transmission	0.370 and 0.207
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1883 / 0 / 171
Goodness-of-fit on F^2	1.073
Final R indices [I>2sigma(I)]	R_1 = 0.0236, wR_2 = 0.0627
R indices (all data)	R_1 = 0.0240, wR_2 = 0.0630
Absolute structure parameter	0.45(3)
Largest diff. peak and hole	0.294 and -0.388 e.$Å^{-3}$

Table 2 Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters ($Å^2$ × 10^3) for compound 3. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br	3084(1)	3689(1)	6423(1)	38(1)
S(1)	2826(2)	9662(1)	7047(1)	33(1)
Cl(1)	2113(1)	6928(1)	3063(1)	34(1)
N(3)	3051(6)	7550(3)	8144(2)	38(1)
N(2)	2946(6)	6882(3)	7520(1)	33(1)
N(1)	2771(4)	7273(3)	6390(1)	26(1)
C(4)	2282(5)	7349(4)	3895(2)	27(1)
C(3)	2334(5)	8820(4)	4088(2)	31(1)
C(9)	2864(5)	7754(3)	7003(2)	27(1)
C(7)	2658(4)	8397(3)	5917(2)	25(1)
C(2)	2479(5)	9145(3)	4750(2)	31(1)
C(8)	2667(5)	9750(3)	6195(2)	32(1)
C(1)	2531(4)	8025(3)	5217(2)	25(1)
C(5)	2343(5)	6205(4)	4350(2)	30(1)
C(6)	2469(5)	6548(3)	5010(2)	27(1)
O(1)	3871(14)	2729(8)	4869(4)	77(2)
O(2)	782(17)	2756(12)	4937(9)	130(5)

106 **Table 3 Bond lengths [Å] and angles [°] for compound 3**

Distances		Angles		Torsion angles	
S(1)-C(9)	1.713(3)	C(9)-S(1)-C(8)	89.64(16)	C(5)-C(4)-C(3)-C(2)	0.8(5)
S(1)-C(8)	1.745(4)	N(2)-N(3)-H(3A)	108(2)	Cl(1)-C(4)-C(3)-C(2)	-179.6(2)
Cl(1)-C(4)	1.744(3)	N(2)-N(3)-H(3B)	109(3)	N(3)-N(2)-C(9)-N(1)	179.6(4)
N(3)-N(2)	1.409(4)	H(3A)-N(3)-H(3B)	107(4)	N(3)-N(2)-C(9)-S(1)	-1.5(6)
N(3)-H(3A)	0.96(5)	C(9)-N(2)-N(3)	118.3(3)	C(7)-N(1)-C(9)-N(2)	178.7(4)
N(3)-H(3B)	0.92(5)	C(9)-N(2)-H(2A)	118(3)	C(7)-N(1)-C(9)-S(1)	-0.2(4)
N(2)-C(9)	1.315(4)	N(3)-N(2)-H(2A)	120(3)	C(8)-S(1)-C(9)-N(2)	-178.7(4)
N(2)-H(2A)	0.87(4)	C(9)-N(1)-C(7)	114.8(3)	C(8)-S(1)-C(9)-N(1)	0.3(3)
N(1)-C(9)	1.324(4)	C(9)-N(1)-H	121(2)	C(9)-N(1)-C(7)-C(8)	0.1(4)
N(1)-C(7)	1.397(4)	C(7)-N(1)-H	123(2)	C(9)-N(1)-C(7)-C(1)	-179.7(3)
N(1)-H	0.83(4)	C(3)-C(4)-C(5)	121.2(3)	C(4)-C(3)-C(2)-C(1)	-1.2(5)
C(4)-C(3)	1.378(5)	C(3)-C(4)-Cl(1)	119.1(2)	N(1)-C(7)-C(8)-S(1)	0.1(4)
C(4)-C(5)	1.383(4)	C(5)-C(4)-Cl(1)	119.6(2)	C(1)-C(7)-C(8)-S(1)	179.8(3)
C(3)-C(2)	1.386(5)	C(4)-C(3)-C(2)	118.8(3)	C(9)-S(1)-C(8)-C(7)	-0.2(3)
C(7)-C(8)	1.339(4)	N(2)-C(9)-N(1)	124.5(3)	C(3)-C(2)-C(1)-C(6)	1.0(4)
C(7)-C(1)	1.470(4)	N(2)-C(9)-S(1)	123.5(2)	C(3)-C(2)-C(1)-C(7)	-179.1(3)
C(2)-C(1)	1.384(5)	N(1)-C(9)-S(1)	112.0(2)	C(8)-C(7)-C(1)-C(2)	2.3(5)
C(1)-C(6)	1.391(4)	C(8)-C(7)-N(1)	111.2(3)	N(1)-C(7)-C(1)-C(2)	-178.0(3)
C(5)-C(6)	1.384(5)	C(8)-C(7)-C(1)	128.1(3)	C(8)-C(7)-C(1)-C(6)	-177.8(3)
		N(1)-C(7)-C(1)	120.7(3)	N(1)-C(7)-C(1)-C(6)	1.9(5)
		C(1)-C(2)-C(3)	121.3(3)	C(3)-C(4)-C(5)-C(6)	-0.1(5)
		C(7)-C(8)-S(1)	112.3(3)	Cl(1)-C(4)-C(5)-C(6)	-179.7(3)
		C(2)-C(1)-C(6)	118.8(3)	C(4)-C(5)-C(6)-C(1)	-0.1(5)
		C(2)-C(1)-C(7)	120.3(3)	C(2)-C(1)-C(6)-C(5)	-0.4(5)
		C(6)-C(1)-C(7)	120.9(3)	C(7)-C(1)-C(6)-C(5)	179.7(3)
		C(4)-C(5)-C(6)	119.3(3)		
		C(5)-C(6)-C(1)	120.6(3)		

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Table 4 Hydrogen bonds for compound 3 [Å and °]

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H...Br	0.83(4)	2.43(4)	3.222(3)	160(3)
N(2)-H(2A)...Br	0.87(4)	2.96(4)	3.635(3)	136(3)
N(3)-H(3A)...Br#1	0.96(5)	2.75(5)	3.612(4)	150(3)
N(3)-H(3B)...Br#1	0.92(5)	2.76(5)	3.652(4)	165(4)

Symmetry transformations used to generate equivalent atoms:
#1 x-1/2,-y+1,-z+3/2 ; #2 x+1/2,-y+1,-z+3/2

Table 5 Antibacterial activity of the title compound 3

Compound	***P.m.***	***B.s.***	***E.c.***	***S.a.***	***P.p.***	***P.a.***	***S.t.***	***M.l.***	***S.f.***	***K.p.***
3	17	19	08	24	17	23	11	18	21	07
Standard Levofloxacin	30	20	30	25	30	28	30	25	30	25

Activity is presented in millimeter (mm)

Pasteurella multocida ***(P.m.)****, Bacillus subtilis* ***(B.s.)****, Escherichia coli* ***(E.c.)****, Staphylococcus aureus* ***(S.a.)****, Pseudomonas putida* ***(P.p.)****, Pseudomonas aeruginosa* ***(P.a.)****, Salmonella typhi* ***(S.t.)****, Micrococcus luteus* ***(M.l.)***, *Shigella flexineri* ***(S.f.)*** and *Klebsiella pneumonae* ***(K.p.)***.

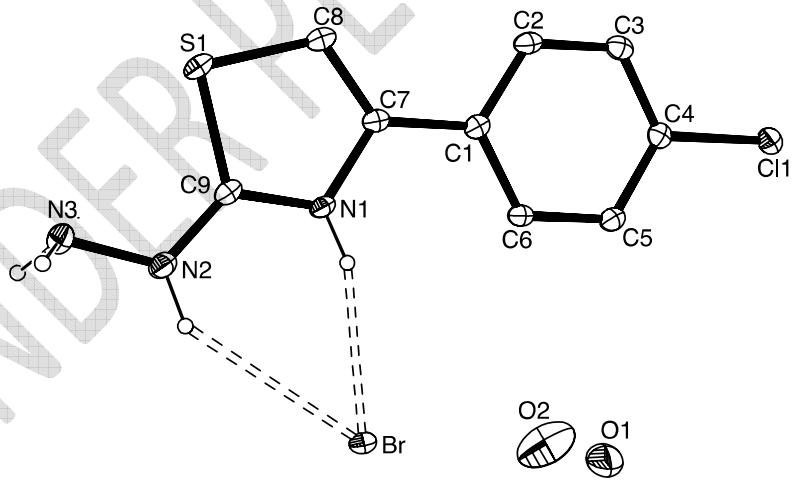


Fig. 1 Thermal ellipsoid plot for compound 3. Ellipsoids are at the 20% probability level. Hydrogen atoms attached to the disordered water molecules were not located.

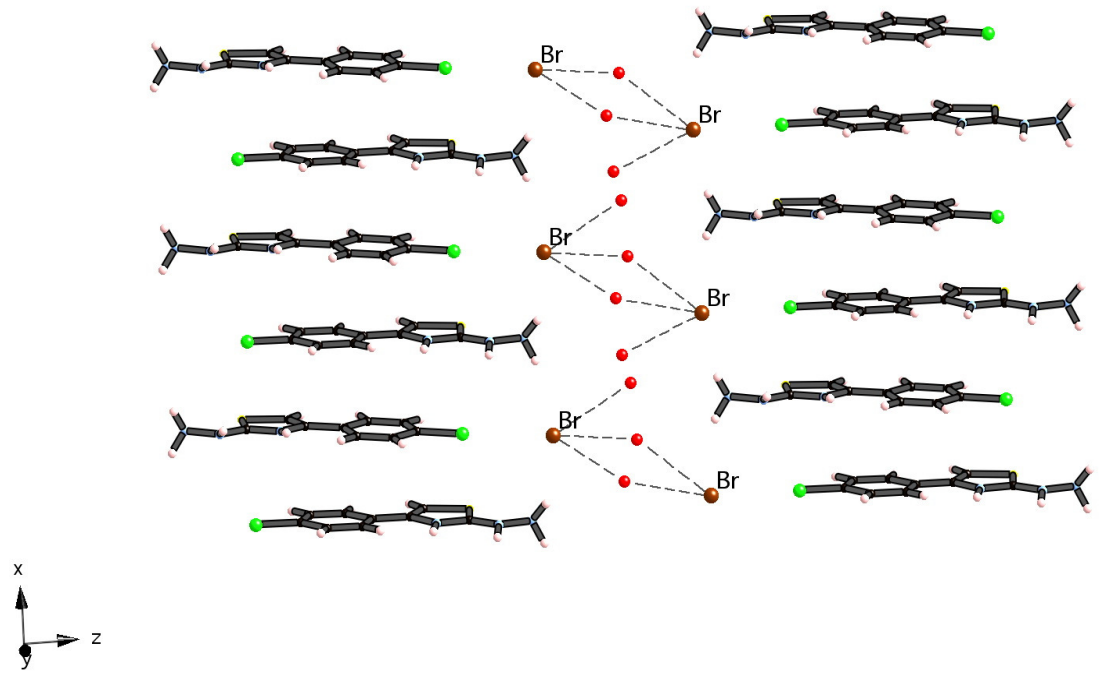


Fig. 2 Partial crystal packing diagram of compound 3 showing disordered water molecules, and the off-set π-stacking arrangement of the planar phenylthiaxole groups extending along the x-axis.

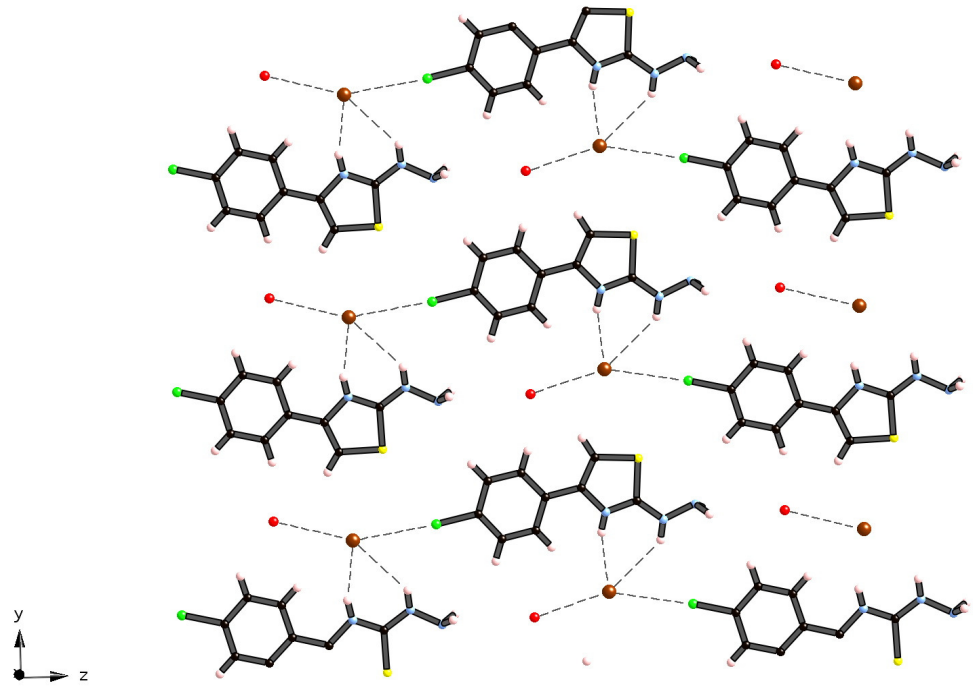


Fig. 3 Partial crystal packing diagram of compound 3 showing the hydrogen bond contacts involving the bromide counterion in the y-z plane.

2.4. Biological Screening

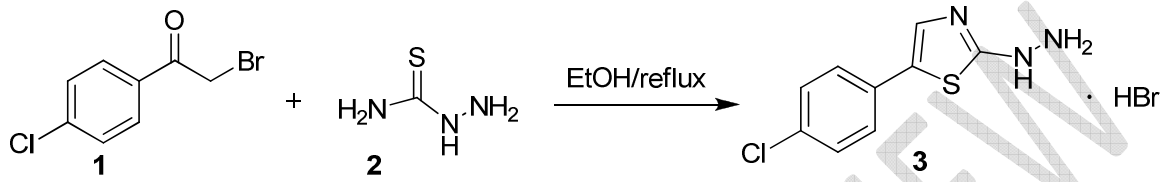
2.4.1. Antibacterial activity

In vitro evaluation of antibacterial activity of the newly synthesized compounds was carried out by agar well diffusion assay against ten different Gram positive and Gram negative bacteria [24]. Antibacterial activity was determined by using the Mueller Hinton Agar (MHA). The fresh inoculums of these bacteria were prepared and diluted by sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5Mc-Farland. A homogeneous bacterial lawn was developed by sterile cotton swabs. The inoculated plates were bored by 6 mm sized borer to make the wells. The sample dilutions were prepared by dissolving sample (1.0 mg) in 1.0 mL of DMSO used as negative control in this bioassay. The equimolar concentration of Levofloxacin (1.0 mg/mL), a broad spectrum antibiotic (positive control) was prepared. These plates were incubated at 37 °C for 24 hours. Antibacterial activity of the prepared compound was determined by measuring the diameter of zone of inhibition (mm, ± standard deviation) and presented by subtracting the activity of the negative control in Table 5.

3. RESULTS AND DISCUSSION

The synthetic pathway that leads to the formation of the title compound **3** is sketched in Scheme-1. By adopting the literature procedure [22], single step condensation of 2-bromo-1-(4-chlorophenyl)ethanone with thiosemicarbazide in absolute ethanol afforded the title compound in good yield. The compound **3** was fully characterized by analytical and spectral data. In general, in IR spectrum, strong bands at 3447-3289 cm^{-1} and 1653 cm^{-1} were assigned to the N-H and C=N group, respectively. The disappearance of methylene stretching frequency around 3000 cm^{-1} also indicated the formation of required product. The ^{1}H NMR spectrum displayed a distinctive singlet at 7.62 ppm for thiazole proton and two other singlets at 10.05 and 5.62 ppm attributed to

N-H and NH_2 groups respectively, confirmed the formation of target molecule. The target compound was further confirmed by recording ^{13}C NMR spectrum and the signals appeared in the spectrum account for all the C-atoms present in the compound **3**.



Scheme 1. Synthesis of title compound (3)

The molecular structure was further confirmed by single crystal X-ray diffraction studies. Experimental details are given in Table 1, while atomic coordinates with equivalent isotropic temperature factors are presented in Table 2. Selected bond distances, angles and dihedral angles are given in Table 3, while H-bond geometries are tabulated in Table 4. A thermal ellipsoid plot at 20% probability level for compound **3** is presented in Fig. 1. Crystals of compound **3** are racemically twinned as indicated by the absolute structure parameter, which refined to 0.45(3). Disordered water molecules form a column running along the x-axis (Fig. 2) and make weak contacts with the bromide counterion (O1…Br; 3.329(7) Å, O2…Br; 3.50(1) Å), however there are no significant contacts with the hydrazine nitrogen's (N2 and N3) or with the thiazole nitrogen (N1). The phenylthiazole rings are essentially coplanar and are π-stacked along the x-axis with an interplanar distance of ca. 3.42 Å (Fig. 2). The bromide counterion forms hydrogen bonds with imidazole nitrogen N1 and the hydrazine nitrogen N2 within the y-x plane (Table 4), while there are hydrogen bonds between the bromide ion and the hydrogens attached to N3 which project above and below this plane (Fig. 3).

3.1. Antibacterial evaluation of target compound

In vitro antibacterial screening of the newly synthesized compound **3** was carried out by agar well diffusion method against ten different Gram positive and Gram negative bacteria and the results are summarized in Table 5. The results revealed that the synthesized compound showed moderate to good antibacterial efficacy against different bacterial strains. The title compound exhibited strong activity against *B. subtilis* and *S. aureus* respectively, as compared to standard.

4. CONCLUSION

In summary, 1-(5-(4-chlorophenyl)thiazol-2-yl)hydrazine hydrobromide has been made conveniently in single step reaction and successfully characterized by spectroscopic techniques and single crystal X-ray diffraction data. The title compound has also been screened for antibacterial activity which exhibited strong efficacy against *B. subtilis* and *S. aureus* respectively. The synthesized thiazole scaffold may be used as an important building block in heterocyclic chemistry.

SUPPLEMENTARY DATA

CCDC 867531 contains the supplementary crystallographic data for this paper. Copies of the data can be obtained free of charge by e-mailing data_request@ccdc.cam.ac.uk or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, Fax: +44(0)1223-336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk

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COMPETING INTERESTS

The authors declare that they have no competing interest.

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