ISSN 0022-4766, Journal of Structural Chemistry, 2020, Vol. 61, No. 7, pp. 1111-1116. © Pleiades Publishing, Ltd., 2020.
Text © The Author(s), 2020, published in Zhurnal Strukturnoi Khimii, 2020, Vol. 61, No. 7, pp. 1173-1178.

SYNTHESIS, CRYSTAL STRUCTURE, ANTI-LUNG CANCER ACTIVITY OF 2-(4-FLUOROPHENYL)-5-(5-IODO-2-METHYLBENZYL)THIOPHENE

L.-P. Zhou[1], F. Qiu[2], Z.-F. Zhang[3], C.-M. Wang[2], Y.-L. Dong[4], and J.-P. Liu[2]*

New heterocycle compound 2-(4-fluorophenyl)-5-(5-iodo-2-methylbenzyl)thiophene (**1**), designed using 5-iodo-2-methylbenzoic acid (**2**) as the starting material is successfully obtained via the multiple synthesis route and finally characterized by IR, ^{1}H NMR, and single crystal X-ray crystallography. In addition, the in vitro anticancer activity of compound **1** on three human lung cancer cells (H20, H2227, and H69) is further determined, which suggests that compound **1** may be a potential anticancer agent.

DOI: 10.1134/S0022476620070148

Keywords: heterocycles, X-ray, anticancer activity.

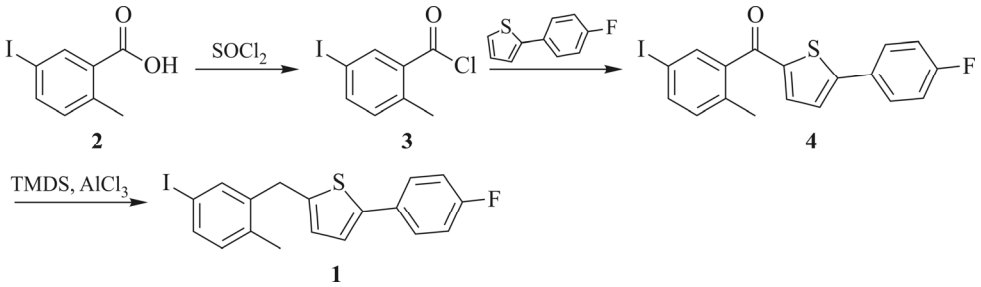
INTRODUCTION

Cancer is a disease in which cells grow and proliferate in an uncontrolled manner. Cancer evokes a high level of mortality regardless of recent advances in the development of clinically authorized anticancer agents [1, 2]. The development of resistance to chemotherapeutic agents and associated side effects are major obstacles to effectively treat cancer [3, 4]. Thus, it is necessary to identify and develop new anticancer agents with the improved efficacy and reduced side effects to complement the present chemotherapeutic strategies [5].

Type-2 diabetes mellitus is a long-term metabolic disorder and a chronic disease with worldwide prevalence [6]. It is characterized by high blood sugar, resistance to insulin, and a deficiency in insulin secretion [7]. Canagliflozin is an antidiabetic drug of the gliflozin class or subtype 2 sodium-glucose cotransporter 2 (SGLT-2) inhibitors; it is used to improve the glycemic control in patients with type-2 diabetes [8]. 2-(4-Fluorophenyl)-5-(5-iodo-2-methylbenzyl)thiophene (**1**) being one of the most important intermediates in the synthesis of canagliflozin fascinated us [9]. Thus, much attention has been devoted to the synthesis, characterization, and crystal structure of compound **1** which have never been reported before.

The present work deals with the synthesis and characterization of title compound **1**. Compound **1** was synthesized by 5-iodo-2-methylbenzoic acid (**2**) as the starting material yielding 5-iodo-2-methylbenzoyl chloride (**3**), then reacted with 2-(4-fluorophenyl)thiophene to obtain (5-(4-fluorophenyl)thiophen-2-yl)(5-iodo-2- methylphenyl)methanone (**4**), and title compound **1** was prepared after the reduction of compound **4** with TMDS and $AlCl_3$ (Scheme 1). In addition, the in vitro anticancer activity of compound **1** on three human lung cancer cells (H20, H2227, and H69) was further determined. Finally, molecular docking studies were utilized to further clarify its structure-activity relationship.

[1]The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, P. R. China. [2]The First Clinical Medical of Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia, P. R. China; *jiping_liu12@sina.com. [3]Tongliao City Hospital, Tongliao, Inner Mongolia, P. R. China. [4]Zhejiang University School of Medicine, Yiwu, Zhejiang, P. R. China. Original article submitted June 28, 2019; revised October 8, 2019; accepted October 10, 2019.



Scheme 1. Synthesis route of compound **1**.

EXPERIMENTAL

Apparatus and materials. IR spectra (400-4000 cm^{-1}) were obtained using a Brucker Equinox-55 spectrophotometer. ^1H NMR spectra were obtained using a Varian Inova-400 spectrometer (at 400 MHz). Mass spectra were obtained using a micrOTOF-Q II mass spectrometer. The melting points were taken on a XT-4 micro melting apparatus, and the thermometer was uncorrected.

Synthesis and characterization of compounds 3, 4 and 1. To a mixture of DMF (140 g, 1.915 mol) and chlorobenzene (50 mL) compound **2** (10.0 g, 0.0382 mol) was added. $SOCl_2$ (5.9 g, 0.0496 mol) was added slowly under the nitrogen atmosphere to the resulting reaction mixture at room temperature, then the temperature was raised to 65-70 °C and the mixture was stirred for 4 h. After the completion of the reaction (based on TLC), the reaction mass was concentrated under vacuo at 60-70 °C and the resulting residue was added to chlorobenzene (60 mL) to afford compound **3** as a solution in chlorobenzene.

2-(4-Fluorophenyl)thiophene (6.792 g, 0.0381 mol) was added to the above solution of **3**. The resulting reaction mixture was cooled to 10-20 °C followed by the addition of $AlCl_3$ (5.58 g, 0.0418 mol). The obtained reaction mixture was heated to 50-55 °C for 4 h.

After the completion of the reaction (based on TLC), the reaction mixture was cooled to 10 °C and then $AlCl_3$ (5.08 g, 0.0418 mol) was added, followed by slow addition of TMDS (21.8 g, 0.162 mol). The obtained reaction mixture was heated to 45-50 °C for 4 h. After the completion of the reaction (based on TLC), the reaction mass was cooled to room temperature and was then added into ice-cold water (150 mL) at 0-5 °C. The precipitated solid was collected by filtration and washed with ice cold MeOH (20 mL) and dried to furnish 11.48 g (73.68%) of title compound **1**, 55.66-62.68 °C. IR (KBr, cm^{-1}): 2915, 1509, 1232, 502. ^1H NMR (DMSO-d_6, 400 MHz): δ 7.62-7.59 (m, 3H), 7.52-7.50 (m, 1H), 7.295 (d, 1H), 7.22-7.18 (m, 2H), 7.00 (d, 1H), 6.835 (d, 1H), 4.12 (s, 1H), 2.22 (s, 3H). HRMS (ESI^+), *m*/*z* calcd for $C_{18}H_{14}FIS$: 430.9743 [$M+Na^+$]. Found: 430.9728.

Crystal structure determination. A suitable single crystal of compound **1** (obtained by slow volatilization of its CH_2Cl_2 solution) was carefully selected under an optical microscope and glued on thin glass fibers. The intensity data on **1** were collected on an Oxford Xcalibur E diffractometer. The empirical absorption corrections were applied to the data using the SADABS system. This structure was solved by a direct method and refined by the full-matrix least-squares method on F^2 using the SHELXS-97 program [10]. All non-hydrogen atoms of **1** were refined anistropically, and all the hydrogen atoms attached to carbon atoms were fixed at their ideal positions. Pertinent crystal data and structural refinement results for compound **1** were summarized in Table 1.

Antitumor activity. The anticancer activity of compound **1** against three human lung cancer cells (H20, H2227, and H69) were determined by the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide] assay [11]. In this study, the cells were plated on 96-wells at $5 \cdot 10^3$. After attachment (24 h), the cells reaching 70-80% confluency were treated for 48 h with each compound at different concentrations or 1% dimethyl sulfoxide (DMSO) as a negative control. After 48 h incubation, 20 μL of the MTT solution (5 mg/mL in phosphate buffer saline (PBS)) was added and incubated for additional

TABLE 1. Crystal Data and Structure Refinements for Compound **1**

Parameter	**1**
Formula	$C_{18}H_{14}FIS$
M_r	408.25
Crystal system	Monoclinic
Space group	$P2_1/c$
a, b, c, Å	5.5339(6), 7.8350(8), 36.835(5)
β, deg	92.983(10)
V, Å^3	1594.9(3)
Z	4
Crystal size, mm	0.48×0.4×0.36
D_{calc}, g/cm^3	1.700
μ(MoK_α), mm^{-1}	2.139
θ range, deg	2.215 to 25.348
Reflections collected	6176
No. unique data (R_{int})	2925 (0.0730)
No. data with $I \geq 2\sigma(I)$	2214
R_1, wR_2 (all data)	0.0943, 0.2392
CCDC	1866597

4 h. Subsequently, the medium was aspirated carefully, and 150 μL of DMSO were added. After incubation for 15 min, the optical density was measured at 490 nm using a FlexStation 3 benchtop multimode microplate reader (Molecular Devices, USA). This assay measures the amount of formazan produced from MTT by dehydrogenase enzymes of metabolically active cells. Thus, the quantity of formazan produced is directly proportional to the number of living cells. Absorbance values of the treated cells were compared with the absorbance values of untreated cells. The IC_{50} value was determined from the non-linear regression equation. The results are presented as the average percentage viability to the negative control (1% DMSO).

Simulation details. The structure of compound **1** was optimized by density functional theory (DFT) with a B3LYP/6-31G(*d*) basis set. The structure optimization and energy calculations were performed with the GAUSSIAN 09 program. We chose Discovery Studio 3.0 (DS 3.0) as the platform to perform the molecular docking simulation. DS 3.0 is a wildly used commercial software to perform molecular docking between small molecule and protein. The advantages of Discovery Studio 3.0 are that it has been customized to better support scoring function development with high precision. The ligand structure was taken from the crystal structure that has been obtained from the X-ray measurement and downloaded from the protein data bank (PDB), using ligand preparation tools before the docking program was performed. The receptor protein was 5JSN (PDB ID) that has been widely used for the drug screening and molecular docking simulation since it has protein residues and a double helix structure at the same time. The Libdock, CDOCKER, and ligand pose scoring tools have been used to prepare the ligand and receptor structures for the molecular docking simulation; the length of the grid box is set to 40 which is large enough to cover the entire docking pocket and contains the protein moieties and double helix chains.

RESULTS AND DISCUSSION

Molecular structure. The structure of compound **1** ($C_{18}H_{14}FIS$) was obtained by the single crystal X-ray diffraction analysis. The result shows that compound **1** has the monoclinic crystal system with the $P2_1/c$ space group. The molecular structural unit of compound **1** is shown in Fig. 1. Within its structural unit, atoms of the six-membered ring (six-membered ring: C(1)–C(6)) and the adjacent five-membered ring (five-membered ring: C(7)–C(10) and S(1)) are nearly located in the same plane. The dihedral angle of the five-membered ring (C(7)–C(10) and S(1)) and the adjacent six-membered rings

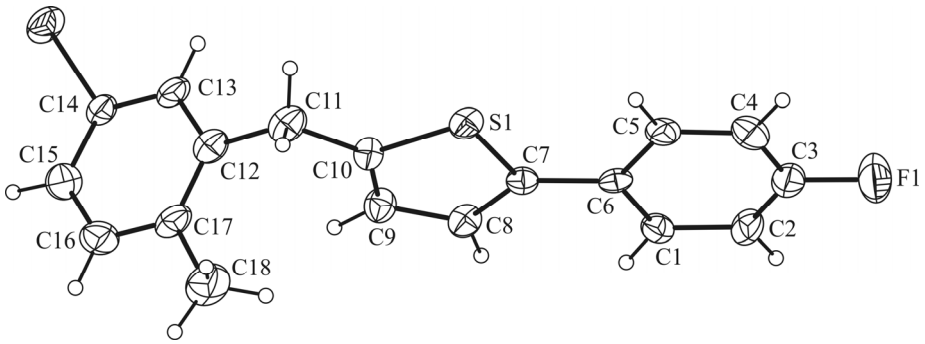


Fig. 1. Molecular structural unit for compound **1**.

(C(12)–C(17)) is 75.86°. The C–C, C–F, C–S, and C–I bond lengths are 1.329(17)-1.511(16) Å, 1.368(16) Å, 1.720(11)-1.741(11) Å, and 2.090(12) Å, respectively. These bond distances of **1** are all in their normal ranges and they are similar to the known related compounds.

In addition, the C–H···S intermolecular hydrogen bond could be observed in the packing structure of compound **1** (H···S: 2.7200 Å; C···S: 3.125(15) Å; ∠C–H···S: 107.00°). Fig. 2 exhibited the crystal packing structure of compound **1** along the *a* axis.

Anticancer activity. The interesting structural features of **1** encouraged us to test its cytotoxicity against three human lung cancer cells (H20, H2227, and H69) along with normal mouse embryonic fibroblast (NIH 3T3) cells by the MTT assay method. Compounds were dissolved in DMSO and blank samples containing the same volume of DMSO were taken as controls to identify the solvent activity in this cytotoxicity experiment. Cisplatin was used as a positive control to assess the cytotoxicity of the test compounds. The results were analyzed by means of cell inhibition expressed as IC_{50} values and are summarized in Table 2. The IC_{50} values of compound **1** showed that it exhibited a significant activity against H20, H2227, and H69 cell lines, which is almost equal to the activity of the wellknown anticancer drug, cisplatin. The results of the in vitro cytotoxic activity studies have also indicated that the IC_{50} value of **1** against NIH 3T3 (normal cells) is found to be above 369 μM, which confirmed that the compound was very specific against cancer cells and even less toxic than cisplatin (IC_{50} = 199 μM).

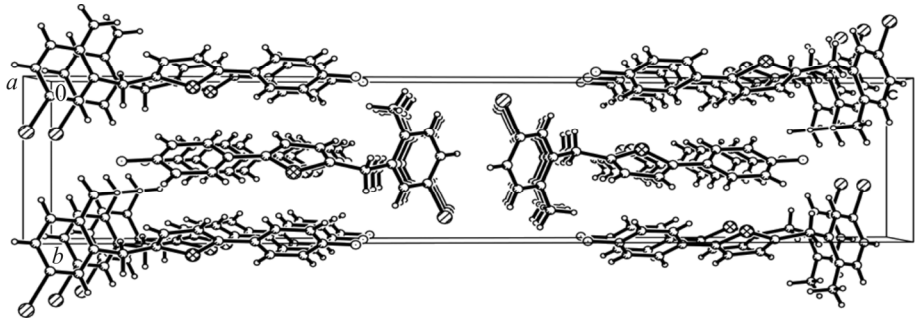


Fig. 2. Crystal packing structure of compound **1** along the *a* axis.

TABLE 2. Cytotoxic Activity of Compound **1** and Cisplatin

Compound	Half maximum inhibitory concentration, μM (IC_{50})			
	H20	H2227	H69	NIH 3T3
1	13.8±1.2	11.1±2.0	13.2±2.2	369±6
Cisplatin	13.5±0.6	13.2±0.6	11.1±1.2	199±12

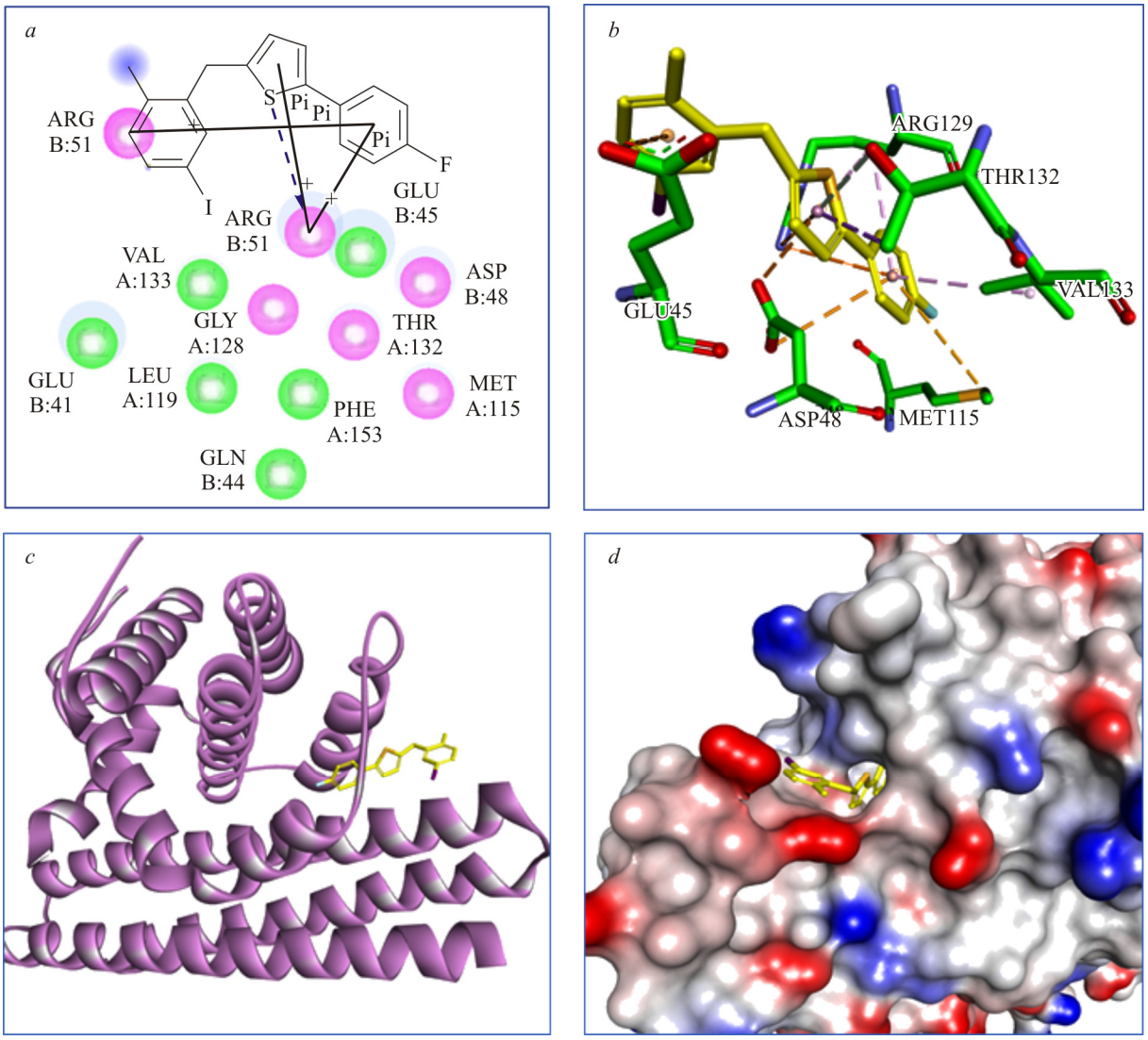


Fig. 3. The summary view of the molecular docking of the synthesized compound with Bcl-2 (PDB: 5JSN). The most favorable binding mode found through the molecular docking and scoring ligand pose module, represented by the 2D interaction map (*a*). Interactions among the compound and Bcl-2. The compound was presented as a yellow (see the electronic version) stick model of the potential binding pose. The residues involved in the interactions were presented as green (see the electronic version) sticks (*b*). The potential binding mode of the compound and the protein. The protein was shown as a violet (see the electronic version) ribbon (*c*). The compound binding modes were presented by a protein surface model. The protein surface was shown as ionizable (*d*).

Molecular docking. The molecular docking approach is a tool that could help us to reveal how the ligand interacts with the receptor protein at the molecular level. To understand the experimental phenomenon observed by the experimental results described above, the target protein NF-κB (PDB: 5JSN) has been chosen to probe possible interactions between the synthesized compound and NF-κB. As shown in Fig. 3, we exhibited both 2D and 3D interaction modes of the compound and Bcl-2. It was predicted that the compound inserted into a pocket containing polar residues (Glu45, Asp48, Arg51, Arg129, and Thr132 (Fig. 3*a*)). The compound showed a potential binding affinity to Bcl-2 through forming π–π interactions in the Bcl-2 binding pocket, which provided the most binding contribution (Fig. 3*b*). In Fig. 3*c*, *d*, we showed the ribbon and surface binding modes of the complex to reveal the potential binding mode and mechanism. The energy unfavorable binding mode shows that the stability of the binding is weak when oxygen-containing functional groups interact with the receptor. This result is in qualitative agreement with the observations in the experiment.

CONCLUSIONS

In conclusion, we synthesized a novel heterocycle derivative and characterized them via IR, ^{1}H NMR, HRMS, and single crystal X-ray crystallography. The MTT assay shows that complex **1** may act as a novel anticancer drug in the future for its in vitro anticancer activities against three human lung cancer lines (H20, H2227, and H69), which is further supported by the molecular docking simulations.

FUNDING

This work was supported by grants from Health and Family Planning Commission of Inner Mongolia Autonomous Region (201703143) and Inner Mongolia Autonomous Region Natural Science Foundation (2018MS08113).

ADDITIONAL INFORMATION

Authors contributed equally to this work.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. T. Itani and T. Ishihara. *Obes*. *Sci*. *Pract*., **2018**, *4*, 477.
2. D. Zhang, H. Q. Zhang, S. Zhao, Z. G. Li, and S. X. Hou. *Int*. *J*. *Electrochem*. *Sc*., **2019**, *14*, 4659.
3. S. Guo, R. Chen, H. Li, T. Zhang, and Y. Liu. *Int*. *J*. *Softw*. *Eng*. *Know*., **2019**, *29*, 139.
4. A. A. Abdelsameea and S. L. Kabil. *N.-S*. *Arch*. *Pharmacol*., **2018**, *391*, 945.
5. J. Su, Z. Sheng, L. Xie, G. Li, and A. X. Liu. *IEEE T*. *Commun*., **2019**, *67*, 2527.
6. N. Inagaki, S. I. Harashima, and H. Iijima. *Expert Opin*. *Pharmaco*., **2018**, *19*, 895.
7. D. Matsutani, M. Sakamoto, Y. Kayama, N. Takeda, R. Horiuchi, and K. Utsunomiya. *Cardiovasc*. *Diabetol*., **2018**, *17*, 73.
8. D. H. Esprit and A. Koratala. *Nephrology (Carlton)*, **2018**, *23*, 493.
9. S. L. Kabil and N. M. Mahmoud. *Eur*. *J*. *Pharmacol*., **2018**, *828*, 135.
10. G. M. Sheldrick. SHELXL-97, Program for Solution Crystal Structure and Refinement. University of Göttingen, Germany, **1997**.
11. L. Kupcsik. *Methods Mol*. *Biol*., **2011**, *740*, 13.