Synthesis, Crystal Structure and Antitumor Activities of Ethyl (R)-2-(Biphenyl-4-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate Postprint

Authors: HU Chun-Hong, WEI Dui-Dui, ZHAO Jing-Si, HU Min-Min, QI Sheng-Xiao, SUN Xiao-Fei, HE Dian

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Abstract

The title compound, ethyl (R)-2-(biphenyl-4-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4- b]indole-1-carboxylate (C27H24N2O3) has been synthesized, and its structure was characterized by 1H-NMR, 13C-NMR, ESI-MS and single-crystal X-ray diffraction. It crystallizes in the orthorhombic system, space group Pbca with a = 16.9950(8), b = 9.5445(4), c = 28.3188(3) Å, V = 4593.6(3) Å3, Z = 8, T = 294.64(10) K, (MoK) = 0.08 mm-1, Dc = 1.228 g/cm3, F(000) = 1792.0 and GOOF = 1.036. 11836 reflections were measured (7.04 2 52.04°), and 4506 were unique (Rint = 0.0393, Rsigma = 0.0546) and used in all calculations. The final R = 0.0576 (I > 2 (I)) and wR = 0.1563 (all data). The preliminary biological tests show that the title compound has a good antitumor activity against Hela in vitro with the IC50 value of 4.71 mol/L.

Full Text

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HU Chun-Hong, WEI Dui-Dui, ZHAO Jing-Si, HU Min-Min, QI Sheng-Xiao, SUN Xiao-Fei, HE Dian

Institute of Medicinal Chemistry, School of Pharmaceutical Science, Lanzhou University, Lanzhou 730000, China

ABSTRACT

The title compound, ethyl (R)-2-(biphenyl-4-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxylate (C H N O), has been synthesized and its structure characterized by ^{1}H-NMR, ^{13}C-NMR, ESI-MS and single-crystal X-ray diffraction. It crystallizes in the orthorhombic system, space group Pbca with a = 16.9950(8), b = 9.5445(4), c = 28.3188(3) Å, V = 4593.6(3) Å^{3}, Z = 8, T = 294.64(10) K, (MoK) = 0.08 mm ^{1}, Dc = 1.228 g/cm^{3}, F(000) = 1792.0 and GOOF = 1.036. 11836 reflections were measured (7.04 2 52.04°), and 4506 were unique (Rint = 0.0393, Rsigma = 0.0546) and used in all calculations. The final R = 0.0576 (I > 2 (I)) and wR = 0.1563 (all data). Preliminary biological tests show that the title compound exhibits good antitumor activity against HeLa cells in vitro with an IC value of 4.71 mol/L.

Keywords: crystal structure; synthesis; antitumor activity

1. INTRODUCTION

Fascaplysin (Scheme 1), a natural product originally isolated from the marine sponge *Fascaplysinopsis Bergquist* [1], is known to possess antimicrobial, antimalarial, and anti-acetylcholinesterase activities [2-4]. Moreover, fascaplysin has been reported to block the growth of cancer cells, presumably through the inhibition of cyclin-dependent kinase 4 (CDK4), an early cell cycle enzyme misregulated in most cancers [5]. However, fascaplysin shows high toxicity at the cellular level, which could be attributed to its planar structure that allows it to bind and intercalate into DNA [6].

In view of these limitations, many efforts have been devoted to the synthesis of a series of non-planar fascaplysin-based derivatives [7-11]. Our laboratory is also engaged in this research. The main goal of our current study is to develop potent, non-planar CDK4-specific analogues of fascaplysin that do not intercalate or interact with the minor groove of double-stranded DNA. Our strategy involves opening the pyrrole ring of fascaplysin while retaining the -carboline scaffold to design derivatives with low toxicity and high efficiency. The title compound was designed as a novel fascaplysin derivative showing promising antitumor activity. In this paper, we describe the synthesis of this compound using tryptamine as the starting material (Scheme 2), and focus on its crystal structure and antitumor activity.

2. EXPERIMENTAL

2.1 Instruments and Reagents

All organic solvents and materials were obtained from commercial suppliers and used without further purification. Melting points were determined using an electrothermal PIF YRT-3 apparatus without correction. ^{1}H-NMR and ^{13}C-NMR

(ppm) spectra were measured on a Varian Mercury (400 MHz) spectrometer using TMS as the internal standard. Mass spectra were recorded on a VGZAB-HS (70 eV) spectrometer with an ESI source for ionization. Crystallographic data were collected using a Super Nova, Dual, Cu at zero, Eos diffractometer.

2.2 General Procedure

Tryptamine (1, 1.46 g, 10 mmol) was selected as the starting material for this experiment and was converted into compound 2 (white solid, 90% yield) via the Pictet-Spengler reaction [12, 13]. Compound 3 (0.520 g, 2.4 mmol) dissolved in 5 mL dry 1,4-dioxane and 2 N aqueous sodium hydroxide (2 mL) were added to a solution of compound 2 (0.51 g, 2 mmol) in a mixed solvent of 2 N aqueous sodium hydroxide (2 mL) and 1,4-dioxane (2 mL), followed by stirring at 0 °C for 20 minutes. The reaction mixture was then stirred at room temperature for 20 hours, acidified with concentrated hydrochloric acid to obtain a solid product, which was washed with water and dried to yield compound 4 (light yellow solid, 85% yield).

Subsequently, thionyl chloride (0.24 g, 2 mmol) was slowly added dropwise to a solution of compound 4 in 6 mL anhydrous ethanol and stirred for 30 minutes in an ice bath. The mixture was then stirred for 8 hours at room temperature and monitored by TLC. After filtration, the filtrate was concentrated under reduced pressure, and the residue was purified via silica gel column chromatography (ethyl acetate/petroleum ether = 1:3) to obtain compound 5 (white solid, 72% yield), m.p.: 201.4–201.8 °C. ^{1}H-NMR (400 MHz, DMSO-d , TMS, ppm): 11.05 (s, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 7.3 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.52 (t, J = 7.6 Hz, 2H), 7.43 (dt, J = 7.3, 6.9 Hz, 3H), 7.12 (t, J = 7.7 Hz, 1H), 7.01 (t, J = 7.4 Hz, 1H), 6.05 (s, 1H), 4.30–4.20 (m, 2H), 4.03 (dd, J = 13.9, 3.9 Hz, 1H), 3.59 (dd, J = 17.8, 7.8 Hz, 1H), 2.95–2.74 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d , TMS, ppm): 171.17, 169.09, 142.36, 139.77, 137.11, 134.82, 129.61, 128.55, 128.18, 127.47, 127.38, 127.19, 126.29, 122.23, 119.38, 118.58, 112.22, 108.93, 62.12, 53.42, 44.66, 21.63, 14.63. ESI-MS: Calcd. for C H N O [M+Na] 447.1787. Found: 447.1748.

Reagents and conditions: i) OHCCOOH, pH 3-4, H O/KOH, 8 h; ii) 1,4-dioxane/NaOH (2 mol/L) (1:1), 20 h; iii) SOCl , EtOH, reflux.

2.3 Structure Determination

A clear white crystal of the compound was obtained by recrystallization from methanol-ethyl acetate (v/v = 1:6). A crystal with dimensions of 0.23 mm × 0.20 mm × 0.15 mm was selected for X-ray diffraction analysis. Data were collected on a Super Nova, Dual, Cu at zero, Eos diffractometer equipped with graphite-monochromated MoK radiation (= 0.7107 Å) at 294.64(10) K. The structure was solved by direct methods with SHELXS [14] and refined with the SHELXL [15] refinement package using least-squares minimization. All hydrogen atoms were positioned geometrically and refined using a riding model,

with Uiso(H) = nUeq(carrier atom), where n = 1.5 for methyl hydrogen groups and 1.2 for C(H), C(H, H) and all N(H) groups. The final R (reflections) = 0.0551, wR = 0.1061 (w = 1/[^{2}(Fo^2) + (0.0492P)^2 + 1.21P], where P = (Fo^2 + 2Fc^2)/3), S = 1.036, (Δ/)max = 0.000, (Δ/)max = 0.12 and (Δ/)min = -0.18 e/Å^3.

2.4 Pharmacological Tests

The bioactivities of the title compound were evaluated by MTT assay using HeLa, A549, Hep-G2 and MCF-7 cell lines [16]. All cell lines were maintained at 37 °C in 5% CO in RPMI-1640 medium supplemented with 10% fetal calf serum. After 24 h, solutions of the compound at different concentrations were added to 96-well plates with fascaplysin as the positive control. After 48 h, 10 L of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 h. The medium was then removed and 150 L DMSO was added to dissolve the blue-colored formazan over 10 minutes. Absorbance was measured at 570 nm to calculate the inhibition rate (%) and determine the IC values.

3. RESULTS AND DISCUSSION

The structure of the title compound 5 was confirmed by ^{1}H NMR, ^{13}C NMR, EI-MS and elemental analysis. The ^{1}H NMR spectrum exhibited a characteristic singlet at 11.05 ppm assigned to the NH proton. The ^{13}C NMR spectrum displayed distinctive peaks at 171.17 and 169.09 ppm, corresponding to carbonyl carbons. The ESI-mass spectrum showed the molecular ion peak as the base peak at m/z 447.1748 [M+Na] . The structure was further determined by single-crystal X-ray diffraction analysis, which revealed that compound 5 crystallizes in the orthorhombic space group Pbca. Selected bond lengths, bond angles and torsional angles are shown in Tables 1 and 2, while hydrogen bond data are given in Table 3 .

The molecular structure with atomic numbering scheme is shown in Fig. 1 [Figure 1: see original paper], and Fig. 2 [Figure 2: see original paper] depicts the molecular packing and hydrogen bonds in the unit cell.

As shown in Table 1 , for compound 5, the bond lengths of C(2)-N(1) (1.377(3) Å), C(3)-N(1) (1.372(4) Å) and C(12)-N(2) (1.345(3) Å) are shorter than typical C-N bonds (1.47 Å) but longer than typical C=N bonds (1.35 Å), confirming that these bonds possess some double-bond or conjugated character. The bond angles of N(2)-C(12)-C(13) (117.4°), O(1)-C(12)-N(2) (121.7°) and O(1)-C(12)-C(13) (120.9°) sum to nearly 360°, indicating sp^2 hybridization of the C(12) atom. The crystal structure is non-planar, as evidenced by torsion angles C(2)-C(1)-C(25)-O(2) of -115.2° and N(2)-C(1)-C(25)-O(2) of 7.2°. Moreover, the dihedral angle between benzene rings C(13)-C(18) and C(19)-C(24) is 27.25°(96). Additionally, the indole ring plane (N(1)-C(2)) makes dihedral angles of 46.34°(78) and 19.10°(83) with benzene rings C(13)-C(18) and C(19)-C(24), respectively, demonstrating that these two benzene planes and the indole

ring plane are not coplanar.

Furthermore, intermolecular hydrogen-bonding interactions N(1)-H(1) ··· O(1) in the crystal link two molecules together. These are further connected to form an extensive network via C(11)-H(11B) ··· (H(11B)-Cg(5) = 2.95 Å), C(17)-H(17) ··· (H(17)-Cg(1) = 2.86 Å), C(17)-H(17) ··· (H(17)-Cg(3) = 2.95 Å) and weak - stacking interactions. Among these, the hydrogen bonds between nitrogen atoms as donors and oxygen atoms as acceptors, N(1)-H(1) ··· O(1), with an N(1) to O(1) distance of 2.907 Å, contribute significantly to structural stabilization.

4. BIOLOGICAL ACTIVITIES

Four human tumor cell lines (A549, HeLa, HepG2 and MCF-7) were used for cytotoxicity testing, and the in vitro antitumor activities of the title compound were evaluated by MTT assay with fascaplysin as a positive control. As described in Table 4 , the compound displays varying degrees of inhibition against A549, HeLa, HepG2 and MCF-7 with IC values of 10.45 ± 1.5, 4.71 ± 0.88, 10.85 ± 1.49 and 10.19 ± 1.80 mol/L, respectively. Notably, compound 5 exhibits superior inhibitory activity against HeLa cells. Therefore, this target derivative shows promise as a novel antitumor agent, and further structural optimization is currently underway.

Table 1. Selected Bond Lengths (Å) and Bond Angles (°) for the Target Compound

Bond/Angle	Value
O(1)-C(12)	1.231(3)
O(2)-C(25)	1.189(3)
O(3)-C(25)	1.327(3)
O(3)-C(26)	1.457(3)
N(1)-C(2)	1.377(3)
N(1)-C(3)	1.372(3)
N(2)-C(1)	1.455(3)
N(2)-C(11)	1.480(3)
N(2)-C(12)	1.345(3)
C(1)-C(2)	1.498(3)
C(1)-C(25)	1.519(3)
C(2)-N(1)	1.377(3)
C(3)-N(1)	1.372(4)
C(7)-C(8)	1.404(3)
C(8)-C(9)	1.424(3)
C(9)-C(10)	1.496(3)
C(10)-C(11)	1.510(4)
C(12)-C(13)	1.495(3)
C(12)-N(2)	1.345(3)

Bond/Angle	Value
C(25)-O(3)-C(26)	117.1(2)
C(3)-N(1)-C(2)	108.1(2)
C(9)-C(10)-C(11)	115.67(19)
O(1)-C(12)-N(2)	117.88(19)
C(1)-N(2)-C(11)	125.4(2)
O(1)-C(12)-C(13)	121.7(2)
C(12)-N(2)-C(1)	117.4(2)
N(2)-C(12)-C(13)	120.9(2)
C(12)-N(11)-C(11)	108.53(18)
C(14)-C(13)-C(12)	123.9(2)
N(2)-C(1)-C(2)	110.1(2)
C(14)-C(13)-C(18)	125.8(2)
N(2)-C(1)-C(25)	129.4(3)
C(18)-C(13)-C(12)	108.1(2)
C(2)-C(1)-C(25)	121.6(2)
C(15)-C(16)-C(19)	131.5(2)
N(1)-C(2)-C(1)	108.9(2)
C(9)-C(2)-N(1)	121.7(2)
C(9)-C(2)-C(1)	120.0(2)
N(1)-C(3)-C(4)	118.5(2)
N(1)-C(3)-C(8)	121.4(2)
C(2)-C(9)-C(10)	122.0(3)
C(8)-C(9)-C(10)	120.6(3)
C(17)-C(16)-C(19)	120.6(3)
C(20)-C(19)-C(16)	125.0(2)
C(24)-C(19)-C(16)	110.5(2)
C(19)-C(20)-C(21)	108.1(3)
O(2)-C(25)-C(1)	120.9(2)
O(3)-C(25)-C(1)	122.0(3)
O(2)-C(26)-C(27)	120.6(3)

Table 2 . Selected Torsional Angles (°) for the Target Compound

Torsional Angle	Value
N(1)-C(2)-C(9)-C(10)	-177.4(2)
N(2)-C(1)-C(2)-N(1)	167.1(3)
N(2)-C(1)-C(2)-C(9)	-8.1(3)
N(2)-C(1)-C(25)-O(2)	7.2(4)
C(12)-N(2)-C(11)-C(10)	-127.4(3)
C(12)-C(13)-C(14)-C(15)	-176.4(3)
C(12)-C(13)-C(18)-C(17)	177.5(2)
C(15)-C(16)-C(17)-C(18)	4.2(4)

Torsional Angle	Value
C(15)-C(16)-C(19)-C(20)	27.0(4)
C(1)-N(2)-C(11)-C(10)	-64.5(3)
C(1)-N(2)-C(12)-O(1)	1.6(4)
C(1)-N(2)-C(12)-C(13)	-179.5(2)
C(2)-C(1)-C(25)-O(2)	-115.2(3)
C(11)-N(2)-C(1)-C(25)	-84.4(3)
C(11)-N(2)-C(12)-O(1)	169.4(2)
C(11)-N(2)-C(12)-C(13)	-11.6(4)
C(25)-C(1)-C(2)-N(1)	-69.1(3)
C(12)-N(2)-C(1)-C(2)	-150.4(3)
C(12)-N(2)-C(1)-C(25)	84.6(3)
C(25)-O(3)-C(26)-C(27)	-179.8(3)
C(26)-O(3)-C(25)-C(1)	180.2(2)

Table 3. Hydrogen Bond Lengths (Å) and Bond Angles (°)

D-H ··· A	d(D-H)	d(H ··· A)	d(D ··· A)
N(1)-H(1) ··· O(1)#1	-	-	2.907(3)
C(11)-H(11B) ··· Cg(5)#2	-	-	3.777(3)
C(17)-H(17) ··· Cg(1)#3	-	-	3.459(3)
C(17)-H(17) ··· Cg(3)#4	-	-	3.756(3)

Symmetry codes: #1: -x, -y+1, -z+1; #2: x, 1+y, z; #3: x, -1+y, z; #4: x, -1+y, z.

Cg(1): N(1), C(3), C(8), C(9), C(2); Cg(3): C(3)-C(8); Cg(5): C(19)-C(24)

Table 4 . Inhibition of Cell Growth (M)

Compound	HepG-2	HeLa	A549	MCF-7
Compound 5	10.45 ± 1.5	4.71 ± 0.88	10.85 ± 1.49	10.19 ± 1.80
Fascaplysin	1.05 ± 0.08	0.24 ± 0.05	0.75 ± 0.04	0.93 ± 0.08

Fig. 1 [Figure 1: see original paper]. Structure of the title compound

Fig. 2 [Figure 2: see original paper]. Three-dimensional structure of the title compound showing hydrogen bonds

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Note: Figure translations are in progress. See original paper for figures.