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A new quinoline derivative with cytotoxic activity from *Streptomyces* sp. neau50

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ABSTRACT

A new quinoline derivative, methyl 8-(3-methoxy-3-methylbutyl)-2-methylquinoline-4-carboxylate (**1**), was isolated from the endophytic strain *Streptomyces* sp. neau50, and the structure was elucidated by extensive spectroscopic analysis. Compound **1** showed cytotoxicity against human lung adenocarcinoma cell line A549 with an IC_{50} value of 29.3 μg mL^{-1}.

Endophytes are microorganisms that spend the whole or part of their lifecycle colonizing inter-and/or intra-cellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease.[1] They can be found in virtually all terrestrial plants and play important roles for the growth of hosts. More recently, endophytes have been considered to be a prolific source of pharmacologically active natural products with potential medicinal or agrochemical applications.[2–5] As part of our continuous screening for more active secondary metabolites from endophytic microorganisms, a new quinoline derivative, methyl 8-(3-methoxy-3-methylbutyl)-2-methylquinoline-4-carboxylate (**1**), was obtained from the fermentation broth of endophytic actinomycete *Streptomyces* sp. neau50.[6–8] Details of the isolation, structure determination, and the biological activity of compound **1** are present here.

Compound **1** (Fig. 1, Table 1)[9] was obtained as yellow oil and showed an $[M+H]^+$ ion at *m/z* 302.1750 in the HRESIMS, which was consistent with the molecular formula $C_{18}H_{23}NO_3$ (calcd for 302.1751), and required 8 degrees of unsaturation. The IR spectrum of **1** demonstrated an ester absorption band at 1731 cm^{-1}. Its ^1H NMR spectrum exhibited two aliphatic methyl singlets at δ 1.30 (6H, s), an aromatic methyl at δ 2.76 (3H, s), two methoxy groups at δ 3.35 (3H, s), δ 4.02 (3H, s), two methylene signals at δ 1.89 (2H, m) and δ 3.27 (2H, m) and a singlet aromatic proton at δ 7.74 (1H, s) in addition to three aromatic protons at δ 7.47 (1H, dd, J = 8.4, 7.0 Hz), δ 7.57 (1H, d, J = 7.0 Hz), δ 8.47 (1H, dd, J = 8.4, 1.2 Hz). The ^{13}C NMR, DEPT135 and HMQC spectra of **1** displayed 5 methyl resonances (two oxygenated ones) at δ_C 25.4 (C-4′, 5′, 2-Me), 49.3 (C-6′), 52.5 (C-10), two methylenes at δ_C 40.7 (C-2′), 26.8 (C-1′), 4 sp^2 methines at δ_C 122.5 (C-3), 123.2 (C-5), 126.8 (C-6), 129.0 (C-7), 5 sp^2 quaternary carbons at δ_C 123.3 (C-4a), 135.3 (C-4), 141.7 (C-8), 147.4 (C-8a), 156.9 (C-2) in addition to one carbonyl at 167.2 (C-9). The presence of a methocarbonyl unit in **1** was evident from the methyl resonance at δ_H 4.02 (H_3-10) to a carbonyl at δ_C 167.2 (C-9) in the HMBC spectrum (Fig. 2). The HMBC correlations between δ_H 1.30 (H-4′, 5′) and C-3′ (δ_C 74.8), C-2′ (δ_C 40.7), δ_H 3.35 (H-6′) and C-3′ (δ_C 74.8) in combination with the crossing peak of δ_H 1.89 (H-2′) and δ_H 3.26 (H-1′) in the ^1H–^1H COSY spectrum (Fig. 2) illustrated the presence of 3-methoxy-3-methylbutyl moiety. Except the aromatic methyl at δ_H 2.75/δ_C 25.3, the remaining 9 aromatic carbons and the N atom revealed in the molecular formula of $C_{18}H_{23}NO_3$ in the HRESIMS suggested **1** had the quinoline skeleton. By detailed comparison of the ^1H and ^{13}C NMR data of **1** with those of 4-quinoline carboxylic acid[11], the similar NMR data of the aromatic moieties further confirmed the above conclusion. The full assignment of **1** was supported by the HMBC correlations. The observed long range correlations from δ_H 2.76 (2-Me) to δ_C 156.9 (C-2) and 122.5 (C-3) in the HMBC spectrum showed that a methyl group was attached to C-2. The HMBC correlation from δ_H 7.74 (H-3) to δ_C 167.2 (C-9) indicated that the carbonyl unit was in C-4. As shown in Figure 2, the linkage of the 3-methoxy-3-methylbutyl moiety with the quinoline skeleton was established by the correlations between δ_H 1.89 (H_2-2′) and δ_C 141.7 (C-8), δ_H 3.27 (H_2-1′) and δ_C 141.7 (C-8), 129.0 (C-7), 147.4 (C-8a). Consequently, the structure of **1** was established to be methyl 8-(3-methoxy-3-methylbutyl)-2-methylquinoline-4-carboxylate.

The antitumor and antimicrobial activities were evaluated using the CCK-8 colorimetric method[12] and the agar diffusion

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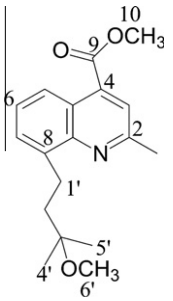

Figure 1. Structure of compound **1**.

Table 1
^{1}H and ^{13}C NMR data of compound **1** in $CDCl_3$

Position	δ_H (J in Hz)	δ_C
2		156.9 s
3	7.74 (1H, s)	122.5 d
4		135.3 s
4a		123.3 s
5	8.47 (1H, dd, 8.4, 1.2)	123.2 d
6	7.47 (1H, dd, 8.4, 7.0)	126.8 d
7	7.57 (1H, d, 7.0)	129.0 d
8		141.7 s
8a		147.4 s
9		167.2 s
10	4.02 (3H, s)	52.5 q
1′	3.27 (2H, m)	26.8 t
2′	1.89 (2H, m)	40.7 t
3′		74.8 s
4′	1.30 (3H, s)	25.4 q
5′	1.30 (3H, s)	25.4 q
6′	3.35 (3H, s)	49.3 q
2-CH_3	2.76 (3H, s)	25.4 q

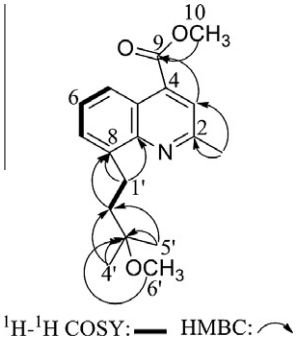

Figure 2. Key HMBC and ^{1}H–^{1}H COSY correlations for compound **1**.

assay[13,14], respectively. The results demonstrated that the compound **1** showed no activity against the *Staphylococcus epidermidis* or *Candida albicans* even at a concentration of 10 mg mL^{-1}, however, it exhibited potent inhibitory activity against human lung adenocarcinoma cell line A549 with an IC_{50} value of 29.3 μg mL^{-1}. Although compound **1** possessed a simple structure and showed a weaker cytotoxicity than positive control adriamycin (4.2 μg mL^{-1}), the 3-methoxy-3-methylbutyl group rarely occurs in natural and synthetic compounds,[15,16] especially in quinolines,[17] implying that **1** is an interesting compound needed further investigation. Furthermore, quinolines display a wide range of biological properties, including antioxidant, antiproliferation, antiinflammation, anticancer activities, etc., and various substituted quinolines were synthesized.[18–20] Therefore, further studies on the bioactivity of compound **1** will be undertaken in laboratory. In conclusion, the results described in this study suggest that compound **1** could be a potent lead as antitumor agents.

Acknowledgements

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6. *Strain material*: The producing strain *Streptomyces* sp. neau50 was isolated from healthy soybean root in Harbin, Heilongjiang province, China, by using moist incubation and desiccation (MI&D) method[10] and was characterized according to the 16S rRNA gene sequence (Accession No: GQ494994 in GenBank).
7. *Fermentation*: The strain *Streptomyces* sp. neau50 was maintained on the medium containing glucose 10 g, maltose 3 g, yeast extract 3 g, $K_2HPO_4{\cdot}3H_2O$ 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, NaCl 0.5 g, KNO_3 1 g and agar 20 g in 1.0 L tap water, pH 7.0. The seed medium consisted of glucose 4 g, maltodextrin 10 g, yeast extract 4 g, $CaCO_3$ 2 g in 1.0 L water and pH 7.2–7.4. All the media were sterilized at 121 °C for 20 min. Slant culture was incubated for 6–7 days at 28 °C. Fermentation was carried out in 50 L first seed fermentor (containing 30 L fermentation broth), 500 L second fermentor (containing 300 L fermentation broth) successively. The producing medium was composed of glucose 1%, soluble amylum 4%, yeast extract 0.5%, soybean powder 2.5%, peptone 0.5%, $CaCO_3$ 0.2%, $MgSO_4{\cdot}7H_2O$ 0.8%, $FeSO_4{\cdot}7H_2O$ 0.6%, $ZnSO_4{\cdot}7H_2O$ 0.2%, $MnSO_4{\cdot}H_2O$ 0.2%, $CoCl_2{\cdot}6H_2O$ 0.05%, $Na_2MoO_4{\cdot}2H_2O$ 0.2%, and pH 7.0 before sterilization. The fermentation was conducted at 28 °C for 7 days stirred at 100 r min^{-1} with an aeration rate of 30 m^3 of air per hour.
8. *Extraction and isolation*: A total of 300 L broth was filtered. The resulting cake was washed with water, and both filtrate and wash were discarded. The washed cake was extracted twice for about 24 h with 100 L of EtOH. The EtOH extract was diluted to about 30% EtOH and subjected to a HP-20 resin column eluting with 30%, 40%, 50%, 60%, 70%, 80% EtOH (each concentration eluted 2 bed volumes). The eluents eluting with 70% and 80% EtOH were pooled and concentrated in vacuo at 50 °C to give a mixture. Then one-fifth of the mixture was subjected to chromatography on a silica gel column, and successively eluted with a stepwise gradient of petroleum ether/acetone (100:0–90:10, v/v) to afford four fractions (fraction I–IV) based on the TLC profiles. The fraction III was chromatographed on a silica gel column using petroleum ether/acetone (90:10–60:40, v/v). During this step, three fractions (A1-A3) were obtained. Fraction A3 was subjected to a Sephadex LH-20 and eluted with EtOH and detected by TLC to give four fractions (B1–B4). Fraction B2 was separated by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm i.d.; 1.5 mL/min; 254 nm; Agilent, Palo Alto, CA, USA) eluting with CH_3CN/H_2O (80:20, v/v) to afford compound **1** (t_R 9.37 min, 18 mg).
9. *Methyl 8-(3-methoxy-3-methylbutyl)-2-methylquinoline-4-carboxylate* (**1**): Yellow oil; UV λ_{max} nm (log ε) in EtOH: 209 (4.14), 245 (4.09), 319 (3.49); IR ν_{max} (KBr) cm^{-1}: 3443, 2922, 2851, 1731, 1647, 1595, 1383; ESI-MS *m/z*: 302 $[M+H]^+$; HRESIMS *m/z*: 302.1750 $[M+H]^+$ ($C_{18}H_{24}NO_3$ requires 302.1751); for ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1.
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12. *Cytotoxicity assay*: Human lung adenocarcinoma cell line A549 was routinely cultured in DMEM containing 10% calf serum at 37 °C for 4 h, in a humidified atmosphere of 5% CO_2 incubator. The adherent cells at their logarithmic growth stage were digested, and were inoculated onto 96-well culture plate at a density of 1.0×10^4 cells/well for the determination of proliferation. Test samples were added to the medium, and incubation was continued for 72 h. Coloration substrate, cell counting kit-8 (CCK-8, Dojindo, Tokyo, Japan), was added to the medium followed by further incubation for 3 h. Absorbance at 450 nm with a 600 nm reference was measured thereafter. Media and DMSO control wells, in which compound was absent, were included in all the experiments in order to eliminated the influence of DMSO. Adriamycin (Zhejiang Hisun Pharmaceutical Co., Ltd, China) was used as the positive control. The inhibitory rate of cell proliferation was calculated by the following formula: Growth inhibition (%) = $(OD_{control} - OD_{treated})/OD_{control} \times 100\%$. The cytotoxicity of compound on tumor cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells).
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