Synthesis and Anticancer Activity Of One Novel Thiopyrano[4,3-*d*]Pyrimidine Derivative Bearing A Indole-hydrazone

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Abstract. A novel thiopyrano[4,3-*d*]pyrimidin derivative bearing a indole-hydrazone was synthesized and structure was confirmed by ^{1}H NMR and MS. Moreover, this compound was evaluated for its cytotoxicity against the H460 cell lines and inhibitory activity against mTOR kinase at a concentration of 10uM. The result indicated that this compound exhibited moderate activity against cancer cell line as well as mTOR kinase. At 10μM level, the inhibition rate of compound **1** against H460 cell lines and mTOR kinase was 39.6% and 47.0%.

Introduction

Every year, there were millions of people died of cancer in the world. Moreover, the latest data from International Agency for Research on Cancer (IARC), indicated that the cancer incidence, mortality, and prevalence were on the rise. And the agency based on the GLOBOCAN 2012 estimates predicted that in 2025 the number of new cancer cases per year will reach 19.3 million [1]. Therefore, looking for safe and effective cancer treatments remains desirable.

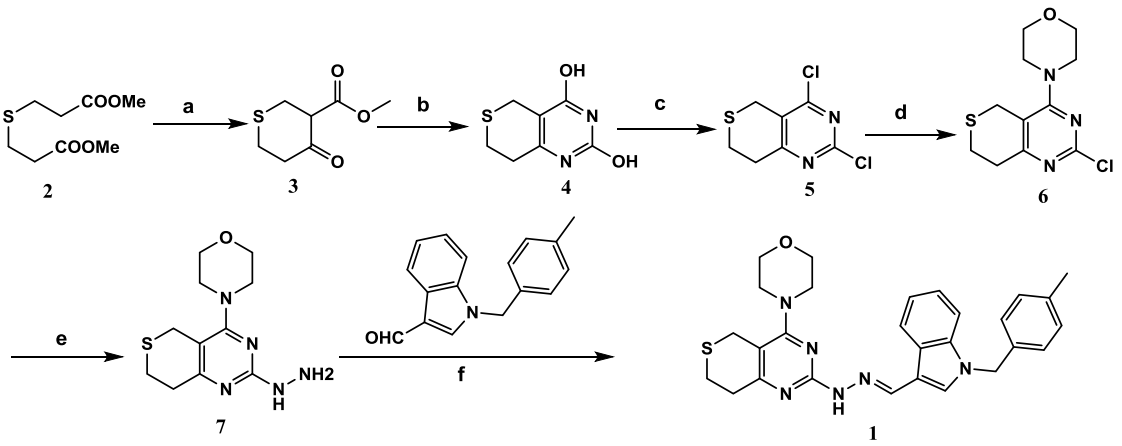
Previous studies have found that the PI3K-Akt-mTOR signalling pathway plays an important role in the development of tumors [2].Therefore, developing inhibitors of this pathway has become one of the research hotspots in molecular targeted therapy for the treatment of human cancer [3]. After years' effort of the researchers, many small molecule inhibitors of this pathway were reported such as GDC-0941[4-10]. Compound BMCL-200908069-1 is an mTOR inhibitor with IC_{50} value of 0.27μM [5]. Structure-activity relationships (SARs) showed that aryl hydrazones and morpholine-triazine moieties were essential for the mTOR activity. Therefore, in this study, we introduced an indole-hydrazone to design and synthesis a novel thiopyrano[4,3-*d*]pyrimidine derivative(**1**). And the target compound was evaluated for its cytotoxicity against H460 cell lines and inhibitory activity against mTOR kinase.

Materials and methods

All melting points were obtained on a Büchi Melting Point B-540 apparatus and were uncorrected. NMR spectra were performed using Bruker 400 MHz spectrometerswith TMS 210 as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS. All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified. Yields were not optimized.TLC analysis was carried out on silica gel plates GF254 .

Synthesis of compounds

The structures and the synthetic route were shown in Scheme 1.



Scheme.1 The synthetic route of compound **1**

Reagents and conditions: (a) NaH, THF, rt, 3 h; (b) 3 equiv urea, C_2H_5ONa, EtOH, reflux, 24 h; (c) $POCl_3$, reflux, 3 h; (d) 2.4 equiv morpholine, MeOH, rt, 1.5 h; (e) 80% $NH_2NH_2.H_2O$, 85 ℃, 1 h; (f) EtOH, reflux, 2h.

Methyl 4-oxotetrahydro-2*H*-thiopyran-3-carboxylate (3). To the mixture of NaH (60%, 8.19g, 0.205mol) and anhydrous THF (100 mL), a solution of dimethyl 3,3'-thiodipropionate (31.8 g, 0.154 mol) in anhydrous THF (40 mL) was added via a dropping funnel over 1 h at room temperature under N_2. After stirring for 2 h at r.t, the reaction was complete by TLC analysis (20%EtOAc in petroleum ether). The mixture was transferred to a beaker, dilute hydrochloric acid was added slowly with stirring maintaining the temperature below 20 ℃, the final pH was 6–7. Then extracted with CH_2Cl_2 (3×50mL). The combined organic layers were washed with brine(3×50mL), and dried over Na_2SO_4, the filtrate was concentrated under reduced pressure to afford **3** as a yellow viscous oil (26.8g, 99.8%) and was used for next step without further purification. ESI-MS m/z: $[M+H]^+$ 175.2

7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine-2,4-diol (4). Finely cut sodium metal (16.2g, 0.704mol) was added into 300mL anhydrous ethanol at ice bath with stirring. After the sodium metal was completely consumed, urea (32.7g, 0.545mol) and **3** (31.3g, 0.180mol) was added to the solution. The mixture was refluxed for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was poured onto ice water , then adjusted the pH to 6-7 with acetic acid under ice bath , filtration, the filter cake was washed with ice-water, dried to obtain a white solid (13.6g, 41.2%). m.p. >300 ℃. ESI-MS *m/z*: $[M-H]^-$ 183.2, ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 10.75 (s, 1H), 3.39 (s, 2H), 2.80 (t, *J* = 5.8 Hz, 2H), 2.57 (t, *J* = 5.6 Hz, 2H).

2,4-dichloro-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidine (5). A mixture of **4** (3.03g, 0.016 mol), $POCl_3$ (20mL) was refluxed for 3 h. The reaction mixture was slowly added to ice/water with vigorous stirring yielding a precipitate. The mixture was then filtered to yield **5** as a yellow solid (3.12g, 85.7%). m.p. 87.1-87.9 ℃. ^{1}H NMR (400 MHz, CDCl3) δ 3.81 (s, 2H), 3.23 (t, *J* = 5.9 Hz, 2H), 2.96 (t, *J* = 6.0 Hz, 2H).

4-(2-chloro-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4-yl)morpholine (6). To the mixture of **5** (7.40 g, 0.033 mol) and MeOH (150 mL), morpholine (7 mL, 0.08 mol) was added drop-wise at room temperature. The reaction mixture then was stirred at room temperature for 1.5 h. The mixture was then filtered, washed with water and MeOH, to yield the title compound as a white solid (6.84 g, 75.3%). m.p. 161.1-162.4 ℃. ESI-MS *m/z*: $[M+H]^+$ 272.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 2H), 3.23 (t, J = 5.9 Hz, 2H), 2.96 (t, J = 6.0 Hz, 2H).

4-(2-hydrazinyl-7, 8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine(7). A solution of 80% hydrazine hydrate ($NH_2NH_2.H_2O$, 110 mL) and compound **6** (3.47g, 0.013mol) was stirred at 85 ℃ for 1h. The precipitate was collected by filtration and washed with water, and dried to give **7** as white solid (1.73 g, 50.7%). m.p. 172.1-173.2 ℃. ESI-MS m/z: $[M+H]^+$ 268.2. ^{1}H NMR (400 MHz, DMSO) δ 7.66 (s, 1H), 4.07 (s, 2H), 3.73 – 3.66 (m, 4H), 3.54 (s, 2H), 3.18 (s, 4H), 2.89 (t, *J* = 6.1 Hz, 2H), 2.82 (t, *J* = 6.0 Hz, 2H).

(*E*)-4-(2-(2-((1-(4-methylbenzyl)-1*H*-indol-3-yl)methylene)hydrazinyl)-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]pyrimidin-4-yl)morpholine(1). A mixture of compound **7** (53mg,0.20 mmol),1-(4-methylbenzyl)-1*H*-indole-3-carbaldehyde (0.20mmol) and one drop of glacial acetic acid in absolute ethanol (10 mL) was refluxed for 2h. The mixture was cooled, separated by filtration and washed with EtOH to afford the target compound as pale yellow solid (77.6 mg, 78%). m.p. 140.5-142.8 ℃. ESI-MS m/z: $[M+H]^+$ 499.2. ^{1}H NMR (400 MHz, DMSO) δ 10.60 (s, 1H), 8.49 (d, *J* = 7.5 Hz, 1H), 8.24 (s, 1H), 7.77 (s, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.17 – 6.99 (m, 6H), 5.36 (s, 2H), 3.76 (s, 4H), 3.59 (s, 2H), 3.34 (s, 4H), 2.91 (d, *J* = 5.9 Hz, 4H), 2.23 (s, 3H).

Biological Evaluation

Cytotoxicity assay in vitro . The cytotoxic activities of compound **1** were evaluated with human lung cancer (H460) cell lines by the standard MTT assay in vitro, with GDC-0941 as the positive controls. The cancer cell lines was cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS).Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO2 at 37 ℃ for 24 h. The test compound at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5μg/mL and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader.

mTOR Kinase Assay [10]. The mTOR kinase activity of the compound **1** was determined using LANCE® Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay following the manufacturer's instructions, with compounds mTOR inhibitors BMCL-200908069-1 and PI103 as positive controls. Briefly, mTOR enzyme (10 nM), ATP (21.6 uM), ULight-4E-BP1 Peptide (100 nM) and test compounds were diluted in kinase buffer (50 mM HEPES pH 7.5,1 mM EGTA, 3 mM MnCl2, 10 mM MgCl2, 2 mM DTT and 0.01%Tween-20). The reactions were performed in white 384-well Optiplates (PerkinElmer, MA, USA) at room temperature for 1 h and stopped by adding EDTA to 16 mM. Eu-anti-phospho-4E-BP1 (Thr37/46) Antibody (PerkinElmer, MA, USA) was then added to each well to a final concentration of 2 nM. The intensity of the light emission was measured with an EnVision® Multilabel Reader (PerkinElmer, MA, USA) in TR-FRET mode (excitation at 320 nm and emission at 665 nm).

Results

The in vitro anticancer activity of the target compound was studied on the H460 cell and mTOR kinase. The test result was listed in Table 1.

Table 1 The inhibition rate of H460 cell and **mTOR** kinase of **1** at 10μM levels

Compound	Inhibition rate at 10μM (%)	
	H460	**mTOR**
1	39.6	47.0
BMCL-200908069-1	-	76.1
PI103	-	98.6
GDC-0941	58.7	-

From Table 1, we could see that at a concentration of 10uM, the inhibition rate of compound **1** against H460 cell lines was 39.6%, and the inhibition rate against mTOR kinase was 47.0%. Although the inhibition rate is lower than positive controls GDC-0941, BMCL-200908069-1, and PI103, it still indicate that the introduction of indole-hydrazone is essential to this kind of compounds. This result suggested that thiopyrano[4,3-*d*]pyrimidine derivative bearing a indole-hydrazone is worthy to study further.

Conclusions

In a conclusion, a novel of thiopyrano[4,3-*d*]pyrimidine derivative bearing a indole-hydrazone moiety is designed, synthesized and evaluated for their activity against the cancer cell lines (H460) and mTOR kinase. The result indicated that the target compound exhibited moderate cytotoxicity and inhibitory activity against H460 cell lines and mTOR kinase, respectively. Further modification and SAR studies based on thiopyrano[4,3-*d*]pyrimidine derivatives are ongoing in our group.

Acknowledgments

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