Design and Synthesis of 7-Azaindole Derivatives and Their Antitumor and Analgesic Activities

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(Received on 3rd June 2019, accepted in revised form 12th October 2019)

Summary: To develop effective anti-tumor and analgesic drugs, a series of novel 7-azaindole derivatives were designed and synthesized through a four-step reaction. 18 target compounds were obtained and characterized through Nuclear Magnetic Resonance and High Resolution Mass Spectrometry. Their anti-proliferative activities and analgesic effect were evaluated. When the 1-position was a methylsulfonyl group and the 5-position was a nitro group, compound **4f** demonstrated the best activity. Furthermore, there was a dramatic difference between the IC_{50} values of compound **4f** in tumor and in healthy cell line. The IC_{50} values of compound **4f** in MCF7 breast cancer cell line was 5.781 μmol/L and 8.077 μmol/L in HepG2 hepatoma carcinoma cell line, but more than 100 μmol/L in HL7702 liver cell line. Preliminary results showed that compounds **3a**, **3g** and **4i** had significant analgesic effects in mice, which were stronger than aspirin. These compounds have good prospects for new drug development.

Keywords: Analgesic activity, antitumor activity, 7-azaindole derivatives, synthesis

Introduction

Indole derivatives are an important class of heterocyclic compounds, and a large number of studies have shown that indole derivatives demonstrate extremely broad physiological activities [1-3]. They are used in a variety of applications, including pesticides [4], medicine, dyes [5], perfumes, and antifouling coatings. Their unique structure determines their antibacterial [6-7], anti-inflammatory and analgesic [8-10], anti-tumor[11-16], anti-malarial[17-18], anti-viral[19-22], antioxidant[23] and anticonvulsant properties[24-25]. The indole unit is widely present in the structure of anti-tumor drugs; for example, camptothecin contains azaindole in its structure. Variolins and meriolins[26-27] are natural compounds which contain the 7-azaindole moiety and have been shown to induce tumor cell apoptosis by inhibiting cyclin-dependent kinases. Lee *et al* designed, synthesized and evaluated 7-azaindole derivatives, and showed that the derivatives had significant antitumor activity [28-32]. Pang and Wu synthesized pyrrole derivatives, and showed that they had an analgesic effect [33-34].

In the current study, 7-azaindole was used as a raw material. When the 4-position was occupied by a chloro group and the 1-position by a different group, the target compounds **3a**~**3i** were designed and synthesized. When the 4-position was occupied by a chloro group, the 5-position by a nitro group and the 1-position by a different group, the target compounds **4a**~**4i** were designed and synthesized. Initially, 7-azaindole reacted with m-chloroperoxybenzoic acid (m-CPBA) to obtain 7-azaindole-N-oxide·3-chlorobenzoate (**1**); this compound reacted with phosphorus oxychloride to form 4-chloro-7-azaindole (**2**); then an acylation reaction occurred to obtain 4-chloro-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (**3i**); finally, 4-chloro-5-nitro-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (**4i**) was synthesized through nitrification[35]. A total of 18 compounds were synthesized, and all compounds were evaluated for their activity in inhibiting proliferation in MCF7, HepG2, TE1, and HL7702 cells in vitro and their analgesic effect in mice.

Experimental

General Information

Methyl tert-butyl ether (MTBE), 7-azaindole, m-CPBA, phosphorus oxychloride, 4-dimethylpyridine (DMAP), triethylamine (Et_3N), tetrabutylammonium nitrate (TBAN), trifluoroacetic anhydride (TFAA), and camptothecin were purchased from Energy Chemical, China. Unless otherwise stated, all materials obtained from commercial suppliers were used without further purification. The MCF7 human breast cancer cell line, the HepG2 human hepatoma cell line, the TE1 human esophageal cancer cell line, and the HL7702 human liver cell line were purchased from ATCC, USA. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Hyclone, USA. Fetal bovine serum

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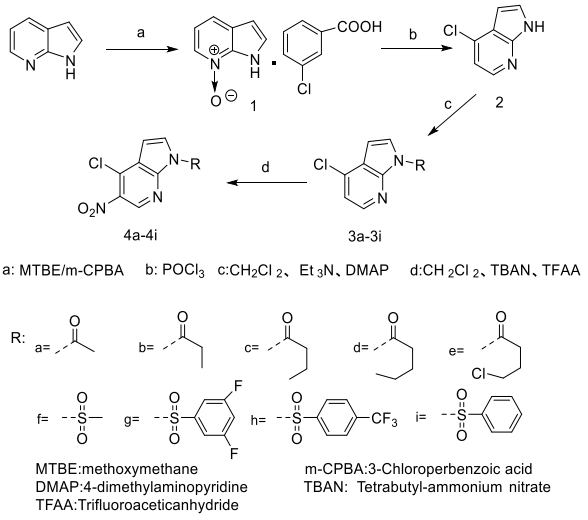
was purchased from Gibco, USA. Trypsin (2.5 g/L) was obtained from Genview, USA. Aspirin enteric-coated tablets (CFDA approval number J20130078) were purchased from Bayer Health Care Co., Ltd. Kunming mice were provided by Changchun high-tech Medical Animal Experimental Research Center (license number: SCXK 2015-0001). The cell viability assay kit was purchased from Promega, USA. A Bruker Avance 500 MHz NMR spectrometer (Bruker, Germany) was used. An API 4000 electrospray ionization mass spectrometer (AB SCIEX, USA) was used, along with a full-wavelength multi-function microplate reader (Biotek, USA). An MCO-15AC CO_2 constant temperature incubator was used (SANYO, Japan). All animal experiments are performed according to Laboratory animal-Guideline for ethical review of animal welfare (National standards of People's Republic of China GB/T 35892-2018).

Synthesis



Scheme-1: Synthetic routes of the title compounds.

Synthesis of compound **1**

Both MTBE (5 mL) and 7-azaindole (0.55 g, 4.7 mmol) were added to the reaction flask and dissolved by stirring. m-CPBA (1.1 g, 6.4 mmol) was added in batches. The reaction temperature was maintained below 20°C, and the reaction continued for 3 h, after which 9 mL of n-heptane was added under stirring, and a large amount of solid precipitated. The mixture was filtered and dried to give 1.25 g of compound **1**.

Synthesis of compound **2**

Phosphorus oxychloride (4.0 g, 26.1 mmol) and compound **1** (1.2 g, 4.1 mmol) were added to the reaction flask, and the reaction temperature was increased to 90~100 °C and stirred for 24 h. Phosphorus oxychloride was evaporated and the sample was diluted using 2 mL of CH_3CN, before 2 mL of H_2O were slowly added. The sample was adjusted to pH 9 using ammonia water, and the mixture was filtered and dried to give 0.53 g of compound **2**.

Synthesis of compounds **3a**~**3i**

Compound **2** (0.52 g, 3.4 mmol), DMAP (0.05 g, 0.4 mmol), and CH_2Cl_2 (5 mL) and Et_3N (0.55 g, 5.4 mmol) were added to a reaction flask. Benzenesulfonyl chloride (0.70 g, 4.0 mmol) was slowly added and the temperature was maintained below 30°C. The reaction mixture was then stirred at room temperature for 24 h, the pH was adjusted to 1~2 using 2% HCl, and the sample was extracted 3 times using CH_2Cl_2. The sample was then combined with the organic phases, and washed using a saturated $NaHCO_3$ solution and H_2O. The sample was then dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated and purified to give 0.75 g of compound **3i**. Compounds **3a**~**3h** used the same synthetic method.

Synthesis of compounds **4a**~**4i**

Compound **3i** (0.73 g, 2.5 mmol), TBAN (1.0 g, 3.3 mmol) and CH_2Cl_2 (2 mL) were added to a reaction flask. TFAA (0.90 g, 4.3 mmol) was slowly added dropwise at 0 °C. After the addition, the mixture was stirred at room temperature for 48 h, and evaporated to dryness. CH_3CN (1 mL) was added, and the sample was stirred, filtered and dried to give 0.50 g of compound **4i**. Compounds **4a**~**4h** used the same synthetic method.

7-Hydroxy-1H-pyrrolo[2,3-b]pyridinium 3-chlorobenzoate (**1**): off-white solid, yield 92%. m.p.: 132.1–132.5 °C. ^{1}H-NMR ($CDCl_3$) δ 13.25 (s, 1H), 12.73 (s, 1H), 8.32 (d, *J*=6.2 Hz, 1H), 8.10 (s, 1H), 7.99 (d, *J*=7.7 Hz, 1H), 7.83 (d, *J*=7.9 Hz, 1H), 7.52 (d, *J*=8.0 Hz, 1H), 7.49 (d, *J*=3.3 Hz, 1H), 7.37 (t, *J*=7.9 Hz, 1H), 7.13 (dd, *J*=7.6, 6.5 Hz, 1H), 6.62 (d, *J*=3.3 Hz, 1H).

4-Chloro-1H-pyrrolo[2,3-b]pyridine (**2**): light gray solid, yield 85%. m.p.: 111.9–114.7 °C. ^{1}H-NMR ($CDCl_3$) δ 11.34 (s, 1H), 8.23 (d, *J*=5.2 Hz, 1H), 7.42 (d, *J*=2.3 Hz, 1H), 7.14 (d, *J*=5.2 Hz, 1H), 6.63 (d, *J*=2.8 Hz, 1H).

1-(4-chloro-1H-pyrrolo[2,3-b]pyridin-1-yl)ethan-1-one (**3a**): white solid, yield 38%. m.p.: 88.1–90.1 °C. ^{1}H-NMR ($CDCl_3$) δ 8.25 (d, *J*=5.2 Hz, 1H), 8.01 (d, *J*=4.0 Hz, 1H), 7.21 (d, *J*=5.2 Hz, 1H), 6.70 (d, *J*=4.0 Hz, 1H), 3.04 (s, 3H). MS (ESI) calcd. for $C_9H_7ClN_2ONa$ $[M+Na]^+$: 217.0139, found: 217.0142.

1-(4-chloro-1H-pyrrolo[2,3-b]pyridin-1-yl)propan-1-one (**3b**): white solid, yield 48%. m.p.: 80.3–82.2 °C. ^{1}H-NMR ($CDCl_3$) δ 8.25 (d, *J*=5.3 Hz, 1H), 8.04 (d, *J*=4.1 Hz, 1H), 7.21 (d, *J*=5.3 Hz, 1H), 6.71 (d, *J*=4.1 Hz, 1H), 3.54 (q, *J*=7.3 Hz, 2H), 1.34 (t, *J*=7.3 Hz, 3H). MS (ESI) calcd. for $C_{10}H_9ClN_2ONa$ $[M+Na]^+$: 231.0296, found: 231.0300.

1-(4-chloro-1H-pyrrolo[2,3-b]pyridin-1-yl)butan-1-one (**3c**): yellow solid, yield 40%. m.p.: 29.8–30.3 °C. ^{1}H-NMR ($CDCl_3$) δ 8.25 (d, *J*=5.3 Hz, 1H), 8.03 (d, *J*=4.1 Hz, 1H), 7.21 (d, *J*=5.3 Hz, 1H), 6.70 (d, *J*=4.1 Hz, 1H), 3.50 (t, *J*=7.3 Hz, 2H), 1.86 (d, *J*=7.4 Hz, 2H), 1.08 (t, *J*=7.4 Hz, 3H). MS (ESI) calcd. for $C_{11}H_{11}ClN_2ONa$ $[M+Na]^+$: 245.0452, found: 245.0456.

1-(4-chloro-1H-pyrrolo[2,3-b]pyridin-1-yl)pentan-1-one (**3d**): white solid, yield 57%. m.p.: 51.5–52.5 °C. ^{1}H-NMR ($CDCl_3$) δ 8.26 (d, *J*=5.3 Hz, 1H), 8.03 (d, *J*=4.1 Hz, 1H), 7.21 (d, *J*=5.3 Hz, 1H), 6.70 (d, *J*=4.1 Hz, 1H), 3.52 (t, *J*=7.4 Hz, 2H), 1.85–1.76 (m, 2H), 1.50 (d, *J*=7.5 Hz, 2H), 0.99 (t, *J*=7.4 Hz, 3H). MS (ESI) calcd. for $C_{12}H_{13}ClN_2ONa$ $[M+Na]^+$: 259.0609, found: 259.0612.

4-chloro-1-(4-chloro-1H-pyrrolo[2,3-b]pyridin-1-yl)butan-1-one (**3e**): white solid, yield 65%. m.p.: 42.3–43.2 °C. ^{1}H-NMR ($CDCl_3$) δ 8.26 (d, *J*=5.3 Hz, 1H), 8.01 (d, *J*=4.1 Hz, 1H), 7.22 (d, *J*=5.3 Hz, 1H), 6.71 (d, *J*=4.1 Hz, 1H), 3.76–3.70 (m, 4H), 2.35–2.29 (m, 2H). MS (ESI) calcd. for $C_{11}H_{10}Cl_2N_2ONa$ $[M+Na]^+$: 279.0062, found: 279.0065.

4-chloro-1-(methylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (**3f**): yellow solid, yield 16%. m.p.: 96.7–98.1 °C. ^{1}H-NMR ($CDCl_3$) δ 8.36 (d, *J*=5.3 Hz, 1H), 7.67 (d, *J*=4.0 Hz, 1H), 7.29 (d, *J*=5.3 Hz, 1H), 6.73 (d, *J*=4.0 Hz, 1H), 3.58 (s, 3H). MS (ESI) calcd. for $C_8H_7ClN_2O_2SNa$ $[M+Na]^+$: 252.9809, found: 252.9815.

4-chloro-1-((3,5-difluorophenyl)sulfonyl)-1H-pyrrolo[2,3-b]pyridine (**3g**): yellow solid, yield 60%. m.p.: 122.2–124.0 °C. ^{1}H-NMR ($CDCl_3$) δ 8.28 (d, *J*=5.3 Hz, 1H), 7.76–7.68 (m, 2H), 7.65 (d, *J*=4.1 Hz, 1H), 7.20–7.17 (m, 1H), 6.99–6.95 (m, 1H), 6.69 (d, *J*=4.1 Hz, 1H). MS (ESI) calcd. for $C_{13}H_7ClF_2N_2O_2SNa$ $[M+Na]^+$: 350.9777, found: 350.9780.

4-chloro-1-((4-(trifluoromethyl)phenyl)sulfonyl)-1H-pyrrolo[2,3-b]pyridine (**3h**): white solid, yield 56%. m.p.: 142.0–143.2 °C. ^{1}H-NMR ($CDCl_3$) δ 8.35 (d, *J*=8.2 Hz, 2H), 8.31 (d, *J*=5.2 Hz, 1H), 7.84–7.68 (m, 3H), 7.22 (d, *J*=5.2 Hz, 1H), 6.74 (d, *J*=3.9 Hz, 1H). MS (ESI) calcd. for $C_{14}H_8ClF_3N_2O_2SNa$ $[M+Na]^+$: 382.9839, found: 382.9846.

4-chloro-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (**3i**): brown solid, yield 75%. m.p.: 109.3–110.7 °C. ^{1}H-NMR ($CDCl_3$) δ 8.31 (d, *J*=5.3 Hz, 1H), 8.21–8.16 (m, 2H), 7.77 (d, *J*=4.0 Hz, 1H), 7.58 (d, *J*=7.5 Hz, 1H), 7.49 (t, *J*=7.8 Hz, 2H), 7.20 (d, *J*=5.3 Hz, 1H), 6.71 (d, *J*=4.0 Hz, 1H). MS (ESI) calcd. for $C_{13}H_9ClN_2O_2SNa$ $[M+Na]^+$: 314.9963, found: 314.9968.

1-(4-chloro-5-nitro-1H-pyrrolo[2,3-b]pyridin-1-yl)ethan-1-one (**4a**): white solid, yield 90%. m.p.: 118.0–120.1 °C. ^{1}H-NMR ($CDCl_3$) δ 9.00 (s, 1H), 8.18 (d, *J*=4.1 Hz, 1H), 6.89 (d, *J*=4.1 Hz, 1H), 3.07 (s, 3H). MS (ESI) calcd. for $C_9H_6ClN_3O_3Na$ $[M+Na]^+$: 261.9990, found: 261.9993.

1-(4-chloro-5-nitro-1H-pyrrolo[2,3-b]pyridin-1-yl)propan-1-one (**4b**): white solid, yield 37%. m.p.: 74.7–76.6 °C. ^{1}H-NMR ($CDCl_3$) δ 8.98 (s, 1H), 8.18 (d, *J*=4.1 Hz, 1H), 6.88 (d, *J*=4.1 Hz, 1H), 3.53 (q, *J*=7.3 Hz, 2H), 1.35 (t, *J*=7.3 Hz, 3H). MS (ESI) calcd. for $C_9H_6ClN_3O_3Na$ $[M+Na]^+$: 276.0146, found: 276.0150.

1-(4-chloro-5-nitro-1H-pyrrolo[2,3-b]pyridin-1-yl)butan-1-one (**4c**): white solid, yield 36%. m.p.: 74.7–76.6 °C. ^{1}H-NMR ($CDCl_3$) δ 9.06–8.92 (m, 1H), 8.16 (dd, *J*=7.6, 5.6 Hz, 1H), 6.86 (dd, *J*=8.1, 5.8 Hz, 1H), 3.51–3.41 (m, 2H), 1.93–1.81 (m, 2H), 1.11–1.07 (m, 3H). MS (ESI) calcd. for $C_{11}H_{10}ClN_3O_3Na$ $[M+Na]^+$: 276.0146, found: 276.0150.

1-(4-chloro-5-nitro-1H-pyrrolo[2,3-b]pyridin-1-yl)pentan-1-one (**4d**): white solid, yield 16%. m.p.: 40.5–41.7 °C. ^{1}H-NMR ($CDCl_3$) δ 8.99 (s, 1H), 8.18 (d, *J*=4.1 Hz, 1H), 6.87 (d, *J*=4.1 Hz, 1H), 3.51 (t, *J*=7.4 Hz, 2H), 1.86–1.75 (m, 2H), 1.50 (d, *J*=7.5 Hz, 2H), 0.99 (t, *J*=7.4 Hz, 3H). MS (ESI) calcd. for $C_{12}H_{12}ClN_3O_3Na$ $[M+Na]^+$: 304.0459, found: 304.0462.

4-chloro-1-(4-chloro-5-nitro-1H-pyrrolo[2,3-b]pyridin-1-yl)butan-1-one (**4e**): yellow solid, yield 37%. m.p.: 61.2–63.0 °C. ^{1}H-NMR ($CDCl_3$) δ 8.99 (s, 1H), 8.16 (d, *J*=4.1 Hz, 1H), 6.89 (d, *J*=4.1 Hz, 1H), 3.76–3.70 (m, 4H), 2.39–2.29 (m, 2H). MS (ESI) calcd. for $C_{11}H_9Cl_2N_3O_3Na$ $[M+Na]^+$: 323.9913, found: 323.9918.

4-chloro-1-(methylsulfonyl)-5-nitro-1H-pyrrolo[2,3-b]pyridine (**4f**): white solid, yield 42%. m.p.: 207.0–208.0 °C. ^{1}H-NMR ($CDCl_3$) δ 9.00 (s, 1H), 7.78

(d, *J*=4.0 Hz, 1H), 6.85 (d, *J*=4.0 Hz, 1H), 3.57 (s, 3H). MS (ESI) calcd. for $C_8H_7ClN_3O_4S$ $[M+H]^+$: 275.9840, found: 275.9839.

4-chloro-1-((3,5-difluorophenyl)sulfonyl)-5-nitro-1H-pyrrolo[2,3-b]pyridine (**4g**): white solid, yield 27%. m.p.: 147.1–148.8 °C. ^{1}H-NMR ($CDCl_3$) δ 9.03 (s, 1H), 7.89 (s, 1H), 7.78 (s, 2H), 7.11 (s, 1H), 6.92 (s, 1H). MS (ESI) calcd. for $C_{13}H_7ClF_2N_3O_4S$ $[M+H]^+$: 373.9808, found: 373.9808.

4-chloro-5-nitro-1-((4-(trifluoromethyl)phenyl)sulfonyl)-1H-pyrrolo[2,3-b]pyridine (**4h**): white solid, yield 56%. m.p.: 167.6–168.2 °C. ^{1}H-NMR ($CDCl_3$) δ 8.93 (s, 1H), 8.30 (d, *J*=8.3 Hz, 2H), 7.87 (d, *J*=4.0 Hz, 1H), 7.75 (d, *J*=8.4 Hz, 2H), 6.84 (d, *J*=4.0 Hz, 1H). MS (ESI) calcd. for $C_{14}H_8ClF_3N_3O_4S$ $[M+H]^+$: 405.9871, found: 405.9871.

4-chloro-5-nitro-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridine (**4i**): light yellow solid, yield 60%. m.p.: 166.7–169.3 °C. ^{1}H-NMR ($CDCl_3$) δ 9.00 (s, 1H), 8.21 (d, *J*=7.9 Hz, 2H), 7.94 (d, *J*=4.0 Hz, 1H), 7.65 (t, *J*=7.4 Hz, 1H), 7.55 (t, *J*=7.8 Hz, 2H), 6.87 (d, *J*=4.0 Hz, 1H). MS (ESI) calcd. for $C_{13}H_9ClN_3O_4S$ $[M+H]^+$: 338.0002, found: 338.0005.

Antitumor cell proliferation activity experiment in vitro

Eighteen compounds were evaluated for anti-tumor cell proliferation activity by using MTS method, CPT as the positive control. The cell lines were MCF7, HepG2, TE1 and HL7702. Tumor cells in logarithmic growth phase were seeded in 96-well culture plates with 100 μL of DMEM containing 10% fetal bovine serum at 7000 cells/well. The culture conditions were at 37 °C, 5% CO_2 concentration. After the cells were attached for 24 h, samples of different concentrations were added to each well (concentrations were 120 μmol/L, 60 μmol/L, 30 μmol/L, 15 μmol/L, 7.5 μmol/L, 3.75 μmol/L), The experiment continues to culture for 24 h. Replace the old medium, then add 20 μL of MTS solution per well, incubated for 1 h in the dark, and measured the OD value at 490 nm to calculate the survival rate and IC_{50} value of different cells.

Determination of the analgesic effect of compounds by hot plate method

Experimental method according to the literature[36], Female Kunming mice were placed on a hot plate (55±1 °C) to test the pain threshold, The mouse licked its rear feet was used as an indicator, each mouse was tested once. Remove the mice that like to jump or licking time is less than 5 seconds or more than 30 seconds, 200 eligible mice were screened and randomly divided into 20 groups, ten mice in each group, one group is blank group, one group is positive control group, eighteen administration groups. Continued to raise for 1 day, each mouse was tested for pain threshold again before administration. This threshold was averaged with the first pain threshold as the normal pain threshold before administration. After 30 minutes of intraperitoneal administration, the mice were placed on a hot plate to observe the licking time. If licking time is more than 1 minute will still be counted as 1 minute.

Results and Discussion

Synthesis

In the current study, 7-azaindole was modified and 18 compounds were designed and synthesized. Among them, 16 new compounds, consisting of **3a**~**3h** and **4a**~**4h**, have not been previously reported. The structures of all compounds were confirmed through ^{1}H-NMR and HR-MS.

Initially, 7-azaindole was used as a starting material to synthesize the target compounds through four steps of oxidation, chlorination, acylation and nitration. During the synthesis of compounds **3a**~**3i**, because of the steric hindrance of the secondary amine of azaindole, the acylation reaction was difficult, and more by-products were produced. Consequently, the post-processing was optimized and improved. The organic phase was firstly washed using hydrochloric acid and sodium bicarbonate. A silica gel/sodium sulfate system was used to dry the products instead of Na_2SO_4, and the target product was purified through ethyl acetate/petroleum ether column chromatography. In the process of preparing compounds **4b**, **4d**, and **4e**, the nitration reaction was incomplete because of the presence of the acylation group, and these products were purified through column chromatography; the other compounds can obtain higher purity products based on **4i** post-treatment.

Biological Activity

Anti-proliferative effect in tumor cells in vitro

The results of an assessment of the anti-proliferative effects of the compounds **4a**~**4i** in MCF7, HepG2, TE1 and HL7702 cells in vitro are shown in Table-1. The IC_{50} values of compounds **3a**~**3i** in the tumor cell lines were all greater than 100 μmol/L, and the anti-tumor cell proliferation activity demonstrated by compounds in the **4** series was stronger than that of the **3** series compounds.

Table-1: Inhibitory activity of compounds on tumor cells in vitro.

	IC_{50}/(μmol/L)			
	MCF7	HepG2	TE1	HL7702
4a	20.412	14.479	41.832	37.084
4b	52.916	36.122	26.409	34.712
4c	89.721	85.088	76.139	51.340
4d	76.677	81.543	>100	>100
4e	51.323	58.241	69.766	31.220
4f	5.781	8.077	26.087	>100
4g	27.289	19.188	29.383	12.378
4h	23.536	14.800	29.416	15.436
4i	6.420	11.090	28.437	22.313
CPT	>100	>100	>100	>100

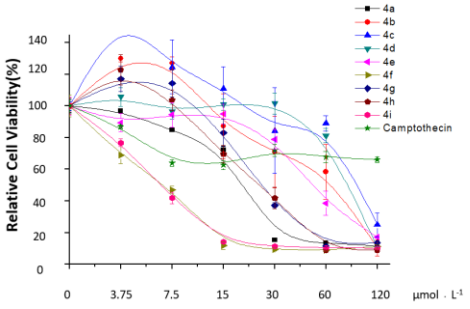


Fig 1 Relative Cell activity of compounds in MCF7

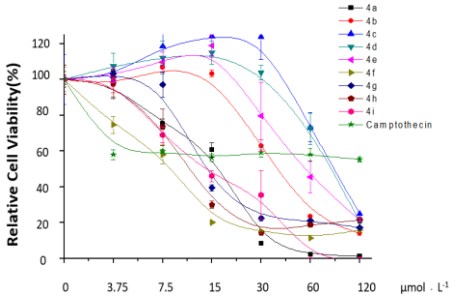


Fig 2 Relative Cell activity of compounds in HepG2

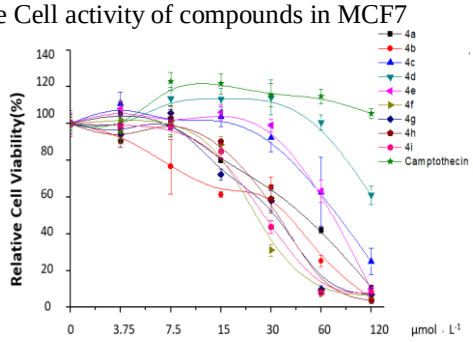


Fig 3 Relative Cell activity of compounds in TE1

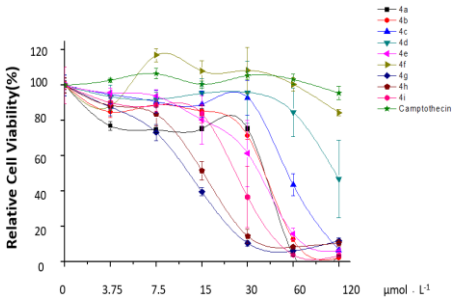


Fig 4 Relative Cell activity of compounds in HL7702

Table-2: Effect of compounds on pain response in mice induced by heat stimulation.

Group	Dose (mg/Kg)	Pain threshold		Analgesic ratio (%)
		pre-dose	post-dose	
Blank group		24.03±4.706	26.40±6.065	
Aspirin group	200	16.50±4.437	25.35±6.086	53.58
3a	50	19.39±5.051	56.83±11.938	193.16
3b	50	24.31±4.678	28.34±11.619	16.59
3c	50	21.21±5.384	26.23±7.233	23.70
3d	50	21.53±5.055	18.60±4.298	-13.61
3e	50	19.48±2.997	28.40±4.865	45.73
3f	50	18.71±5.167	24.86±6.121	32.83
3g	50	24.16±6.424	44.20±12.686	82.94
3h	50	22.79±4.385	33.26±7.595	45.98
3i	50	23.50±7.632	26.12±6.534	11.14
4a	50	21.20±5.790	21.72±5.993	2.45
4b	50	19.40±6.617	21.57±8.525	11.18
4c	50	25.71±8.446	38.67±10.959	50.39
4d	50	22.64±5.207	27.06±7.719	19.51
4e	50	23.49±5.046	32.76±8.269	39.43
4f	50	20.87±4.470	23.09±5.595	10.66
4g	50	23.09±7.491	31.56±9.334	36.72
4h	50	24.42±6.611	35.77±7.245	46.46
4i	50	21.44±6.321	45.45±12.280	111.99

The target compounds **4a**~**4i** showed some inhibitory activity against MCF7, HepG2 and TE1 cells. Compound **4f** had a very significant inhibitory effect on the growth of MCF7 and HepG2 cells. The IC_{50} value of compound **4f** in a healthy liver cell line was greater than 100 μmol/L, which indicates that **4f** had little cytotoxicity in healthy cells. Compound **4i**, a benzsulfamide showed the most growth inhibitory effect(IC_{50}=6.42μmol/L) in the growth of MCF7 cells, but the compound **4i** has apparently showed cytotoxicity on HL7702 cells,_indicates that the presence of benzenesulfonyl increases cytotoxicity. The results showed that the introduction of a nitro group might represent a pharmacophore which could improve the antitumor activity of this series of compounds, and the methylsulfonyl group and the benzenesulfonyl group also enhanced the antitumor activity.

The analgesic effect of the compounds assayed through a hot plate method

The analgesic effects of 18 compounds, assayed through a hot plate method, are shown in Table-2.

The preliminary analgesic activity test results showed that after intraperitoneal administration (50 mg/kg), most compounds significantly increased the pain threshold in mice compared with the blank group and the aspirin group. The results suggest that some compounds had obvious analgesic effects in mice, especially compounds **3a**, **3g** and **4i**, with an increased pain threshold rate of 193.16%, 82.94% and 111.99%, respectively. The compounds in the **3a**~**3d**, compound **3a**, bearing the shortest carbon chain acetyl group, showed the most potent analgesic effects, and compound **3d**, bearing the longest carbon chain valeryl group, showed the worst analgesic effects, and with the increase of carbon chain, the overall performance of the analgesic effect is weakened. These analgesic effects were much higher than those in the positive control group (aspirin, 200 mg/kg, 53.58%). However, there was no obvious relationship between structure and analgesic effect.

Conclusion

Eighteen 7-azaindole derivatives were synthesized using 7-azaindole as raw material. We tried to ascertain potent compounds for anti-proliferative activities against tumor cell lines and the analgesic effects on mice. Compound **4f** has the best activity. Furthermore, there is a dramatic difference between the IC_{50} values of compound **4f** against tumor cell lines and normal cell line. Preliminary results showed that compounds **3a**, **3g** and **4i** had significant analgesic effects on mice, and the analgesic effects were stronger than aspirin. But the compound **4i** has apparently showed cytotoxicity on HL7702 cells, **3a** and **3g** have a good prospect for new drug development.

Acknowledgements

This work was supported by the National Science Foundation of China (81370497), the Science and Technology Development Funds of Jilin Province(20160204018YY) and the Project of The Education Department of Jilin Province (JJKH20180867KJ). We thank Conn Hastings, PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

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