



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Design, synthesis and biological evaluation of the novel isoindolinone derivatives

Jiang Liu, Luyao Wang, Na Guo*, Yu-ou Teng and Peng Yu*

Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology
Tianjin Key Laboratory of Industry Microbiology, College of Biotechnology
Sino-French Joint Lab of Food Nutrition/Safety and Medicinal Chemistry, Tianjin University of Science & Technology, Tianjin, China

ABSTRACT

Isoindolinones have a wide range of biological activities, such as antitumor, anticonvulsant and so on. Some isoindolinone derivatives have been studied as antitumor drug molecules. In this paper, we would like to report the design, synthesis and biological activities of a series of novel 2-benzyl-6-substituted-ethoxy-isoindolinone derivatives which have not been reported yet. All the target compounds (9-12) were characterized by ¹H NMR and ESI-MS and were tested for their cytotoxicity using HT-29, K562, HepG2 cell line. Preliminary result showed that tert-butyl 4-(2-(2-benzyl-3-oxoisoindolin-5-yl)oxy) ethyl) piperazine-1-carboxylate (11) demonstrated antitumor activity against HepG2 cancer cell with an IC₅₀ of 5.89 μM.

Key words: Isoindolinone; antitumor; HepG2 cancer cell; Synthesis

INTRODUCTION

Isoindolinones, belonging to the alkaloids family, are found in many natural products such as vitedoamine A, chilenine, lennoxamine, magallanesine and nuevamine [1-6]. These compounds possess a lot of biological activities such as anxiolytic/anticonvulsant, TNF α -inhibitory, antiangiogenic, 5-HT antagonistic/antidepressant [7-11], PARP-1-inhibitory [12], histone deacetylase inhibitory [13] and cytotoxicity activity.

Cancer is a global public health problem. The World Health Organization (WHO) estimates that there are about 10 million new cancer patients each year. At present, cancer mortality rate is still on the rise. The existing chemotherapy drugs in clinical with the using of anti-cancer drugs, the resistance of existing chemotherapy drugs have gradually emerged in clinic, thus there is an urgent need for new effective anti-cancer drugs.

In this paper, we would like to report the design and synthesis of novel anticancer isoindolinone derivatives. The target compounds (9-12) (Fig. 1) were synthesized in eight steps with nucleophilic substitution reaction and cyclization as the key steps. Biological activity test indicated that tert-butyl 4-(2-(2-benzyl-3-oxoisoindolin-5-yl)oxy) ethyl) piperazine-1-carboxylate (11) has a micromolar IC₅₀ value against HepG2 cell.

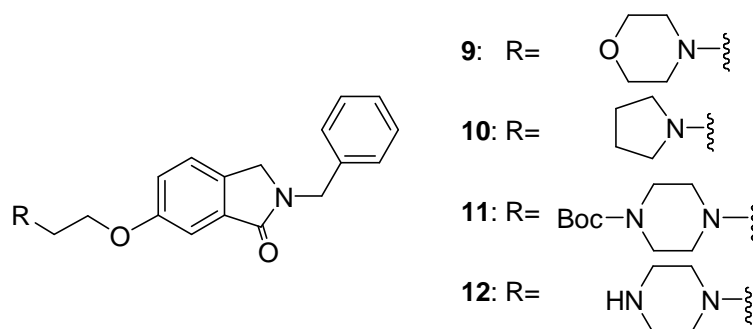


Fig. 1 Structures of compounds 9-12

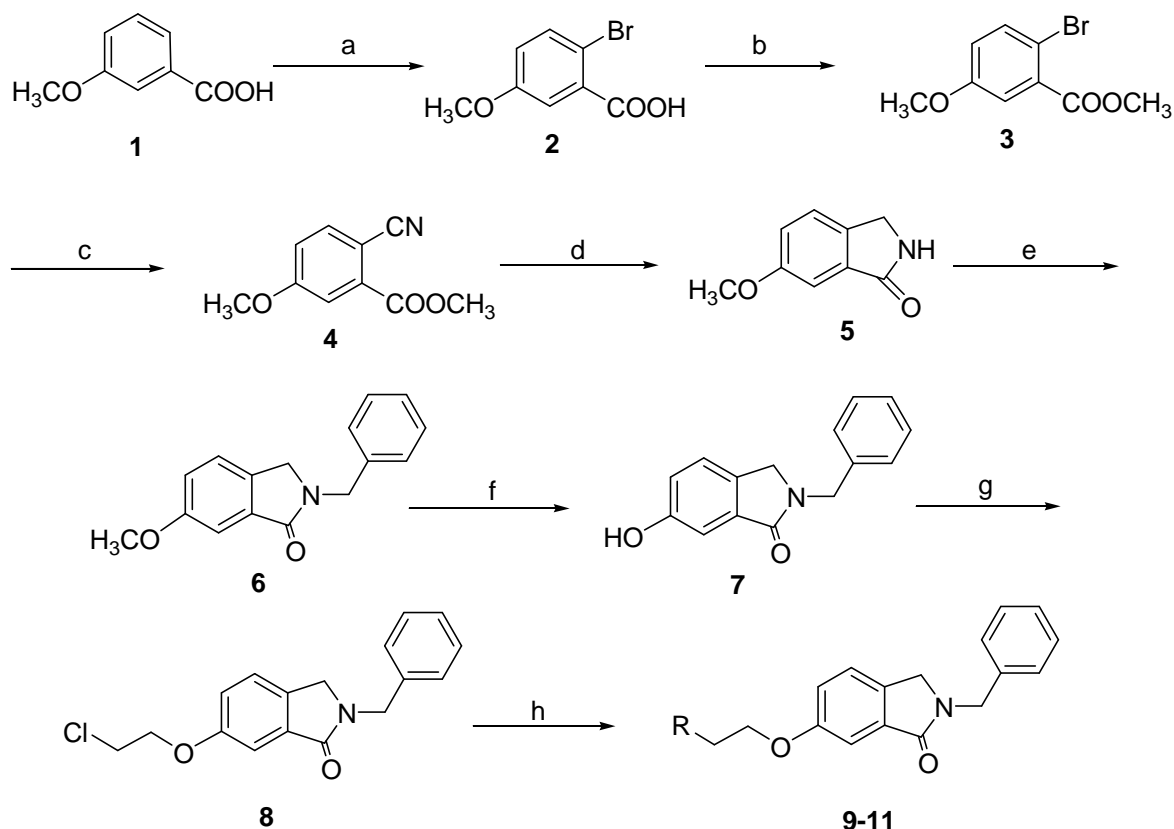
EXPERIMENTAL SECTION

2.1 Material and measurements

All reagents and solvents used in this paper were of reagent grade. Reaction temperatures were controlled using oil bath temperature modulator. Thin layer chromatography (TLC) was performed using E. Merck silica gel 60 GF₂₅₄ precoated plates (0.25 mm) and visualized using UV. Silica gel (particle size 200-400 mesh) was used for flash chromatography. ¹H NMR spectra was recorded on Bruker AM-400 NMR spectrometers in deuterated chloroform or deuterated DMSO. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane as internal standard.

2.2 Chemistry

The synthetic route of target compounds 9-12 was illustrated in Scheme 1.



Scheme 1. Synthetic route of isoindolinone derivatives

Reagents and conditions: (a) AcOH, Br₂, 60 °C; (b) H₂SO₄, 80 °C; (c) CuCN, DMF, 140 °C; (d) Ni, H₂, NaOH, 58 °C; (e) NaH, DMF; (f) BBr₃; -78 °C; (g) K₂CO₃, DMF; (h) K₂CO₃, DMF.

2.2.1 2-Bromo-5-methoxybenzoic acid (2)

To the 3-methoxybenzoic acid (10.0 g, 65.73 mmol) in acetic acid (70 mL) was added dropwise Br₂ (11.5 g, 72.3 mmol) at 0 °C. After stirring for 48 h, the mixture was poured into cold water, then collected the precipitated solid. The compound 2 was obtained by filtration (8.0 g, 53%).

2.2.2 Methyl 2-bromo-5-methoxybenzoate (3)

To the compound **2** (8.0 g, 32.78 mmol) in methanol (240 mL) was added conc. H₂SO₄ (8.0 mL) dropwise at 0°C, then the mixture was stirred at 80°C. After 8 h, the solvent was removed under vacuum to afford the crude which was purified by flash column chromatography (petroleum ether/ethyl acetate 20:1) to afford compound **3** (7.4 g, 83%).

2.2.3 Methyl 2-cyano-5-methoxybenzoate (4)

Compound **3** (4.5 g, 18.36 mmol) was dissolved in dry N,N-Dimethylacetamide (25 mL), then CuCN (5.76 g, 64.27 mmol) was added in portions at 0°C. The mixture was heated to 140°C and stirred for 1 h before cooling to room temperature. The mixture was filtered, the filtrate was diluted with water, extracted with dichloromethane, dried with anhydrous sodium sulfate. The solvent was removed under vacuum to provide the crude product, which was purified by flash column chromatography (petroleum ether/ethyl acetate 20:1) to afford compound **4** (2.0 g, 53%).

2.2.4 6-Methoxyisoindolin-1-one (5)

To a solution of compound **4** (1.0 g, 5.23 mmol) in ethanol (10 mL) was added 30% Raney Ni (0.1 g) and the hydrogen. The mixture was heated to 80°C, stirred for 5 h until the reaction was completed. The Raney Ni was removed, then NaOH (1 mol/L) was added dropwise until the pH was around 8-9. The ethanol was removed under vacuum, the mixture was extracted with ethyl acetate, the organic layer was dried and concentrated under vacuum to give the crude product, which was purified by flash column chromatography (petroleum ether/ethyl acetate 1:1) to afford compound **5** (0.6 g, 50%).

2.2.5 2-Benzyl-6-methoxyisoindolin-1-one (6)

To a solution of compound **5** (0.1 g, 0.612 mmol) in dry N,N-Dimethylformamide (5 mL) was added NaH (36.0 mg, 1.22 mmol) at 0°C, then benzyl chloride (93.0 mg, 0.74 mmol) was added and stirred for 0.5 h at room temperature. The reaction mixture was quenched with aq. NH₄Cl and then extracted with dichloromethane. The organic layer was dried with anhydrous sodium sulfate and concentrated under vacuum to provide the crude product. Compound **6** (110 mg, 71%) was obtained by flash column chromatography (petroleum ether/ethyl acetate 15:1).

2.2.6 2-Benzyl-6-hydroxyisoindolin-1-one (7)

Compound **6** (0.2 g, 0.78 mmol) was dissolved in dry CH₂Cl₂ (20 mL), BBr₃ (0.59 g, 2.37 mmol) was then added slowly at -78°C, stirred over night at room temperature. After completion of the reaction, the mixture was quenched with methanol. Removal of organic solvent under vacuum gave a residue that was purified by column chromatography (petroleum ether/ethyl acetate/dichloromethane 1:1:1) to provide compound **7** (0.15 g, 80%).

2.2.7 2-Benzyl-6-(2-chloroethoxy) isoindolin-1-one (8)

To a solution of compound **7** (0.1 g, 0.42 mmol) in dry N, N-dimethylformamide (2 mL) was added potassium carbonate (0.17 g, 1.25 mmol), stirred for 10 min, then 1-bromo-2-chloroethane (0.9 g, 0.63 mmol) was added, stirred for another 12 h at 90°C, the mixture was diluted with brine, extracted with dichloromethane, the organic layer was combined, dried with anhydrous sodium sulfate, concentrated under vacuum, providing compound **8** (0.11 g, 87%) by column chromatography (petroleum ether/ethyl acetate 10:1).

2.2.8 2-Benzyl-6-(2-morpholinoethoxy) isoindolin-1-one (9)

To a solution of compound **8** (0.11 g, 0.36 mmol) in dry N, N-dimethylformamide (2 mL) was added potassium carbonate (0.15 g, 1.09 mmol), stirred for 10 min, then morpholine (0.5 g, 0.55 mmol) was added, stirred for another 2 h at 80°C, the mixture was diluted with brine, extracted with dichloromethane, the organic layer was combined, dried with anhydrous sodium sulfate, removal of solvent under vacuum to provide a residue which was purified by column chromatography (petroleum ether/ethyl acetate 1:1) to give compound **9** (0.08 g, 75%).

2.2.9 2-Benzyl-6-(2-(pyrrolidin-1-yl) ethoxy) isoindolin-1-one (10)

Compound **10** was obtained (0.1 g, 62%) by the method the same as compound **9**.

2.2.10 Tert-butyl 4-(2-(2-benzyl-3-oxoisoindolin-5-yl)oxy) ethyl) piperazine-1-carboxylate (11)

Compound **11** was obtained (0.12 g, 40%) by the method the same as compound **9**.

2.2.11 2-Benzyl-6-(2-(piperazin-1-yl) ethoxy) isoindolin-1-one (12)

To a solution of compound **11** (0.1 g, 0.22 mmol) in dry dichloromethane (4 mL) was added trifluoroacetic acid (1 mL) slowly, stirred for 2 h, then added aq. NaOH (1M) dropwise to adjust the pH to 7-8 following dilution with brine, extraction with dichloromethane. The combined organic layer was dried with anhydrous sodium sulfate,

concentrated, and purified by column chromatography (dichloromethane/methanol 1:1) to give compound **12** (0.05 g, 64%).

2.3 Biological assay

The antiproliferative effect of these compounds was determined against K562, HepG2 and HT-29 cells by using MTT assay. The cells were diluted to a density of 5×10^4 cells/mL and added 100 μ L to each well of the 96-well plates with a multichannel pipet. After incubating for 24 hours, 0.5 μ L compounds were added and then cells were further incubated for 48 hours (final concentrations of each compound: 0.1, 0.3, 1, 3 and 10 μ M). The culture plates were incubated for 4 h after which 20 μ L MTT was added to each well, then the medium were removed from the wells and 100 μ L DMSO added into each well. After leaving for further 10 min to dissolve the formazan crystals formed, the optical density (OD) was measured at 490 and 630 nm. Cell viability was calculated from measurements of OD value according to the corresponding formula and a graph is plotted of Cell viability (y-axis) against drug concentration (x-axis). The inhibitory concentration by 50% (IC₅₀) values of sample compounds toward test cell proliferation were presented in Table 1.

RESULTS AND DISCUSSION

3.1 Characterization of compounds 2-12 by ¹H-NMR

3.1.1 2-Bromo-5-methoxybenzoic acid (2)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 3.78 (s, 3H), 7.04-7.01 (m, 1H), 7.25(d, $J=2.8$, 1H), 7.58 (d, $J=8.8$, 1H), 13.35(s, 1H).

3.1.2 Methyl 2-bromo-5-methoxybenzoate (3)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 3.80 (s, 3H), 3.93 (s, 3H), 6.89-6.86 (m, 1H), 7.30(d, $J=2.8$, 1H), 7.50 (d, $J=9.2$, 1H).

3.1.3 Methyl 2-cyano-5-methoxybenzoate (4)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 3.92 (s, 3H), 4.00 (s, 3H), 7.15-7.12 (m, 1H), 7.61(d, $J=1.6$, 1H), 7.72 (d, $J=8.8$, 1H).

3.1.4 6-Methoxyisoindolin-1-one (5)

¹H NMR (d₆-DMSO 400 MHz): δ /ppm 3.81 (s, 3H), 4.28 (s, 2H), 7.17-7.14 (m, 2H), 7.47-7.45(m, 1H).

3.1.5 2-Benzyl-6-methoxyisoindolin-1-one (6)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 3.89 (s, 3H), 4.22 (s, 2H), 4.82 (s, 2H), 7.10-7.08 (m, 1H), 7.40-7.26(m, 7H).

3.1.6 2-Benzyl-6-hydroxyisoindolin-1-one (7)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 4.23 (s, 2H), 4.69 (s, 2H), 7.05-6.97 (m, 1H), 7.06(s, 1H), 7.29-7.24(m, 3H), 7.36-7.32(m, 3H), 9.74(s, 1H).

3.1.7 2-Benzyl-6-(2-chloroethoxy) isoindolin-1-one (8)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 3.66 (t, $J=6.0$ Hz, 1H), 3.84(t, $J=5.6$ Hz, 1H), 4.20 (s, 2H), 4.30(t, $J=5.6$ Hz, 1H), 4.36(t, $J=6.0$ Hz, 1H), 4.80(s, 2H), 7.14-7.11(m, 1H), 7.32-7.29(m, 5H), 7.35-7.32(m, 1H), 7.37-7.35(m, 1H).

3.1.8 2-Benzyl-6-(2-morpholinoethoxy) isoindolin-1-one (9)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 2.60-2.58 (m, 4H), 2.83 (t, $J = 5.6$ Hz, 2H), 3.74 (t, $J = 4.8$ Hz, 2H), 4.18-4.16 (m, 4H), 4.78 (s, 2H), 7.09-7.07 (m, 1H), 7.37-7.24 (m, 7H).

3.1.9 2-Benzyl-6-(2-(pyrrolidin-1-yl) ethoxy) isoindolin-1-one (10)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 1.85-1.82 (m, 4H), 2.66 (m, 4H), 2.96 (t, $J = 5.6$ Hz, 2H), 4.20-4.18 (m, 4H), 4.81 (s, 2H), 7.14-7.16 (m, 1H), 7.40-7.29 (m, 6H), 7.40(d, $J = 2.4$ Hz, 1H).

3.1.10 *Tert*-butyl 4-(2-(2-benzyl-3-oxoisoindolin-5-yloxy) ethyl) piperazine-1-carboxylate (11)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 1.46 (s, 9H), 2.55-2.52 (m, 4H), 2.85 (t, $J = 5.6$ Hz, 2H), 3.47-3.45 (m, 4H), 4.18 (t, $J = 5.6$ Hz, 2H), 4.20 (s, 2H), 4.80 (s, 2H), 7.10-7.01 (m, 1H), 7.33-7.26(m, 7H).

3.1.11 2-Benzyl-6-(2-(piperazin-1-yl) ethoxy) isoindolin-1-one (12)

¹H NMR (CDCl₃ 400 MHz): δ /ppm 2.50-2.46 (m, 4H), 2.69 (t, $J=5.6$ Hz, 2H), 2.76-2.75 (m, 4H), 4.15 (t, $J = 5.6$ Hz, 2H), 4.28 (s, 2H), 4.72 (s, 2H), 7.17-7.14 (m, 1H), 7.27-7.22 (m, 6H), 7.29 (d, $J=7.2$ Hz, 1H).

3.2 Anticancer activity assay

The target compounds (**9-12**) were tested for their in vitro anticancer activity against K562, HepG2 and HT-29 cells by MTT based assay. The results were listed in Table 1. Compounds **11** showed obvious activity against HepG2 cell.

Table1. Inhibition Activity of the compound **9-12**

Tested cell	Samples (IC ₅₀ μM)			
	9	10	11	12
K562	>10	>10	>10	>10
HepG2	>10	>10	5.89	>10
HT-29	>10	>10	>10	>10

CONCLUSION

In summary, we report the design and synthesis of a series of novel isoindolinone derivatives. The structures of these targets compounds and all of intermediates were confirmed by ¹H NMR. Biological activity test indicated that compound **11** has good antitumor activity against HepG2 cell. In order to improve the antitumor activity, further modification base on compound **11** was undergoing in our lab.

Acknowledgments

The authors sincerely thank the financial support from the Tianjin Municipal Science and Technology Commission (14JCQNJC13200) and the National Natural Science Foundation of China (81302649).

RERERENCES

- [1] M Lamblin; A Couture; E Deniau; P Grandclaudon. *Tetrahedron*, **2007**, 63 : 2664.
- [2] A Daïch; S Marchalin; P Pigeon; B Decroix. *Tetrahedron Lett.* **1998**, 39 : 9187.
- [3] T Taniguchi; K Iwasaki; M Uchiyama; O Tamura; H Ishibashi. *Org. Lett.* **2005**, 7 : 4389.
- [4] FG Fang; GB Feigelson; SJ Danishefsky. *Tetrahedron Lett.* **1989**, 30: 2743.
- [5] R Alonso; L Castedo; D Dominguez. *Tetrahedron Lett.* **1985**, 26: 2925.
- [6] G Kim; P Jung; LA Tuan. *Tetrahedron Lett.* **2008**, 49: 2391.
- [7] TL Stuk; BK Assink; RC Bates; DT Erdman; V Fedij; SM Jennings; JA Lassig; RJ Smith; TL Smith. *Org. Proc. Res. Dev.* **2003**, 7: 851.
- [8] Luzzio, F. A.; Zacherl, D. P. *Tetrahedron Lett.* **1999**, 40: 2087.
- [9] FA Luzzio; AV Mayorov; SSW Ng; EA Kruger; WD Figg. *J. Med. Chem.* **2003**, 46: 3793.
- [10] M Norman; DJ Minick; GC Rigdon. *J. Med. Chem.* **1996**, 39: 149.
- [11] DK Luci; EC Lawson; S Ghosh; WA Kinney; CE Smith; J Qi; Y Wang; LK Minor; BE Marynoff. *Tetrahedron Lett.* **2009**, 50: 4958.
- [12] GME Papeo; MY Krasavin; P Orsini; A Scolaro. *PCT Int. Appl* **2014WO2014064149A1**.
- [13] S Lee; C Shinji; K Ogura; M Shimizu; S Maeda; M Sato; M Yoshida; Y Hashimoto; H Miyachi. *Bioorg. Med. Chem. Lett.* **2007**, 17: 4895.
- [14] Y Zhou; B Shi; KL Han; QN Guo; Y Yang; B Song; Y Teng; P Yu. *J. Chem. Pharm. Res.* **2013**, 5(12): 1024.