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Research Article

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Design, synthesis and biological evaluation of hydantoin derivatives

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ABSTRACT

*A series of hydantoin derivatives were designed and synthesized about 25-30% overall yields. Two of the six newly synthesized compounds, compound **3, 5** have not been reported before. Compounds **2-6** were obtained by Aldol reaction，and those structures were confirmed by ^{1}H and ^{13}C NMR. All the newly synthesized derivatives were subjected to evaluate their cytotoxic properties against human tumor cell lines, HepG2. Results indicated that compounds **2** and **4** exhibited significant anti-tumor activities against liver cancer (HepG2)cell lines. Among, compound **2** with the IC_{50} values of 3.37 μM against cancer cell lines HepG2.*

Keywords: Hydantoin derivatives, synthesis, anti-tumor activity, MTT

INTRODUCTION

In recent years, a large number of studies have shown that, Bcl-2 proteins that control cell wither the death of the final regulation, its overexpression induced cell inappropriate health may be one of the main reason for tumor formation and chemotherapy resistance, to Bc1-2 protein as a target for anticancer drug design and development projects has become former research focus [1]. WL-276 (Fig. 1), a rhodanine derivative which synthesized by Wang and other groups [2,3,4], not only inhibit the Bcl-2, and for resistance to potency prostate cancer PC-3 cells showed strong inhibitory activity [5, 6,7].

In this paper, we would like to report the design, synthesis and biological evaluation of hydantoin derivatives (an electronic isostere of rhodanine). The target compounds (compound **2-6**) were synthesized in three steps through Aldol reaction [8], nucleophilic substitution reaction [9], and so on.

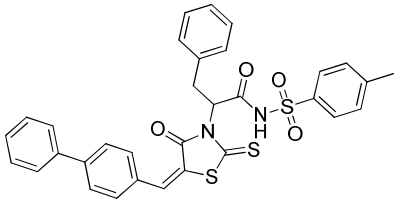

Fig. 1 WL-276

EXPERIMENTAL SECTION

2.1 Materials and measurements

Used in this article, all reagents and solvents were of analytical grade. The reaction temperature control uses the oil bath temperature modulator. Thin layer chromatography (TLC) with silica gel 60 GF_{254} E. Merck precoated plates (0.25 mm) was visualized using UV. 0.1 for flash chromatography on silica gel (particle size 100-200 mesh).^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 NMR spectrometers in deuterated chloroform and deuterated DMSO. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane as internal standard.

2.2 Synthesis route of hydantoin derivatives

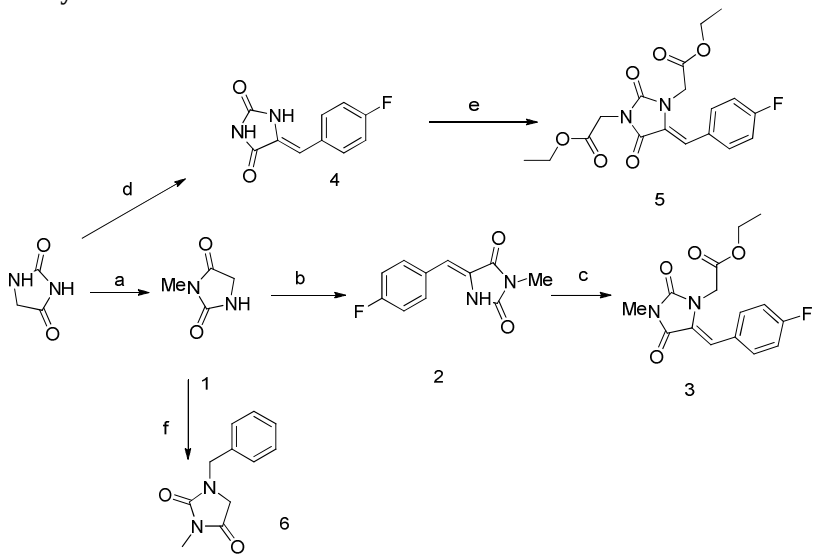

Fig 1: Synthesis route of hydantoin derivatives

Reagents and conditions：*(a)1,1-dimethoxyethyl(dimethyl)amine,toluene;(b) 60%KOH(aq),EtOH;(c) Ethyl chloroacetate,NaH, DMF;（d）60%KOH(aq), EtOH; (e) Ethyl chloroacetate, NaH, DMF; (f) Benzyl chloride, NaH, DMF;*

2.2.1 Synthesis of 3-methylimidazolidine-2,4-dione (compound **1)**

Hydantoin (10.00 g, 99.92 mmol) and 1,1-dimethoxyethyl(dimethyl)amine (16.00 g, 119.91 mmol) were added in toluene at 0℃. After stirring for 10 min, the reaction mixture was heated to reflux for 4 h. The precipitate formed was collected by filtration, washed with water and dried under vacuum to get compound **1** (10.00 g, 88%).

2.2.2 Synthesis of (Z)-5-(4-fluorobenzylidene)-3-methylimidazolidine-2,4-dione (compound **2)**

To a solution of compound **1** (1.00 g, 8.76 mmol) and 4-Fluorobenzaldehyde(1.31 g, 10.52 mmol) in 10 mL of glacial acetic acid, 2 drops of concentrated hydrochloric acid was added at 0℃, the reaction was stirred at the room temperature for 3 h. The reaction mixture was poured into ice-water and filtered. The filter cake was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 30:1-20:1) to get compound **2** (1.70 g, 88%).

2.2.3 Synthesis of (Z)-ethyl 2-(5-(4-fluorobenzylidene)-3-methyl-2,4-dioxoimidazolidin-1-yl)acetate (compound **3)**

To a solution of compound **2** (1.00 g, 4.54 mmol) in DMF (10 mL) was added NaH(0.20 g, 5.45 mmol) and ethylchloroacetate (0.67 g, 5.45 mmol).The mixture was stirred for 3h at 40℃.After the solvent was evaporated, the residue was dissolved in water and extracted with ethyl acetate, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 10:1) to afford compound **3** (1.15 g, 82%)**.**

2.2.4 Synthesis of (Z)-5-(4-fluorobenzylidene)imidazolidine-2,4-dione (compound **4)**

To a solution of Hydantoin (1.00 g, 8.76 mmol) and 4-Fluorobenzaldehyde(1.31 g, 10.52 mmol) in 10 mL of glacial acetic acid, 2 drops of concentrated hydrochloric acid was added at 0℃, the reaction was stirred at the room temperature for 3 h. The reaction mixture was poured into ice-water and filtered. The filter cake was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 30:1-20:1) to get compound **4** (1.70 g, 88%).

2.2.5 Synthesis of (Z)-diethyl 2,2'-(4-(4-fluorobenzylidene)-2,5-dioxoimidazolidine -1,3-diyl)diacetate (compound **5)**

To a solution of compound **4** (1.00 g, 4.54 mmol) in DMF (10 mL) was added NaH(0.47 g, 11.60 mmol) and ethylchloroacetate(1.43 g, 11.60 mmol).The mixture was stirred for 3 h at 40℃.After the solvent was evaporated, the residue was dissolved in water and extracted with ethyl acetate, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 10:1) to afford compound **5** (1.60 g, 87%).

2.2.6 Synthesis of 1-benzyl-3-methylimidazolidine-2,4-dione (compound **6)**

To a solution of compound **1** (1.00 g, 8.76 mmol) in DMF (10 mL) was added NaH (0.42 g, 10.52 mmol) and Benzyl chloride (1.33 g, 10.52 mmol).The mixture was stirred for 3 h at 40℃.After the solvent was evaporated, the residue was dissolved in water and extracted with ethyl acetate, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 10:1) to afford compound **6 (**1.50 g, 84%**).**

2.3 Biological assay.

The cell lines HepG2 was plated in 96-well plates at a density of 5×10^3 cells per well and cultured at 37℃ in 5% CO_2 for 24 h. Cells were treated with different concentrations of compounds and incubated at 37 ℃ for an additional 48 h. MTT assay was performed using Thermo microplate reader. The DMSO-treated controls were calculated as a cell viability value of 100%. The IC_{50} values were obtained by nonlinear regression using GraphPad Prism 4.0. IC_{50} measurements for each compound were done three times.

RESULTS AND DISCUSSION

3.1 Characterize Hydantoin derivatives by ^{1}H and ^{13}C NMR.

3.1.1 3-methylimidazolidine-2,4-dione by ^{1}H and ^{13}C NMR (compound 1).

^{1}H NMR (400 MHz, DMSO-d6): 2.82(s, 3H), 3.89(d, J= 0.8Hz, 2H), 8.00(s,1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 28.8, 46.0, 157.4, 172.0;

3.1.2 (Z)-5-(4-fluorobenzylidene)-3-methylimidazolidine-2,4-dione by ^{1}H and ^{13}C NMR (compound 2).

^{1}H NMR (400 MHz, DMSO-d_6): 2.96(s, 3H), 6.51(s, 1H),7.60(s,4 H),10.82(s,1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.3, 115.2, 115.4, 124.8, 130.4, 129.8, 154.1, 162.1, 163.3 ;

3.1.3 (Z)-ethyl 2-(5-(4-fluorobenzylidene)-3-methyl-2,4-dioxoimidazolidin-1-yl) acetate by ^{1}H and ^{13}C NMR (compound 3).

^{1}H NMR (400 MHz, DMSO-d_6): 1.16(t, J = 7.2 Hz, 3H), 3.19 (s, 3H), 4.01(d, J = 6.8 Hz, 2H),4.22(s, 2H),6.89(s, 1H),7.12(d, J = 8 Hz, 2H),7.52(d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.1, 29.4, 49.1, 61.0, 107.5, 115.4, 122.4, 129.8, 130.4, 155.3, 161.5, 162.1, 167.5,

3.1.4 (Z)-5-(4-fluorobenzylidene)imidazolidine-2,4-dione by ^{1}H and ^{13}C NMR (compound 4).

^{1}H NMR (400 MHz, DMSO-d_6): 6.39 (s, 1H), 7.58(s, 4H), 10.93(m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 115.2,

115.4, 124.6, 129.8, 130.4, 154.4, 162.1, 163.6

3.1.5 (Z)-diethyl 2,2'-(4-(4-fluorobenzylidene)-2,5-dioxoimidazolidine-1,3-diyl) diacetate by ^{1}H and ^{13}C NMR (compound 5).

^{1}H NMR (400 MHz, DMSO-d_6): 1.25(t, J = 7.2 Hz, 3H), 1.35(t, J = 7.2 Hz, 3H), 4.01(d, J = 6.8 Hz, 2H), 4.31(d, J = 6.8 Hz, 2H),4.29(s, 2H), 4.41(s, 2H),6.94(s, 1H),7.14(d, J = 8 Hz, 2H),7.55(d, J = 8.4 Hz, 2H);^{13}C NMR (100 MHz, DMSO-d_6) δ 14.1, 45.4, 49.1, 61.0, 107.5, 115.4, 122.4, 129.8, 130.4, 155.3, 161.3, 162.1, 167.5

3.1.6 1-benzyl-3-methylimidazolidine-2,4-dione by ^{1}H and ^{13}C NMR (compound 6).

^{1}H NMR (400 MHz, DMSO-d_6): 2.86(s, 3H), 3.85(s, 2H), 4.48(s, 2H), 7.26-7.35(m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.1, 46.7, 53.9, 128.5, 127.0, 127.9, 136.4, 154.9, 171.0

3.2 Anticancer activity assay.

All the above compounds were tested for their anticancer activity against HepG2 by MTT based assay. The results were presented in Table 1.

Table 1 Inhibition activity of hydantoin derivatives

Tested cells	Compounds (IC_{50}, μM)					
	1	**2**	**3**	**4**	**5**	**6**
HepG2	15.47	3.37	71.34	20.47	>100	>100

CONCLUSION

We report the design and synthesis of novel hydantoin derivatives. Several steps among this route were optimized, such as nucleophilic substitution reaction, Aldol reaction and so on. All the target compounds were synthesized in three steps with the overall yield of 25%-30%, respectively. The structures of these novel targets and part of intermediates were confirmed by ^{1}H and ^{13}C NMR.

Biological activity test indicated that compound **2** has good antitumor activity against HepG2 cells. In order to improve the antitumor activity, further modification based on compound **2** was undergoing in our lab.

Acknowledgments

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