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Synthesis, Anticancer Evaluation and Docking Study of 3-Benzyloxyhydantoin Derivatives

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Abstract: A series of 3-benzyloxyhydantoin derivatives were designed and synthesized by introducing hydroxyurea pharmacophore into hydantoin rigid scaffold. The cytotoxic activities of the target compounds were evaluated in *vitro* against three cancer cell lines. Compounds **5b, 5c, 5e, 5g, 6c** and **6g** displayed high activity on all of the three cancer cell lines and the most promising compounds were **5g, 6g** with IC_{50} values of 0.04 and 0.01μM. Binding of derivatives for the ribonucleotide reductase (RR) was investigated by use of molecular docking studies. Our findings show that modification at the C5 position of hydantoin with isopropyl or isobutyl was favorable to increasing binding affinity to the active site of the RR receptor and antiproliferative activity.

Xi Mai

Keywords: Molecular modeling, ribonucleotide reductase, anticancer, synthesis, MTT Assay, benzyloxyhydantoin derivatives.

1. INTRODUCTION

As we all know, because of its low cure rate and high mortality, tumor has become one of the most terrible diseases and is also one of the leading causes of death around the world [1-3]. Leukemia is a malignant disorder of blood progenitor cells affecting a significant segment of the population, especially children. In fact, leukemia is the most frequent childhood cancer, with 26% of all cases, and 20% mortality [4]. For the therapy of leukemia various drugs such as hydroxyurea (HU), cyclophosphamide, vincristine, doxorubicin, cisplatin, cytarabine, mercaptopurine, methotrexate, imatimb, ect are available in the market. Long term use of these drugs causes several side effects such as arrest of bone marrow, nephrotoxicity, neurotoxicity and gastrointestinal reaction [5]. In addition, the treatments for leukemia may become ineffective and complex as patients develop resistance to front-line therapies [6]. Therefore, it is essential to search for new chemotherapeutic or adjuvant agents for the treatment of leukemia. The past two decades have seen a dramatic change in cancer treatment paradigms. For example, clofarabine, a ribonucleotide reductase(RR) inhibitor, is one of such major advances [7]. Eukaryotic ribonucleotide reductase catalyzes nucleoside diphosphate conversion to deoxynucleoside diphosphate. Crucial for rapidly dividing cells, RR is an attractive therapeutic target of cancer, and over the past years, many chemotherapeutics inhibiting different subunits of RR have been developed and tested clinically for their anticancer activities [8-11].

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Hydantoins and their derivatives have been investigated for more than 140 years [12] and the hydantoin is a common 5-membered ring containing a reactive cyclic urea core which is present in various biologically active compounds [13]. Hydantoin derivatives possess a variety of pharmacological and biological activities, including antitumor [14-17], antimicrobial [18, 19], anti-inflamatory [20], antiplatelet [21], antiarrythmia [22, 23], antidiabetes [24, 25] and anti-HIV [26]. In particular, they have also been found to act as antagonists of leukocyte cell adhesion and allosteric inhibitors of the protein–protein interaction [27] and show antiproliferative effects toward human carcinoma cells [28]. So the derivatives of hydantoin have attracted much interest in drug discovery [29].

We have recently reported preliminary results on the antiproliferative action by 3-benzyloxyhydantoins [30]. The results unraveled that 3-benzyloxyhydantoin analogs possessed potent antitumor activity against the human leukemia cell line K562 and murine leukemia cell line L1210. Inspired by these results, we have designed a set of new series of more constrained 3-benzyloxyhydantoin derivatives containing different substituents on the benzene ring of benzyloxy and different side chains at C5 position of hydantoin (hydrogen, methyl, isobutyl, isopropyl) and synthesized in a convenient route. Computational docking studies were performed to help explaining the possible interactions that might take place between the proposed tested derivatives and the RR enzyme in comparing to hydroxyurea (HU) as a lead compound. HU, which quenches tyrosine radicals in the active site of RR and inhibits DNA synthesis in proliferating cells [31], has been widely used in the treatment of many neoplastic diseases [32], such as chronic, resistant, myelo-

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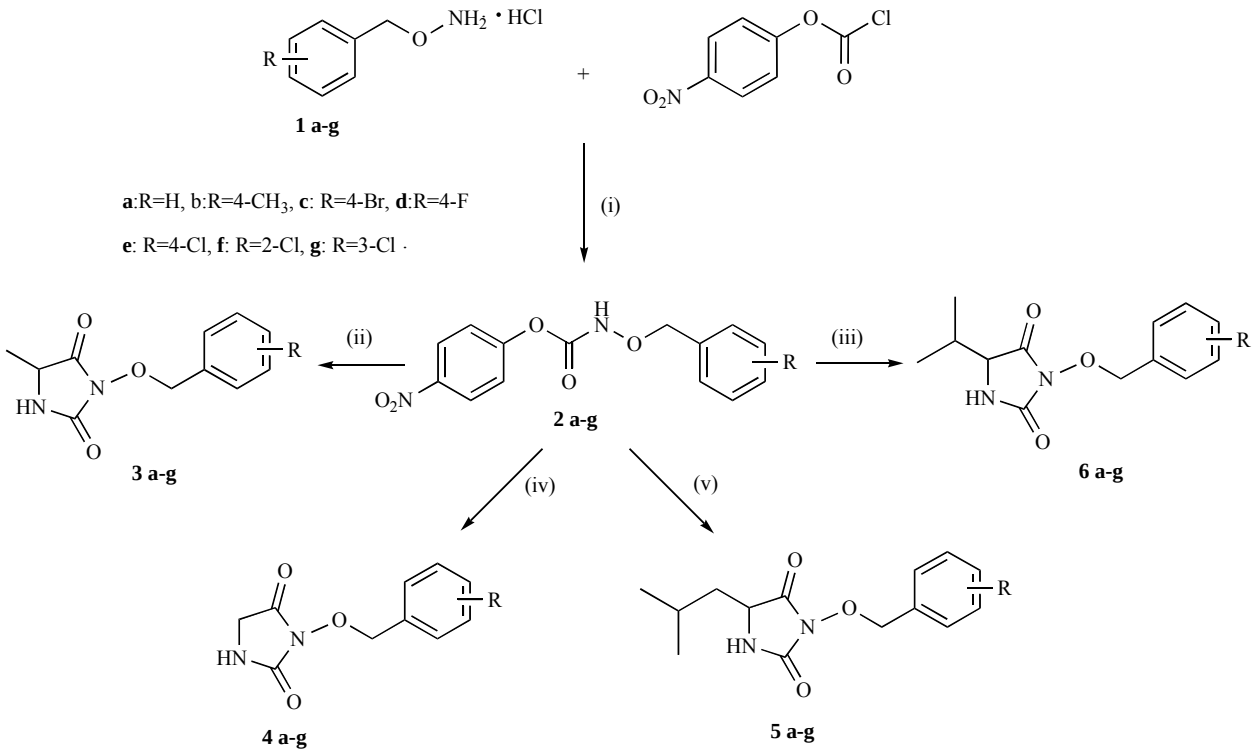

Scheme 1. Reagents and conditions: (i) pyridine, dry dichloromethane, reflux; (ii) alanine methyl ester hydrochloride, dry triethylamine, dry dichloromethane, rt; (iii) glycine methyl ester hydrochloride, dry triethylamine, dry dichloromethane, rt; (iv) leucine methyl ester hydrochloride, dry triethylamine, dry dichloromethane, rt; (v) valine methyl ester hydrochloride, dry triethylamine, dry dichloromethane, rt.

cytic leukemia [33-35], cervical carcinoma [36], oesophageal carcinoma [37] ect. The benzyloxyhydantoin derivatives designed in this study are also potential HU analogues which contain benzyloxy substituted hydroxyurea pharmacophore (3- benzyloxy- 1, 3- diamino- 2- ketone moiety in hydantoin ring). Hence, they may be arranged to yield potent and hopefully selective anticancer drugs by introducing hydroxyurea pharmacophore into hydantoin rigid scaffold. The target compounds were evaluated for their anticancer activity *in vitro* against human leukemia cell line K562, murine leukemia cell line L1210 and human laryngreal carcinoma cell line HEP-2 by the 3-(4, 5-dimethylthiahiazol-2-y1)-2, 5-diphenytetrazolium bromide (MTT) assay. Meanwhile, the technology of molecular docking was used to target compounds, which revealed many useful informations.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The target compounds were synthesized following the procedures as shown in Scheme **1**. The key starting compounds *O*-benzyl-hydroxylamine analogues **1a-1g** were prepared by the procedures reported by us [30, 38]. The *O*-benzyl- hydroxylamine analogues were treated with 4-nitrophenyl chloroformate in dry dichloromethane (DCM) to give **2a-2g** [30, 38-41]. Finally, the synthesis of the hydantoin derivatives **3a-3g, 4a-4g, 5a-5g** and **6a-6g** were accomplished by reaction of amino acids methyl ester hydrochloride with **2a-2g** in dry dichloromethane in the presence of anhydrous triethylamine (Scheme **1**).

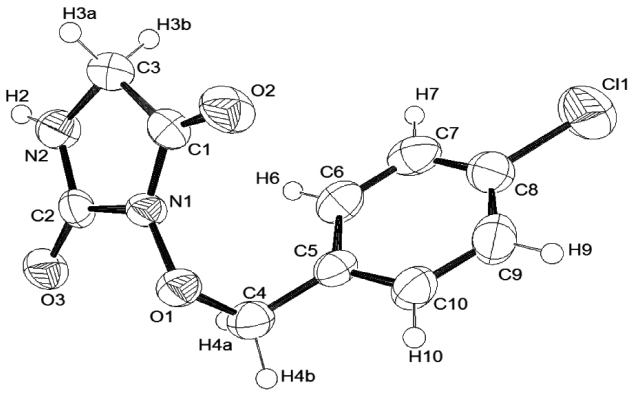

Fig. (1). An ORTEP-3 projection, showing the atomic labelling and the 30% probability ellipsoids of the compound **4e**.

All the target compounds were fully characterized on the basis of spectroscopic techniques (^{1}H NMR, ^{13}C NMR, IR and MS). In addition, the structure of **4e** was also confirmed by X-ray diffraction analysis. The good quality crystal of **4e** was grown by the slow evaporation technique using acetic ester and petroleun ether (4:1) as solvent. (Fig. **1**) showed the ORTEP diagram with 30% probability, main crystallographic parameters were presented in supplementary files. In the title crystal structure, the two carbonyl bond lengths were 1.204(3) (C1=O2), 1.218(2) (C2=O3) respectively, which was in the mean value range of 1.19 - 1.23 Å found for carbonyl bonds [42,43], but the latter was longer than former. The reason of this phenomenon was probably that the electron donation from the ring N atoms to the carbonyl groups

Table 1. ***In vitro*** **cytotoxicity results of target compounds against L1210, K562 and HEP-2 cancer cell lines.**

Compound No.	IC_{50}(μM)		
	L1210 Cells	K562 Cells	HEP-2 Cells
3a	22.19	21.63	>200
3b	>200	10.96	>200
3c	>200	12.95	20.91
3d	25.24	22.39	>200
3e	15.12	>200	>200
3f	21.82	19.14	>200
3g	46.94	3.80	>200
4a	25.08	>200	0.048
4b	>200	4.24	>200
4c	21.10	12.49	>200
4d	25.29	>200	>200
4e	34.24	8.49	116.8
4f	9.50	5.67	>200
4g	>200	9.87	>200
5a	>200	>200	78.86
5b	4.26	10.58	69.63
5c	10.99	9.70	0.15
5d	31.01	7.83	nd
5e	3.56	10.02	22.84
5f	>200	15.99	0.10
5g	7.87	15.67	0.04
6a	10.84	>200	0.70
6b	6.10	3.96	>200
6c	3.46	7.39	17.48
6d	7.23	>200	>200
6e	5.59	>200	>200
6f	5.30	13.60	nd
6g	7.62	17.82	0.01
HU	21.67	7.27	192.0

a) nd=not determined

would be expected to lengthen the C=O bonds and shorten the ring C-N bonds, C2=O3 being affected from both sides while C1=O2 being affected from only one side [44]. This was also reflected in the ranking order of bond lengths of hydantoin ring which was C1-C3 [1.492(3)]> C3-N2 [1.446(3)] > C2-N1 [1.399(3)] > C1-N1 [1.368(3)] > C2-N2 [1.330(3)]. There are two significant planar parts in the structure: the benzene and the hydantoin plane (O1, O2, O3, N1, N2, C1, C2, C3). The benzene plane forms a dihedral angle of 47.14° with the hydantoin plane. The N-H…O and C-H…O hydrogen bonds link the molecules into a continous parallel structure.

2.2. Antitumor Evaluation

The *in vitro* antitumor activity of the synthesized compounds **3a-3g, 4a-4g, 5a-5g, 6a-6g** against three different cancer cell lines, including human leukemia cell line K562, murine leukemia cell line L1210 and human laryngreal carcinoma cell line HEP-2, were assayed by the improved MTT method [45] and the results are listed in Table **1**.

From the obtained results we concluded that all target compounds showed moderate to excellent cytostatic potency against the different cancer cells compared to the reference compound HU. In general, most of them displayed good inhibitory effects on L1210 and K562, especially **3a, 3d, 3f, 3g, 4c, 4e, 4f, 5b, 5c, 5d, 5e, 5g, 6b, 6c, 6f** and **6g** which demonstrated high activity on both L1210 and K562 cell lines. Compounds **3c, 4a, 5c, 5e, 5f, 5g, 6a, 6c, 6g** showed significant activity on HEP-2 cell with IC_{50} values of 20.91, 0.048, 0.15, 22.84, 0.10, 0.04, 0.70, 17.48, 0.01μmol/L respectively. Interestingly, the substitute R of most potent compounds (**5c, 5e, 5f, 5g, 6g**) against HEP-2 cell were bromine and chlorine. Hence, it was anticipated that substitution of halogens substituents, such as chlorine, bromine on the benzene ring may be essential for increasing antitumor activity. In general, the compounds with hydrophobic isopropyl and isobutyl groups at the C-5 position of hydantoin demonstrated better activity, especially the compounds **5b, 5c, 5e, 5g, 6c** and **6g** showed highly active to inhibit proliferation of all the three cancer cell lines.

2.3. Docking Study

Similar to our previous docking study, the subsite B in Saccharomyces cerevisiae R1 (ScR1) domain of RR (PDB id- 2ZLF) [38, 46] was selected as the active site. Molecular modeling simulation study demonstrated that there were good complementarities between the target derivatives and the hydrophobic pocket of the enzymatic cavity (Fig. **2**). Binding energies of all tested hydantoin compounds were in range from -5.44 to -6.85 kcal/mol and were less than HU (Table **2**).

The LEU716, ARG717, GLN692, LYS723, SER726, MET727, LYS693, ILE696 and TYR730 of RR protein were found to be directly interacted with the target compounds. Most of the compounds, such as **3a** in (Fig. **2**), were oriented in the active site of the protein in a way that placed the aromatic ring into the hydrophobic pocket comprising the residues MET727, LYS693, ILE696 and TYR730. However, the compounds **5a**, **5c**, **6a** and **6g** were oriented in a way that placed the hydantoin ring into the above hydrophobic pocket and all of them showed good binding energy which were -6.16, -6.52, -6.56, -6.56 respectively. This may be attributed to lipophilic substituents: isopropyl and isobutyl groups at C5 position of hydantoin which comfirms the preference of the lipophilic substituents on the hydantoin ring. These derivatives showed high activity on HEP-2 cell lines with IC_{50} values of 78.86, 0.15, 0.70, 0.01μmol/L respectively, especially **6g** exhibited the best activity on HEP-2 cell lines.

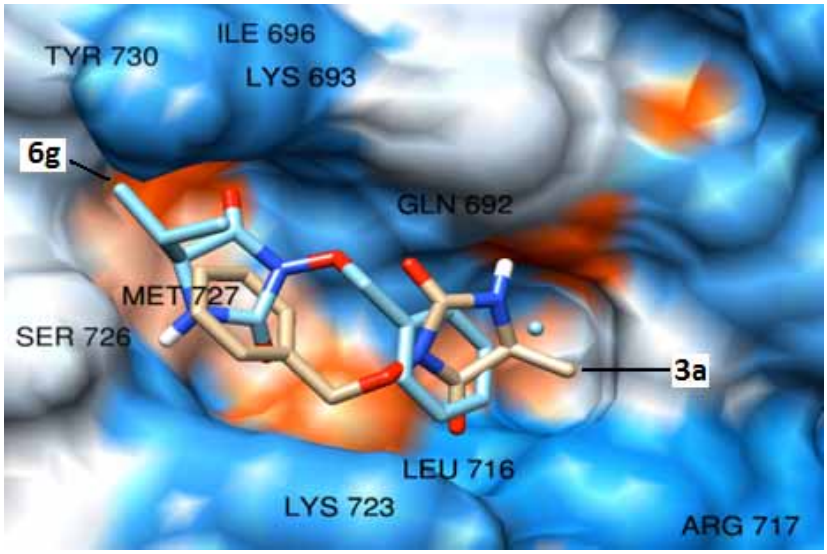

Fig. (2). The docking modes of compounds **3a**, **6g** at ScR1. The compounds are color coded as carbon beige (**3a**) and nattier blue (**6g**), nitrogen blue, oxygen red and hydrogen white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

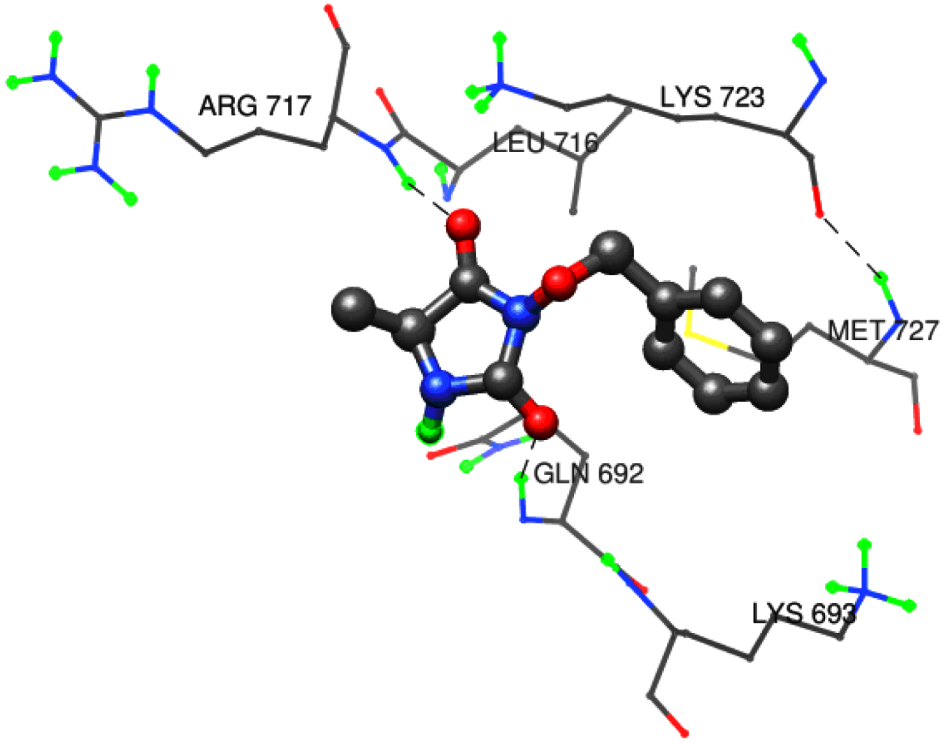

Fig. (3). Plausible binding modes for compound **3a** in the binding site of ScR1. The compound is color coded as carbon dim gray, nitrogen blue, oxygen red and hydrogen green. The hydrogen bonds are drawn in black dash line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

According to Table **2**, similar H-bonds have been observed for compounds **3a-3g** and **4a-4g** except for **4c**. The hydrogen bonding interactions of representative compound **3a** were showed in (Fig. **3**), two hydrogen bonds were identified. One is a H-bond formed between the C1- carbonyl oxygen of hydantoin and amide proton from ARG717 in RR. The other bond was formed between the C3- carbonyl oxygen of hydantoin and amide proton from GLN692 in enzyme. However, **5a-5g** and **6a-6g** showed more diversified hydrogen bonding interaction with more kinds of amino acid including LYS693, LYS723, SER691, LEU716 besides GLN-692 and ARG-717. The binding modes of representative compound **5g** in (Fig. **4**) revealed key hydrogen binding interactions: the H-bond of the oxygen of C3- carbonyl in hydantoin with amide hydrogen of LYS 693 and the H-bond of the hydrogen in 4-imino group of hydantoin with the the oxygen in SER691.

Better affinities to the active site of RR were gained by the compounds **5a-5g, 6a-6g** which have better antitumor

Table 2. ***In Silico*** **docking results.**

Compound No.	Binding Energy(Kcal/mol)	H-bond
HU	-3.09	GLN692(1.942Å), GLN692(2.216Å), ARG717(1.821Å)
3a	-6.35	GLN692(1.651Å), ARG717(1.830Å)
3b	-5.77	GLN692(1.675Å), ARG717(1.836Å)
3c	-6.68	GLN692(1.701Å), ARG717(1.822Å)
3d	-5.82	GLN692(1.663Å), ARG717(1.849Å)
3e	-6.35	GLN692(1.660Å), ARG717(1.975Å)
3f	-5.95	GLN692(1.656Å), ARG717(1.904Å)
3g	-6.31	GLN692(1.657Å), ARG717(1.832Å)
4a	-5.44	GLN692(1.763Å), ARG717(1.809Å)
4b	-5.99	GLN692(1.786Å), ARG717(2.020Å)
4c	-6.31	GLN692(1.730Å), LEU716(1.781Å)
4d	-6.53	GLN692(2.284Å), ARG717(1.911Å)
4e	-6.04	GLN692(1.676Å), ARG717(1.835Å)
4f	-5.81	GLN692(2.108Å), ARG717(1.955Å)
4g	-5.94	GLN692(1.689Å), ARG717(1.853Å)
5a	-6.16	LYS693(2.406Å), SER691(2.302Å)
5b	-6.61	GLN692(1.759Å)
5c	-6.52	LYS723(2.211Å), ARG717(2.558Å)
5d	-6.12	GLN692(1.650Å)
5e	-6.32	GLN692(1.807Å), LEU716(2.064Å)
5f	-6.64	LYS693(2.363Å), SER691(2.305Å)
5g	-6.56	LYS693(2.300Å), SER691(2.247Å)
6a	-6.19	GLN692(2.271Å)
6b	-6.85	GLN692(1.655Å), ARG717(1.871Å)
6c	-6.08	GLN692(2.465Å)
6d	-5.95	GLN692(1.775Å), ARG717(1.767Å)
6e	-6.46	GLN692(1.686Å), ARG717(1.950Å)
6f	-6.37	GLN692(1.830Å)
6g	-6.56	LYS723 (2.035Å)

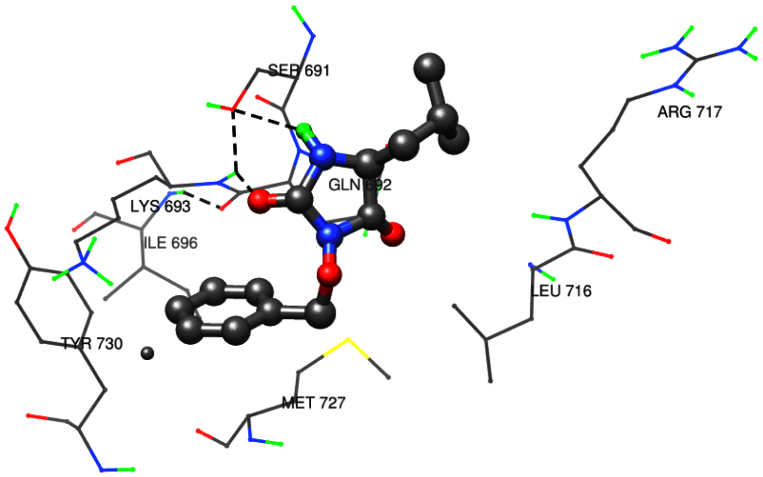

Fig. (4). Plausible binding modes for compound **5g** in the binding site of ScR1. The compound is color coded as carbon dim gray, nitrogen blue, oxygen red and hydrogen green. The hydrogen bonds are drawn in black dash line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

activity. They showed binding energy less than -6.00 except for compound **6d** and compound **6b** exhibited the highest affinity and attained the score of −6.85 kcal/mol. This is probably the reason that compound **6b** displayed the best inhibitory effects on K562 with IC_{50} values of 3.96, yet, compound **6d** exhibited inactive on K562 and HEP-2. The active compounds on all of the three cancer cell lines **(5b, 5c, 5e, 5g, 6c** and **6g**) fitted well in the active site of RR and attained the score of -6.61 to -6.08 kcal/mol. This suggested that the introduction of isopropyl and isobutyl groups was favorable not only to increasing antiproliferative activity, but also to increasing binding affinity to the active site of the RR receptor.

3. CONCLUSION

In summary, a series of 3-benzyloxyhydantoin derivatives was designed and synthesized by a convenient method. The anticancer activity of the target compounds was estimated by studying their inhibitory effects on human leukemia cell line K562, murine leukemia cell line L1210 and human laryngreal carcinoma cell line HEP-2. Binding of 3-benzyloxyhydantoin derivatives for the RR was investigated by use of molecular docking studies. Some of the prepared compounds showed potent antitumor activities against different cancer cell lines. Structure activity relationship (SAR) analysis and molecular moleding studies revealed that slightly larger lipophilic substituents such as isopropyl and isobutyl at C5 position of hydantoin were favorable to increasing binding affinity to the active site of the RR receptor and antiproliferative activity. In particular, the promising compounds **5b, 5c, 5e, 5g, 6c** and **6g,** which were the compounds of 5-isopropyl and 5-isobutyl benzyloxyhydantoin series, displayed high activity on all of the three cancer cell lines. These ndings have encouraged us to continue the development and testing of novel hydantoin derivatives and to conduct further studies to investigate SAR and their mechanisms of action.

4. EXPERIMENTAL

4.1. Docking Studies

Docking studies were performed using a free Autodock 4.0 [47]. The X-ray structures of ScR1-P6 complex (Protein Data Bank ID: 2ZLF) was retrieved from the Protein Data Bank [38]. The three-dimensional structures of the proposed compounds were constructed and energy minimizations were performed with the Chem-Draw/Chem3D [48]. A grid of 40, 40, and 40 points in x-, y-, and z-direction, respectively, with grid spacing of 0.375Å was built centered on the center of mass of the catalytic site of considered receptor. The population size was set to 150 and the individuals were initialized randomly. Docking was performed with the Lamarckian Genetic Algorithm using a medium of 2,500,000 energy evaluations per docking. The maximum number of top individual the automatically survived set to 1, maximum number of generations to 27,000, mutation rate to 0.02 and crossover rate to 0.8. The number of GA runs was set to 100. Calculated AutoDock binding free energies were used to score the ligands. The top scoring (most negative, thus favorable to binding) of the ligands were selected and arranged in Table **1**. All complexes pictures were rendered employing the UCSF Chimera software [49].

4.2. Chemistry

Melting points were determined using a capillary method and were uncorrected. Infrared spectra were recorded on a Shimadzu FTIR 8400 spectrometer (KBr pellets). ^{1}H- NMR and ^{13}C-NMR spectra were recorded on either a Bruker AV 400 MHz or a Bruker AV 600 MHz spectrometer. Chemical shifts (δ values) and coupling constants (J values) were reported in parts per million (ppm) and Hertz (Hz), respectively. Chemical shifts were relative to TMS except for solvents that were used, and the signals were quoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The mass spectra (EI or ESI) were recorded on either a Agilent 1100 MSD/TOF or on a Waters 2695 LC-ZQ4000. Crystal data were collected by a Bruker APEX- area-detector diffractometer. Column chromatography was carried out on silica gel (200-300 mesh). Unless otherwise stated, materials were obtained from commercial suppliers and purified according to the methods of chemical reagents. Alanine methyl ester hydrochloride, glycine methyl ester hydrochloride, leucine methyl ester hydrochloride and valine methyl ester hydrochloride were prepared following the procedures reported by Zeng [50].

4.3. General Procedure for the Synthesis of 1-(4-Nitrophenyl)- *N*- benzyloxy Carbamate Analogues 2a-2g

The synthesis of 1-(4-nitrophenyl)- *N*- benzyloxy carbamate analogues **2a-2g** has been described by us in previous papers and the spectroscopic data for compounds **2a-2e** and **2g** are in agreement with those in the papers [30, 39]. The spectroscopic data of **2f** were as follows.

4.3.1. 4-Nitrophenyl-N-[O-(2-chlorobenzylhydroxy)] carbamate (2f)

O-(2-Chlorobenzyl)hydroxylamine hydrochloride (19.3g, 0.1mol) was reacted according to general procedure to give **2f** as a white solid (23.1g, 72%): mp 182°C-183°C; IR (KBr): ν = 3267, 3074, 2870, 1732, 1543, 1481, 752 cm^{-1}; MS (ESI): m/z $[M+Na]^+$ 345.08.

4.4. General Procedure for the Synthesis of Hydantoin Analogues 3a-3g

Alanine methyl ester hydrochloride (9 mmol) was suspended in dry DCM (20 ml), dry triethylamine (36 mmol) was added slowly. 4-Nitrophenyl -*N* -(O -benzylhydroxy) carbamate **(2a-2g)** (9mmol) was dissolved in dry DCM (40 ml) and then added dropwise in the solution. The mixture was stirred at room temperature for 12 h. Upon completion, the reaction mixture was successively washed with 1 M $NaHCO_3$ (3×30ml), 1M HCl (30ml) and H_2O (2×30 ml). The DCM layer was dried by anhydrous $MgSO_4$, filtered and concentrated under vacuum to give yellow oil substance, which was purified by crystallization from anhydrous ether/petroleun ether (4:1) to afford the expected compounds.

4.4.1. 3-Benzyloxy-5-methylhydantoin (3a)

Yield: 36%; m.p.: 104-107°C; IR (KBr): ν = 3314, 1771, 1724, 1416, 760, 706 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.48 (d, *J* = 3.0 Hz, 3H), 7.37 (d, *J* = 2.5 Hz, 2H), 6.61 (s, 1H), 5.13 (s, 2H), 3.96 (d, *J* = 6.9 Hz, 1H), 1.34 ppm (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 169.38, 154.23, 133.62, 130.40, 129.81, 128.88, 77.43, 51.39, 17.73 ppm; HRMS-ESI: m/z calcd. for $C_{11}H_{13}N_2O_3$ $[M+H]^+$: 221.0926, found: 221.0918.

4.4.2. 3-(4-Methylbenzyloxy)-5-methylhydantoin (3b)

Yield: 17%; m.p.: 90-92°C; IR (KBr) cm^{-1}: ν = 3329, 1740, 1709, 1423, 810, 750 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.38 (s, 2H), 7.19 (s, 2H), 6.32 (s, 1H), 5.10 (s, 2H), 4.00 (s, 1H), 2.36 (s, 3H), 1.38 ppm (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.92, 153.73, 139.46, 130.28, 130.17, 129.21, 77.04, 51.02, 21.33, 17.45 ppm; HRMS-ESI: m/z calcd. for $C_{12}H_{15}N_2O_3$ $[M+H]^+$: 235.1083, found: 235.1078.

4.4.3. 3-(4-Bromobenzyloxy)-5-methylhydantoin (3c)

Yield: 43%; m.p.: 134-136°C; IR (KBr): ν = 3346, 1732, 1705, 1593, 1489, 1420, 812, 720 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.51 (d, *J* = 8.2 Hz, 2H), 7.37 (d, *J*=8.1 Hz, 2H), 6.11 (s, 1H), 5.09 (s, 2H), 4.02(d, *J* = 6.9 Hz, 1H), 1.39 ppm (d, *J*=7.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.78, 153.39, 132.35, 131.75, 131.53, 123.72, 77.02, 51.05, 17.48 ppm; HRMS-ESI: m/z calcd. for $C_{11}H_{12}BrN_2O_3$ $[M+H]^+$: 299.0031, found: 299.0028.

4.4.4. 3-(4-Fluorobenzyloxy)-5-methylhydantoin (3d)

Yield: 18%; m.p.: 98-100°C; IR (KBr) : ν = 3306, 1736, 1713, 1620, 1585, 1497, 920, 768 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.50 (t, *J* = 7.3 Hz, 1H), 7.35-7.48 (m, 1H), 7.16 (t, *J* = 14.9 Hz, 1H), 7.07 (t, *J* = 18.1 Hz, 1H), 6.53 (s, 1H), 5.20 (s, 2H), 4.01-3.99 (m, 1H), 1.38 ppm (d, *J*=7.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.92, 153.65, 132.46, 131.65, 131.57, 124.30, 120.85, 115.41, 77.04, 51.06, 17.33 ppm; HRMS-ESI: m/z calcd. for $C_{11}H_{12}FN_2O_3$ $[M+H]^+$: 239.0832, found: 239.0823.

4.4.5. 3-(4-Chlorobenzyloxy)-5-methylhydantoin (3e)

Yield: 14%; m.p.: 136~137°C; IR (KBr): ν = 3344, 1709, 1597, 1489, 802, 690 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.44 (dd, J_1=6.6 Hz, J_2=1.8 Hz, 2H), 7.36 (dd, J_1=6.6 Hz, J_2=1.8 Hz, 2H), 5.50 (s, 1H), 5.12 (s, 2H), 4.04-4.01 (m, 1H), 1.41 ppm (d, *J*=7.2 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 168.68, 153.17, 135.48, 131.78, 131.33, 128.77, 77.02, 51.03, 17.55 ppm; HRMS-ESI: m/z calcd. for $C_{11}H_{13}ClN_2O_3$ $[M+H]^+$: 255.0536, found: 255.0533.

4.4.6. 3-(2-Chlorobenzyloxy)-5-methylhydantoin (3f)

Yield: 22%; m.p.: 148~149°C; IR (KBr): ν = 3329, 1740, 1709, 1402, 760 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.59 (dd, J_1=7.0 Hz, J_2=2.2 Hz, 1H), 7.40 (dd, J_1=7.6 Hz, J_2=1.7 Hz, 1H), 7.34-7.30 (m, 2H), 5.75 (s, 1H), 5.29 (dd, J_1=13.2 Hz, J_2=11.4 Hz, 2H), 4.03 (q, J_1= 13.8 Hz, J_2=7.2 Hz, 1H), 1.42 ppm (d, *J*= 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 168.71, 153.35, 134.81, 131.99, 131.60, 130.69, 129.59, 126.99, 77.03, 51.05, 17.42 ppm; HRMS-ESI: m/z calcd. for $C_{11}H_{12}ClN_2O_3$ $[M+H]^+$: 255.0536, found: 255.0531.

4.4.7. 3-(3-Chlorobenzyloxy)-5-methylhydantoin (3g)

Yield: 27%; m.p.: 108~110°C; IR (KBr): ν = 3294, 1774, 1724, 1574, 1402, 1477, 1369, 891, 802 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.51 (s, 1H), 7.41-7.28 (m, 3H), 6.05 (s, 1H), 5.13 (s, 2H), 4.06 (s, 1H), 1.43 ppm (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.83, 153.54, 135.26, 134.37, 129.87, 129.56, 127.93, 77.03, 51.09, 17.45 ppm. HRMS-ESI: m/z calcd. for $C_{11}H_{12}ClN_2O_3$ $[M+H]^+$: 255.0536, found: 255.0526.

4.5. General Procedure for the Synthesis of Hydantoin Analogues 4a-4g

Glycine methyl ester hydrochloride (9 mmol) and 4-nitrophenyl - *N* - (O -benzylhydroxy) carbamate **(2a-2g)** (9mmol) were reacted according to the procedure of **3a-3g** to give **4a-4g** as a white solid**.**

4.5.1. 3-Benzyloxyhydantoin (4a)

Yield: 20%; m.p.: 107~109°C; IR (KBr): ν = 3248, 1728, 1450, 1201, 741, 698 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.49 (s, 3H), 7.38 (s, 2H), 6.57 (s, 1H), 5.12 (s, 2H), 3.86 ppm (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 166.04, 154.99, 133.67, 130.23, 129.82, 128.96, 77.44, 44.79 ppm; HRMS-ESI: m/z calcd. for $C_{10}H_{11}N_2O_3$ $[M+H]^+$: 207.0770, found: 207.0763.

4.5.2. 3-(4-Methylbenzyloxy)hydantoin (4b)

Yield: 28%; m.p.: 118~121°C; IR (KBr): ν = 3263, 1709, 1447, 1201, 813, 730 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.45 (s, 2H), 7.26 (s, 2H), 5.15 (s, 2H), 4.52 (s, 1H), 3.94 (s, 2H), 2.43 ppm (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 169.56, 157.47, 142.28, 133.24, 132.91, 132.05, 80.58, 43.96, 23.92 ppm; HRMS-ESI: m/z calcd. for $C_{11}H_{13}N_2O_3$ $[M+H]^+$: 221.0926, found: 221.0917.

4.5.3. 3-(4-Bromobenzyloxy)hydantoin (4c)

Yield: 18%; m.p.: 160~162°C; IR (KBr): ν = 3256, 1732, 1701, 1450, 1201, 802, 730 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.52 (d, *J*=8.4 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 5.38 (s, 1H), 5.10 (s, 2H), 3.94 ppm (s, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 163.97, 158.48, 132.83, 130.75, 130.49, 129.57, 122.57, 121.96, 76.84, 40.64 ppm; HRMS-ESI: m/z calcd. for $C_{10}H_{10}BrN_2O_3$ $[M+H]^+$: 284.9875, found: 284.9869.

4.5.4. 3-(4-Fluorobenzyloxy)hydantoin (4d)

Yield: 12%; m.p.: 74~76°C; IR (KBr): ν = 3306, 1747, 1720, 1651, 1551, 1512, 1201, 833, 700 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.48 (dd, J_1=8.5 Hz, J_2=5.4 Hz, 1H), 7.39 (dd, J_1= 8.4 Hz, J_2=5.4 Hz, 1H), 7.10-7.06 (m, 2H), 6.15 (s, 1H), 5.11 (s, 1H), 4.81 (s, 1H), 4.02 (d, *J*=4.8 Hz, 1H), 3.92 ppm (s, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 164.22, 159.54, 154.05, 131.91, 130.97, 129.26, 115.66, 115.53, 77.03, 41.30 ppm; HRMS-ESI: m/z calcd. for $C_{10}H_{10}FN_2O_3$ $[M+H]^+$: 225.0675, found: 225.0687.

4.5.5. 3-(4-Chlorobenzyloxy)hydantoin (4e)

Yield: 17%; m.p.: 158~159°C; IR (KBr): ν = 3260, 1732, 1701, 1450, 1201, 806, 730 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.45 (d, *J*=4.8 Hz, 2H), 7.37 (dd, J_1=6.6 Hz, J_2=1.8

Hz, 2H), 5.43 (s, 1H), 5.12 (s, 2H), 3.94 ppm (d, *J*=1.2 Hz, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 165.15, 153.74, 135.48, 131.80, 131.20, 128.83, 77.23, 44.35 ppm; HRMS-ESI: m/z calcd. for $C_{10}H_{10}ClN_2O_3$ $[M+H]^+$: 241.0380, found: 241.0372.

4.5.6. 3-(2-Chlorobenzyloxy)hydantoin (4f)

Yield: 20%; m.p.: 118~119°C; IR (KBr): *v* = 3337, 1732, 1709, 1439, 1201, 890, 760 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.60 (t, *J*=7.2 Hz, 1H), 7.41 (dd, J_1=7.2 Hz, J_2=1.8 Hz, 1H), 7.34-7.31 (m, 2H), 5.94 (s, 1H), 5.29 (s, 2H), 3.93 ppm (d, *J*=6.0 Hz, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 165.26, 154.09, 134.66, 131.83, 131.57, 130.68, 129.62, 127.08, 77.03, 44.42 ppm; HRMS-ESI: m/z calcd. for $C_{10}H_{10}ClN_2O_3$ $[M+H]^+$: 241.0380, found: 241.0373.

4.5.7. 3-(3-Chlorobenzyloxy)hydantoin (4g)

Glycine methyl ester hydrochloride (1.13g, 9 mmol) and 4- nitrophenyl-*N*-[*O*-(3-chlorobenzylhydroxy)] carbamate (2.90g, 9mmol) **(2g)** were reacted to give crude product as yellow oil substance. The crude product was purified by silica gel chromatography using ethyl acetate/ n-hexane (2:5) as eluent, solvent was eliminated from the elution under reduced pressure and then recrystallized in ether/petroleun ether (4:1) to give **4g** as a white solid. Yield: 24%; m.p.: 68~70°C; IR (KBr): *v* = 3306, 1747, 1720, 1651, 1547, 1439, 1210, 795, 710 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.51 (s, 1H), 7.48 (s, 1H), 7.39-7.35 (m, 2H), 6.23 (s, 1H), 5.10 (s, 2H), 3.93 ppm (d, *J*=6.0 Hz, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 165.37, 154.14, 137.11, 134.59, 130.05, 129.52, 129.01, 127.78, 77.25, 41.31 ppm; HRMS-ESI: m/z calcd. for $C_{10}H_{10}ClN_2O_3$ $[M+H]^+$: 241.0380, found: 241.0371.

4.6. General Procedure for the Synthesis of Hydantoin Analogues 5a-5g

Leucine methyl ester hydrochloride (9 mmol) and 4-nitrophenyl - *N* - (O -benzylhydroxy) carbamate **(2a-2g)** (9mmol) were reacted as described for **3a-3g**. Purification by recrystallization from AcOEt and petroleum ether to give **5a-5g** as a white solid**.**

4.6.1. 3-Benzyloxy-5-isobutylhydantoin (5a)

Yield: 17%; m.p.: 106~108°C; IR (KBr): *v* = 3233, 2954, 1782, 1736, 1420, 1202, 745, 698 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.50(dd, J_1=1.2 Hz, J_2=3.6 Hz, 2H), 7.38(t, *J*=1.8 Hz, 3H), 5.75(s, 1H), 5.15(s, 2H), 3.97-3.94 (m, 1H), 1.75-1.68 (m, 2H), 1.45-1.40 (m, 1H), 0.96 (d, *J*=6.6 Hz, 3H), 0.93 ppm (d, *J*=6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.47, 153.33, 133.33, 130.09, 129.44, 128.52, 77.23, 53.79, 40.72, 29.72, 24.92, 22.97 ppm. HRMS-ESI: m/z calcd. for $C_{14}H_{19}N_2O_3$ $[M+H]^+$: 263.1396, found: 263.1380.

4.6.2. 3-(4-Methylbenzyloxy)-5-isobutylhydantoin (5b)

Yield: 13%; m.p.: 103~104°C; IR (KBr): *v* = 3233, 2959, 1782, 1732, 1466, 1202, 810, 740 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.37 (t, *J*= 8.4 Hz, 2H), 7.18 (d, *J*=7.8 Hz, 2H), 6.16 (s, 1H), 5.10 (s, 2H), 3.96-3.93 (m, 1H), 2.36 (s, 3H), 1.74-1.66 (m, 2H), 1.44-1.39 (m, 1H), 0.96 (d, *J*=6.6 Hz, 3H), 0.93 ppm (d, *J*=6.6 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 168.66, 153.89, 139.41, 130.29, 130.17, 129.19, 77.24, 53.76, 40.68, 24.80, 23.00, 21.47 ppm; HRMS-ESI: m/z calcd. for $C_{15}H_{21}N_2O_3$ $[M+H]^+$: 277.1552, found: 277.1535.

4.6.3. 3-(4-Bromobenzyloxy)-5-isobutylhydantoin (5c)

Yield: 30%; m.p.: 108~111°C; IR (KBr): *v* = 3190, 2955, 1778, 1736, 1470, 1202, 810, 710 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.52 (s, 2H), 7.39 (s, 2H), 5.73 (s, 1H), 5.10 (s, 2H), 3.98 (s, 1H), 1.73 (s, 2H), 1.45 (s, 1H), 0.96 ppm (s, 3H), 0.95 ppm (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.51, 153.34, 132.41, 131.74, 131.55, 123.71, 77.34, 53.83, 40.72, 24.88, 22.97, 21.65 ppm; HRMS-ESI: m/z calcd. for $C_{14}H_{18}BrN_2O_3$ $[M+H]^+$: 341.0501, found: 341.0483.

4.6.4. 3-(4-Fluorobenzyloxy)-5-isobutylhydantoin (5d)

Yield: 12%; m.p.: 109~110°C; IR (KBr): *v* = 3240, 2963, 1778, 1732, 1512, 1423, 1210, 829, 710 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.50-7.47 (m, 2H), 7.08-7.05 (m, 2H), 6.01 (s, 1H), 5.11 (s, 2H), 3.99-3.96 (m, 1H), 1.74-1.71 (m, 2H), 1.45(dd, J_1=9.6Hz, J_2=8.4Hz, 1H), 0.97(d, *J*=6.6 Hz, 3H), 0.94 ppm (d, *J*=6.6 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 168.59, 164.24, 162.59, 153.64, 132.05, 129.28, 115.61, 115.47, 77.03, 53.82, 40.68, 24.86, 22.98, 21.60 ppm; HRMS-ESI: m/z calcd. for $C_{14}H_{18}FN_2O_3$ $[M+H]^+$: 281.1301, found: 281.1307.

4.6.5. 3-(4-Chlorobenzyloxy)-5-isobutylhydantoin (5e)

Yield: 19%; m.p.: 104~106°C; IR (KBr): *v* = 3221, 2959, 1774, 1724, 1663, 1431, 1201, 814, 719 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.44 (dd, J_1= 6.6 Hz, J_2=1.8 Hz, 2H), 7.35 (dd, J_1=6.6 Hz, J_2=2.4 Hz, 2H), 5.90 (s, 1H), 5.11 (s, 2H), 3.98-3.96 (m, 1H), 1.75-1.70 (m, 2H), 1.46-1.43(m, 1H), 1.01(d, *J*=6.6 Hz, 3H) 0.95 ppm (dd, J_1=6.6 Hz, J_2=6.0 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 168.65, 153.82, 135.45, 131.86, 131.30, 128.75, 77.25, 53.83, 40.66, 24.81, 23.00, 21.60 ppm; HRMS-ESI: m/z calcd. for $C_{14}H_{18}ClN_2O_3$ $[M+H]^+$: 297.1006, found: 297.0996.

4.6.6. 3-(2-Chlorobenzyloxy)-5-isobutylhydantoin (5f)

Yield: 21%; m.p.: 85~86°C; IR (KBr): *v* = 3190 , 2959, 1774, 1728, 1431, 1201, 748 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.60-7.58 (m, 2H), 7.42-7.39 (m, 2H), 5.98 (d, *J*=6.0 Hz, 1H), 5.27(t, *J*=16.2 Hz, 2H), 3.99-3.96 (m, 1H), 1.76-1.71 (m, 2H), 1.48 (dd, J_1=8.4 Hz, J_2=8.4 Hz, 1H), 0.97 (d, *J*=6.6 Hz, 3H), 0.94 ppm (d, *J*=6.6 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 168.48, 153.52, 134.81, 132.10, 130.86, 129.67, 127.08, 126.97, 77.23, 53.81, 40.63, 24.87, 22.99, 21.59 ppm; HRMS-ESI: m/z calcd. for $C_{14}H_{18}ClN_2O_3$ $[M+H]^+$: 297.1006, found: 297.0992.

4.6.7. 3-(3-Chlorobenzyloxy)-5-isobutylhydantoin (5g)

Yield: 28%; m.p.: 98~100°C; IR (KBr): *v* = 3240 , 2955, 1728, 1427, 1201, 787, 709 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.48 (d, *J*=6.6 Hz, 1H), 7.41 (d, *J*=7.4 Hz, 1H), 7.37-7.31 (m, 2H), 5.67 (s, 1H), 5.11 (d, *J*=6.0 Hz, 2H), 3.98 (dd, J_1=9.3 Hz, J_2=3.1 Hz, 1H), 1.76-1.71 (m, 2H), 1.46 (t, *J* =9.0 Hz, 1H), 0.97 (d, *J*=6.0 Hz, 3H), 0.95 ppm (d, *J*=6.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.53, 153.56, 135.34, 134.36, 129.86, 129.30, 129.03, 127.95, 77.33, 53.87, 40.71, 24.79, 22.90, 21.79 ppm; HRMS-ESI: m/z

calcd. for $C_{14}H_{18}ClN_2O_3$ $[M+H]^+$: 297.1006, found: 297.0999.

4.7. General Procedure for the Synthesis of Hydantoin Analogues 6a-6g

Valine methyl ester hydrochloride (9 mmol) and 4- nitrophenyl-*N*-[*O*-(3-chlorobenzylhydroxy)] carbamate (9mmol) **(2a-2g)** were reacted as described for **3a-3g** to give crude product as yellow oil substance. The crude product was firstly purified by recrystallization from ether and petroleun ether, and then recrystallized from acetone and water to afford the expected compounds.

4.7.1. 3-Benzyloxy-5-isopropylhydantoin (6a)

Yield: 11%; m.p.: 91~92°C; IR (KBr): ν = 3248, 2970, 1771, 1720, 1462, 1416, 1201, 748, 698 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.50 (t, J_1=9.6 Hz, 2H), 7.37 (t, J_1=6.6 Hz, 3H), 5.37 (s, 1H), 5.15 (t, J =15.6 Hz, 2H), 3.84 (dd, J_1=1.2 Hz, J_2=1.2 Hz, 1H), 2.18-2.15 (m, 1H), 0.98 (d, J=6.6 Hz, 3H), 0.94 ppm (d, J=6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 167.64, 154.30, 135.23, 133.44, 129.93, 129.37, 128.80, 128.52, 77.03, 57.43, 52.13, 29.90, 18.59 ppm; HRMS-ESI: m/z calcd. for $C_{13}H_{17}N_2O_3$ $[M+H]^+$: 249.1239, found: 249.1228.

4.7.2. 3-(4-Methylbenzyloxy)-5-isopropylhydantoin (6b)

Yield: 15%; m.p.: 74-76 °C; IR (KBr): ν = 3233, 2962, 1771, 1724, 1462, 1416, 1201, 802, 695 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.38 (d, J=7.8 Hz, 2H), 7.18 (d, J=8.4 Hz, 2H), 5.26 (s, 1H), 5.10 (dd, J_1= 10.2 Hz, J_2=9.6 Hz, 2H), 3.84 (d, J=3.6 Hz, 1H), 2.35 (s, 3H), 2.17 (t, J=3.6 Hz, 1H), 0.98 (d, J=6.6 Hz, 3H), 0.84 ppm (d, J=7.2 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 167.55, 154.08, 139.37, 130.36, 130.06, 129.20, 77.02, 60.29, 30.05, 21.33, 18.36 ppm; HRMS-ESI: m/z calcd. for $C_{14}H_{19}N_2O_3$ $[M+H]^+$: 263.1396, found: 263.1389.

4.7.3. 3-(4-Bromobenzyloxy)-5-isopropylhydantoin (6c)

Yield: 12%; m.p.: 70-73°C; IR(KBr): ν = 3287, 2966, 1790, 1724, 1462, 1408, 1157, 849, 798 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.51 (t, J=9.0 Hz, 2H), 7.38 (d, J=8.4 Hz, 2H), 6.13 (d, J=6.6 Hz, 1H), 5.08 (dd, J_1=9.6 Hz, J_2=4.2 Hz, 2H), 3.87 (dd, J_1=4.2 Hz, J_2=1.2 Hz, 1H), 2.21-2.16 (m, 1H), 1.00 (d, J=6.6 Hz, 3H), 0.85 ppm (d, J=6.6 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 167.78, 154.63, 132.45, 131.72, 131.47, 123.65, 77.08, 60.41, 30.10, 18.31, 15.91 ppm; HRMS-ESI: m/z calcd. for $C_{13}H_{15}BrN_2O_3$ $[M]^+$: 326.0266, found: 326.0261.

4.7.4. 3-(4-Fluorobenzyloxy)-5-isopropylhydantoin (6d)

Yield: 15%; m.p.: 97-100°C; IR (KBr): ν = 3132, 1728, 1427, 1218, 941, 763 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.52 (s, 1H), 7.37 (s, 1H), 7.17 (s, 1H), 7.08 (s, 1H), 5.72 (s, 1H), 5.22 (s, 2H), 3.86 (s, 1H), 2.18 (s, 1H), 1.00 (s, 3H), 0.87 ppm (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 167.63, 154.26, 132.29, 131.45, 130.27, 127.58, 124.23, 115.52, 77.04, 60.37, 30.12, 18.27, 16.02 ppm; HRMS-ESI: m/z calcd. for $C_{13}H_{16}FN_2O_3$ $[M+H]^+$: 267.1145, found: 267.1139.

4.7.5. 3-(4-Chlorobenzyloxy)-5-isopropylhydantoin (6e)

Yield: 14%; m.p.: 100-101°C; IR (KBr): ν = 3252, 2966, 1786, 1728, 1493, 1408, 1201, 853, 805 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.40 (d, J=1.7 Hz, 1H), 7.36 (dd, J_1=7.3 Hz, J_2=1.4 Hz, 1H), 7.34-7.32 (m, 1H), 7.30 (d, J=7.2 Hz, 1H), 5.80 (d, J=7.2 Hz, 1H), 5.10 (dd, J_1=14.4 Hz, J_2=10.2 Hz, 2H), 3.88 (dd, J_1=3.6 Hz, J_2=1.8 Hz, 1H), 2.21-2.18 (m, 1H), 1.00 (d, J=6.6 Hz, 3H), 0.86 ppm (d, J=6.6 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 167.77, 154.60, 135.38, 132.43, 131.34, 130.55, 128.86, 123.64, 77.06, 60.39, 30.08, 18.30, 15.88 ppm; HRMS-ESI: m/z calcd. for $C_{13}H_{16}ClN_2O_3$ $[M+H]^+$: 283.0849, found: 283.0840.

4.7.6. 3-(2-Chlorobenzyloxy)-5-isopropylhydantoin (6f)

Yield: 24%; m.p.: 111~113°C; IR (KBr): ν = 3233, 2959, 1774, 1724, 1477, 1427, 1201, 764, 680 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.39 (d, J=1.2 Hz, 1H), 7.36 (d, J=1.2 Hz, 1H), 7.34-7.33 (m, 1H), 7.33-7.30 (m, 1H), 6.61 (s, 1H), 5.10 (dd, J_1=13.2 Hz, J_2=10.2 Hz, 2H), 3.88 (dd, J_1=4.2 Hz, J_2=1.2 Hz, 1H), 2.21-2.18 (m, 1H), 1.02 (d, J=7.2 Hz, 3H), 0.86 ppm (d, J=7.2 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 167.65, 154.45, 135.37, 134.35, 129.86, 129.81, 129.49, 127.84, 77.04, 60.42, 30.12, 18.32, 15.92 ppm; HRMS-ESI: m/z calcd. for $C_{13}H_{16}ClN_2O_3$ $[M+H]^+$: 283.0849, found: 283.0835.

4.7.7. 3-(3-Chlorobenzyloxy)-5-isopropylhydantoin (6g)

Yield: 25%; m.p.: 73~74°C; IR (KBr): ν = 3256, 2970, 1771, 1720, 1410, 1201, 791, 687 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.43(dd, J_1=4.2 Hz, J_2=1.8 Hz, 2H), 7.35 (dd, J_1=1.8 Hz, J_2=4.2 Hz, 2H), 6.13 (s, 1H), 5.10 (dd, J_1=15.0 Hz, J_2=10.8 Hz, 2H), 3.86 (dd, J_1=4.2 Hz, J_2=1.2 Hz, 1H), 2.20-2.17 (m, 1H), 1.00 (d, J=7.2 Hz, 3H), 0.85 ppm (d, J=6.6 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 167.58, 154.27, 135.37, 134.36, 130.30, 129.49, 128.65, 127.85, 77.03, 60.40, 30.10, 18.32, 15.91 ppm. HRMS-ESI: m/z calcd. for $C_{13}H_{16}ClN_2O_3$ $[M+H]^+$: 283.0849, found: 283.0856.

4.8. Cytotoxicity Assay *in Vitro*

The human leukemia cell line K562, murine leukemia cell line L1210 and human laryngreal carcinoma cell line HEP-2 were purchased from Nanjing Keygen Biotech. Co., Ltd., China. The three cell lines were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 80 IU /ml penicillin G and 100 IU /ml streptomycin sulfate in a 5% CO_2-95% humidity incubator at 37°C. Each compound was dissolved with DMSO to obtain a serial concentration of 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, 1×10^{-6} and 1×10^{-7}M. The improved MTT assay was chosen to sdetermine the IC_{50} of the target compounds **3a-3g, 4a-4g, 5a-5g** and **6a-6g**. Tumor cells (1×10^6 cells /ml) and the test samples were inoculated in 96-well culture plates with the quantity of 90μl /well and 10μl /well respectively. After cultured for 48 h, 20μl of MTT (5mg /mL) was added to each well and then the mixture was incubated for 4h. After that, the cultured cells were mixed with 100μl of triple solution (10% SDS, 5% isobutanol, 0.01 M HCl) and incubated for 10 h at 37°C. Finally the absorbance of each well was measured at 570 nm. Each experiment was performed at least 3 times.

CONFLICT OF INTEREST

The authors confirms that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Main crystallographic parameters of **4e**. ^{1}H-NMR and ^{13}C-NMR for target compouds **3a-3g, 4a-4g, 5a-5g** and **6a-6g**.

Supplementary material is available on the publishers Web site along with the published article.

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