INTRODUCTION

Hydroxyurea (HU) which is an effective ribonucleoside reductase inhibitor has been of manifold pharmacological activities, so it has been used as antineoplastic agent to treat chronic myelocytic leukemia, melanoma and other malignant tumor diseases. Hydroxyurea is also useful in therapy of sickle cell anemia and in combined antiretroviral therapy, where it is most effective if taken in combination with reverse transcriptase inhibitors (ddI, AZT, ddC or d4T). Our recent research indicated that the stronger hydrophobic nature of the hydroxyurea derivatives might favour the cytotoxic activity and the benzyl groups at the O-position of hydroxyurea is associated with enhanced cytotoxic activity. The disadvantages associated with hydroxyurea’s physicochemical properties, e.g., high hydrophilicity (log P: -1.80) and small molecular size, may be overcome by the structural modification. This background prompted us to further explore novel hydroxyurea derivatives having substituents at O-position and N3-position. We designed and synthesized 15 target compounds by used D-glucosamine or L-amino acid moiety as substituents to modify hydroxyurea, respectively. Among them, 13 compounds were novel structures except for 5a and 7a. The compounds 6(a-e) and 7(a-e) are racemic mixtures. The specific rotations of the compounds 5(a-e) were not to be determined because of their low in vitro antitumor activity. In this paper, we report the synthesis and in vitro antitumor activity of benzyloxyurea and the benzyloxyhydantoin derivatives.

EXPERIMENTAL

All the starting materials were obtained from commercial source and used without further purification unless stated. D-glucosamine, L-methionine methyl ester hydrochloride and L-phenylalanine methyl ester hydrochloride were prepared following the procedures reported by Zhang. Melting points were determined using capillary method and were uncorrected. The infrared spectra were recorded on a Shimadzu FT-IR 8400 spectrometer (KBr discs). 1H and 13C NMR spectra were recorded on a Bruker AV 400 MHz spectrometer. Mass spectra were recorded on Agilent 1100 MSD/TOF and Waters 2695 LC-ZQ 4000 system. Crystal data were collected by a Bruker APEX-II area-detector Diffractometer. 

General procedure for preparation of intermediates 2(a-e): To anhydrous ethanol (150 mL) freshly cut sodium (7 g, 0.3 mol) was added in small portions. When the metal has dissolved, cooled to room temperature, the solution of acetoxime (21.9 g, 0.3 mol) was added slowly into the reaction mixture and stirred for another 5 h at room temperature. 30 mL of water was added, the white precipitate was extracted with ether (3 × 150 mL) and the combined organic phases were dried with MgSO4. After filtering, the solvent was evaporated to get 2(a-e) as yellow oily liquid. The yields were reported in Table-1. 

General procedure for preparation of O-benzylhydroxylamines 3(a-e): The obtained yellow oily liquid 2(a-e)
above was added gradually with stirring to a solution of concentrated hydrochloric acid at room temperature for 2 h. Then the reaction mixture was stirred under reduced pressure. The formed solid product was filtered off, washed with ethanol and ethyl acetate to afford 3(a-e) as white solid. The yields and melting points of the intermediates were shown in Table-1.

### Preparation of intermediates 4(a-e): O-Benzylhydroxyhydrochloride (0.1 mol) was suspended in dry dichloromethane (DCM) (200 mL) and pyridine (0.1 mol). 4-Nitrophenylchloroformate (0.1 mol) was added dropwise in dichloromethane (100 mL) while stirring at room temperature for 45 min. After the addition was completed, the reaction mixture was heated to reflux and stirred for about 12 h, then cooled to room temperature, diluted with dichloromethane (200 mL), washed sequentially with 1 M HCl (3 × 200 mL), 1 M NaHCO₃ (3 × 200 mL), H₂O (2 × 200 mL) and saturated sodium chloride solution (200 mL). The organic phase was dried with anhydrous MgSO₄, filtered, evaporated under vacuum and dried to afford the target compound.

N-Benzoxynoaminoforandine-4-nitrophenylester (4a)

Preparation of glucosamine derivatives of benzoxycarbonylurea 5(a-e): To a solution of the intermediates 4(a-e) (0.01 mol) in anhydrous DMSO (5 mL), D-glucosamine (0.01 mol) prepared before hand was added at room temperature with stirring for 2 days. After adding dichloromethane (70 mL) into the reaction mixture until white solid appeared, the mixture was placed in a refrigerator overnight. The formed solid product was filtered off, washed with dichloromethane and ether for several times. After vacuum drying in reduced pressure, the target compound was obtained as light yellow powder.

| Compounds | R     | Yield (%) | m.p. (°C) |
|-----------|-------|-----------|-----------|
| 2a        | H     | 95        | nd⁹       |
| 2b        | 4-CH₃ | 88        | nd        |
| 2c        | 4-Br  | 91        | nd        |
| 2d        | 2-F   | 97        | nd        |
| 2e        | 3-Cl  | 92        | nd        |
| 3a        | H     | 46        | 230-233   |
| 3b        | 4-CH₃ | 42        | 224-225   |
| 3c        | 4-Br  | 57        | 202-204   |
| 3d        | 2-F   | 37        | 172-174   |
| 3e        | 3-Cl  | 44        | 203-204   |

⁹nd= not determined. Compounds 2(a-e) were liquid (oil) at room temperature.
Preparation of L-methionine derivatives of benzyloxy-urea (6a-e): L-Methionine methyl ester hydrochloride (0.018 mol) was suspended in dry dichloromethane (15 mL), dry triethylamine (0.036 mol) was added slowly. The intermediates 4a-e (0.018 mol) was dissolved in dry dichloromethane (85 mL) and then added dropwise in the solution. The mixture was stirred at room temperature for 3-4.5 h. After the completion of the reaction, the reaction mixture was successively washed with 1 M NaOH (3 × 50 mL), 1 M HCl (50 mL) and H<sub>2</sub>O (2 × 50 mL). The dichloromethane layer was dried by anhydrous MgSO<sub>4</sub>, filtered and vaporated under reduced pressure, then recrystallized to get the target compound.

3-(Benzyl)-5-[2-(methylsulfonyl)methyl]imidazolidine-2,4-dione (6a): White crystals 0.60 g, yield: 14%; m.p. 123-125 °C. IR (KBr, v<sub>max</sub>, cm<sup>-1</sup>): 3295.5 (NH), 1766.7 (C=O), 1724.2 (C=O); 1<sup>H</sup>NMR (400 MHz, CDCl<sub>3</sub>): δ 7.50-7.39 (5H, m, Ar-H), 5.99 (1H, s, NH), 5.16 (2H, s, OCH<sub>2</sub>), 4.10 (1H, q, J<sub>1</sub> = 4.3 Hz, J<sub>2</sub> = 3.4 Hz, CH<sub>2</sub>), 2.60-2.54 (2H, m, CH<sub>2</sub>S), 2.14 (2H, m, CH<sub>2</sub>), 2.09 (3H, s, SCH<sub>3</sub>); 13<sup>C</sup>NMR (100 MHz, CDCl<sub>3</sub>): δ 168.05, 153.85, 133.13, 130.04, 129.46, 128.54, 79.22, 54.32, 30.28, 29.67, 15.21; EI-MS: m/z [M + Na]<sup>+</sup> 303.55.

3-(4-Methylbenzyl)-5-[2-(methylsulfonyl)ethyl]imidazolidine-2,4-dione (6b): White crystals 0.60 g, yield: 10%; m.p. 92-94 °C. IR (KBr, v<sub>max</sub>, cm<sup>-1</sup>): 3244.0 (NH), 1770.5 (C=O), 1735.8 (C=O); 1<sup>H</sup>NMR (400 MHz, CDCl<sub>3</sub>): δ 7.73 (2H, dd, J<sub>1</sub> = 7.0 Hz, ArCH<sub>2</sub>S), 2.36 (3H, s, Ar-CH<sub>3</sub>), 2.08 (3H, s, SCH<sub>3</sub>); 13<sup>C</sup>NMR (400 MHz, CDCl<sub>3</sub>): δ 167.90, 153.53, 139.46, 137.68, 129.22, 127.02, 79.04, 54.43, 30.31, 29.84, 21.42, 15.19; EI-MS: m/z [M + Na]<sup>+</sup> [M + H]<sup>+</sup> 297.30.

5-Benzyl-3-(4-methylbenzyl)imidazolidine-2,4-dione (7a): White crystals 1.57 g, yield 27%; m.p. 142-144 °C. IR (KBr, v<sub>max</sub>, cm<sup>-1</sup>): 3328.9 (NH), 3255.6 (NH), 3240.2 (NH), 1782.1 (C=O), 1770.3 (C=O); 1<sup>H</sup>NMR (400 MHz, CDCl<sub>3</sub>): δ 7.34-7.29 (4H, m, Ar-H), 6.39 (1H, s, NH), 6.37 (1H, s, NH), 4.80 (2H, s, OCH<sub>2</sub>), 4.57 (1H, q, J<sub>1</sub> = 7.9 Hz, J<sub>2</sub> = 6.5 Hz, CH<sub>2</sub>), 3.76 (3H, s, OCH<sub>3</sub>), 2.43 (2H, t, J = 7.4 Hz, CH<sub>2</sub>S), 2.13 (2H, q, J<sub>1</sub> = 6.8 Hz, J<sub>2</sub> = 7.0 Hz, CH<sub>2</sub>), 2.07 (3H, s, SCH<sub>3</sub>); 13<sup>C</sup>NMR (100 MHz, CDCl<sub>3</sub>): δ 172.44, 157.24, 148.04, 134.51, 130.00, 127.88, 127.31, 124.73, 75.61, 54.11, 57.18, 30.34, 29.83, 15.38; EI-MS: m/z [M + Na]<sup>+</sup> 369.68.

Preparation of L-phenylalanine derivatives of benzyloxy-urea (7a-e): Prepared as described for 6a-e, starting from L-phenylalanine methyl ester hydrochloride (0.018 mol) and the intermediates 4a-e (0.018 mol). Purification by recrystallization from anhydrous methanol: CHCl<sub>3</sub>(5:5).

5-Benzyl-3-(benzyl)imidazolidine-2,4-dione (7b): White crystals 1.16 g, yield: 19%; m.p. 122-124 °C. IR (KBr, v<sub>max</sub>, cm<sup>-1</sup>): 3301.9 (NH), 1782.1 (C=O), 1708.8 (C=O); 1<sup>H</sup>NMR (400 MHz, CDCl<sub>3</sub>): δ 8.74-7.17 (9H, m, Ar-H), 5.18 (1H, s, NH), 4.98 (2H, s, OCH<sub>2</sub>), 4.14 (1H, q, J<sub>1</sub> = 2.6 Hz, J<sub>2</sub> = 1.8 Hz, CH), 3.24 (1H, q, J = 3.8 Hz, J = 7.0 Hz, ArCH<sub>2</sub>), 2.72 (1H, q, J = 9.1 Hz, J = 7.0 Hz, ArCH<sub>2</sub>), 2.71 (1H, q, J = 9.2 Hz, J = 7.0 Hz, ArCH<sub>2</sub>), 2.36 (3H, s, ArCH<sub>3</sub>); 13<sup>C</sup>NMR (100 MHz, CDCl<sub>3</sub>): δ 167.21, 152.99, 134.61, 133.31, 130.00, 129.29, 129.01, 128.51, 127.66, 79.29, 56.17, 37.76; EI-MS: m/z [M + Na]<sup>+</sup> 333.73.
Methyl-2-[(3-chlorobenzyl)oxy]carbamoylamino]-3-phenylpropanoate (7e): Viscous light yellowish oil 2.05 g; yield: 34 %. IR (KBr, ν₉₅₀, cm⁻¹): 3294.2 (NH), 1735.8 (C=O), 1674.1 (C=O); ¹H NMR (400 MHz, CDCl₃): δ 7.31-7.10 (9H, m, 2Ar-H), 6.10 (1H, d, J = 8.0 Hz, NH), 5.70 (1H, s, NH), 4.91 (1H, q, J₁ = 10.3 Hz, J₂ = 7.5 Hz, CH), 4.68 (2H, s, OCH₂), 3.73 (3H, s, OCH₃), 3.23 (1H, q, J₁ = 3.5 Hz, J₂ = 7.0 Hz, ArCH₂), 3.13 (1H, t, J = 5.0 Hz, ArCH₂); ¹³C NMR (100 MHz, CDCl₃): δ 172.01, 153.39, 148.06, 137.14, 134.49, 129.99, 128.66, 127.79, 127.15, 126.77, 125.47, 75.66, 56.15, 52.39, 37.48, 385.70.

X-Ray crystallography: Colourless granular single crystals of the compound 7a suitable for X-ray data collection were obtained by slow evaporation of the mixed solvent acetone and N-hexane (2:5) at 4 °C for 3 months. The X-ray data were collected on a diffractometer equipped with graphite-monochromated MoKα radiation (λ = 0.71073 Å) at 298 (2) K. The structure was determined by direct methods and refined on F² by full-matrix least-squares using the program SHELXTL-97. An X-ray diffraction study of the compound showed that it was crystallized in a monoclinic system with the P2₁/c space group. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were calculated and allowed to ride. Computer programs: structure solution, SHELXS-97, refinement, SHELXS-97, molecular diagrams, ORTEP.

In vitro Antitumor activity (MTT assay): The murine leukemia cell line L1210 and human K562 leukemia cell line were purchased from Nanjing Keygen Biotech. Co., Ltd, China. The cells were cultured in improved RPMI-1640 medium supplemented with 10 % heat-inactivated FBS, 100 IU/mL penicillin G and 100 IU/mL streptomycin sulfate at 37 °C in a CO₂ incubator in a humidified atmosphere containing 5 % CO₂ atmosphere.

The in vitro antitumor activity of the target compounds against K562 and L1210 was evaluated by the improved MTT assay using hydroxyurea as a positive control. In brief, cells (2 × 10⁴ cells/mL) in logarithmic growth phase were plated in 96-well plates (90 µL/well). Test compounds were prepared prior to the experiment by dissolving in 0.1 % DMSO and diluted with medium. 10 µL of culture medium containing different concentrations of the drugs was added to the wells. Cells in the control wells received the same volume of medium containing 0.1 % DMSO. The cells were then incubated for 2 days. After that, 20 µL MTT reagent (5 mg/mL) was added and the cell cultures were incubated for another 4 h at 37 °C. The formazan produced by the viable cells was solubilized by addition of 100 µL mixed solvent of 10 % (v/v) sodium dodecylsulfonate, 5 % (v/v) isobutanol and 0.012 M HCl. The suspension was placed in the dark incubator at 37 °C overnight and the optical density was measured at 570 nm by the ELISA reader. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Synthesis: The intermediates 4(a-e) were prepared in Scheme-I. Acetoxime was chosen as the starting reagent. To obtain intermediates with different substituents on phenyl group, various benzyl chloride or benzyl bromide bearing different substituents on phenyl 1(a-e) were used for the preparation of the intermediates 4(a-e). The benzoxoxyurea derivatives having D-glucosamine moiety 5(a-e) were prepared by the reaction of D-glucosamine with the intermediates 4(a-e) (Scheme-II). Synthesis of the compounds 6(a-e) were achieved by the reaction of L-methionine ester hydrochloride with the intermediates 4(a-e) (Scheme-III). The compounds 7(a-e) were prepared by the reaction of L-phenylalanine ester hydrochloride with the intermediates 4(a-e) (Scheme-IV). When 4a,4b were coupled with L-methionine ester and L-phenylalanine ester, 4d was coupled with L-phenylalanine ester, the hydantoin derivatives 6a, 6b, 7a, 7b and 7d were achieved by a facile intramolecular cyclization. All the target compounds were characterized by spectroscopic data as IR, MS, ¹H and ¹³C NMR. The exact stereostructure of compound 7a was determined by X-ray crystal structure analysis.

X-Ray crystal structure analysis: The crystallographic data of 7a are summarized in Table-2. The selected bond lengths, angles and torsion angles are given in Table-3. The molecular structure with the atom numbering by ORTEP drawing is displayed in Fig. 1. The skeleton of 7a consists of a five-membered hydantoin ring, a benzoxoxy group attached to the hydantoin ring atom N2 and a benzyl group attached to the hydantoin ring atom C3.

In the hydantoin ring, the length of the carbonyl bond [d(C=O) = 1.194(5) Å and 1.214(5) Å] is in the normal range of 1.19-1.23 Å as well as other hydroxyurea derivatives. The C-N bond lengths [1.345(5)-1.467(5) Å, mean 1.389(5) Å] are longer than a typical C=N double bond (mean 1.269 Å).
TABLE-2
CRYSTAL AND EXPERIMENTAL DATA FOR 7a

| Item                                      | Data                        |
|-------------------------------------------|-----------------------------|
| Empirical formula                         | C6H14N2O3                   |
| Formula mass/g mol⁻¹                      | 296.32                      |
| Temperature                               | 298 K                       |
| Wavelength                                | 0.71073 Å                   |
| Crystal system, space group               | Monoclinic, P21/c           |
| Unit cell dimensions                      | a = 4.5437(5) Å            |
|                                           | b = 32.226 (2) Å          |
|                                           | α = 90 °                  |
|                                           | β = 92.7440(10)°         |
|                                           | γ = 90 °                  |
| Volume                                    | 1470.3 (2) Å               |
| Z, calculated density                     | 4, 1.339 g cm⁻³            |
| Absorption coefficient                    | 0.10 mm⁻¹                  |
| Fmax                                       | 624                          |
| Crystal size                              | 0.45 mm x 0.40 mm x 0.38 mm |
| θ range for data collection               | 2.4-20.9°                   |
| Limiting indices                          | -5 ≤ h ≤ 5, -25 ≤ k ≤ 38, -11 ≤ l ≤ 11 |
| Reflections collected/unique              | 7194/2578 [R(int) = 0.052]  |
| Absorption correction                     | Multi-scan                  |
| Max. and min. transmission                | 0.966 and 0.959             |
| Refinement method                         | Full-matrix least-squares on F² |
| Goodness-of-fit on F²                      | 1.03                         |
| Final R indices [I > 2σ(I)]               | R₁ = 0.078, wR₁ = 0.219     |
| Largest diff. peak and hole               | 0.46 and -0.20 e Å⁻³       |

TABLE-3
SELECTED BOND DISTANCES (Å), ANGLES (°) AND TORSION ANGLES (°) FOR 7a

| Bond distances (Å) | Angle (°)                  |
|--------------------|----------------------------|
| N1-C1              | 1.330 (5)                  |
| N1-C3              | 1.467 (5)                  |
| N2-C1              | 1.383 (5)                  |
| N2-O1              | 1.373 (4)                  |
| C3-C11             | 1.480 (6)                  |
| C1-N1-C3           | 112.8 (3)                  |
| N1-C3-C2           | 101.9 (3)                  |
| N1-C1-N2           | 106.5 (4)                  |
| O1-N2-C1-N1        | 110.0 (3)                  |
| C2-C3              | 1.522 (6)                  |
| N2-C2              | 1.374 (5)                  |
| O1-C4              | 1.442 (5)                  |
| C4-C5              | 1.490 (6)                  |
| C11-C12-C13        | 1.514 (6)                  |
| N2-C2-C3           | 171.4 (4)                  |
| C3-C11-C12         | 110.4 (3)                  |
| N2-C2-C3           | 104.7 (4)                  |
| C4-C5              | 1.514 (6)                  |
| C11-C12-C13        | 114.5 (4)                  |
| N2-C2-C3           | 104.7 (4)                  |
| C4-C5              | 171.4 (4)                  |
| C11-C12-C13        | 114.5 (4)                  |

but shorter than a C-N single bond [mean 1.443(4) Å], indicating electron delocalization in the hydantoin ring. The dihedral angle between the two coplanar benzene rings is

Scheme-III: Synthesis of compounds 6(a-e)

Scheme-IV: Synthesis of compounds 7a, 7b, 7d a: R = H; b: R = 4-CH₃; c: R = 4-Br; d: R = 2-F; e: R = 3-Cl

Scheme-V: Synthesis of compounds 6(a-e)

Scheme-VI: Synthesis of compounds 7a, 7b, 7d a: R = H; b: R = 4-CH₃; c: R = 4-Br; d: R = 2-F; e: R = 3-Cl
Fig. 1. Molecular structure and labeling of compound 7a, showing 50% probability ellipsoids.

60.5(1)°. The hydantoin ring atoms C1/C2/C3/N1/N2/O2/O3 are in a plane that is almost perpendicular with respect to the benzene ring (C12-C17). The two rings forms a dihedral angle of 78.0(1)°. But the hydantoin ring is almost parallel with respect to the other phenyl group (C5-C10) and the dihedral angle is 17.7(2)°. Furthermore, the C2 atom of the hydantoin ring is antiperiplanar with respect to the C12 atom of the benzene ring. The corresponding C2-C3-C11-C12 torsion angle amounting to -179.2(4)°. The N-O bond is almost coplanar with the hydantoin ring and twisted by about 170° out of the hydantoin plane. In the crystal packing diagram, molecules linked to chains by pairs of intermolecular N-H...O hydrogen bonds lead to inversion dimers (Fig. 2).

Fig. 2. Perspective view of the three-dimensional structure of 7a.

**in vitro Antitumor activity:** The target compounds were evaluated for their cytotoxicity in vitro against human leukemia cell line K562 and murine leukemia cell line L1210 by the MTT assay using hydroxyurea as the reference drug. Antitumor potency of the target compounds was indicated by the inhibitory ratio and IC<sub>50</sub> values summarized in Tables 4 and 5.

The data of the inhibitory ratio against K562 and L1210 cells were expressed as means values ± standard deviation and statistically evaluated by Dunnett’s t-test, but p values > 0.05 were not considered significant except 7e compared with hydroxyurea against K562. But of the benzoylurea and benzoylhydantoin derivatives 6(a-e) and 7(a-e), all the compounds showed inhibitory activity against K562 cells with IC<sub>50</sub> values ranged from 23.38-774.49 μM. The IC<sub>50</sub> ratios of hydroxyurea over 6(a-e) and 7(a-e) range from 2.6 to 85, indicating that the compounds are 2.6 to 85 fold more potent than hydroxyurea against K562 cells. Among them, 7e exhibited the most potent activity (IC<sub>50</sub> is 23.38 μM) and showed 85-fold more potency on K562 cells compared to hydroxyurea. L-Phenylalanine derivatives 7b, 7e and 7e showed inhibitory activity against L1210 cell lines. Among them, the best cytostatic activity was also found for compound 7e (IC<sub>50</sub> is 83.11 μM). When the N3-H of benzoylurea derivatives was substituted by glucosamine, we found decrease in inhibitory activity (5a-e).

The C log P values (hydrophobic parameters) of the title compounds calculated by ChemDraw Ultra 8.0 were also shown in Table-4. The benzoylurea derivatives 6, 7 with higher C log P showed higher cytotoxicity than the corresponding benzoylurea derivatives 5 with lower C log P. The result is agreement with the result in our previous paper.<ref>

**Conclusion**

We have synthesized a series of benzoylurea and benzoylhydantoin derivatives and tested for their anticancer activity on K562 and L1210 cell lines. Compounds 7e and 7b were identified as most potent having IC<sub>50</sub> values 23.38 and 52.21 μM, respectively. In case of compounds bearing chlorine and bromine on benzyl were found to be more potent (6c, 6e, 7c and 7e) than others. The glucosamine substituent at N3-position was observed to be less potent 5(a-e). The L-phenylalanine ester substituent at N3-position was allowed with markedly increasing the activity against the tumor cells 7(a-e).

**ACKNOWLEDGEMENTS**

The financial support from the National Key S & T Special Project of China: Grand New Drug R & D (Grant No. 2009ZX-09103-087) and the Grand Science and Technology Project of Jiangxi Province (2012BBG70094-2) is gratefully acknowledged.

**TABLE-4**

| Compounds                | R    | Molar concentration | Inhibitory ratio (%) | IC<sub>50</sub> (μM) | C log P |
|--------------------------|------|---------------------|----------------------|-----------------------|--------|
|                          |      | 10<sup>-5</sup> M   | 10<sup>-4</sup> M   | 10<sup>-3</sup> M   | 10<sup>-2</sup> M |
| Hydroxyurea              | –    | 48.79 ± 10.99       | 41.14 ± 24.56       | 34.43 ± 26.35       | 10.11 ± 7.46 | 7.76 ± 7.01 | 164.57 | -1.8  |
| 5a                       | H    | 21.75 ± 6.78        | 9.99 ± 1.22         | 2.93 ± 1.05         | 3.62 ± 2.56  | 3.82 ± 1.54 | 2301.19 | -0.04 |
| 5b                       | 4-CH | 26.03 ± 1.69        | 1.59 ± 1.78         | 0.92 ± 1.40         | 0.92 ± 2.16  | 0.52 ± 0.66 | 2420.42 | 0.46  |
| 5c                       | 4-Br | 28.22 ± 6.44        | 2.55 ± 2.08         | 0.64 ± 1.06         | 2.84 ± 5.40  | -1.06 ± 3.07 | 2240.08 | 0.82  |
| 5d                       | 2-F  | 26.48 ± 0.77        | 4.21 ± 3.29         | 3.47 ± 3.42         | 5.36 ± 0.81  | 1.91 ± 2.31 | 1802.05 | 0.1   |
| 5e                       | 3-Cl | 31.89 ± 4.08        | 7.51 ± 3.67         | 0.97 ± 3.72         | 1.20 ± 3.16  | 0.85 ± 3.51 | 1754.08 | 0.67  |
| 6a                       | H    | 70.07 ± 3.11        | 8.84 ± 0.45         | 1.03 ± 1.64         | 3.67 ± 4.78  | -1.03 ± 3.34 | 564.47  | 0.99  |
| 6b                       | 4-CH | 81.01 ± 1.63        | 13.73 ± 2.23        | 4.84 ± 1.91         | 1.58 ± 2.64  | 1.52 ± 1.54 | 328.72  | 1.49  |
| 6c                       | 4-Br | 87.98 ± 7.84        | 25.46 ± 1.86        | 2.79 ± 3.56         | 2.11 ± 3.05  | 0.79 ± 1.56 | 217.19  | 2.48  |
| 6d                       | 2-F  | 67.30 ± 0.75        | 8.80 ± 2.55         | 0.93 ± 4.40         | 4.74 ± 3.71  | -0.23 ± 4.36 | 584.70  | 1.76  |
| 6e                       | 3-Cl | 93.97 ± 3.55        | 12.32 ± 7.00        | 2.97 ± 2.58         | 1.36 ± 4.41  | 1.62 ± 5.23 | 245.05  | 2.33  |
| 7a                       | H    | 78.98 ± 0.82        | 21.69 ± 2.51        | 1.88 ± 2.61         | 1.02 ± 0.79  | 0.25 ± 1.96 | 326.48  | 2.26  |
| 7b                       | 4-CH | 97.91 ± 1.19        | 65.68 ± 5.04        | -0.13 ± 7.35        | -2.32 ± 6.90 | -3.82 ± 5.95 | 87.42   | 2.76  |
| 7c                       | 4-Br | 88.32 ± 2.35        | 44.20 ± 11.81       | 5.04 ± 3.00         | -1.00 ± 3.44 | -3.39 ± 4.77 | 160.71  | 3.75  |
| 7d                       | 2-F  | 89.35 ± 0.11        | 26.15 ± 0.84        | -0.37 ± 1.80        | -1.72 ± 2.44 | -0.46 ± 2.31 | 252.03  | 2.41  |
| 7e                       | 3-Cl | 98.33 ± 1.32        | 70.17 ± 2.57        | 0.96 ± 8.93         | -1.94 ± 9.37 | -7.26 ± 2.74 | 83.11   | 3.6   |
The authors also thank Center of Analysis and Testing of Nanchang University for assistance with the MS testing of compounds.

## REFERENCES

1. M.P. Pujari, A. Barrientos, F.M. Muggia and R.T. Koda, *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci.*, **694**, 185 (1997).

2. A. Ferster, P. Tahriri, C. Vermylen, G. Sturbois, F. Corazza, P. Fondu, C. Devalck, M.F. Dresse, W. Feremans, K. Hunnink, M. Toppet, P. Philippet, C.V. Geet and E. Sariban, *Blood*, **97**, 3628 (2001).

3. W.Y. Gao, D.G. Johns and H. Mitsuy a, *Mol. Pharmacol.*, **46**, 767 (1994).

4. F. Lori, A. Malykh, A. Cara, D. Sun, J.N. Weinstein, J. Lissiewicz and R.C. Gallo, *Science*, **266**, 801 (1994).

5. S.D. Malley, J.M. Grange, F. Hameli-Sangsari and J.R. Vila, *Proc. Natl. Acad. Sci. USA*, **91**, 11017 (1994).

6. X. Mai, X.S. Lu, H.Y. Xia, Y.S. Cao, Y.J. Liao and X.L. Lv, *Chem. Pharm. Bull. (Tokyo)*, **58**, 94 (2010).

7. T. George, M. Mark, and R. Photon, Word Intellectual Property Organization, WO03/082301A1 (2003).

8. I. Perkovic, I. Butula, Z. Rajic, D. Hadjipavlou-Litina, E. Pontiki and B. Zorc, *Croat. Chem. Acta*, **83**, 151 (2010).

9. L. Zhang, Chinese Selected Doctoral Dissertations and Master's Theses Full-Text Databases (Doctor) (2005).

10. G.M. Sheldrick, SHELXTL Version 5.03, Institut für Anorganische Chemie, University of Göttingen, Göttingen, Germany (1997).

11. J.J. Zhou, X.F. Yue, J.X. Han and W.Y. Yang, *Chin. J. Pharm.*, **24**, 455 (1993).

12. C. Campestre, P. Tortorella, M. Agamennone, S. Preziuso, A. Biasone, E. Nuti, A. Rossello and C. Gallina, *Eur. J. Med. Chem.*, **43**, 1008 (2008).

13. X. Mai, H.Y. Xia, Y.S. Cao, X.S. Lu and X.N. Fang, *Acta Crystallogr.*, **E65**, o442 (2009).

14. X. Mai, H.-Y. Xia, Y.-S. Cao, W. Tong and G.-G. Tu, *Acta Crystallogr.*, **E65**, o2983 (2009).

15. J.J. Wei, X. Mai, H.Y. Xia, T. Wei and L. Lin, *Z. Kristallogr. NCS*, **225**, 553 (2010).

16. W.E. Thiessen, H.A. Levy and B.D. Flaug, *Acta Crystallogr. B*, **34**, 2495 (1978).

### TABLE 5

| Compounds | Inhibitory ratio (%) | IC_{50} (µM) |
|-----------|----------------------|-------------|
|           | R | 10^{-3} M | 10^{-4} M | 10^{-5} M | 10^{-6} M | 10^{-7} M |
| Hydroxyurea | – | 30.6±4.38 | 17.69±7.28 | -0.16±3.99 | -2.21±3.07 | -6.76±5.52 | 1987.94 |
| 5a | H | 28.19±5.10 | -0.27±4.11 | -1.41±2.85 | -3.02±3.49 | 0.68±3.60 | 2788.34 |
| 5b | 4-CH_{3} | 29.58±1.05 | -6.08±4.90 | -2.00±4.38 | -0.24±2.96 | -2.62±6.29 | 3197.09 |
| 5c | 4-Br | 26.09±2.22 | 3.03±2.36 | -3.09±2.99 | 1.09±0.74 | -1.16±1.05 | 2710.40 |
| 5d | 2-F | 21.51±1.81 | -2.15±4.70 | -1.37±4.35 | 0.36±2.66 | -0.90±3.16 | 3367.92 |
| 5e | 3-Cl | 21.36±0.87 | 0.52±2.43 | -1.74±2.79 | 0.78±3.35 | -1.41±4.05 | 3204.49 |
| 6a | H | 69.13±1.74 | 14.13±7.04 | 2.43±6.65 | 3.76±8.10 | 1.89±3.16 | 456.41 |
| 6b | 4-CH_{3} | 81.16±1.78 | 9.94±2.26 | 3.29±3.19 | 3.76±8.10 | 3.51±4.96 | 332.80 |
| 6c | 4-Br | 94.61±5.74 | 30.23±2.37 | 1.79±4.35 | 3.80±3.19 | -2.18±3.71 | 172.41 |
| 6d | 2-F | 94.1±4.24 | 7.48±3.75 | -0.94±3.58 | 5.56±1.58 | -0.03±2.52 | 389.37 |
| 6e | 3-Cl | 95.35±0.36 | 9.37±2.82 | 3.08±3.56 | 4.43±6.72 | 3.28±3.74 | 285.89 |
| 7a | H | 72.88±3.16 | 30.48±7.41 | -0.10±2.61 | -0.88±1.34 | -3.71±2.50 | 3204.49 |
| 7b | 6-CH_{3} | 97.37±0.53 | 69.11±8.77 | 18.63±6.38 | 14.72±14.30 | 10.79±11.41 | 23.38 |