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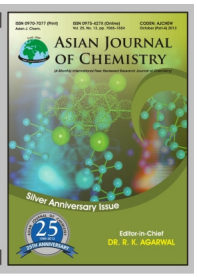

Synthesis, Crystal Structure and *in vitro* Antitumor Activity of Benzyloxyurea and Benzyloxyhydantoin Derivatives

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A series of benzyloxyurea and benzyloxyhydantoin derivatives were synthesized by substituting different benzyl groups on O-position and glucosamine, amino acid ester on N3-position of hydroxyurea. Their structures were elucidated by using spectrometry along with X-ray crystal structures analysis and *in vitro* antitumor activity against the human leukemia cell line K562 and murine leukemia cell line L1210 was evaluated by MTT assay. The L-phenylalanine ester substituent at N3-position was allowed with markedly increasing the activity against the tumor cells. The most promising compounds were **7e** and **7b**.

Key Words: Benzyloxyurea, Benzyloxyhydantoin, Glucosamine, Amino acid ester, Antitumor.

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[1]. Hydroxyurea is also useful in therapy of sickle cell anemia[2] and in combined antiretroviral therapy, where it is most effective if taken in combination with reverse transcriptase inhibitors (ddI, AZT, ddC or d4T) [3-5].

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[6]. 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**[7] and **7a**[8]. 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[9]. Melting points were determined using capillary method and were uncorrected. The infrared spectra were recorded on a Shimadzu FT-IR 8400 spectrometer (KBr discs). ^{1}H and ^{13}C 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) in anhydrous ethanol (100 mL) was added and stirred for 2 h at room temperature. **1(a-e)** (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 $MgSO_4$. 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)**

TABLE-1
YIELDS AND MELTING POINTS OF INTERMEDIATES **2(a-e)** AND **3(a-e)**

Compounds	R	Yield (%)	m.p. (ºC)
2a	H	95	nd[a]
2b	4-CH_3	88	nd
2c	4-Br	91	nd
2d	2-F	97	nd
2e	3-Cl	92	nd
3a	H	46	230-233
3b	4-CH_3	42	224-225
3c	4-Br	57	202-204
3d	2-F	37	172-174
3e	3-Cl	44	203-204

[a]nd= not determined. Compounds **2(a-e)** were liquid (oil) at room temperature.

above was added gradually with stirring to a solution of concentrated hydrochloric acid at room temperature for 2 h. Then the reaction mixture was distilled 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-Benzylhydroxylamine hydrochloride (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), H_2O (200 mL), 1 M $NaHCO_3$ (3 × 200 mL), H_2O (2 × 200 mL) and saturated sodium chloride solution (200 mL). The organic phase was dried with anhydrous $MgSO_4$, filtered, evaporated under vacuum and dried to afford the target compound.

N-Benzyloxy-aminoformate-4-nitrophenylester (4a): Light yellow crystals 21.52 g, yield: 75 %; m.p. 95-97 ºC. IR (KBr, ν_{max}, cm^{-1}): 3275.9 (NH), 1723.3 (C=O); MALDI-MS: *m/z* $[M + Na]^+$ 311.0639 (calcd. (%) for $C_{14}H_{12}N_2O_5$ + Na: 311.0644).

N-(4-Methylbenzyloxy)aminoformate-4-nitrophenylester (4b): Light yellow crystals 22.57 g, yield: 75 %; m.p. 107-109 ºC. IR (KBr, ν_{max}, cm^{-1}): 3278.8 (NH), 1728.1 (C=O); MALDI-MS: *m/z* $[M + Na]^+$ 325.0794 (calcd. (%) for $C_{15}H_{14}N_2O_5$ + Na: 325.0800).

N-(4-Bromobenzyloxy)aminoformate-4-nitrophenylester (4c): White crystals 23.04 g, yield: 63 %; m.p. 146-149 ºC. IR (KBr, ν_{max}, cm^{-1}): 3271.0 (NH), 1728.1 (C=O); MALDI-MS: *m/z* $[M + Na]^+$ 390.0121 (calcd. (%) for $C_{15}H_{14}N_2O_5$ + Na: 388.9749).

N-(2-Fluorobenzyloxy)aminoformate-4-nitrophenylester (4d): Light yellow crystals 23.45 g, yield: 77 %; m.p. 118-120 ºC. IR (KBr, ν_{max}, cm^{-1}): 3294.2 (NH), 1735.8 (C=O); MALDI-MS: *m/z* $[M + Na]^+$ 329.0542 (calcd. (%) for $C_{14}H_{11}FN_2O_5$ + Na: 329.0550).

N-(3-Chlorobenzyloxy)aminoformate-4-nitrophenylester (4e): Light yellow crystals 23.73 g, yield: 74 %; m.p. 88-90 ºC. IR (KBr, ν_{max}, cm^{-1}): 3271.0 (NH) 1720.4 (C=O); MALDI-MS: *m/z* $[M + Na]^+$ 345.0249 (calcd. (%) for $C_{14}H_{11}ClN_2O_5$ + Na: 345.0254).

Preparation of glucosamine derivatives of benzyloxyurea 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.

1-(Benzyloxy)-3-[2,4,5-trihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3-yl]urea (5a): Light yellow powder 1.22 g, yield: 41 %; m.p. 136-142 ºC. IR (KBr, ν_{max}, cm^{-1}): 3309.6 (OH), 1648.1 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45-7.36 (5H, m, Ar-H), 6.05 (1H, s, NH), 6.03 (1H, s, NH), 4.77 (2H, s, OCH_2), 3.61-3.47 (m, 2-glucosamine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.30, 136.12, 128.68, 128.35, 128.16, 90.94, 77.48, 72.24, 71.11, 70.88, 61.13, 54.38; MALDI-MS: *m/z* $[M + H]^+$ 329.1344 (calcd. (%) for $C_{14}H_{20}N_2O_7$+H: 329.1349).

1-[(4-Methylbenzyl)oxy]-3-[2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran–3-yl]urea (5b): Light yellow powder 1.22 g, yield: 60 %; m.p. 122-126 ºC. IR (KBr, ν_{max}, cm^{-1}): 3310.6 (OH), 1649.0 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.31 (2H, dd, J_1 = 7.7 Hz, J_2 = 10.0 Hz, Ar-H), 7.19 (2H, dd, J_1 = 8.3 Hz, J_2 = 5.4 Hz, ArH), 6.54 (1H, s, NH), 5.96 (1H, d, J = 7.7Hz, NH), 4.89 (2H, s, OCH_2), 3.63-3.14 (m, 2-glucosamine), 2.31 (3H, s, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.30, 137.50, 137.19, 128.92, 128.73, 90.94, 85.34, 77.6.4, 71.82, 71.14, 63.35, 61.10, 20.78; MALDI-MS: *m/z* $[M + H]^+$ 343.1496 (calcd. (%) for $C_{15}H_{22}N_2O_7$ + H: 343.1505).

1-[(4-Bromobenzyl)oxy]-3-[2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl]urea (5c): Light yellow powder 2.41 g, yield: 66 %; m.p. 137-140 ºC. IR (KBr, ν_{max}, cm^{-1}): 3306.7 (OH), 1651.0 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.57 (2H, dd, J_1 = 8.3 Hz, J_2 = 5.6 Hz, Ar-H), 7.40 (2H, dd, J_1 = 8.3 Hz, J_2 = 13.5 Hz, ArH), 6.60(1H, s, NH), 6.03 (1H, d, J = 7.6Hz, NH), 4.95 (2H, s, OCH_2), 3.65-3.16 (m, 2-glucosamine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.28, 135.94, 131.25, 131.01, 121.34, 90.91, 85.44, 76.46, 71.79, 71.15, 63.33, 61.09; MALDI-MS: *m/z* $[M+H]^+$ 407.0455 (calcd. (%) for $C_{14}H_{19}BrN_2O_7$+H: 407.0454).

1-[(2-Fluorobenzyl)oxy]-3-[2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl]urea (5d): Light yellow powder 1.47 g, yield: 47 %; m.p. 108-114 ºC. IR (KBr, ν_{max}, cm^{-1}): 3325.0 (OH), 1648.1 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.62-7.17 (4H, m, Ar-H), 6.57 (1H, s, NH), 6.02 (1H, d, J = 7.9 Hz, NH), 4.83 (2H, s, OCH_2), 3.62-3.14 (m, 2-glucosamine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.28, 158.97, 131.67, 130.77, 130.27, 124.43, 115.44, 90.91, 85.43, 71.81, 71.19, 68.17, 63.33, 61.12; MALDI-MS: *m/z* $[M+H]^+$ 347.1258 (calcd. (%) for $C_{14}H_{19}FN_2O_7$+H: 347.1255).

1-[(3-Chlorobenzyl)oxy]-3-[2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl]urea (5e): Light yellow powder 2.07 g, yield: 63 %; m.p. 114-118 ºC. IR (KBr, ν_{max}, cm^{-1}): 3310.6 (OH), 1651.0 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.57-7.35 (4H, m, Ar-H), 6.59 (1H, s, NH), 6.05 (1H, d, J = 8.0 Hz, NH), 4.95 (2H, s, OCH_2), 3.65-3.12 (m, 2-

glucosamine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.30, 139.09, 132.98, 130.24, 127.97, 127.06, 126.97, 90.92, 85.56, 76.39, 71.83, 71.19, 63.34, 61.12; MALDI-MS *m/z* [M + H]$^+$: 363.0950 (calcd. (%) for $C_{14}H_{19}ClN_2O_7$ + H: 363.0959).

Preparation of L-methionine derivatives of benzyloxyurea 6(a-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 **4(a-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_2O (2 × 50 mL). The dichloromethane layer was dried by anhydrous $MgSO_4$, filtered and vaporated under reduced pressure, then recrystallized to get the target compound.

3-(Benzyloxy)-5-[2-(methylsulfanyl)ethyl]imidazolidine-2,4-dione (6a): White crystals 0.79 g, yield: 14 %; m.p. 123-125 ºC. IR (KBr, ν_{max}, cm^{-1}): 3259.5 (NH), 1766.7 (C=O), 1724.2 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50-7.39 (5H, m, Ar-H), 5.99 (1H, s, NH), 5.16 (2H, s, OCH_2), 4.10 (1H, q, J_1 = 4.3 Hz, J_2 = 3.4 Hz, CH), 2.60-2.54 (2H, m, CH_2S), 2.14 (2H, m, CH_2), 2.09(3H, s, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.05, 153.85, 133.33, 130.04, 129.46, 128.54, 79.22, 54.32, 30.28, 29.67, 15.21; ESI-MS: *m/z* [M + Na]$^+$ 303.55.

3-(4-Methylbenzyloxy)-5-[2-(methylsulfanyl)ethyl]-imidazolidine-2,4-dione (6b): White crystals 0.60 g, yield: 10 %; m.p. 92-94 ºC. IR (KBr, ν_{max}, cm^{-1}): 3244.0 (NH), 1770.5 (C=O), 1720.4 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (2H, dd, J_1 = 7.8 Hz, J_2 = 13.0 Hz, Ar-H), 7.18 (2H, dd, J_1 = 7.5 Hz, J_2 = 6.2 Hz, Ar-H), 5.58 (1H, s, NH), 5.15 (2H, s, OCH_2), 4.08 (1H, q, J_1 = 4.3 Hz, J_2 = 3.4 Hz, CH), 2.55 (2H, m, CH_2S), 2.36 (3H, s, Ar-CH_3), 2.35 (2H, s, CH_2), 2.08 (3H, s, SCH_3); ^{13}C NMR (400 MHz, $CDCl_3$): 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; ESI-MS: *m/z* [M+Na]$^+$ 317.55.

Methyl-2-({[(4-bromobenzyl)oxy]carbamoyl}amino)-4-(methylsulfanyl)butanoate (6c): White crystals 4.61 g, yield: 65 %; m.p. 76-77 ºC. IR (KBr, ν_{max}, cm^{-1}): 3348.2 (NH), 3213.2 (NH), 1739.7 (C=O), 1651.0 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (2H, d, *J* = 8.2 Hz, Ar-H), 7.28 (2H, d, *J* = 7.7 Hz, Ar-H), 6.28 (1H, s, NH), 6.26 (1H, s, NH), 4.78 (2H, s, OCH_2), 4.59 (1H, q, J_1 = 7.6 Hz, J_2 = 6.4 Hz, CH), 3.77 (3H, s, OCH_3), 2.44 (2H, t, *J* = 7.4 Hz, CH_2S), 2.13 (2H, q, J_1 = 7.4 Hz, J_2 = 6.3 Hz, CH_2), 2.08 (3H, s, SCH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.46, 159.42, 134.33, 131.86, 130.98, 123.00, 54.12, 52.53, 51.72, 31.90, 29.84, 15.45; ESI-MS: *m/z* M^+ 391.58.

Methyl-2-({[(2-fluorobenzyl)oxy]carbamoyl}amino)-4-(methylsulfanyl)butanoate (6d): White crystals 2.13 g, yield: 36 %; m.p. 74-75 ºC. IR (KBr, ν_{max}, cm^{-1}): 3328.9 (NH), 3255.6 (NH), 1720.4 (C=O), 1635.5 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19-7.09 (4H, m, Ar-H), 6.12 (2H, s, 2NH), 4.94 (2H, s, OCH_2), 4.60 (1H, q, J_1 = 7.9 Hz, J_2 = 6.4 Hz, CH), 3.76 (3H, s, OCH_3), 2.44 (2H, t, *J* = 7.2 Hz, CH_2S), 2.16 (2H, q, J_1 = 6.6 Hz, J_2 = 7.0 Hz, CH_2), 2.09 (3H, s, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.32, 159.56, 153.79, 132.39, 132.03, 131.16, 124.34, 115.74, 72.66, 54.17, 51.73, 30.41, 29.82, 15.40; ESI-MS: *m/z* [M + Na]$^+$ 353.68.

Methyl-2-({[(3-chlorobenzyl)oxy]carbamoyl}amino)-4-(methylsulfanyl)butanoate (6e): Viscous light yellowish oil 4.92 g, yield: 79 %. IR (KBr, ν_{max}, cm^{-1}): 3425.3 (NH), 1735.8 (C=O), 1658.7 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.29 (4H, m, Ar-H), 6.39 (1H, s, NH), 6.37 (1H, s, NH), 4.80 (2H, s, OCH_2), 4.57 (1H, q, J_1 = 7.9 Hz, J_2 = 6.5 Hz, CH), 3.76 (3H, s, OCH_3), 2.43 (2H, t, *J* = 7.4 Hz, CH_2S), 2.13 (2H, q, J_1 = 6.6 Hz, J_2 = 7.0 Hz, CH_2), 2.07 (3H, s, SCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.44, 157.24, 148.04, 134.51, 130.00, 127.88, 127.31, 125.43, 75.61, 54.11, 51.78, 30.34, 29.83, 15.38; ESI-MS: *m/z*[M+Na]$^+$ 369.68.

Preparation of L-phenylalanine derivatives of benzyloxyurea 7(a-e): Prepared as described for **6(a-e)**, starting from L-phenylalanine methyl ester hydrochloride (0.018 mol) and the intermediates **4(a-e)** (0.018 mol). Purification by recrystallization from anhydrous methanol: CH_2Cl_2 (5:5).

5-Benzyl-3-(benzyloxy)imidazolidine-2,4-dione (7a): White crystals 1.57 g, yield 27 %; m.p. 142-144 ºC. IR (KBr, ν_{max}, cm^{-1}): 3271.0 (NH), 1766.7 (C=O), 1720.4 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.17 (10H, m, 2Ar-H), 5.27 (1H, s, NH), 5.01 (2H, s, OCH_2), 4.15 (1H, q, J_1 = 2.6 Hz, J_2 = 1.8 Hz, CH), 3.24 (1H, q, J_1 = 3.8 Hz, J_2 = 7.0 Hz, $ArCH_2$), 2.72 (1H, q, J_1 = 9.1 Hz, J_2 = 7.0 Hz, $ArCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): 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; ESI-MS: *m/z* [M + H]$^+$ 297.30.

5-Benzyl-3-[(4-methylbenzyl)oxy]imidazolidine-2,4-dione (7b): White crystals 1.16 g, yield: 19 %; m.p. 122-124 ºC. IR (KBr, ν_{max}, cm^{-1}): 3301.9 (NH), 1782.1 (C=O), 1708.8 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.17 (9H, m, 2Ar-H), 5.18 (1H, s, NH), 4.98 (2H, s, OCH_2), 4.14 (1H, q, J_1 = 2.6 Hz, J_2 = 4.6 Hz, CH), 3.24 (1H, q, J_1 = 3.5 Hz, J_2 = 7.0 Hz, $ArCH_2$), 2.71 (1H, q, J_1 = 9.2 Hz, J_2 = 7.0 Hz, $ArCH_2$), 2.36 (3H, s, $ArCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.38, 153.36, 139.41, 134.57, 130.10, 129.40, 129.21, 128.93, 127.61, 127.03, 79.18, 56.17, 37.65, 21.33; ESI-MS: *m/z* [M + Na]$^+$ 333.73.

Methyl-2-({[(4-bromobenzyl)oxy]carbamoyl}amino)-3-phenylpropanoate (7c): White crystals 0.33 g, yield: 5 %; m.p. 125-127 ºC. IR (KBr, ν_{max}, cm^{-1}): 3240.2 (NH), 1782.1 (C=O), 1739.7 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26-7.09 (9H, m, 2Ar-H), 6.08 (1H, d, *J* = 8.0 Hz, NH), 5.33 (1H, s, NH), 4.76 (1H, q, J_1 = 5.8 Hz, J_2 = 7.0 Hz, CH), 4.66 (2H, s, OCH_2), 3.73 (3H, s, OCH_3), 3.23 (1H, d, *J* = 3.6 Hz, $ArCH_2$), 3.13 (1H, d, *J* = 5.8 Hz, $ArCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.99, 153.24, 135.73, 134.40, 131.86, 130.82, 129.45, 128.91, 127.63, 123.64, 78.44, 56.17, 52.38, 37.56; ESI-MS: *m/z* [M + Na]$^+$ 429.62.

5-Benzyl-3-[(2-fluorobenzyl)oxy]imidazolidine-2, 4-dione (7d): White crystals 0.81 g, yield: 13 %; m.p. 131-133 ºC. IR (KBr, ν_{max}, cm^{-1}): 3325.0 (NH), 1797.5 (C=O), 1724.2 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.06 (9H, m, 2 Ar-H), 5.74 (1H, s, NH), 5.11 (1H, q, J_1 = 10.8 Hz, J_2 = 11.0 Hz, CH), 5.02 (2H, s, OCH_2), 3.22 (1H, q, J_1 = 3.4 Hz, J_2 = 7.0 Hz, $ArCH_2$), 2.81 (1H, q, J_1 = 8.7 Hz, J_2 = 6.9 Hz, $ArCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.35, 160.31, 153.30, 134.52, 132.25, 131.48, 129.47, 128.89, 127.58, 124.27, 121.00, 115.62, 72.48, 56.20, 37.57; ESI-MS: *m/z* [M + H]$^+$ 315.73.

Methyl-2-({[(3-chlorobenzyl)oxy]carbamoyl}amino)-3-phenylpropanoate (7e): Viscous light yellowish oil 2.05 g, yield: 34 %. IR (KBr, ν_{max}, cm^{-1}): 3294.2 (NH), 1735.8 (C=O), 1674.1 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): 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, J_1 = 10.3 Hz, J_2 = 7.5 Hz, CH), 4.68 (2H, s, OCH_2), 3.73 (3H, s, OCH_3), 3.23 (1H, q, J_1 = 3.5 Hz, J_2 = 7.0 Hz, $ArCH_2$), 3.13 (1H, t, *J* = 5.0 Hz, $ArCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): 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; ESI-MS: *m/z* $[M + Na]^+$ 385.70.

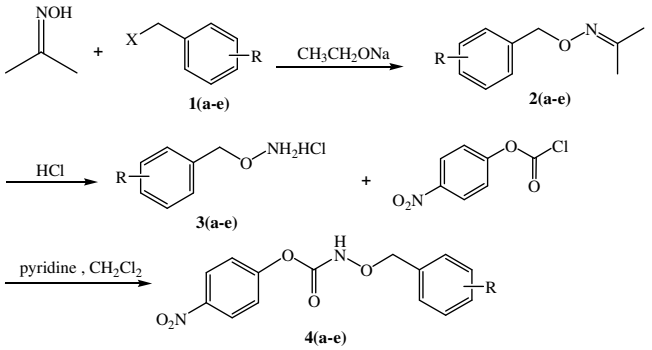
X-Ray crystallography: Colourless granular single crystals of the compound **7a** suitable for X-ray data collection were obtained by slow evaporation of the mixed solvent acetone and N-hexane (2:5) at 4 ºC for 3 months. The X-ray data were collected on a diffractometer equipped with graphite-monochromated MoK_α radiation (λ = 0.71073 Å) at 298(2) K. The structure was determined by direct methods and refined on F^2 by full-matrix least-squares using the program SHELXTL-97[10]. 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 ºC in a CO_2 incubator in a humidified atmosphere containing 5 % CO_2 atmosphere.

The *in vitro* antitumor activity of the target compounds against K562 and L1210 was evaluated by the improved MTT assay[11] using hydroxyurea as a positive control. In brief, cells (2×10^5 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 ºC. The formazan produced by the viable cells was solubilized by addition of 100 µL mixed solvent of 10 % (m/v) sodium dodecylsulfonate, 5 % (v/v) isobutanol and 0.012 M HCl. The suspension was placed in the dark incubator at 37 ºC overnight and the optical density was measured at 570 nm by the the ELISA reader. The experiment was performed in triplicate.

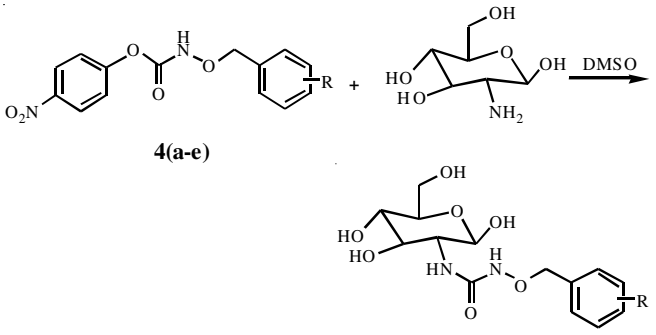
RESULTS AND DISCUSSION

Synthesis: The intermediates **4(a-e)** were prepared in **Scheme-I**. Acetoxime was chosen as the starting reagent. To obtain intermediates with different subtituents 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 benzyloxyurea 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[12]. All the target compounds were characterized by spectroscopic data as IR, MS, ^{1}H and ^{13}C NMR. The exact stereostructure of compound **7a** was determined by X-ray crystal structure analysis.



a: R = H, X = Cl; **b:** R = 4-CH_3, X = Cl; **c:** R = 4-Br, X = Br; **d:** R = 2-F, X = Cl; **e:** R = 3-Cl, X = Cl

Scheme-I: Synthesis of compounds **4(a-e)**

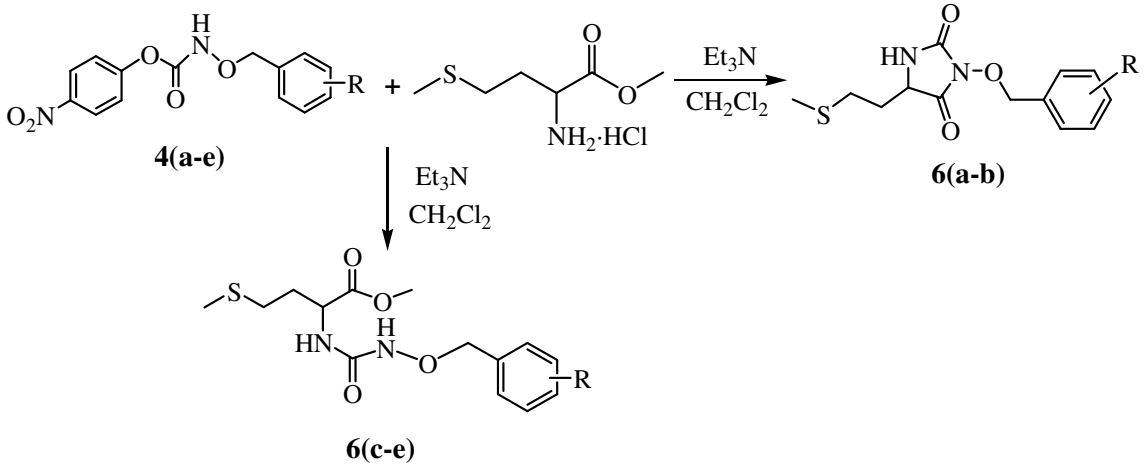


a: R = H; **b:** R = 4-CH_3; **c:** R = 4-Br; **d:** R = 2-F; **e:** R = 3-Cl

Scheme-II: Synthesis of compounds **5(a-e)**

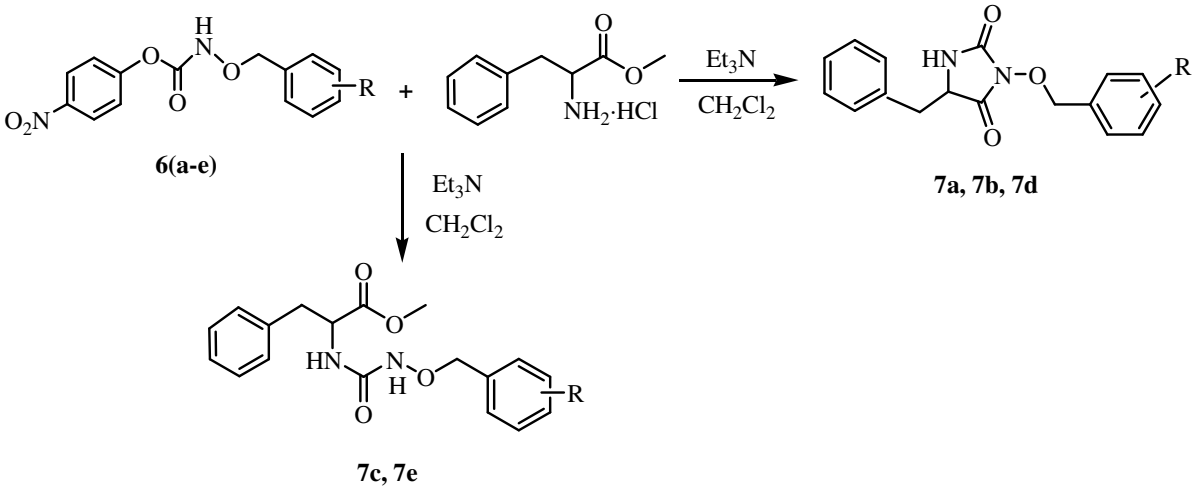
X-Ray crystal structure analysis: The crystallographic data of **7a** are summarized in Table-2. The selected bond lengths, angles and torsion angles are given in Table-3. The molecular structure with the atom numbering by ORTEP drawing is displayed in Fig. 1. The skeleton of **7a** consists of a five-membered hydantoin ring, a benzyloxy 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[6,13-15]. The C-N bond lengths [1.330(5)-1.467(5) Å, mean 1.389(5) Å] are longer than a typical C=N double bond (mean 1.269 Å),



a: R = H; **b:** R = 4-CH_3; **c:** R = 4-Br; **d:** R = 2-F; **e:** R = 3-Cl

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



a: R = H; **b:** R = 4-CH_3; **c:** R = 4-Br; **d:** R = 2-F; **e:** R = 3-Cl

Scheme-IV: Synthesis of compounds **7(a-e)**

TABLE-2
CRYSTAL AND EXPERIMENTAL DATA FOR **7a**

Item	Data
Empirical formula	$C_{17}H_{16}N_2O_3$
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 α = 90 °
	b = 32.226 (2) Å β = 92.7440(10)°
	c = 10.0532 (9) Å γ = 90 °
Volume	1470.3 (2) $Å^3$
Z, calculated density	4, 1.339 g cm^{-3}
Absorption coefficient	0.10 mm^{-1}
$F_{(000)}$	624
Crystal size	0.45 mm × 0.40 mm × 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 F^2
Goodness-of-fit on F^2	1.03
Final R indices [I > 2σ(I)]	R_1 = 0.078, wR_2 = 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**

N1-C1	1.330 (5)	C2-C3	1.522 (6)
N1-C3	1.467(5)	N2-C2	1.374 (5)
N2-C1	1.383 (5)	O1-C4	1.442(5)
N2-O1	1.373(4)	C4-C5	1.490(6)
C3-C11	1.480(6)	C11-C12	1.514(6)
C1-N1-C3	112.8(3)	C2-N2-C1	114.0(3)
N1-C3-C2	101.9(3)	N2-C2-C3	104.7(4)
N1-C1-N2	106.5(4)	N2-O1-C4	110.0(3)
O1-C4-C5	105.7(3)	C3-C11-C12	114.5(4)
C3-C11-C12-C13	157.2(4)	N1-C3-C11-C12	-64.1(5)
C2-C3-C11-C12	-179.2(4)	C4-C5-C10-C9	176.9(6)
O1-N2-C2-C3	171.7(4)	O1-N2-C1-N1	-173.4(3)
C1-N2-C2-O3	-177.9(4)	C2-N2-C1-O2	176.2(4)
C2-N2-O1-C4	102.7(5)	C4-C5-C6-C7	-178.0(5)
C1-N2-O1-C4	-88.6(5)	C3-N1-C1-O2	176.2(4)
C1-N1-C3-C11	-123.5(4)	C3-C11-C12-C17	-22.4(7)
O3-C2-C3-N1	-179.9(4)	C11-C12-C13-C14	-179.8(5)
N2-O1-C4-C5	168.6(4)	N2-C2-C3-C11	122.1(4)
O1-C4-C5-C6	101.3(5)	O1-C4-C5-C10	-78.0(6)

but shorter than a C-N single bond [mean 1.443(4) Å], indicating electron delocalization in the hydantoin ring[16]. The dihedral angle between the two coplanar benzene rings is

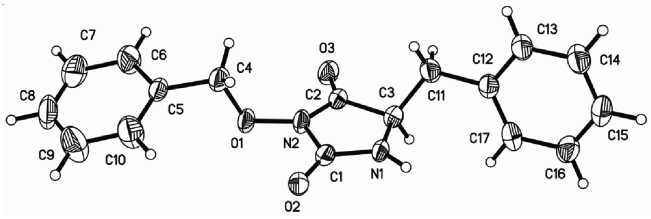


Fig. 1. Molecular structure and labeling of compound **7a**, showing 50 % probability ellipsoids

60.5(1)º. 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)º. 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).

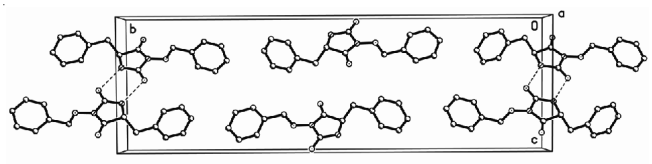


Fig. 2. Perspective view of the three-dimensional structure of **7a**

***in vitro* Antitumor activity:** The target compounds were valuated 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_{50} 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 benzyloxyurea and benzyloxyhydantoin derivatives **6(a-e)** and **7(a-e)**, all the compounds showed inhibitory activity against K562 cells with IC_{50} values ranged from 23.38-774.49 μM. The IC_{50} 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_{50} is 23.38 μM) and showed 85-fold more potency on K562 cells compared to hydroxyurea. L-Phenylalanine derivatives **7b**, **7c** and **7e** showed inhibitory activity against L1210 cell lines. Among them, the best cytostatic activity was also found for compound **7e** (IC_{50} is 83.11 μM). When the N3-H of benzyloxyurea 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 benzyloxyurea derivatives **6, 7** with higher C log P showed higher cytotoxity than the corresponding benzyloxyurea derivatives **5** with lower C log P. The result is agreement with the result in our previous paper[6].

Conclusion

We have synthesized a series of benzyloxyurea and benzyloxyhydantoin derivatives and tested for their anticancer activity on K562 and L1210 cell lines. Compounds **7e** and **7b** were identied as most potent having IC_{50} 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 obsearved 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)**.

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TABLE-4
CYTOTOXIC EFFECTS AGAINST L1210 CELLS AND HYDROPHOBIC PARAMETERS OF THE TITLE COMPOUNDS

Compounds	R	Inhibitory ratio (%)					IC_{50} (μM)	C log P
		10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M		
Hydroxyurea	–	48.79 ± 10.99	41.14 ± 24.56	31.43 ± 26.35	10.11 ± 7.46	7.76 ± 7.01	164.57	-1.8
5a	H	21.75 ± 6.78	0.99 ± 1.22	2.93 ± 1.05	3.62 ± 2.56	3.82 ± 1.54	2301.19	-0.04
5b	4-CH_3	26.03 ± 1.69	1.50 ± 1.78	1.02 ± 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	4.37 ± 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_3	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_3	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

TABLE-5
CYTOTOXIC EFFECTS AGAINST K562 CELLS OF THE TITLE COMPOUNDS

Compounds	R	Inhibitory ratio (%)					IC_{50} (μM)
		10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	
Hydroxyurea	–	30.63±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	3.73±1.29	1.89±4.84	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	59.41±4.24	7.48±3.75	-0.94±3.58	5.56±1.58	-0.03±2.52	774.49
6e	3-Cl	95.35±0.36	9.37±2.82	3.08±3.56	-4.43±0.72	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	389.37
7b	4-CH_3	97.37±0.74	60.54±2.80	11.25±21.12	5.00±7.26	3.58±2.16	52.51
7c	4-Br	88.91±4.77	47.22±9.15	10.89±15.00	-1.59±18.45	1.56±13.52	115.26
7d	2-F	88.93±5.33	32.40±12.30	2.36±10.21	5.91±15.93	-0.93±14.75	175.08
7e	3-Cl	97.73±0.53	69.11±8.77	18.63±6.38	14.72±14.30	10.79±11.41	23.38

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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. Hunninck, M. Toppet, P. Philippet, C.V. Geet and E. Sariban, *Blood*, **97**, 3628 (2001).
3. W.Y. Gao, D.G. Johns and H. Mitsuya, *Mol. Pharmacol*., **46**, 767 (1994).
4. F. Lori, A. Malykh, A. Cara, D. Sun, J.N. Weinstein, J. Lisziewicz and R.C. Gallo, *Science*, **266**, 801 (1994).
5. S.D. Malley, J.M. Grange, F. Hamedi-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. Flaig, *Acta Crystallogr. B*, **34**, 2495 (1978).