Synthesis, Antitumor Evaluation and Crystal Structure of Hydroxyurea Derivatives

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A series of hydroxyurea derivatives have been synthesized and elucidated by means of FT-IR, ^{1}H-, ^{13}C-NMR and MS. The exact stereostructures of representative compounds have been determined by X-ray crystal structure analysis. In the crystals, inversion dimers linked by pairs of N–H···O hydrogen bonds occurred, and further N–H···O links led to chains of molecules. *In vitro* antitumor activities against Tca8113 human tongue cancer cells and L1210 murine leukemia cells were evaluated. A total of 8 of the 12 compounds had higher inhibitory activities than hydroxyurea against L1210 cells. Among them, the most promising compounds were 3e, 3d, 3a and 2d.

Key words synthesis; antitumor evaluation; crystal structure; hydroxyurea

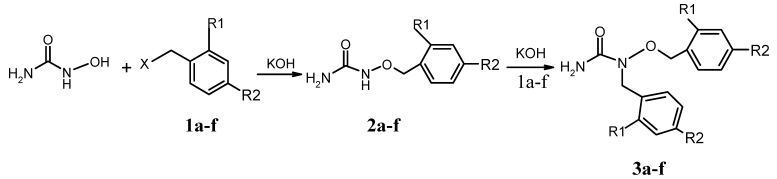
Hydroxyurea (HU) has been used in cancer chemotherapy for many years. It has been of manifold pharmacological interest, so it has been used for the treatment of melanoma, chronic myelocytic leukemia, and recurrent, metastatic, or inoperable ovarian cancer. HU is also used in therapy of squamous cell carcinomas in the head and neck and relapsed metastasis ovarian cancer,[1)] and people have found that it has certain effect on sickle cell anemia,[2)] beta-thalassemia,[3)] and psoriasis.[4)] It is also reported that HU has been used for the treatment of AIDS in combination with didanosine, showing no viral rebound after one year treatment.[5)] HU causes an immediate inhibition of DNA synthesis by acting on the R_2 subunit of the ribonucleotide reductase.[6—8)] Therapeutic application of HU has several disadvantages such as short half-life (1.9—3.9 h) in patients due to its small molecular size (MW=76.06) and extremely polar nature ($\mathrm{Clog}\,P_{o/w}=-1.80$), the necessity of using a high dosage (80 mg/kg every third day or 20—30 mg/kg daily), and the rapid development of resistance.[1,9—11)]

In this study, structure modification of HU based on increasing its hydrophobic nature and molecular size has been adopted to obtain a more potent compound. A series of monosubstituents and disubstituents of HU with different benzyls were synthesized and their structures were elucidated using spectrometry along with X-ray crystal structures analysis for representative compounds. The antitumor activity tests *in vitro* for human tongue cancer cell line and murine leukemia cell line were evaluated.

Results and Discussion

Synthesis The target compounds were prepared using the reaction sequence in Chart 1. The compounds **2a**—**f** were synthesized in good yield by condensation of HU with various benzyls (**1a**—**f**) in the presence of potassium hydroxide under reflux. The condensation of **2a**—**f** with HU afforded compounds **3a**—**f**. The chemical structures of the synthesized compounds (**2a**—**f** and **3a**—**f**) were confirmed by spectroscopic methods, and exact stereostructures of compounds **2a** and **3f** have been determined by X-ray crystal structure analysis.

X-Ray Crystal Structure Analysis The crystallographic data of **2a** are summarized in Table 1. The selected bond lengths, angles and torsion angles are given in Table 2. ORTEP drawings of the compounds **2a** and **3f**[12)] are illustrated in Fig. 1. Crystallographic data and the structure analysis of compound **3f** have been outlined in the previous paper.[12)] Conformations of the C8=O2 double bond and N1–O1 bond in **2a** are the opposite of each other, similar to that observed in **3f** (C1=O2 double bond and N1–O1 bond), *N*-hydroxyurea[13—17)] and other hydroxyurea derivates.[18)] The length of the carbonyl bond (C8=O2) in **2a** is in the normal range of 1.19—1.23 Å, similar to that observed in **3f**,[12)] but obviously shorter than *N*-hydroxyurea, 1-hydroxy-1-methylurea and 1-hydroxy-3-methylurea (>1.25 Å). This may be related to the hydroxyl etherification. The average distance between the carbon atom and the coordination nitrogen atom is 1.300 (3) Å. The group N–(C=O)–N urea planar forms a dihedral angle of 71.48 (15)° with the benzyl group, and the N–O bonds are twisted by about 20° out of the N–(C=O)–N



a: R_1=H, R_2=H, X=Cl; b: R_1=H, R_2=CH_3, X=Cl; c: R_1=H, R_2=CH_3O, X=Cl;

d: R_1= Cl, R_2=H, X=Cl; e: R_1=H, R_2=Br, X=Br; f: R_1=F, R_2=H, X=Cl;

Chart 1. Synthesis and Structures of Compounds **2a**—**f** and **3a**—**f**

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Table 1 Crystal and Experimental Data

Empirical formula: $C_8H_{10}N_2O_2$
Formula weight=166.18
Wavelength=0.71073 Å
Crystal system: monoclinic
Space group: $P2_1/c$
a=12.456 (3) Å
b=5.0081 (13) Å
c=13.681 (4) Å
β=96.017 (4)°
Volume=848.7 (4) Å^3
Z=4
D_x=1.301 g/cm^3
Absorption coefficient: 0.10 mm^{-1}
F(000)=352
Crystal size: 0.80×0.41×0.16 mm
θ range for data collection: 2.3 to 26.8°
Limiting indices: $-14\leq h\leq 14$, $-5\leq k\leq 5$, $-16\leq l\leq 16$
Reflections collected/unique: 4629/1453 [R_{int}=0.020]
$2\theta_{max}$=50.0° with Mo$K\alpha$
Absorption correction: multi-scan
Max. and min. transmission: 0.99 and 0.94
Goodness-of-fit on F^2: 1.04
Final R indices [$I>2\sigma(I)$]: R_1=0.057, wR_2=0.166
$(\Delta\rho)_{min}=-0.42$ e Å^{-3}
$(\Delta\rho)_{max}$=0.46 e Å^{-3}
$(\Delta/\sigma)_{max}<0.001$
No. of reflections used=1173
Measurement: Bruker APEX-II area-detector diffractometer
Program system: SHELXL 97
Structure determination: direct method
Refinement: full matrix least-squares on F^2

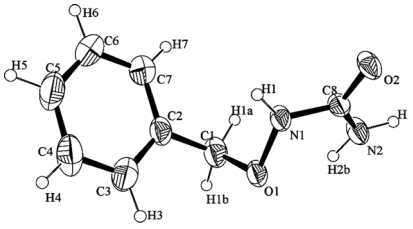
Table 2. Selected Bond Distances (Å), Angles (°), and Torsion Angles (°) for **2a**

C1–O1	1.441 (3)	N1–C8	1.274 (3)
C8–N2	1.326 (3)	O1–N1	1.406 (2)
C8–O2	1.220 (3)		
C2–C1–O1	105.3 (2)	N1–O1–C1	107.48 (18)
C8–N1–O1	112.71 (17)	O2–C8–N1	115.16 (19)
N1–C8–N2	116.4 (2)	O2–C8–N2	128.36 (19)
C1–C2–C3–C4	179.5 (3)	N2–C8–N1–O1	−20.0 (3)
C1–C2–C7–C6	−179.5 (3)	O1–C1–C2–C3	55.5 (3)
C2–C1–O1–N1	71.5 (2)	O1–C1–C2–C7	−125.1 (3)
C1–O1–N1–C8	105.1 (2)	O2–C8–N1–O1	163.84 (19)

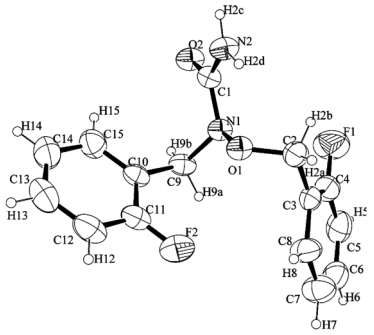
urea planes. In the crystal structure, molecules are linked to antiparallel chains running along the b axis by intermolecular N–H···O hydrogen bonding (Fig. 2). The hydrogen bonding parameters are summarized in Table 3.

Antitumor Evaluation The prepared compounds were evaluated for their cytotoxicity against cancer *in vitro* using HU as positive control. Firstly, compounds **2a**—**d** and **3a**—**d** were evaluated for their cytotoxic activity *in vitro* against human tongue cancer cell line (Tca8113). The results are summarized in Table 4. As shown in Table 4, compounds **3a**, **3b** and **3d** are the most active compounds to Tca8113 cells with IC_{50} values ranging from $5.29\times10^{-4}\times2.87$ mM, with **3d** exhibiting the most potent activity (IC_{50} is 5.29×10^{-4} mM). Because of the good results, we continued to synthesize the hydroxyurea derivatives **2e**—**f** and **3e**—**f**, and evaluated the cytotoxicity by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for all the target products using murine leukemia cell line L1210. The ranking order of the cytotoxicity of all the compounds is **3e**>**3d**>**3a**>**3d**>**2d**>**3d**>**3c**>**3d**>**3b**>**3d**>**3f**>**3d**>**2e**>**3d**>**HU**>**3d**>**2a**>**3d**>**2c**>**3d**>**2b**>**3d**>**2f**. Among them, the most promising compounds were **3e**, **3d**, **3a** and **2d**. The IC_{50} ratios of HU over compounds **3e**, **3d**, **3a** and **2d** range from 10 to 40, indicating that the four compounds are 10 to 40 fold more potent than HU against L1210 cells. **3c**, **3b** and **3f** also had remarkable activity.

The approximate partition coefficient Clog P of each de-



2a



3f

Fig. 1. ORTEP Structures of **2a** and **3f**, Showing 30% Probability Ellipsoids

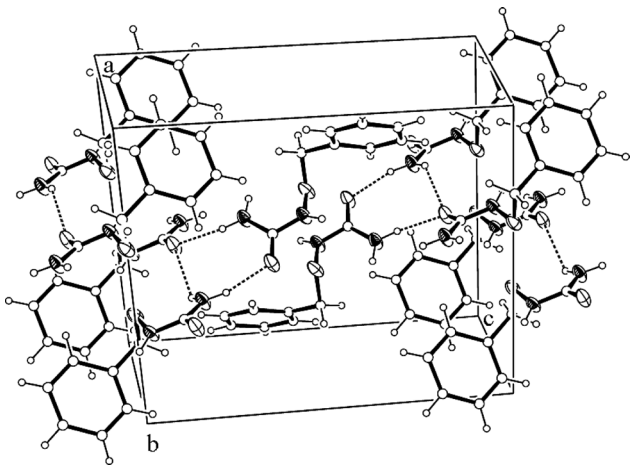


Fig. 2. Perspective View of the Three-Dimensional Structure of **2a**

Table 3. Hydrogen Bonding Geometry (Å, °) of **2a**

D–H···A	D–H	H···A	D···A	D–H···A
N2–H2B···O2^{i}	0.86	2.29	2.950 (3)	134
N2–H2A···O2^{ii}	0.86	1.90	2.733 (2)	163

Symmetry codes: (i) $x, y+1, z$; (ii) $-x+1, y+1/2, -z+5/2$.

Table 4. Cytotoxicity Results of Compounds

Compounds	Tca8113 (IC_{50} mM)	L1210 (IC_{50} μM)	Clog *P*
HU	31.03	71.70	−1.8
2a	3.51×10^{4}	161.10	1.704
2b	15.64	481.09	2.203
2c	20.10	195.73	1.623
2d	31.36	6.90	2.417
2e	nd[a)]	66.59	2.567
2f	nd	959.73	1.847
3a	2.87	6.24	2.994
3b	1.85	21.68	3.992
3c	8.48×10^{2}	15.17	2.832
3d	5.29×10^{-4}	1.94	4.42
3e	nd	1.81	4.72
3f	nd	28.84	3.28

a) nd=not determined.

rivative compound was calculated, and the values are included in Table 4. The higher Clog *P* value corresponds to the stronger hydrophobic or weaker hydrophilic nature of the compound. After the chemical modification, all of the HU derivatives possessed higher Clog *P* values than HU. Notably, disubstituents **3** with higher Clog *P* showed higher cytotoxity than the corresponding monosubstituents **2** with lower Clog *P*, suggesting that the stronger hydrophobic nature of the HU derivatives might favor the cytotoxic activity.

In conclusion, the desired HU derivatives were prepared. From the data of antitumor activity tests *in vitro*, some of them showed high or medium cytotoxicity against the cancer cell lines Tca8113 and L1210. Among them, the most promising compounds were **3e**, **3d**, **3a** and **2d**. To assess the potentials of these new compounds as cancer chemotherapeutic agents, further *in vivo* activity and toxicity studies are needed. The results obtained from this study can be used as guidelines for further development.

Experimental

Materials The starting compounds (**1a**—**f**) were purchased from Shanghai Darei Finechemical Co., Ltd., China. Hydroxyurea was purchased from Lingyi Furei Finechemical Co., Ltd., China. All reagents were obtained from commercial sources and used without further purification unless stated. Methanol was dried over calcium chloride and distilled. Acetone was dried over magnesium sulphate and distilled.

Apparatus Melting points (mp) were determined using a capillary method and were uncorrected. IR spectra were recorded on a Shimadzu FT-IR 8400 spectrometer (KBr pellets). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AV 400 MHz spectrometer. Mass spectra were recorded on a Waters 2695 LC- ZQ4000 system. Crystal data were collected by a Bruker APEX-II area-detector diffractometer.

Cell Lines The human tongue cancer cell line Tca8113 was provided by the Institute of Medical Sciences in Jiangxi province, China and the lymphocytic murine leukemia cell line L1210 was purchased from Nanjing Keygen Biotech. Co., Ltd., China. The two cell lines were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/ml penicillin G and 100 IU/ml streptomycin sulfate at 37 °C in a humidified atmosphere containing 5% CO_2 atmosphere.

Production of 2a—f. General Procedure Potassium hydroxide (34 mmol) and benzyl chloride or benzyl bromide with different substituents on phenyl (**1a**—**f**) (26 mmol) were added to a solution of hydroxyurea (26 mmol) in methanol (80 ml). The reaction mixture was refluxed and checked by TLC until HU was consumed; solvent was removed under reduced pressure at 35 °C. The resulting crude solid was filtered and washed in chloroform, then recrystallized in acetone and chloroform (5 : 2) to get **2a**—**f** as colorless crystals.

1-(Benzyloxy)urea (**2a**): Yield 80%. mp 140—142 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.75 (2H, s, –CH_2–), 6.38 (2H, s, –NH_2), 7.40 (5H, m, Ar-H), 9.06 (1H, s, –NH–). ^{13}C-NMR (DMSO-d_6) δ: 77.73, 128.43, 128.66, 129.16, 137.06, 161.27. IR (KBr) cm^{-1}: 3394.5 (NH), 3222.8 (NH), 1631.7 (C=O), 1107.1 (C–O). MS *m*/*z*: 167.1 $(M+H)^+$ (Calcd for $C_8H_{10}N_2O_2$: 166.18).

4-Methylbenzyloxyurea (**2b**): Yield 82%. mp 124—126 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.31 (3H, s, –CH_3), 4.67 (2H, s, –CH_2–), 6.31(2H, s, –NH_2–), 7.17 (2H, d, J=8.0 Hz, Ar-H), 7.30 (2H, d, J=7.6 Hz, Ar-H), 8.98(1H, s, –NH–). ^{13}C-NMR (DMSO-d_6) δ: 21.26, 77.57, 129.19, 129.31, 133.96, 137.66, 161.22. IR (KBr) cm^{-1}: 3384.8 (NH), 3197.8 (NH), 2916.2 (–CH_3), 1662.5 (C=O), 1118.6 (C–O). MS *m*/*z*: 181.1 $(M+H)^+$ (Calcd for $C_9H_{12}N_2O_2$: 180.20).

4-Methoxylbenzyloxyurea (**2c**): Yield 84%. mp 121—123 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.76 (3H, s, –OCH_3), 4.63 (2H, s, –CH_2–), 6.27 (2H, s, –NH_2–), 6.92 (2H, d, J=8.4 Hz, Ar-H), 7.34 (2H, d, J=8.4 Hz, Ar-H), 8.94 (1H, s, –NH–). ^{13}C-NMR (DMSO-d_6) δ: 55.54, 77.38, 114.02, 128.90, 130.93, 130.98, 161.18. IR (KBr) cm^{-1}: 3404.1 (NH), 3201.6 (NH), 2837.1 (–OCH_3), 1664.5 (C=O), 1172.6 (C–O). MS *m*/*z*: 197.2 $(M+H)^+$ (Calcd for $C_9H_{12}N_2O_3$: 196.20).

2-Chlorobenzyloxyurea (**2d**): Yield 72%. mp 130—132 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.72 (2H, s, –CH_2–), 6.42 (2H, s, –NH_2–), 7.38 (3H, t, J=4.8 Hz, Ar-H), 7.52 (1H, s, Ar-H), 9.05 (1H, s, –NH–). ^{13}C-NMR (DMSO-d_6) δ: 76.67, 127.60, 128.25, 128.78, 130.48, 133.34, 139.71,161.22. IR (KBr) cm^{-1}: 3406.1 (NH), 3197.8 (NH), 1662.5 (C=O), 1112.9 (C–O). MS *m*/*z*: 201.1 $(M+H)^+$ (Calcd for $C_8H_9ClN_2O_2$: 200.62).

4-Bromobenzyloxyurea (**2e**): Yield 76%. mp 163—164 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.68 (2H, s, –CH_2–), 6.37 (2H, s, –NH_2–), 7.38 (2H, d, J=8.4 Hz, Ar-H), 7.56 (2H, d, J=8.0 Hz, Ar-H), 9.01 (1H, s, –NH–). ^{13}C-NMR (DMSO-d_6) δ: 76.75, 121.59, 131.34, 131.52, 136.55, 161.18. IR (KBr) cm^{-1}: 3394.5 (NH), 3197.8 (NH),1666.4 (C=O), 1114.8 (C–O). MS *m*/*z*: 245.2 (M^+) (Calcd for $C_8H_9BrN_2O_2$: 245.07).

1-(2-Fluorobenzyloxy)urea (**2f**): Yield 73%. mp 150—152 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.78 (2H, s, –CH_2–), 6.36 (2H, s, –NH_2–), 7.22 (2H, d, J=7.2 Hz, Ar-H), 7.41 (1H, d, J=6.0 Hz, Ar-H), 7.54 (1H, t, J=9.4 Hz, Ar-H), 9.10 (1H, s, –NH–). ^{13}C-NMR (DMSO-d_6) δ: 71.24, 124.74, 124.77, 130.88, 130.97, 132.14, 132.18, 161.20. IR (KBr) cm^{-1}: 3398.3 (NH), 3226.7 (NH), 1631.7 (C=O), 1230.5 (C–O). MS *m*/*z*: 206.8 $(M+Na)^+$ (Calcd for $C_8H_9FN_2O_2$: 184.17).

Production of 3a—f. General Procedure Potassium hydroxide (17 mmol) and benzyl chloride or benzyl bromide with different substituents on phenyl (**1a**—**f**) (13 mmol) were added to a solution of compounds **2a**—**f** (13 mmol) in methanol (80 ml). After refluxing for 14—18 h (as evidenced by TLC), solvent was removed under reduced pressure at 35 °C. The residue was extracted with ether and the extraction solution was concentrated under reduced pressure. The crude product was column chromatographed on silica using acetone/chloroform (1 : 4) as eluent, solvent was eliminated from the elution under reduced pressure and then recrystallized in an acetone and chloroform mixture (5 : 1.5) to get **3a**—**f** as colorless crystals.

1-Benzyl-1-benzyloxyurea (**3a**): Yield 32%. mp 97—98 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.65 (2H, s, –CH_2–), 4.66 (2H, s, –CH_2–), 5.35 (2H, s, –NH_2), 7.35 (10H, m, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 52.76, 77.04, 127.68, 128.45, 128.72, 128.92, 129.05, 129.31, 134.97, 136.76, 160.94. IR (KBr) cm^{-1}: 3402.2 (NH), 3209.3 (NH), 1654.8 (C=O), 1209.3 (C–O). MS *m*/*z*: 257.2 $(M+H)^+$ (Calcd for $C_{15}H_{16}N_2O_2$: 256.30).

1-(4-Methylbenzyl)-1-(4-methylbenzyloxy)urea (**3b**): Yield 34%. mp 128—130 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (3H, s, –CH_3), 2.30 (3H, s, –CH_3), 4.46 (2H, s, –CH_2–), 4.68 (2H, s, –CH_2–), 6.47 (2H, s, –NH_2), 7.10 (2H, d, J=8.0 Hz, Ar-H), 7.16 (4H, d, J=10.4 Hz, Ar-H), 7.27 (2H, d, J=8.0 Hz, Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 21.15, 21.27, 51.29, 75.99, 129.01, 129.10, 129.20, 129.97, 133.18, 134.95, 136.62, 138.04, 160.75. IR (KBr) cm^{-1}: 3377.1 (NH), 3199.7 (NH), 2947.0 (–CH_3), 1666.4 (C=O), 1118.6 (C–O). MS *m*/*z*: 286.1 $(M+H)^+$ (Calcd for $C_{17}H_{20}N_2O_2$: 284.35).

1-(4-Methoxylbenzyl)-1-(4-methoxylbenzyloxy)urea (**3c**): Yield 36%. mp 99—101 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.33 (6H, s, –OCH_3, –OCH_3), 4.53 (2H, s, –CH_2–), 4.74 (2H, s, –CH_2–), 6.55 (2H, s, –NH_2), 7.31 (8H, m, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 20.64, 20.73, 51.80, 76.56, 128.63, 128.78, 128.91, 129.15, 129.70, 133.03, 134.86, 136.81, 160.86. IR (KBr) cm^{-1}: 3404.1 (NH), 3209.3 (NH), 2945.1(–OCH_3), 1654.8 (C=O), 1209.3 (C–O). MS *m*/*z*: 317.3 $(M+H)^+$ (Calcd for $C_{17}H_{20}N_2O_4$: 316.35).

1-(2-Chlorobenzyl)-1-(2-chlorobenzyloxy)urea (**3d**): Yield 24%. mp 81—83 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.56 (2H, s, –CH_2–), 4.78 (2H, s, –CH_2–), 6.70 (2H, s, –NH_2), 7.35 (8H, m, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 50.97, 75.13, 127.56, 127.60, 128.49, 128.67, 128.73, 128.80, 129.71, 130.50, 133.22, 133.28, 138.54, 140.49, 160.66. IR (KBr) cm^{-1}: 3467.8 (NH),

3195.8 (NH), 1689.5 (C=O), 1010.6 (C–O). MS *m/z*: 325.0 (M^+) (Calcd for $C_{15}H_{14}C_{l2}N_2O_2$: 325.19).

1-(4-Bromobenzyl)-1-(4-bromobenzyloxy)urea (**3e**): Yield 28%. mp 141—142 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.50 (2H, s, $-CH_2-$), 4.70 (2H, s, $-CH_2-$), 6.60 (2H, s, $-NH_2$), 7.21 (2H, d, J=8.4 Hz, Ar-H), 7.36 (2H, d, J=8.4 Hz, Ar-H), 7.50 (2H, d, J=8.4, Ar-H), 7.54 (2H, d, J=8 Hz, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 31.48, 51.06, 75.24, 120.76, 122.08, 131.18, 131.56, 132.08, 135.54, 137.36, 160.64. IR (KBr) cm^{-1}: 3404.1 (NH), 3186.2 (NH), 1670.2 (C=O), 1070.4 (C–O). MS *m/z*: 414.9 (M^+) (Calcd for $C_{15}H_{14}Br_2N_2O_2$: 414.09).

1-(2-Fluorobenzyl)-1-(2-fluorobenzyloxy)urea (**3f**): Yield 25%. mp 110—112 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.82 (2H, s, $-CH_2-$), 4.60 (2H, s, $-CH_2-$), 6.60 (2H, s, $-NH_2$), 7.19 (8H, m, Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 29.78, 45.12, 69.89, 124.60, 124.64, 124.75, 124.79, 129.70, 131.03, 131.07, 131.35, 131.43, 132.64, 132.68, 160.58. IR (KBr) cm^{-1}: 3498.6 (NH), 3226.7 (NH), 1689.5 (C=O), 1224.7 (C–O). MS *m/z*: 293.1 $(M+H)^+$ (Calcd for $C_{15}H_{14}F_2N_2O_2$: 292.28).

X-Ray Crystallography Colorless needle-shaped single crystals of the compound **2a** suitable for X-ray structure analysis were obtained by slow evaporation from the mixed solvent acetone and *N*-hexane (9 : 11) at room temperature for one week. The X-ray data were collected on a diffractometer equipped with graphite-monochromated Mo$K\alpha$ radiation (λ=0.71073 Å) at 296(2) K. The structure was determined by direct methods and refined on F^2 by full-matrix least-squares using the program SHELXTL-97.[19] An X-ray diffraction study of the compound showed that it was crystallized in a monoclinic system with the $P2_1/c$ 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,[19] refinement, SHELXS-97,[20] molecular diagrams, ORTEP.[21]

Pharmacology The newly synthesized compounds were evaluated for their *in vitro* cytotoxicity by growth-inhibition studies using two cancer-cell lines: human tongue cancer cell line (Tca8113) and murine leukemia cell line (L1210). Sulforhodamine B (SRB) assay[22] was used for study of Tca8113 cells, while MTT assay[23] was used of L1210 cells.

SRB Method Tumor cells (1×10^5 cells ml^{-1}) were inoculated in 96-well culture plates (100 μl/well). After culture for 24 h at 37 °C in a 5% CO_2 atmosphere, 50 μl of culture medium containing synthetic compound of various concentrations was added to the wells, the cells were then incubated for 65 h, and the medium was removed by suction. The cells were fixed with 10% cold trichloroacetic acid (TCA) and the plates were kept at 4 °C for 1 h. The TCA was removed by suction, and the plates were rinsed with water repeatedly 5 times and stained with SRB 0.4% (w/v) in 1% acetic acid for 15 min. Excess dye was washed out by 1% acetic acid repeatedly 5 times, air dried and the bound stain was subsequently solubilized with 150 μl Tris base (tris(hydroxy-methyl)aminomethane). The absorbance of SRB solution was measured at 490 nm with a microplate reader.

MTT Method Tumor cells (1×10^6 cells ml^{-1}) were inoculated in 96-well culture plates (100 μl/well). After cultured for 24 h, 50 μl of culture medium containing synthetic compound of various concentrations was added to the wells, then the cells were incubated for 65 h. Twenty microliters of MTT was added at a final concentration of 5 mg/ml and after 4 h incubation, 150 μl of DMSO was added and the optical density was measured at 490 nm.

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