Synthesis, Structure Analysis and Cytotoxicity Studies of Novel Unsymmetrically *N*,*N*′-Substituted Ureas from Dehydroabietic Acid

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A series of novel unsymmetrically *N*,*N*′-substituted ureas were synthesized from dehydroabietic acid and their structures were characterized by IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy and single crystal X-ray diffraction. Three six-membered rings of urea 4c exhibited plane, half-chair and chair configurations, respectively. Their cytotoxicity activities against SMMC7721 liver cancer cells were evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The results showed that the title compounds exhibited highly effective cytotoxicity activities against SMMC7721 cells. Their IC_{50} values are between 8.8 and 14.2 μmol/l. The change of *N*′ substituted groups resulted little difference to the cytotoxicity activities of ureas, which indicated that the cytotoxicity of this kind of ureas depend strongly on the tricyclic hydrophenanthrene structure.

Key words unsymmetrically *N*,*N*′-substituted urea; dehydroabietic acid; crystal structure; cytotoxicity activity

Cancer is the primary cause of death in most of the countries and as a result there is a need for searching cancer-effective compounds.[1)] Natural products played an important role in drugs discovery. They are still major source of new antitumor drugs development, and many natural or natural based antitumor drugs such as taxol and vinblastine were found and clinically used in recent years.[2,3)]

Substituted ureas have been attracted great attention due to a set of valuable properties allowing their use in industry, agriculture, and medicine. Wide range of biological activities made substituted ureas used as antitumor agents, insecticides, plant growth regulators, gastroprotectives as well as tranquillising and anticonvulsant agents. Moreover, ureas substituted with amino acid groups have been shown to be potent human immunodeficiency virus (HIV)-1 protease inhibitors.[4—6)]

Dehydroabietic acid (DHA) is a natural occurring diterpenic resin acid, which can be easily isolated from commercial disproportionated rosin by crystallization of the 2-aminoethanol salt.[7)] DHA and its derivatives exhibited wide range of biological activities, such as antibacterial, antifungal, gastroprotective, cytotoxic, antisecretory, antipepsin and anti-penetrant activities.[8,9)] DHA was reported to have properties of enhancing the inhibitory activity of an anticancer drug in cervical carcinoma cells, hepatocellular carcinoma cells, or breast cancer cells.[10)] However, the cytotoxicity activities of unsymmetrically *N*,*N*′-substituted ureas from DHA have not yet been reported so far. Encouraged by these research results, DHA was chosen as the raw material in screening for new potential antitumor compounds.

In this report, we described the synthesis a series of unsymmetrically *N*,*N*′-substituted ureas from DHA through 4 steps of reactions. The crystal structure of **4c** was determined. The cytotoxicity activity of these novel ureas was studied, and their structure activity relationship was also investigated.

Experimental

Materials and Methods All chemicals purchased were of reagent grade and used without further purification. Infrared spectrum was recorded as KBr pellets on a Bio-Rad FTS-185 IR spectrophotometer. Melting points were determined by XT5 melting point apparatus. ^{1}H- and ^{13}C-NMR spectrum was recorded on a DPX-300 Bruker AVANCE 300 spectrometer ($CDCl_3$ as solvent).

General Method for Preparation 4 Dehydroabietic acid (0.1 mol) dissolved in 30 ml $CHCl_3$ was gradually added to a solution of 5 ml PCl_3, the reaction was performed for 3 h at 60—65 °C. Then cooled to room temperature and the mixture was filtrated and the solvent was distilled off under vacuum. The synthesized dehydroabietic chloride was slowly added to the 200 ml NH_4OH solution, the mixture was quickly stirred for 2 h at room temperature, and then the precipitates were filtered and crystallized from acetone to give **2**. To a 150 ml 10% NaOH solution, 12 ml Br_2 and 100 ml NaOH and **2** were added with ice cooling, the mixture was stirred for 4 h at room temperature, then the mixture was extracted with ether three times, the solution was dried with Na_2SO_4, the solvent was distilled off and **3** was obtained. 0.1 mol amine was added to 20 ml CH_2Cl_2 solutions, the mixture was stirred at 40 °C for 12 h, the solvents was distilled off under vacuum and crystals were crystallized from acetone to give **4**.

4a: $C_{20}H_{30}N_2O$, IR (cm^{-1}): 3463, 3376 (N–H); 2950 (–CH_3, –CH_2); 1658 (O=C–N); 816 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.27—6.88 (3H, C=C<u>H</u>–); 4.82 (1H, –N<u>H</u>CO); 4.34 (2H, CO–N<u>H</u>$_2$–); 2.81 (1H, –C<u>H</u>(Me)$_2$); 2.90—1.68 (10H, –C<u>H</u>$_2$–); 1.46 (1H, >C<u>H</u>–); 1.28—1.19 (12H, –C<u>H</u>$_3$). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 158.59 (1C, <u>C</u>=O); 146.88, 145.45, 134.69, 126.78, 124.27, 123.76 (6C, <u>C</u>=C); 47.15 (1C, <u>C</u>–N); 33.42 (1C, <u>C</u>–C); 56.27, 30.21 (2C, <u>C</u>H–C); 38.09, 37.68, 37.59, 19.67, 18.81 (5C, <u>C</u>H$_2$–C); 25.11, 23.99, 21.03 (4C, <u>C</u>H$_3$).

4b: $C_{26}H_{34}N_2O$, IR (cm^{-1}): 3367, 3249 (N–H); 2950 (–CH_3, –CH_2); 1649 (O=C–N); 831 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.17—6.87 (3H, C=C<u>H</u>–); 5.80 (1H, –N<u>H</u>CO); 4.53 (1H, CO–N<u>H</u>–); 2.83 (1H, –C<u>H</u>(Me)$_2$); 2.91—1.70 (10H, –C<u>H</u>$_2$–); 1.34 (1H, >C<u>H</u>–); 1.26—1.18 (12H, –C<u>H</u>$_3$). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 155.60 (1C, <u>C</u>=O); 146.88, 145.51, 139.49, 134.67, 128.88, 126.87, 124.32, 123.82, 122.46, 119.77 (12C, <u>C</u>=C); 47.15 (1C, <u>C</u>–N); 33.54 (1C, <u>C</u>–C); 56.73, 30.14 (2C, <u>C</u>H–C); 38.12, 37.71, 37.59, 19.70, 18.97 (5C, <u>C</u>H$_2$–C); 25.11, 24.12, 20.95 (4C, <u>C</u>H$_3$).

4c: $C_{28}H_{38}N_2O$, IR (cm^{-1}): 3367 (N–H); 2960 (–CH_3, –CH_2); 1654 (O=C–N); 831 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 5.87 (1H, –N<u>H</u>CO); 4.46 (1H, CO–N<u>H</u>–); 2.78 (1H, –CH(Me)$_2$); 2.29 (6H, –Ph-C<u>H</u>$_3$); 2.87—1.71 (10H, –C<u>H</u>$_2$–); 1.32 (1H, >C<u>H</u>–); 1.28—1.19 (12H, –C<u>H</u>$_3$). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 154.57 (1C, <u>C</u>=O); 145.82, 144.47, 135.79, 133.52, 130.33, 129.62, 125.73, 125.62, 123.74, 123.52, 123.25, 122.81 (12C, <u>C</u>=C); 46.28 (1C, <u>C</u>–N); 32.44 (1C, <u>C</u>–C); 55.62, 29.14 (2C, <u>C</u>H–C); 37.07, 36.69, 32.44, 17.88, 16.80 (5C, <u>C</u>H$_2$–C); 23.98, 23.02, 22.98, 19.83, 18.65 (6C, <u>C</u>H$_3$).

4d: $C_{26}H_{33}FN_2O$, IR (cm^{-1}): 3367 (N–H); 2960 (–CH_3, –CH_2); 1639 (O=C–N); 831 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.21—6.87 (7H, C=CH–); 6.57 (1H, –NHCO); 4.74 (1H, CO–N<u>H</u>–); 2.78 (1H, –CH(Me)$_2$); 2.94—1.76 (10H, –CH$_2$–); 1.30 (1H, >CH–); 1.27—1.19 (12H, –CH$_3$). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 155.19 (1C, <u>C</u>=O); 160.48, 146.72, 145.65, 135.02, 134.39, 126. 78, 124.31, 123.94, 122.29, 122.18, 115.75, 115.46 (12C, <u>C</u>=C); 47.44 (1C, <u>C</u>–N); 33.47 (1C, <u>C</u>–C); 56.90,

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Table 1. Synthesis of Compounds

Compound	R_1	R_2	mp (°C)
4a	H	H	195.8
4b	H	Ph	234.6
4c	H	2,5-$(CH_3)_2$Ph	227.8
4d	H	*p*-FPh	152.1
4e	H	*o*-FPh	226.2
4f	H	2,6-F_2Ph	245.5
4g	H	3,5-F_2Ph	208.8
4h			244.4
4i			240.4
4j	H	iso-Pr	197.5
4k	H	Bu	171.6

30.14 (2C, C̲H–C); 38.13, 37.79, 35.82, 19.64, 18.95 (5C, C̲H_2–C); 25.01, 24.01, 20.94 (4C, C̲H_3).

4e: $C_{26}H_{33}FN_2O$, IR (cm^{-1}): 3369 (N–H); 2958 (–CH_3, –CH_2); 1639 (O=C–N); 823 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.97—6.87 (7H, C=C̲H–); 6.58 (1H, –N̲HCO); 4.81 (1H, CO–N̲H–); 2.82 (1H, –C̲H(Me)$_2$); 2.91—1.48 (10H, –C̲H_2–); 1.34 (1H, >C̲H–); 1.24—1.21 (12H, –C̲H_3). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 153.81 (1C, C̲=O); 154.47, 146.74, 145.63, 134.52, 127.59, 126.83, 124.45, 123.89, 122.94, 122.84, 121.81, 114.97 (12C, C̲=C); 47.46 (1C, C̲–N); 33.47 (1C, C̲–C); 57.31, 30.18 (2C, C̲H–C); 38.22, 37.72, 19.67, 18.95 (5C, C̲H_2–C); 25.07, 24.01, 23.98, 21.01 (4C, C̲H_3).

4f: $C_{26}H_{32}F_2N_2O$, IR (cm^{-1}): 3353 (N–H); 2954 (–CH_3, –CH_2); 1645 (O=C–N); 823 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.33—6.74 (7H, C=C̲H–); 6.07 (1H, –N̲HCO); 4.51 (1H, CO–N̲H–); 2.83 (1H, –C̲H(Me)$_2$); 2.93—1.69 (10H, –C̲H_2–); 1.32 (1H, >C̲H–); 1.25—1.21 (12H, –C̲H_3). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 154.57 (1C, C̲=O); 163.01, 159.78, 146.72, 145.62, 138.04, 134.45, 131.19, 126.79, 124.28, 123.90, 116.38, 110.44 (12C, C̲=C); 47.31 (1C, C̲–N); 33.46 (1C, C̲–C); 57.12, 30.13 (2C, C̲H–C); 38.17, 37.69, 19.66, 18.98 (5C, C̲H_2–C); 25.02, 24.01, 23.98, 20.99 (4C, C̲H_3).

4g: $C_{26}H_{32}F_2N_2O$, IR (cm^{-1}): 3362 (N–H); 2955 (–CH_3, –CH_2); 1628 (O=C–N); 826 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.28—6.86 (6H, C=C̲H–); 6.65 (1H, –N̲HCO); 4.87 (1H, CO–N̲H–); 2.81 (1H, –C̲H(Me)$_2$); 2.93—1.68 (10H, –C̲H_2–); 1.34 (1H, >C̲H–); 1.31—1.19 (12H, –C̲H_3). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 155.11 (1C, C̲=O); 146.79, 145.54, 139.24, 134.53, 129.02, 126.79, 124.29, 123.84, 122.93, 120.28 (12C, C̲=C); 47.45 (1C, C̲–N); 33.47 (1C, C̲–C); 56.84, 30.15 (2C, C̲H–C); 38.13, 37.77, 37.70, 19.65, 18.95 (5C, C̲H_2–C); 25.04, 24.03, 23.99, 20.88 (4C, C̲H_3).

4h: $C_{25}H_{38}N_2O$, IR (cm^{-1}): 3358 (N–H); 2955 (–CH_3, –CH_2); 1626 (O=C–N); 1529 (C=C); 832 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.183—6.875 (3H, C=C̲H–); 4.203 (4H, CONC̲H_2–); 3.682 (4H, OC̲H_2–); 3.290 (4H, NC̲H_2–); 3.237 (1H, –C̲H(Me)$_2$); 2.829—1.725 (10H, –C̲H_2–); 1.357 (1H, >C̲H–); 1.278—1.222 (12H, –C̲H_3). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 156.73 (1C, C̲=O); 146.93, 145.49, 134.45, 126.70, 124.14, 123.76 (6C, C̲=C); 47.04 (1C, C̲–N); 33.39 (1C, C̲–C); 56.72, 30.12 (2C, C̲H–C); 47.04, 45.15, 44.53, 38.09, 37.79, 37.61, 19.72, 18.84 (10C, C̲H_2–C); 23.90, 22.22, 21.37 (4C, C̲H_3).

4i: $C_{24}H_{36}N_2O_2$, IR (cm^{-1}): 3358 (N–H); 2955 (–CH_3, –CH_2); 1626 (O=C–N); 832 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.18—6.87 (3H, C=C̲H–); 4.20 (4H, CONC̲H_2–); 3.68 (4H, O–C̲H_2–); 3.29 (4H, NC̲H_2–); 3.23 (1H, –C̲H(Me)$_2$); 2.82—1.72 (10H, –C̲H_2–); 1.35 (1H, >C̲H–); 1.28—1.22 (12H, –C̲H_3). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 156.71 (1C, C̲=O); 146.85, 145.64, 134.40, 126.78, 124.24, 123.89 (6C, C̲=C); 47.41 (1C, C̲–N); 33.46 (1C, C̲–C); 57.02, 30.17 (2C, C̲H–C); 66.53, 44.24, 43.45, 38.19, 37.78, 19.73, 18.94 (9C, C̲H_2–C); 24.92, 24.03, 21.19 (4C, C̲H_3).

4j: $C_{23}H_{36}N_2O$, IR (cm^{-1}): 3356 (N–H); 2961 (–CH_3, –CH_2); 1633 (O=C–N); 826 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.15—6.85 (3H, C=C̲H–); 4.30 (1H, –N̲HCO); 3.06 (1H, –N̲HCO); 2.79 (1H, –C̲H(Me)$_2$); 2.86—1.67 (10H, –C̲H_2–); 1.43 (2H, >C̲H–); 1.31—0.88 (18H, –C̲H_3). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 157.40 (1C, C̲=O); 147.06, 145.35, 134.73, 126.77, 124.30, 123.71 (6C, C̲=C); 47.23 (1C, C̲–N); 33.46 (1C, C̲–C); 56.15, 41.48, 30.31 (3C, C̲H–C); 38.15, 37.94, 37.85, 19.81, 18.85 (5C, C̲H_2–C); 25.07, 24.04, 23.98, 23.39, 23.32, 21.29 (6C, C̲H_3).

4k: $C_{25}H_{39}N_2O$, IR (cm^{-1}): 3326 (N–H); 2953 (–CH_3, –CH_2); 1633 (O=C–N); 819 (Ar-H); ^{1}H-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 7.14—6.84 (3H, C=C̲H–); 4.32 (1H, –N̲HCO); 3.78 (1H, –N̲HCO); 2.82 (1H, –C̲H(Me)$_2$); 2.87—1.46 (16H, –C̲H_2–); 1.44 (1H, >C̲H–); 1.45—1.08 (15H, –C̲H_3). ^{13}C-NMR: ($CDCl_3$, δ/ppm, 300 MHz), 157.27 (1C, C̲=O); 146.06, 144.32, 133.75, 125.77, 123.26, 122.69 (6C, C̲=C); 46.26 (1C, C̲–N); 32.45 (1C, C̲–C); 55.03, 31.55 (3C, C̲H–C); 38.56, 37.14, 36.90, 36.82, 29.25, 19.07, 18.83, 17.85 (8C, C̲H_2–C); 24.11, 22.99, 20.28,12.84 (5C, C̲H_3).

Table 2. Crystal Data and Structure Refinement for (**4c**)

Empirical formula	$C_{28}H_{38}N_2O$
Formula weight	418.60
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system	Trigonal
Space group	*P65*
a (Å)	13.0880(19)
b (Å)	13.0880(19)
c (Å)	25.097(5)
α (°)	90.00
β (°)	90.00
γ (°)	120.00
V (Å^3)	3723.0(11)
Density (calculated) (mg m^{-3})	1.117
Absorption coefficient (mm^{-1})	0.067
F (0 0 0)	1362.0
Crystal size (mm)	0.40×0.20×0.10
θ range for data collection (°)	1.80 to 26.00
Limiting indices	$0\leq h\leq 13$, $0\leq k\leq 13$, $-30\leq l\leq 30$
Reflections collected/unique [R_{int}=0.057]	5492/4852
Completeness to θ=25.99 (%)	99.9
Max. and min. transmission	0.9736, 0.9933
Data/restraints/parameters	4852/4/295
Goodness-of-fit on F^2	1.001
Final *R* indices [$I>2\sigma(I)$]	R_1=0.105
	wR_2=0.327
R indices (all data)	R_1=0.1044, wR_2=0.1735
Absolute structure parameter	−5(6)
Largest diff. peak and hole (e·A^{-3})	0.31, −0.25

$R_1=\Sigma||F_o|-|F_c||/\Sigma|F_o|$; $wR_2=\Sigma[w(F_o^2-F_c^2)^2/\Sigma[w(F_o^2)^2]^{1/2}$.

X-Ray Crystallography The crystal structure of **4c** was determined by X-ray single crystal diffraction. XRD data were collected on a Enraf-Nonius CAD-4 diffractometer equipped with Mo$K\alpha$ (λ=0.07103) at 293 K. A single crystal suitable for determination was mounted inside a glass fiber capillary. The structure of the title compound was solved by direct methods and refined by full-matrix least squares on F^2. All the hydrogen atoms were added in their calculated positions and all the non-hydrogen atoms were refined with anisotropic temperature factors. SHELXS97 were used to solve the structure and SHELTL were used to refine the structure.[11,12] The crystallographic details are summarized in Table 2. The selected bond lengths and angles are shown in Table 3.

Pharmacology Test compounds **4a**—**c** were evaluated *in vitro*, for cytotoxicity in SMMC7721 liver cancer cell lines at 1, 10, 15, 25 and 50 mmol/l concentrations using MTT assay, respectively.[13]

Results and Discussion

Synthesis As shown in Chart 1, a series of unsymmetrically *N*,*N*′-substituted ureas were synthesized from DHA through 4 steps of reactions. Although high steric hindrance of tricycle hydrophethane structure to the carboxylic acid (**1**), the reaction activity was greatly improved by conversion of the acid group to chloride intermediate. Previous study revealed that PCl_3 is the best chloride reagent. (**2**) can be obtained from chloride in the presence of NH_4OH at room temperature. Through Hoffman degrading reaction, isocyanate

(**3**) can be obtained. Isocyanate can be converted to unsymmetrically *N*,*N*′-substituted ureas (**4**) with substituted amine.

Structure Analysis Full crystallographic details of **4c** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 683542. White crystals of **4c** suitable for X-ray analysis were obtained by solvent evaporation under room temperature. Its single-crystal structure was determined by X-ray crystallography. As shown in Fig. 1, the molecular structure of **4c** contains four crystallographically unique six-membered rings. Their torsion angles show ring B (C6, C7, C10—C13) and C (C10, C11, C14—C17) exhibits a chair and half-chair configuration, respectively, they form *trans* ring junction with two methyl groups (C18, C19) in axis positions. However, the six atoms in the other two six-membered rings are coplanar. From the view of the crystal packing (Fig. 2), it is found that weak inter-molecular $\pi\cdots\pi$ stacking interactions exist in the structure.

Cytotoxicity Activity In order to investigate whether the synthesized unsymmetrically *N*,*N*′-substituted ureas inhibit the growth of tumor cells, SMMC7721 liver cancer cells were selected as tumor agent. All compounds were subjected to a preliminary screening for cytotoxicity activity at sample concentrations of 1.0, 10 and 25 μM using the MTT assay, respectively. The data of inhibition ratios are listed in Table 4. As can be concluded from the results, the inhibition ratios of all compounds increased with the increment of concentrations, they reached very high activity at the concentration of 25 μM, the inhibition ratios reached about 66—82%, especially compounds **4a**, **4d**, **4g** and **4h** exhibited higher activities than others, the inhibition ratios are near 80%.

Detailed bioassays were carried out for compounds **4a**, **4d**, **4g** and **4h** at sample concentrations of 1.0, 10, 15, 25, 50 μM, respectively. The inhibition ratios and IC_{50} values of these compounds are listed in Table 5. The compounds exhibited high activity at 50 μM, the inhibition ratios reached between 90% and 99.1%, IC_{50} values are between 8.8 and 14.2. Compound **4a** exhibited highest activity against SMMC7721 liver cancer cells with IC_{50} value of 8.8 μM. The title compound is

Table 3. Selected Bond Lengths and Angles for (**4c**)

Bond length [Å]	
O–C20	1.269(9)
N1–C20	1.323(10)
N1–C17	1.464(10)
N2–C20	1.365(10)
N2–C21	1.402(11)
Bond angles [°]	
C20–N1–C17	127.3(7)
C20–N2–C21	127.9(7)
C6–C5–C4	120.3(11)
C8–C13–C12	115.2(8)
N1–C17–C16	109.7(7)
N1–C17–C11	108.4(6)
C16–C17–C11	109.5(6)
N1–C17–C19	103.3(7)
O–C20–N1	124.2(8)
O–C20–N2	120.0(7)
N1–C20–N2	115.7(7)
C22–C21–N2	119.9(9)
N2–C21–C26	119.3(9)
C21–C22–C27	121.8(10)
Torison angles [°]	
C5–C6–C7–C10	−178.3(10)
C20–N1–C17–C16	−54.7(11)
C15–C16–C17–N1	169.7(7)
C17–N1–C20–O	−7.6(15)
C17–N1–C20–N2	176.3(7)
C21–N2–C20–O	6.5(14)
C21–N2–C20–N1	−177.2(8)
N2–C21–C26–C25	173.2(8)

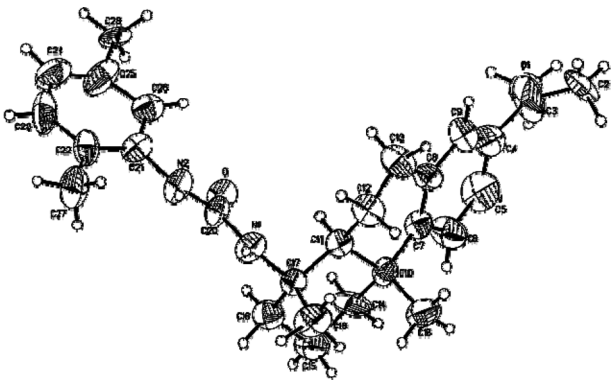

Fig. 1. ORTEP Diagram of Compound **4c** with H Atoms Represented by Small Spheres of Arbitrary Radius

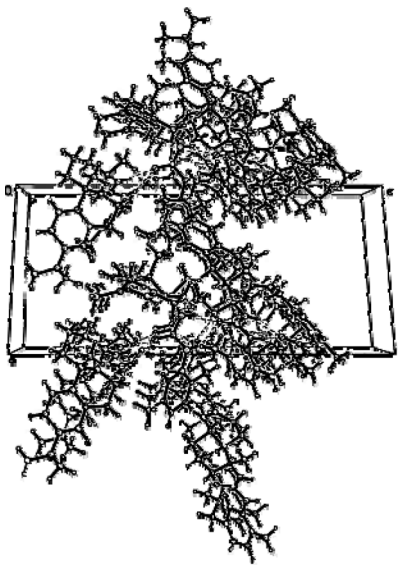

Fig. 2. The Packing Diagram of Compound **4c** in the Unit Cell

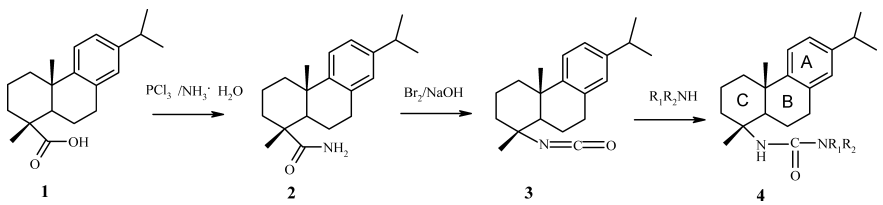

Chart 1. Synthetic Scheme of Unsymmetrically *N*,*N*′-Substituted Ureas

Table 4. Inhibition Ratios of Compounds against SMMC7721 Liver Cancer Cells at Different Concentration

Compounds	Concentration (μM)		
	1.0	10	25
4a	3.4	34.7	81.3
4b	0	16.9	74.6
4c	0	19.5	73.7
4d	2.5	17.8	77.1
4e	0	0	68.6
4f	0	0	69.5
4g	3.4	20.3	76.3
4h	3.4	25.4	78.8
4i	0	0	66.9
4j	0	0	67.8
4k	0	0	70.3

Table 5. Inhibition Ratios of Compounds against SMMC7721 Liver Cancer Cells at Different Concentration

Compounds	Concentration (μM)					IC_{50}
	1.0	10	15	25	50	
4a	3.4	34.7	61.1	81.3	99.1	8.8
4d	2.5	17.8	50.5	77.1	90.4	14.2
4g	3.4	20.3	58.4	76.3	92.3	12.8
4h	3.4	25.4	59.2	78.8	98.1	10.2

a kind of lead compounds of antitumor agent that warrants further investigation.

According to the structure and cytotoxicity activity of these compounds, change of the substituted groups of R_1 and R_2 can little improve the cytotoxicity activity, which indicating that the cytotoxicity activity of this kind of ureas depend strongly on the tricyclic hydrophenanthrene structure.

Possible Mechanism In our previous study,[14] we have explored the possible mechanisms of dehydroabietic derivaties (TBIDOM) on SMMC-7721 liver cancer cells. Pretreatment of SMMC-7721 cells with TBIDOM significantly induced a decrease of Bcl-2 protein expression and an increase of caspase-3 activity and Bax protein expression. Its mechanism may have been related to the decrease in the expression of anti-apoptotic protein, Bcl-2, accompanied by a drop in the mitochondrial membrane potential and the activation of caspase-3, that led to apoptotic body formation and finally apoptosis. We think the ureas possess the same mechanism as TBIDOM.

Conclusions

In summary, we have synthesized a series of unsymmetrically *N*,*N*′-substituted ureas from dehydroabietic acid and their structures were identified by IR, ^{1}H-NMR spectroscopy and single crystal X-ray diffraction. The tricyclic hydrophenanthrene structure of **4c** exhibited classic configuration. Three six-membered rings of molecule **4c** exhibited plane, half-chair and chair configurations, respectively. This kind of compounds will be a new class of antitumor agents because of its strong indication of cytotoxicity activities and natural based properties. The change of *N*′ substituted groups resulted little difference to the cytotoxicity activities of ureas, which indicated that the cytotoxicity of this kind of ureas depend strongly on the tricyclic hydrophenanthrene structure.

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