J Chem Crystallogr (2008) 38:807–813
DOI 10.1007/s10870-008-9396-9

ORIGINAL PAPER

Syntheses, Structural, and Biological Studies of Two New Peptide Compounds, 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl Acetate Hemihydrate and 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl Acetic Acid

Mao-Lin Hu · Ping Yin · Zhi-Cheng Ma · Ali Morsali

Received: 4 November 2007 / Accepted: 2 April 2008 / Published online: 15 April 2008

Abstract Two new peptide compounds, 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate hemihydrate (**1:1/2H$_2$O**) and 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetic acid (**2**), have been synthesized and characterized by elemental analysis, IR, ^{1}H- and ^{13}C NMR spectroscopy. Two new compounds were structurally characterized by single-crystal X-ray diffraction. The thermal stabilities of compounds **1:1/2H$_2$O** and **2** were studied by DSC-TGA techniques. The result of the biological test showed that the compounds **1:1/2H$_2$O** and **2** have certain antitumor activities.

Keywords 2(s)-(5-Fluorouracil-1-aceto)amino-2-isopropylacetate · 2(s)-(5-Fluorouracil-1-aceto)amino-2-isopropyl acetic acid · Crystal structure · Thermal analysis · Antitumor activity

Introduction

5-Fluorouracil (5-FU) is an antimetabolite with good antimicrobial and antitumor activity and most frequently used for treating solid tumors, such as breast, colorectal, and gastric cancers, in either monotherapy or combination therapy with various cytotoxic drugs [1–4]. However, its administration is accompanied by significant toxic side effects and delivery problem [5–9]. Many derivatives of 5-FU have been synthesized to improve the topical delivery of 5-FU and reduce the side effects. In previous investigations, we have successfully obtained 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)acetic acid [10]. To extend our interest in searching for new derivatives of 5-FU with higher bioactivity and take advantage of the concept of bioisosterism, the title compounds containing 5-FU ring, 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate hemihydrate (**1:1/2H$_2$O**) and 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetic acid (**2**), was firstly designed and synthesized.

M.-L. Hu (✉) · P. Yin · Z.-C. Ma
College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325027, People's Republic of China
e-mail: maolin_hu@yahoo.com

A. Morsali
Department of Chemistry, Faculty of Sciences, Tarbiat Modares University, Tehran, 14115-175, Islamic Republic of Iran
e-mail: morsali_a@yahoo.com

Experimental

Materials and Instruments

All chemicals were obtained from a commercial source and used without further purification. The elemental analyses were performed on a Carlo-Erba 1112 Elemental Analyzer. IR spectrum was recorded on a Perkin Elmer 2000 system in the range of 4,000–400 cm^{-1} with KBr disk technique. NMR spectra were obtained with Bruck AM-300 spectrometer with DMSO as solvent. To access the thermal stability of the title compound, DSC-TGA (Differential Scanning Calorimetry and Thermogravimetric Analysis) analyses were carried out on a Universal V4.1D TA Instruments (SDT Q600) under a nitrogen atmosphere. Antimicrobial activities of the compounds **1:1/2H$_2$O** and **2** were evaluated at the National Center for Drug Screening, Shanghai, China. All measurements were made on a Bruker Smart-Apex CCD diffractometer by using a graphite-monochromated Mo$K\alpha$

($\lambda = 0.071073$ nm) radiation with an ω-scan technique. Determination of the crystal class, orientation matrix, and cell dimensions was performed according to the established procedures. Lorentz polarization and absorption corrections were applied. Empirical absorption corrections were performed with SADABS program. Most of the non-hydrogen atoms were located by direct methods, and those remaining were derived by subsequent Fourier syntheses. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were held stationary and included in the final stage of full-matrix least-squares refinement based on F^2 using SHELXL-97 program packages [12]. The final cycle of refinement gave $R = 0.0934$, $wR = 0.2588$ for compound **1:1/2H$_2$O** and $R = 0.0319$, $wR = 0.0790$ 2588 for compound **2** ($w = 1/[\sigma^2(F_o^2) + (0.1183P)^2 + 0.8793P]$, where $P = (F_o^2 + 2F_c^2)/3$). The all H atoms were positioned geometrically and allowed to ride on their parent atoms. The anisotropic displacement parameters, the calculated structure factors, and full lists of bond distances, bond angles and torsion angles are given in the supplementary material. X-ray crystal structure and perspective views of the packing are shown in Figs. 1–4. The reason for the relatively high values of the R factors for compound **1:1/2H$_2$O** is the poor quality of the crystal.

Synthesis of Compounds (1:1/2H$_2$O) and 2

The starting material of 5-fluorouracil-1-acetic acid (5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-1-acetic acid) was prepared from 5-fluorouracil and bromoacetic acid following the method of the reported [11].

Preparation of Compound 1:1/2H$_2$O

The compound **1:1/2H$_2$O** was synthesized from 5-fluorouracil-1-acetic acid, dicyclohexyl carbodiimide (DCC), and 1-hydroxybenzotriazole (HOBT). A solution (25 mL) of *N*,*N*-dimethyl formamide (DMF) with DCC (0.030 mol) was added dropwise to a DMF solution (75 mL) with 5-fluorouracil-1-acetic acid (0.020 mol) and HOBT (0.022 mol) at 273 K. After 5 h reaction at room temperature, 2(s)-amino-2-isopropyl acetate (0.020 mol) and triethylamine (0.020 mol) was added to the above mixture. After 4 h stirring, a white solid 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate hemihydrate (**1**) was obtained after filtration, reduced pressure distillation of DMF, and column chromatography separation, m.p. 115 °C. IR (KBr): $v = 3555$ (bs), 3283 (s), 2969 (s), 1723 (s), 1665 (s), 1380 (m), 1219 (m) cm^{-1}. Anal. Calcd for compound **1:1/2H$_2$O**: C 46.45, H 5.48, N 13.55; found C 46.50, H 5.37, N 13.62.

Preparation of Compound 2

The 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetic acid (**2**), was obtained by hydrolysis with sodium hydroxide solution (1 mol/L) of compound **1:1/2H$_2$O**, d.p. 255 °C. ^{1}H-NMR(DMSO-d$_6$): IR (KBr): $v = 3296$ (s),

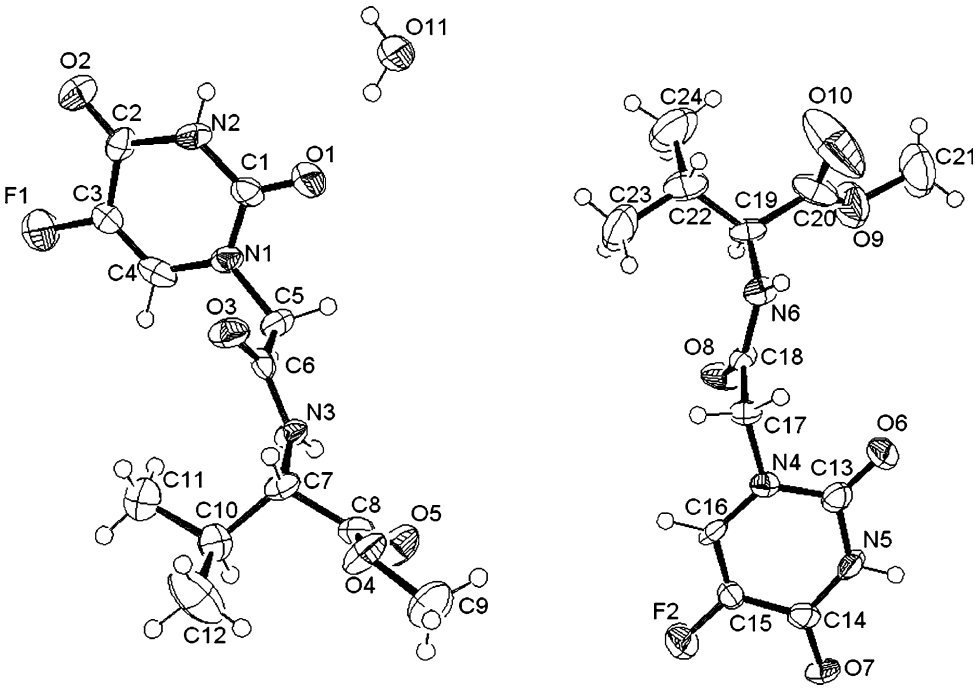

Fig. 1 Ortep plot at the 50% probability level of compound 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate hemihydrate (**1:1/2H$_2$O**)

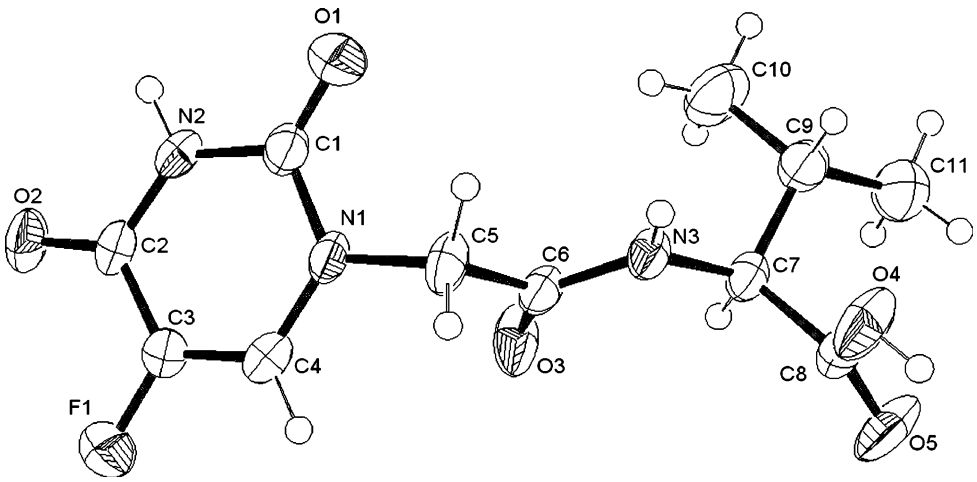

Fig. 2 Ortep plot at the 50% probability level of compound 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetic acid (**2**)

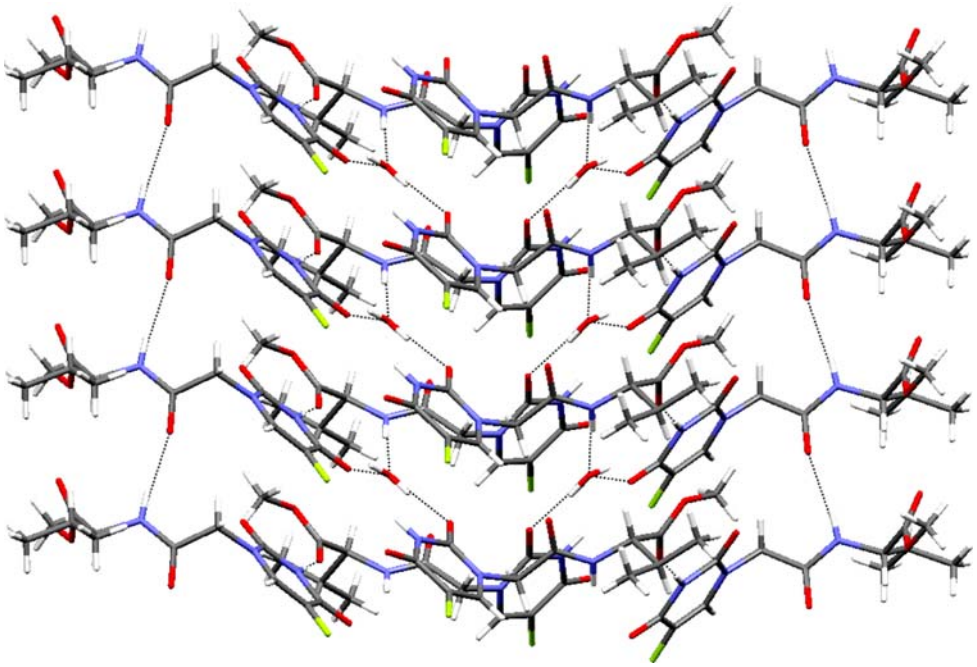

Fig. 3 Depiction of packing and showing the N–H···O and O–H···O hydrogen bonds in compound 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate hemihydrate (**1:1/2H$_2$O**)

3082 (bs), 2977 (s), 1724 (s), 1661 (s), 1386 (m), 1216 (m), 977 (m) cm^{-1}. Anal. Calcd for compound **2**: C 45.99, H 4.88, N 14.34; found C 46.02, H 4.76, N 14.43.

The purified products **1:1/2H$_2$O** and **2** were dissolved in 95% ethanol and single crystals were separated after 10 days.

Results and Discussion

In the infrared spectrum of the title compounds, the broad absorption bands at 3,283 and 2,969 cm^{-1} were assigned to the N–H and the C–H stretching vibration, and the strong ones at 1,723 and 1,665 cm^{-1} were to the stretching vibration of $\nu_{C=O}$ of carbonyl group. The characteristic strong bands at 1,219 cm^{-1} were attributed to the vibration absorption of ν_{C-O-C}. The assignment of ^{1}H- and ^{13}CNMR of compounds **1:1/2H$_2$O** and **2** is shown in Tables 1 and 2.

Single X-ray crystal analysis reveals that compounds **1:1/2H$_2$O** and **2** crystallize in monoclinic system with space groups of C2 and P2$_1$, respectively (Table 3). A displacement ellipsoid plot with the numbering scheme of compounds **1:1/2H$_2$O** and **2** is shown in Figs. 1 and 2, respectively. Figures 3 and 4 display a perspective view of the crystal packing of compounds **1:1/2H$_2$O** and **2**, respectively. The compound **1:1/2H$_2$O** has been formed from three components, two 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate molecules with very slight difference in the bond lengths and angles and one lattice water molecule. In the crystal structure of compounds **1:1/2H$_2$O** and **2**, bond lengths and angles in 5-FU ring are generally normal [13–16]. The C–F bonds length is similar to the earlier reported

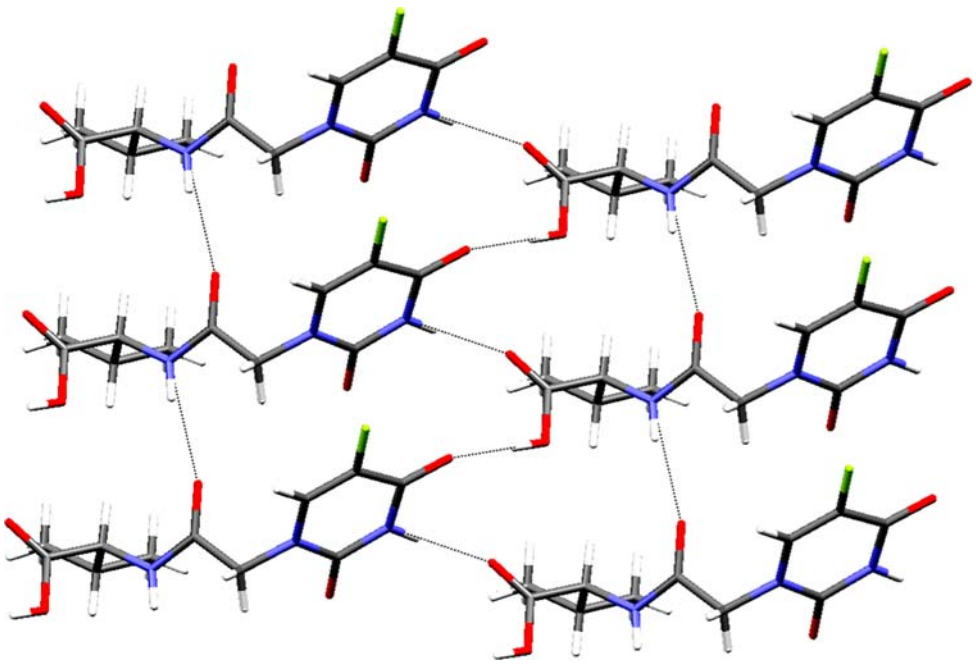

Fig. 4 Depiction of packing and showing the N–H···O and O–H···O hydrogen bonds in compound 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetic acid (**2**)

Table 1 Assignment of the ^{1}H NMR signals of the compounds **1:1/2H$_2$O** and **2**

Compound 1	Assignment	Compound 2	Assignment
3.65 (3H, *s*)	$COOCH_3$	12.71(1H, *s*)	COOH
11.80 (1H, *d*, $^{4}J_{FH} = -5.0$ Hz)	FCCNH	11.80(1H, *d*, $^{4}J_{FH} = 5.0$Hz)	FCCNH
8.54 (1H,*d*, $^{3}J_{HH} = 7.5$ Hz)	CONH	8.40(1H,d, $^{3}J_{HH} = 7.5$ Hz)	CONH
8.01 (1H, *d*, $^{3}J_{FH} = 6.8$ Hz)	FCCH	8.02(1H, *d*, $^{3}J_{FH} = 6.8$ Hz)	FCCH
4.39 (2H, *s*)	CH_2	4.39(2H, *s*)	CH_2
4.22 (1H, *m*, 3*J*HH = 7.2Hz)	NCH	4.19(1H, *m*, $^{3}J_{HH} = 5.7$ Hz)	NCH
2.04 (1H, *m*, $^{3}J_{HH} = 6.6$ Hz)	CCH	2.05(1H, *m*, $^{3}J_{HH} = 6.9$ Hz),	CCH
0.89 (6H, *d*, $^{3}J_{HH} = 6.0$ Hz)	$C(CH_3)_2$	0.89(6H, *d*, $^{3}J_{HH} = 6.6$ Hz)	$C(CH_3)_2$

results [17]. The bond lengths of C3–C4 = 1.437(13) and C14–C15 = 1.341(12) Å in the compound **1:1/2H$_2$O** and C3–C4 = 1.334(4) in the compound **2** are indicative of the considerable double-bond character. The bond lengths of C1–O1 = 1.202(11), C2–O2 = 1.201(10), C13–O6 = 1.205(11), and C14–O7 = 1.220(11) Å in the compound **1:1/2H$_2$O** as well as C1–O1 = 1.198(3) and C2–O2 = 1.231(3) Å in the compound **2** are similar to the earlier reported bond lengths, 1.17 to 1.25 Å [18]. The bond lengths of N3–C6 = 1.332(10) and N6–C18 = 1.338(10) Å in the compound **1:1/2H$_2$O** and N3–C6 = 1.332(3) Å in the compound **2** are shorter than a typical C–N bond length [ca. 1.443 Å], but longer than a typical double C=N bond (ca. 1.269 Å) [19], indicating the electron delocalization at the O–C–N fraction.

Solvate water molecule is found in the lattice of compound **1:1/2H$_2$O** and involved in a hydrogen bonding network (Fig. 3). The water molecule is acting as hydrogen bond donors toward the N atoms of the amide groups of 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate molecules and the lattice water molecules themselves are forming hydrogen bonds with the C=O groups of two different 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate molecules (Table 4). Also the N–H group of amide groups is acting as hydrogen bond donor toward the C=O group of amide groups of adjacent molecules (Table 4). Consequently, the molecules in the compound **1:1/2H$_2$O** are grown by the hydrogen bonds into a two-dimensional network (Fig. 3). In the compound **2**, there are three different hydrogen bonds (Fig. 4). The –OH group of acetic acid fraction is acting as hydrogen bond donor toward the C=O group of 5-fluorouracil rings and the C=O groups of acetic acid fraction themselves are forming hydrogen bonds with the NH groups of 5-fluorouracil rings (Table 5). Also the N–H of amino group is acting as hydrogen bond donor toward the O atoms of the C=O group of –COOH fraction of adjacent molecules (Table 5). Consequently, the molecules in the compound **2** are also grown by the hydrogen bonds into a two-dimensional network (Fig. 4).

Table 2 Assignment of the ^{13}C NMR signals of the compounds **1:1/2H$_2$O** and **2**

Compound 1	Assignment	Compound 2	Assignment
171.89	$COOCH_3$	172.83	COOH
167.13	CONH	166.98	CONH
157.69 (*d*, $^2J_{FC}$ = 25.8 Hz)	FCCO	157.72(*d*, $^2J_{FC}$ = 25.8 Hz)	FCCO
149.80	NCON	149.82	NCON
137.82 (*d*, $^1J_{FC}$ = −228.2 Hz)	FC	139.32(*d*, $^1J_{FC}$ = −228.2 Hz)	FC
4.22 (1H, *m*, 3*J*HH = 7.2 Hz)	NCH	–	–
131.34 (*d*, $^2J_{FC}$ = 33.9 Hz)	FCC	131.42(*d*, $^2J_{FC}$ = 33.9 Hz)	FCC
57.66	$CHCOOCH_3$	–	–
51.89	OCH_3	57.53	CHCOOH
49.52	CH_2	49.60	CH_2
30.38	CCH	30.34	CCH
19.03	CH_3	19.24	CH_3
18.28	CH_3	18.09	CH_3

Table 3 Crystal data and structure refinement for compounds **1:1/2H$_2$O** and **2**

Identification code	**1:1/2H$_2$O**	2
Empirical formula	$C_{24}H_{34}F_2N_6O_{11}$	$C_{11}H_{14}$ FN_3 O_5
Formula weight	620.57	287.25
Temperature	298(2) K	298(2) K
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	C2	$P2_1$
Unit cell dimensions	a = 33.668(10) Å	a = 4.8255(8) Å
	b = 4.856(1) Å	b = 11.2512(19) Å
	c = 17.755(5) Å	c = 12.036(2) Å
	β = 93.572(6)°	β = 99.051(3)°
Volume	2,897.2(15) Å^3	645.33(19) Å^3
Z	4	2
Density (calculated) (g cm^{-3})	1.423	1.478
Absorption coefficient (mm^{-1})	0.121	0.126
F(000)	1,304	300
Crystal size	0.29 × 0.09 × 0.06 mm^3	0.34 × 0.22 × 0.14 mm^3
Theta range for data collection	1.15–25.02°	1.71–25.04°
Index ranges	$-39 \le h \le 40$	$-5 \le h \le 5$
	$-5 \le k \le 5$	$-13 \le k \le 13$
	$-19 \le l \le 21$	$-14 \le l \le 14$
Reflections collected	7,653	4,630
Independent reflections	2,881	1,211
Absorption correction	Empirical from equivalents	Empirical from equivalents
Max. and min. transmission	0.990 and 0.966	0.980 and 0.958
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	2,881/4/400	1,211/1/183
Goodness-of-fit on F^2	1.122	1.115
Final *R* indices [$I > 2\sigma$ (*I*)]	R_1 = 0.0934	R_1 = 0.0319
	wR_2 = 0.2588	wR_2 = 0.0790
R Indices (all data)	R_1 = 0.1143	R_1 = 0.0332
	wR_2 = 0.2791	wR_2 = 0.0799
Largest diff. peak, hole	0.30 and −0.34 e Å^{-3}	0.16 and −0.15 e Å^{-3}

Table 4 Hydrogen bonds for compound **1:1/2H$_2$O** (Å and °)

D-H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
N(2)-H(2)···O(7)#1	0.86	2.30	3.164(10)	176.3
N(3)-H(3)···O(11)#2	0.86	1.98	2.835(11)	174.3
N(5)-H(5)···O(5)#3	0.86	2.12	2.983(9)	178.0
N(6)-H(6)···O(8)#4	0.86	2.08	2.931(9)	170.7
O(11)-H(11D)···O(7)#1	0.82(2)	2.30(9)	2.977(10)	141.0(11)
O(11)-H(11D)···O(5)#5	0.82(2)	2.60(11)	3.094(11)	120.0(11)
O(11)-H(11E)···O(1)	0.82(2)	1.93(3)	2.746(10)	171.0(15)

Symmetry transformations used to generate equivalent atoms: #1 x, y − 1, z + 1; #2 −x + 1, y + 1, −z + 1; #3 − x + 1, y, −z; #4 x, y − 1, z; #5 − x + 1, y−1, −z + 1

Table 5 Hydrogen bonds for compound **2** (Å and °)

D-H···A	d(D-H)	d(H···A)	d(D···A)	<(DHA)
O(4)–H(4)···O(2)#1	0.82	1.97	2.779(3)	169.6
N(2)–H(2)···O(5)#2	0.86	1.97	2.797(3)	160.3
N(3)–H(3)···O(3)#3	0.86	2.10	2.943(3)	166.5

Symmetry transformations used to generate equivalent atoms: #1 x + 1, y − 1,z; #2 x, y + 1, z; #3 x + 1, y, z

In order to examine the thermal stabilities of compounds **1:1/2H$_2$O** and **2**, TGA and DSC were carried out between 30 and 700 °C. The DSC-TGA curves for the compound **1:1/2H$_2$O** are illustrated in Fig. 5 and the hydrated form of compound **1:1/2H$_2$O** was used for TGA and DSC experiments. TGA shows a weight loss in three stages. The first ranges between 75 and 175 °C and showed about 3.4% loss in weight. This might correspond to the loss of crystal water [20–22]. The second of weight loss started at 250 °C and continues up to 320 °C during which there was 89% weight loss, the third completed up to 750 °C with 6% loss in weight. Both the last two stages are due to the degradation of the compound **1:1/2H$_2$O**. In DSC, the compound

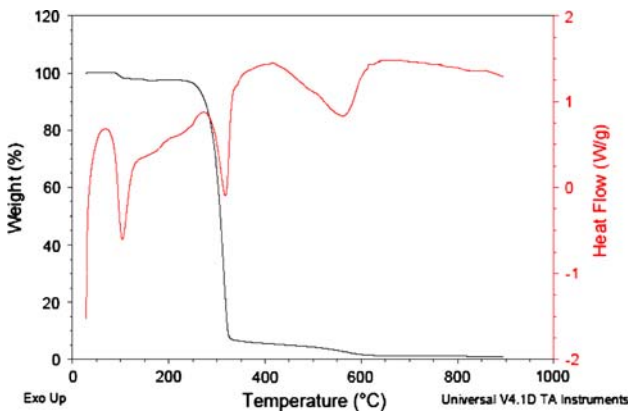

Fig. 5 The DSC-TGA curves for the compound 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetate hemihydrate (**1:1/2H$_2$O**)

first lost their crystal water with an endothermic peak at 110 °C to maintain the equilibrium pressure. A crystal–crystal transition might be taken into account for the small exothermic peak at 200 °C. The two endothermic peaks at 328 and 364 °C represented the pyrolysis of the compound **1:1/2H$_2$O**. The TGA of the compound **2** shows an overall weight loss of about 100% (Fig. 6). It is evident that there is no weight loss up to 240 °C. At higher temperatures there are two main losses. The first one takes place in the 240–425 °C range (75%), the second loss is completed up to 760 °C (25%). Both the two stages are due to the degradation of the compound **2** and in good agreement with the range of the endothermic and exothermic peaks that appear in DSC. The DSC curve exhibits four endothermic peaks (the sharpest one is at 260 °C and the other three are small and well resolved centered at 360, 470 and 575 °C, respectively) and two exothermic peaks (310 and 736 °C). In summary, the thermogravimetric analysis shows that two compounds have a remarkably thermal stability that they only decompose above temperatures of ca 240 °C.

The anticarcinogenic activities of both the compound **1:1/2H$_2$O** and **2** were tested by using microculture tetrazolium assay [23] on HL-60 cells and sulforhodamine B protechromosomic method [24] on BEL-7402 cells.

As shown in Table 6, the compound **1:1/2H$_2$O** has more antitumor activities than compound **2** which is due to the

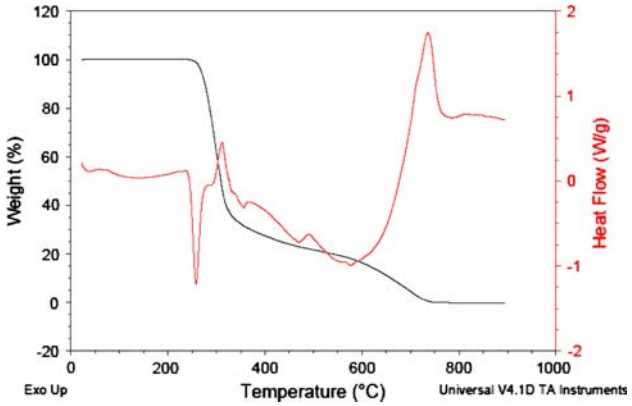

Fig. 6 The DSC-TGA curves for the compound 2(s)-(5-fluorouracil-1-aceto)amino-2-isopropyl acetic acid (**2**)

Table 6 Antitumor activity of compounds **1:1/2H$_2$O** and **2** against Hl-60 and BEL-7402 tumor cells line

Compound	Tumor cell	Concentration (mol/L)				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1:1/2H$_2$O	HL-60	85.2	29.3	4.1	0	0
	BEL-7402	50.9	18.7	5.6	1.2	0
2	HL-60	60.1	15.1	2.2	0	0
	BEL-7402	30.4	9.8	3.1	1.1	0

lower fat-solubility of compound **2**. Obviously, both the compound **1:1/2H$_2$O** and **2** displayed prominent inhibition activity to HL-60 cell at high concentration and relatively lower inhibition against BEL-7402 cell, while the effects of inhibition against the two cells were opposite at low concentrations. Despite the sensitivity difference between these cell lines, it might be indicated that the compound **1:1/2H$_2$O** and **2** possibly had different inhibition mechanisms against various tumor cells. Extensive antitumor screening and intensive mechanism investigation are demanded in this area to elucidate the mechanism and develop more desirable bioactive agents.

Supplementary Material

CIF files have been deposited with the Cambridge Crystallography Data Center. Supplementary data are available from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, on request, quoting the deposition numbers 637496–637497 for compounds **1:1/2H$_2$O** and **2**, respectively.

Acknowledgment We acknowledge financial support by the National Natural Science Foundation of China (No. 20571057).

References

1. Cai Bill TW, Tang XP, Nagorski J, Brauschweiger PG, Wang PG (2003) Bioorg Med Chem 11:4971
2. Wang XY, Li J, Zhang XM, Liu Q, Xu Q, Tan RX (2003) J Inorg Biochem 94:186
3. Huang ZH, Lin ZL, Huang JL (2001) Eur J Med Chem 36:863
4. Shi DQ, Chen Q, Li ZH (2005) Chin J Org Chem 5:549 (in Chinese)
5. Li CZ, Wang LF, Li YZ, Xia CG, Dai RB (2000) Acta Cryst C56:e376
6. Hu ML, Wang WD (2005) Acta Cryst E61:m975
7. Maeda M, Kajimoto N, Yamaizumi Z, Okamoto Y, Nagahara K, Takayanagi H (1997) Tetrahedron Lett 38:6841
8. Shi DQ, Wei J, Tan XS (2005) Chin J Org Chem 12:1602 (in Chinese)
9. Liu YQ, Zhang HJ, Han ST (2002) Chin J Org Chem 4:279 (in Chinese)
10. Xiong J, Lei XX, Hu ML, Yuan JX, Cai XQ (2006) Z Kristallogr NCS 221:37
11. Liu XJ, Chen RY, Liu YY (2002) Chem J Chin Univ 23:1299 (in Chinese)
12. Sheldrick GM (1997) SHELXS-97 and SHELXL-97. Göttingen University, Germany
13. Barnard KR, Bruck M, Huber S, Grittini C, Enemark JH, Gable RW, Wedd AG (1997) Inorg Chem 36:637
14. Hulme AT, Price SL, Tocher DA (2005) J Am Chem Soc 127:1116
15. Hu ML, Yuan JX, Morsali A (2006) Solid State Sci 8:981
16. Yuan JX, Hu ML, Morsail A (2006) Inorg Chem Conmmun 9:277
17. Hu ML, Yin P, Xiong J, Yuan JX (2006) Z Kristallogr NCS 221:39
18. Ji MJ, Zhao ZL, Yan DY (1999) Chin J Struct Chem 18:150
19. Jin ZM, Li L, Li MC, Hu ML, Shen L (2004) Acta Cryst C60:o642
20. Lin SY, Chien JL (2003) Pharm Res 20:1926
21. Hu TC, Wang SL, Chen TF, Lin SY (2002) J Pharm Sci 91:1351
22. Huang YQ, Lin JR, Du HY, Gao LF, Hu Y (2006) Mater Lett 60:3818
23. Mossman T (1983) J Immunol Methods 65:55
24. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren HJ, Bokesch H, Kenney S, Boyd MR (1990) J Natl Cancer Inst 82:1107