The Novel L- and D-Amino Acid Derivatives of Hydroxyurea and Hydantoins: Synthesis, X-ray Crystal Structure Study, and Cytostatic and Antiviral Activity Evaluations

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The novel L- and D-amino acid derivatives of hydroxyurea **5a**−**o** were prepared by aminolysis of *N*-(1-benzotriazolecarbonyl)amino acid amides **4a**−**o** with hydroxylamine. The hydantoin derivatives **6a**−**e,m,p** were synthesized by base-catalyzed cyclization of amides **4**, common precursors for **5** and **6**. X-ray crystal structure analysis shows that the C5 atom in **6e** possesses the *S* configuration, which is consistent with the configuration of the starting reagent, L-leucine. Among L-amino acid derivatives of hydroxyurea, **5h** and **5i** inhibited specifically murine leukemia and human T-lymphocytes (IC_{50} = 10−19 μM) and showed selectivity with respect to normal human fibroblasts (WI 38). D-Amino acid derivatives of hydroxyurea **5m** and **5o** inhibited the growth of all tumor cell lines (IC_{50} = 4.8−83.9 μM), but not the growth of normal fibroblasts (WI 38; IC_{50} > 100 μM). Results on antiviral evaluations showed that *N*-(1-benzotriazolecarbonyl)amino acid amide **4m** and hydantoin **6m** had marked activity against the Davis strain of CMV (**4m**, EC_{50} = 3.2 μg/mL; **6m**, EC_{50} = 4.0 μg/mL). However, these compounds showed also rather expressed cytotoxicity (**4m**, CC_{50} = 43.4 μg/mL; **6m**, CC_{50} = 12.5 $\mu g/mL^{-1}$).

Introduction

Hydroxyurea (hydroxycarbamide, HU) is an antineoplastic agent used to treat leukemia and other malignant tumor diseases.[1] The drug is also useful in therapy of sickle cell anemia[2] and in combined antiretroviral therapy,[3] where it is most effective if taken in combination with reverse transcriptase inhibitors (ddI, AZT, ddC, or d4T). HU inhibits ribonucleotide reductase as well as cellular DNA synthesis during the S phase of cell division.[4] Because HU targets a cellular enzyme instead of a viral enzyme, no viral mutations occur after repeated exposure to the drug. In addition, HU slows down HIV mutations and resistance development.[4] Hydroxyurea is structurally related to hydroxamic acids, important iron chelators and microbial siderophores. Hydroxamic acids possess diverse biological activities, including antibacterial, antifungal, antitumor, and antiinflammatory ones.[5] Finally, some derivatives of hydroxamic acid are currently accepted therapeutic agents (desferrioxamine B, hydroxycarbamide, ibuproxam, oxametacin, bufexamac, adrafinil).[5] On the other hand, hydantoins are well-known anticonvulsive drugs and herbicides.[1]

The main purpose of this study was to investigate the effects of the novel L- and D-amino acid derivatives of hydroxyurea **5**, hydantoins **6**, and their synthetic precursors *N*-(1-benzotriazolecarbonyl)amino acid amides **4** on the proliferation of different human tumor cell lines and to evaluate their potential as antiviral agents.

Chemistry

The L- and D-amino acid derivatives of hydroxyureas (**5a**−**k** and **5l**−**o**) were prepared by aminolysis of *N*-[(1-benzotriazolecarbonyl (Btc)]amino acid amides (**4a**−**o**) with hydroxylamine (Scheme 1). All amides **4a**−**p** and hydroxyureas **5a**−**o** are new compounds prepared according to previously described procedures.[6] The synthesis of common intermediates BtcCl and *N*-Btc amino acids (**2**) has been also described.[7] Three amino acids of the L-series (L-valine, L-leucine, and L-phenylalanine) and one D-amino acid (D-phenylglycine) were chosen as the starting reagents for the synthesis of the desired molecules. To obtain compounds with different lipophilicity, various amines (cyclopentylamine, cyclohexylamine, cyclohexanemethylamine, pyrrolidine, piperidine, and aminodiphenylmethane) were used for the preparation of the amides **4a**−**p**. Intramolecular cyclization of *N*-(1-benzotriazolecarbonyl)amino acid amides **4** with sodium carbonate gave hydantoin derivatives **6**.

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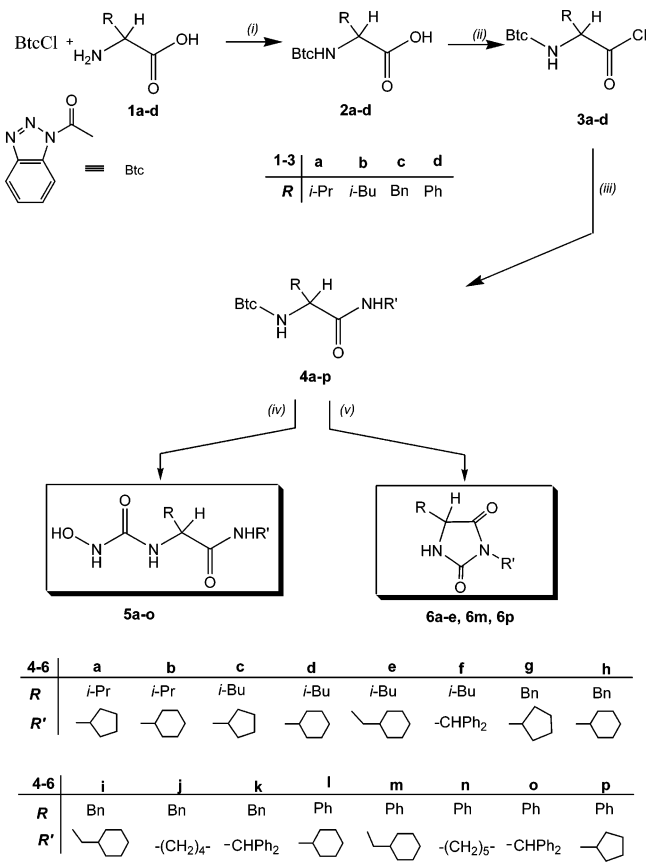
Scheme 1. Synthesis of L- and D-Amino Acid Derivatives of Hydroxyurea **5a**−**k** and **5l**−**o** and Hydantoins **6a**−**e**, **6m**, and **6p**, Respectively[a]



[a] Reagents and conditions: (i) anhydrous dioxane, rt, 24 h; (ii) $SOCl_2$, rt, 24 h or reflux, 1 h; (iii) amine, Et_3N, anhydrous toluene, rt, 24 h; (iv) NH_2OH, anhydrous toluene, rt, 2−10 days; (v) Na_2CO_3, acetone, rt, 2 h.

The L- and D-amino acid derivatives of hydroxyureas **5a**−**k** and **5l**−**o** presented in Scheme 1 were obtained by sequence of chemical transformations, which do not involve a stereogenic center. Therefore, the configuration of that center should be retained in the final molecules. To provide the experimental proof for this generally adopted rule, we have synthesized the L-leucine hydantoin derivative **6e** from **4e**, the common precursors for both products (**5e** and **6e**). Despite many attempts, we have not succeeded in obtaining single crystal of hydroxyureas **5a**−**o** of satisfactory quality for crystallographic analysis. However, we achieved the growth of single crystals of the hydantoin **6e** that were suitable for X-ray crystal structure analysis.

All syntheses were carried out under mild reaction conditions. In preparation of the amides **4a**−**p**, an equimolar ratio of the Btc-amino acid chlorides **3a**−**d** and the corresponding amine was found to be crucial in order to avoid ureido amides formation.

Structures of compounds **4a**−**p**, **5a**−**o,** and **6a**−**e,m,p** were deduced from analysis of their ^1H and ^{13}C NMR spectra (see Tables 6−8 in the Supporting Information) and confirmed by elemental analysis. The chemical shifts are consistent with the proposed structures of the novel compounds **4**−**6**. The chemical shifts assignments are in agreement with the corresponding ones of the structurally related molecules.[6] IR spectra of the amides **4a**−**o** showed absorption bands at 3423−3220 (NH), 1748−1708 (CO bond to benzotriazole), 1679−1627 (amide I), and 1565−1504 cm^{-1} (amide II). IR spectra of hydroxyureas **5a**−**o** showed characteristic bands at 3430−3370 (OH), 3359−3172 (NH), 1659−1600 (urea and amide carbonyls), and 1560−1522 (amide II) cm^{-1}, while IR spectra of hydantoins **6a**−**e,m,p** showed characteristic bands at 3320−3230 (NH), 1775−1760, and 1710−1695 (CO) cm^{-1}.

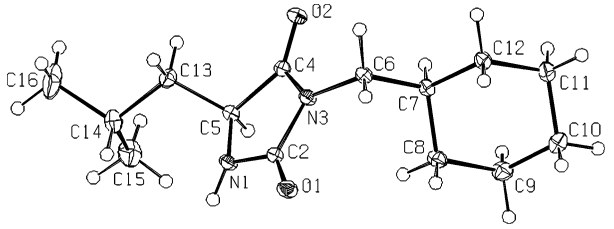


Figure 1. The molecular structure and labeling of compound **6e**. Displacement ellipsoids are drawn at the 40% probability level.

X-ray Crystal Structure Study

The crystal structure of the compound **6e** was determined by X-ray crystal structure analysis. The molecular structure with the atom numbering is displayed in Figure 1.

The skeleton of **6e** consists of a five-membered hydantoin ring, a cyclohexane ring attached via a methylene group to the hydantoin ring atom N3, and an isobutyl group attached to the hydantoin ring atom C5. The absolute configuration of the hydantoin ring C5 atom is *S*, which is in agreement with its assignment in the starting reagent (L-leucine).

The hydantoin ring deviates slightly from planarity; the largest observed deviation of the ring atoms from their mean plane is 0.044(1) Å for the atom C5. The carbonyl oxygen atom O1 lies in the plane of the ring. The dihedral angle between the C2−O1 bond line and the mean plane of the hydantoin ring is 0.4(1)°. On the other hand, the carbonyl oxygen O2 atom is slightly shifted away from the ring plane, with the corresponding dihedral angle of 5.6(1)°. As expected, the exocyclic angles around N3 [C2−N3−C6 = 123.3(1)°, C4−N3−C6 = 125.3(1)°] are greater than the endocyclic angle [C2−N3−C4 = 111.3(1)°]. However, the sum of the angles amounts to 359.9°, indicating no pyramidalization of the N3 atom. The C4 and C5 atoms of the hydantoin ring are in an antiperiplanar position with respect to the atoms C14 and C16 of the isobutyl group. The torsion angles C4−C5−C13−C14 and C5−C13−C14−C16 amount to −177.9(1)° and 177.3(1)°, respectively. The cyclohexane ring thus adopts a chair conformation.

The molecules of **6e** are joined by the N1···O2 hydrogen bond into infinite chains parallel to the *c* axis, with the N···O distance of 2.919(2) Å and the N−H···O angle of 170(2)° (for a crystal packing diagram, see the Supporting Information). These chains are additionally linked by four C−H···O intermolecular hydrogen bonds [C···O distances = 3.266(2)−3.394(2) Å], thus forming a two-dimensional network. The hydrogen-bonded molecules are assembled in such manner that they form cavities along the *a* axis. The formation of cavities may

Table 1. Inhibitory Effects of *N*-(1-Benzotriazolecarbonyl)amino Acid Amides **4a**−**o** (Scheme 1) on the Growth of Malignant Tumor Cell Lines in Comparison with Their Effects on the Growth of Normal Human Fibroblasts (WI 38)

	tumor cell growth IC_{50}[a] (μM)								
compd	L1210	Molt4/C8	CEM	SW 620	Hep-2	MiaPaCa-2	MCF-7	HeLa	WI 38
4a	>200	>200	>200	>100	60.3 ± 1.6	>100	79.3 ± 34.6	96.4 ± 11.5	≥100
4b	108 ± 14	112 ± 14	97 ± 4	>100	60 ± 0.6	>100	≥100	60.9 ± 53	>100
4c	102 ± 13	116 ± 17	94 ± 4	>100	55.7 ± 2.8	≥100	63.7 ± 15.8	82.3 ± 7.5	>100
4d	109 ± 6	107 ± 8	98 ± 7	>100	57.9 ± 1.6	40.2 ± 56.9	63.1 ± 4.1	63.9 ± 21	>100
4e	123 ± 62	164 ± 8	96 ± 5	>100	>100	>100	69.2 ± 22.2	≥100	>100
4f	17 ± 2	139 ± 15	79 ± 50	63.5 ± 31.9	55.3 ± 16.6	69.3 ± 42	>100	60 ± 3.8	62.7 ± 78
4g	68 ± 7	106 ± 60	61 ± 23	>100	95 ± 47	>100	92 ± 97	53.8 ± 7	>100
4h	126 ± 40	70 ± 7	116 ± 30	110 ± 105	64.5 ± 34.6	≥100	20.7 ± 18.6	34.3 ± 15.3	63.2 ± 1.2
4i	77 ± 21	142 ± 43	145 ± 6	>100	81 ± 6.8	≥100	24.7 ± 22.8	>100	>100
4j	133 ± 94	192 ± 11	123 ± 33	>100	≥100	>100	≥100	>100	>100
4k	17 ± 1	18 ± 1	15 ± 0	37.6 ± 3.4	40.5 ± 1.9	23.9 ± 3.5	16.3 ± 15.1	30.2 ± 9	14.3 ± 7.3
4l	95 ± 0	118 ± 14	92 ± 9	>100	69.7 ± 12.8	≥100	57.6 ± 6.5	67 ± 25	>100
4m	98 ± 6	123 ± 15	73 ± 20	>100	68.6 ± 12.8	72.7 ± 19.1	35.6 ± 26.3	79.8 ± 17.4	>100
4n	82 ± 1	94 ± 7	82 ± 9	>100	>100	≥100	67.4 ± 65.2	>100	≥100
4o	114 ± 31	88 ± 3	89 ± 8	51.7 ± 3.6	59.1 ± 13.9	42.5 ± 21.8	34.2 ± 6.9	33.3 ± 9	100 ± 62.5

[a] IC_{50}, the concentration that causes 50% growth inhibition.

Table 2. Inhibitory Effects of Amino Acid Derivatives of Hydroxyurea **5a**−**o** (Scheme 1) on the Growth of Malignant Tumor Cell Lines in Comparison with Their Effects on the Growth of Normal Human Fibroblasts (WI 38)

	tumor cell growth IC_{50}[a] (μM)								
compd	L1210	Molt4/C8	CEM	SW 620	Hep-2	MiaPaCa-2	MCF-7	HeLa	WI 38
5a	>200	>200	>200	>100	33.6	>100	>100	>100	>100
5b	>200	>200	>200	>100	>100	>100	>100	>100	58.8
5c	>100	>100	>100	>100	>100	>100	>100	>100	>100
5d	89 ± 1	153 ± 11	150 ± 0	>100	>100	>100	>100	>100	>100
5e	126 ± 18	185 ± 21	126 ± 8	>100	>100	>100	>100	>100	>100
5f	54 ± 1	107 ± 5	56 ± 15	≥ 100	35.8	63.8	17.8	>100	95.8
5g	145 ± 9	>200	>200	>100	>100	>100	>100	>100	>100
5h	18 ± 1	17 ± 1	19 ± 1	100	94.2	30.6	≥100	58	100
5i	17 ± 0	12 ± 0	10 ± 1	>100	>100	>100	N. D.	>100	>100
5j	>200	>200	192 ± 51	>100	≥100	>100	>100	>100	≥100
5k	18 ± 2	23 ± 0	21 ± 0	66.6	61.3	41.1	43.8	39.8	32.8
5l	>200	>200	>200	>100	>100	84	12.1	≥100	57.3
5m	6.7 ± 0.3	4.8 ± 0.3	17 ± 10	36.7	32	63.8	17.8	26.8	>100
5n	80 ± 2	108 ± 4	125 ± 21	>100	>100	>100	>100	>100	6.7
5o	18 ± 3	17 ± 0	39 ± 3	63.7	83.9	31.8	83.8	42	>100

[a] IC_{50}, the concentration that causes 50% growth inhibition.

Table 3. Inhibitory Effects of Hydantoins **6a**−**e,m,p** (Scheme 1) on the Growth of Malignant Tumor Cell Lines in Comparison with Their Effects on the Growth of Normal Human Fibroblasts (WI 38)

	tumor cell growth IC_{50}[a] (μM)								
compd	L1210	Molt4/C8	CEM	SW 620	Hep-2	MiaPaCa-2	MCF-7	HeLa	WI 38
6a	>200	>200	>200	>100	98	>100	>100	100	>100
6b	>200	>200	>200	>100	100	>100	>100	>100	>100
6c	>200	>200	>200	>100	>100	>100	67.4	>100	>100
6d	>200	>200	>200	>100	69.7	>100	94.3	>100	>100
6e	>200	>200	>200	>100	47	100	29.5	>100	74.1
6m	>200	>200	>200	96.8	82.9	68.9	3.3	>100	78.9
6p	>200	>200	>200	>100	>100	>100	22	>100	>100

[a] IC_{50}, the concentration that causes 50% growth inhibition.

be explained by repulsion between the hydrophobic isobutyl groups of the neighboring hydrogen-bonded molecules.

Biological Results and Discussion

Cytostatic Activity. The compounds **4a**−**o**, **5a**−**o**, and **6a**−**e,m,p** were evaluated for their activities against malignant tumor cell lines: murine leukemia (L1210), human T-lymphocytes (Molt4/C8 and CEM), colon carcinoma (SW 620), laryngeal carcinoma (Hep-2), pancreatic carcinoma (MiaPaCa-2), breast carcinoma (MCF-7), and cervical carcinoma (HeLa) and compared with their effects on the growth of human normal fibroblasts (WI 38) (Tables 1−3).

Of the *N*-(1-benzotriazolycarbonyl)amino acid amide series (**4a**−**o**), compounds **4f** and **4k** showed the best cytostatic effects. Compound **4f** showed rather pronounced inhibitory activity against murine leukemia (L1210; IC_{50} = 17 μM), while **4k** inhibited all tumor cell lines with the best effects (IC_{50} = 15−18 μM) on murine leukemia, human T-lymphocytes, and breast carcinoma. However, compound **4k** also exhibited a cytotoxic effect on normal human fibroblasts. All other compounds from this class, except for **4a**, had only slight antiproliferative effects on malignant tumor cells (IC_{50} = 20.7−192 μM).

L-Amino acid derivatives of hydroxyurea **5h** and **5i** showed selective inhibition of the growth of all tested cell lines. Thus, these compounds inhibited specifically

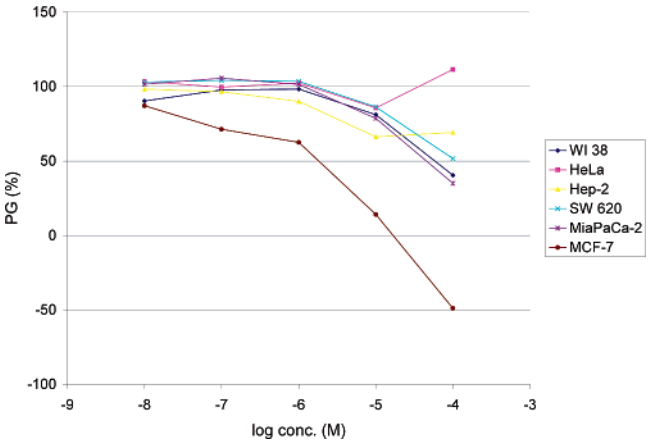


Figure 2. Cytostatic effect of the hydantoin derivative **6m** on different human cell lines. The cells were treated with **6m** at different concentrations, and percentage of growth was calculated. Each point represents a mean value of four parallel samples in three individual experiments.

murine leukemia and human T-lymphocytes (IC_{50} = 10−19 μM), but not normal fibroblasts. Another L-amino acid derivative of hydroxyurea with an aminodiphenylmethyl residue (**5k**) showed rather moderate inhibitory effect against all cell lines (IC_{50} = 18−66.6 μM).

D-Amino acid derivatives of hydroxyurea **5m** and **5o**, as well as previously discussed L-amino acid derivatives of hydroxyurea, **5h** and **5i**, showed selective inhibition regarding normal and tumor cells. Compounds **5m** and **5o** inhibited the growth of all tumor cell lines (IC_{50} = 4.8−83.9 μM), but not the growth of human normal fibroblasts (WI 38; IC_{50} > 100 μM). Of all compounds tested, **5m** showed the most pronounced inhibitory effect against human T-lymphocytes (Molt4/C8; IC_{50} = 4.8 μM).

Among hydantoin series **6a**−**e**,**m**,**p**, the best cytostatic activity was found for compound **6m** (Figure 2). This compound showed marked inhibitory effect against breast carcinoma (MCF-7; IC_{50} = 3.3 μM). However, compound **6m** also had cytotoxic activity against normal WI 38 cells.

Antiviral Activity. Compounds **4a**−**o**, **5a**−**o**, and **6a**−**e,m,p** were evaluated against cytomegalovirus (CMV, AD-169, and Davis strains) and varicella-zoster virus (thymidine kinase-positive strain, TK^+ VZV and thymidine kinase-deficient strain, TK^- VZV) in human embryonic lung (HEL) cells, and their activities were compared with those of ganciclovir (GCV), (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (HPMC, cidovir, CDV), and acyclovir (ACV) (Tables 4 and 5).

Results on antiviral evaluations of *N*-(1-benzotriazolecarbonyl)amino acid amides **4a**−**o** presented in Table 4 showed that **4m** with a cyclohexanemethylamine moiety had rather pronounced activity against the Davis strain of CMV (EC_{50} = 3.2 μg/mL). Amide **4c** exhibited only slight activity against VZV (TK^+ VZV, IC_{50} = 66 μg/mL; TK^- VZV, IC_{50} = 99 $\mu g mL^{-1}$). L- and D-amino acid derivatives of hydroxyureas **5a**−**k** and **5l**−**o** did not display activity against CMV and VZV. Hydantoin **6m** had distinct activity against the Davis strain (EC_{50} = 4 $\mu g mL^{-1}$) and AD-169 strain of CMV (EC_{50} = 7.6 $\mu g mL^{-1}$). This compound exhibited also cytotoxicity (CC_{50} = 12.5 $\mu g mL^{-1}$).

Table 4. Activity of *N*-(1-Benzotriazolylcarbonyl)amino Acid Amides **4a**−**o** Against Cytomegalovirus (CMV, AD-169, and Davis Strains) and Varicella-Zoster Virus (Thymidine Kinase-Positive Strain, TK^+ VZV, and Thymidine Kinase-Deficient Strain, TK^- VZV) in Human Embryonic Lung (HEL) Cell Cultures

	antiviral activity EC_{50}[a] ($\mu g mL^{-1}$)				cytotoxicity ($\mu g mL^{-1}$)	
compd	AD-169 strain	Davis strain	TK^+ VZV OKA strain	TK^- VZV 07/1 strain	cell morphology (MCC)[b]	cell growth (CC_{50})[c]
4a	>200	>200	>80	>200	>200	>200
4b	>80	>16	>3.2	>3.2	≥80	90
4c	>3.2	>80	66	99	≥80	87.3
4d	>16	>16	>16	>16	80	76
4e	>80	>80	>16	>16	200	156
4f	>16	>16	>0.13	>0.64	80	40
4g	>3.2	>3.2	>3.2	>3.2	16	15.5
4h	>16	>16	>3.2	>3.2	80	33.6
4i	>16	>16	>3.2	>3.2	80	37.6
4j	>200	>200	117	106	>200	147.7
4k	>16	>3.2	>0.13	>0.13	80	17.3
4l	>3.2	>16	>0.64	>3.2	≥16	15
4m	>16	3.2	>3.2	>3.2	≥16	43.4
4n	>80	>80	>80	>80	200	188
4o	>16	>16	>3.2	>3.2	80	38.3
GCV	2.03	2.33	ND	ND	>50	87
HPMC	0.76	0.58	ND	ND	>50	32
ACV	ND[d]	ND	0.27	12	>50	>200

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [c] Cytotoxic concentration required to reduce cell growth by 50%. [d] Not determined.

Table 5. Activity of Hydantoins **6a**−**e,m,p** against Cytomegalovirus (CMV, AD-169, and Davis Strains) and Varicella-Zoster Virus (Thymidine Kinase-Positive Strain, TK^+ VZV, and Thymidine Kinase-Deficient Strain, TK^- VZV) in Human Embryonic Lung (HEL) Cell Cultures

	antiviral activity EC_{50}[a] ($\mu g mL^{-1}$)				cytotoxicity ($\mu g mL^{-1}$)	
compd	AD-169 strain	Davis strain	TK^+ VZV OKA strain	TK^- VZV 07/1 strain	cell morphology (MCC)[b]	cell growth (CC_{50})[c]
6a	>100	>100	84	90	>100	>50
6b	45	41	45	29	≥ 100	>50
6c	50	37	33	>20	>100	>50
6d	37	41	18	9	>100	>50
6e	>20	20	>4	>4	100	50
6m	7.6	4.0	15	11	100	12.5
6p	>20	>4	>4	9	≥20	27.4
GCV	0.24	0.34	ND	ND	>400	65.4
HPMC	0.13	0.13	ND	ND	>400	21.3
ACV	ND[d]	ND	0.6	9.4	>400	200

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [c] Cytotoxic concentration required to reduce cell growth by 50%. [d] Not determined.

Compounds **5a**−**o** and **6a**−**e,m,p** were also evaluated for their activity against human immunodeficiency virus type 1 and 2 (HIV-1, HIV-2), while **5b**−**d**,**l**,**n**−**h** were evaluated against herpes simplex virus type 1 and 2 [HSV-1 (KOS), HSV-2 (G)], vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK^- KOS (ACV^r), Coxsackie virus B4, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, and Punta Toro virus. No specific antiviral effects (i.e. minimal antiviral effective concentration ≥ 5-fold lower than minimal cytotoxic concentration) were noted for

any of the compounds against any of these viruses (data not shown).

Conclusions

The principal aim of the presented work was to evaluate the novel *N*-[(1-benzotriazolecarbonyl]amino acid amides **4**, amino acid derivatives of hydroxyurea **5** and hydantoins **6**, for their cytostatic and antiviral activities. The L- and D-amino acid derivatives of hydroxyureas (**5a**−**k** and **5l**−**o**) were prepared by aminolysis of *N*-Btc-amino acid amides (**4a**−**o**) with hydroxylamine. The L-leucine derivative of hydantoin **6e** was synthesized by base-catalyzed cyclization of **4e**. The crystallographic analysis of **6e** showed that the C5 atom of the hydantoin ring possesses *S* configuration, in agreement with the configuration of the starting L-leucine. Therefore, the series of L- and D-amino acid derivatives of hydroxyurea (**5a**−**k** and **5l**−**o**) should also possess the same configuration at the stereogenic center as the starting amino acid.

D-Amino acid derivatives of hydroxyurea **5m** and **5o** showed rather expressed cell-growth inhibitory activities against all examined tumor cell lines (IC_{50} = 4.8−83.9 μM), but not against human normal fibroblasts (WI 38; IC_{50} > 100 μM). Thus, these compounds represent good leads for their structural optimization and further in vivo evaluations.

Results on antiviral evaluations showed that *N*-Btc amino acid amide **4m** and hydantoin **6m** had rather expressed activity against the Davis strain of CMV (**4m**, EC_{50} = 3.2 μgmL^{-1}; **6m**, EC_{50} = 4.0 μgmL^{-1}) but also cytotoxicity (**4m**, CC_{50} = 43.4 μgmL^{-1}; **6m**, CC_{50} = 12.5 μgmL^{-1}).

Experimental Section

General Methods. Melting points were determined on a Boëtius micro-heating stage and were uncorrected. IR spectra were recorded on a FTIR Perkin-Elmer Paragon 500 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 300 and 75.5 MHz for the ^{1}H and ^{13}C nuclei, respectively. Samples were measured in DMSO-d_6 solutions at 20 °C in 5 mm NMR tubes. Chemical shifts (δ) in ppm were referred to TMS. Precoated Merck silica gel 60 F_{254} plates were used for thin-layer chromatography. Spots were visualized by short-wave UV light and iodine vapor. Column chromatography was performed on silica gel (0.063−0.200 mm), with dichloromethane/methanol (97:3) as eluent.

Compounds Preparations. 1-Benzotriazole carboxylic acid chloride, *N*-(1-benzotriazolecarbonyl)amino acids (**2a**−**d**), and their chlorides (**3a**−**d**) were prepared according to the procedures previously published.[7] Hydroxylamine was prepared from hydroxylamine hydrochloride and sodium methoxyde. Amino acids were purchased from Kemika (Zagreb, Croatia) and amines (cyclopentylamine, cyclohexylamine, cyclohexanemethylamine, pyrrolidine, piperidine and aminodiphenylmethane) from Aldrich.

***N*-(1-Benzotriazolecarbonyl)amino Acid Amides (4a−p). General Procedure.** To a cold solution of *N*-(1-benzotriazolecarbonyl)amino acid chloride (**3a**−**d**) (4 mmol) in toluene (40 mL) was added a solution of corresponding amine (4 mmol) and TEA (4 mmol) in toluene (30 mL) dropwise. The reaction mixture was stirred at room temperature for 24 h. The precipitated TEA·HCl was filtered off and the mother liquor was extracted with diluted hydrochloric acid (w = 1%) and water, dried over sodium sulfate, and evaporated in a vacuum.

^{1}H NMR spectral data for *N*-(1-benzotriazolylcarbonyl)-amino acid amides **4a**−**p** are given in Supporting Information (Table 6).

***N*-(1-Benzotriazolecarbonyl)-L-valine Cyclopentylamide (4a).** The analytically pure sample was obtained by triturating with ether and recrystallization from a cyclohexane/acetone mixture: yield 0.500 g (38%); mp 150−153 °C; IR (KBr, ν/cm^{-1}) 3367, 3308, 2964, 2869, 1741, 1679, 1542, 1495; ^{13}C NMR (DMSO-d_6) δ 168.62 (1), 147.98 (4), 145.02 (1′), 130.70 (6′), 129.59 (5′), 125.12 (4′), 119.34 (3′), 112.93 (2′), 58.80 (2), 49.94 (2″), 31.82 (3″), 31.42 (6″) 30.46 (5), 22.83 (4″, 5″), 18.60 (6), 17.78 (7). Anal. ($C_{17}H_{23}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-valine Cyclohexylamide (4b).** The analytically pure sample was obtained by triturating with ether: yield 0.275 g (20%); mp 113−117 °C; IR (KBr, ν/cm^{-1}) 3386, 3279, 3098, 2931, 2856, 1723, 1709, 1642, 1562, 1522; ^{13}C NMR (DMSO-d_6) δ 168.61 (1), 148.41 (4), 145.46 (1′), 131.13 (6′), 130.03 (5′), 125.56 (4′), 119.80 (3′), 113.37 (2′), 59.23 (2), 47.58 (2″), 32.30 (3″), 32.05 (7″), 30.91 (5), 25.07 (5″), 24.30 (4″, 6″), 19.03 (6), 18.19 (7). Anal. ($C_{18}H_{25}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-leucine Cyclopentylamide (4c).** The analytically pure sample was obtained by triturating with ether: yield 0.522 g (38%); mp 116−117 °C; IR (KBr, ν/cm^{-1}) 3290, 3080, 2957, 2869, 1715, 1647, 1554, 1521; ^{13}C NMR (DMSO-d_6) δ 175.16 (1), 170.94 (4), 149.21 (1′), 131.83 (6′), 130.56 (5′), 126.16 (4′), 120.36 (3′), 114.08 (2′), 54.72 (2), 53.25 (2″), 32.76 (3″), 32.54 (6″), 29.15 (5), 25.19 (4″), 24.02 (5″), 23.57 (6), 22.01 (7, 8). Anal. ($C_{18}H_{25}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-leucine Cyclohexylamide (4d).** The analytically pure sample was obtained by triturating with ether: yield 0.615 g (43%); mp 122−123 °C; IR (KBr, ν/cm^{-1}) 3309, 3084, 2931, 2854, 1715, 1646, 1524; ^{13}C NMR (DMSO-d_6) δ 174.52 (1), 169.89 (4), 148.56 (1′), 131.22 (6′), 129.93 (5′), 125.53 (4′), 119.74 (3′), 113.46 (2′), 52.70 (2), 47.67 (2″), 32.25 (3″), 32.00 (7″), 28.85 (5), 26.24 (5″), 25.10 (4″), 24.34 (6″), 23.93 (6), 22.95 (7), 21.43 (8). Anal. ($C_{19}H_{27}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-leucine Cyclohexanemethylamide (4e).** The analytically pure sample was obtained by recrystallization once from ether/petroleum ether and once from cyclohexane: yield 1.114 g (75%); mp 84−86 °C; IR (KBr, ν/cm^{-1}) 3423, 3312, 3010, 2925, 2850, 1743, 1653, 1564, 1515; ^{13}C NMR (DMSO-d_6) δ 171.42 (1), 149.32 (4), 146.00 (1′), 131.87 (6′), 130.52 (5′), 126.14 (4′), 120.33 (3′), 114.09 (2′), 53.44 (2), 45.43 (2″), 37.90 (3″), 30.85 (4″, 6″, 8″), 26.85 (5″), 26.56 (7″), 24.98 (6), 23.61 (7), 21.74 (8). Anal. ($C_{20}H_{29}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-leucine Diphenylmethylamide (4f).** Product **4f** precipitated together with TEA·HCl. It was filtered off and dissolved in a dichloromethane/water mixture. The organic layer was extracted with diluted hydrochloric acid (w = 1%) and water, dried over sodium sulfate, and evaporated in a vacuum. The residue was triturated with ether and gave analytically pure **4f**: yield 1.024 g (58%); mp 153−158 °C; IR (KBr, ν/cm^{-1}) 3328, 3261, 2958, 1716, 1650, 1557, 1522; ^{13}C NMR (DMSO-d_6) δ 173.91 (1), 155.82 (4), 148.30 (1′), 141.60 (3″), 137.84 (9″), 130.80 (6′), 129.50 (5′), 127.77 (5″, 7″, 11″, 13″), 126.89 (4″, 8″), 126.71 (10″, 14″), 126.61 (6″), 126.50 (12″), 125.09 (4′), 119.29 (3′), 113.00 (2′), 56.45 (2), 54.04 (2″), 40.33 (5), 23.53 (6), 22.53 (7), 20.84 (8). Anal. ($C_{26}H_{27}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-phenylalanine Cyclopentylamide (4g).** The analytically pure sample was obtained by triturating with ether: yield 0.891 g (59%); mp 155−158 °C; IR (KBr): ν_{max} 3393, 3287, 2958, 1735, 1675, 1562, 1488; ^{13}C NMR (DMSO-d_6) δ 169.77 (1), 149.00 (4), 145.96 (1′), 138.05 (6), 131.71 (6′), 130.57 (5′), 129.80 (7, 11), 128.65 (8, 10), 126.95 (4′), 126.15 (9), 120.34 (3′), 113.98 (2′), 55.98 (2), 51.06 (2″), 37.95 (5), 32.74 (3″), 32.54 (6″), 24.00 (4″, 5″). Anal. ($C_{21}H_{23}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-phenylalanine Cyclohexylamide (4h).** The analytically pure sample was obtained by triturating with ether: yield 0.673 g (43%); mp 80−84 °C; IR (KBr, ν/cm^{-1}) 3318, 2930, 2854, 1715, 1660, 1530, 1449; ^{13}C NMR (DMSO-d_6) δ 169.36 (1), 149.00 (4), 145.95 (1′), 138.07 (6), 131.70 (6′), 130.57 (5′), 129.81 (7, 11), 128.65 (8, 10), 126.95

(4′), 126.16 (9), 120.35 (3′), 113.98 (2′), 56.03 (2), 48.35 (2″), 37.93 (5), 32.79 (3″, 7″), 25.71 (5″), 25.08 (4″, 6″). Anal. ($C_{22}H_{25}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-phenylalanine Cyclohexanemethylamide (4i).** The analytically pure sample was obtained by triturating with ether: yield 0.892 g (55%); mp 141−143 °C; IR (KBr, ν/cm^{-1}) 3327, 2921, 2850, 1709, 1657, 1541, 1449; ^{13}C NMR (DMSO-d_6) δ 170.33 (1), 149.13 (4), 145.95 (1′), 138.28 (6), 131.75 (6′), 130.52 (5′), 129.74 (7, 11), 128.67 (8, 10), 126.91 (4′), 126.12 (9), 120.31 (3′), 113.98 (2′), 56.27 (2), 45.55 (2″), 37.69 (5), 30.86 (3″), 26.54 (4″, 6″, 8″), 25.94 (5″, 7″). Anal. ($C_{23}H_{27}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-phenylalanine Pyrrolidineamide (4j).** The analytically pure sample was obtained by triturating with ether: yield 0.479 g (33%); mp 102−103 °C; IR (KBr, ν/cm^{-1}) 3220, 2981, 1722, 1633, 1526, 1449; ^{13}C NMR (DMSO-d_6) δ 168.66 (1), 149.00 (4), 145.94 (1′), 137.70 (6), 131.71 (6′), 130.63 (5′), 129.91 (7, 11), 128.74 (8, 10), 127.15 (4′), 126.20 (9), 120.39 (3′), 113.93 (2′), 54.57 (2), 46.32 (2″, 5″), 37.11 (5), 26.16 (3″), 24.12 (4″). Anal. ($C_{20}H_{21}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-L-phenylalanine Diphenylmethylamide (4k).** The analytically pure sample was obtained by triturating with ether: yield 0.951 g (50%); mp 165−166 °C; IR (KBr, ν/cm^{-1}) 3320, 1708, 1653, 1555, 1516, 1450; ^{13}C NMR (DMSO-d_6) δ 170.05 (1), 149.19 (4), 145.93 (1′), 142.57 (3″, 9″), 138.05 (6), 131.70 (6′), 130.59 (5′), 129.84 (7, 11), 128.92 (5″, 7″, 11″, 13″), 128.69 (8, 10), 128.05 (4″, 8″), 127.79 (10″, 14″), 127.62 (6″), 127.54 (12″), 127.00 (4′), 126.18 (9), 120.34 (3′), 113.96 (2′), 56.71 (2), 56.23 (2″), 37.67 (5). Anal. ($C_{29}H_{25}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-D-phenylglycine Cyclohexylamide (4l).** The analytically pure sample was obtained by recrystallization from a cyclohexane/dichloromethane mixture: yield 0.664 g (44%); mp 164−166 °C; IR (KBr, ν/cm^{-1}) 3403, 3296, 2933, 2855, 1741, 1645, 1556, 1497; ^{13}C NMR (DMSO-d_6) δ 168.13 (1), 148.43 (4), 146.06 (1′), 138.65 (5), 131.67 (6′), 130.75 (5′), 129.05 (6, 10), 128.49 (4′), 127.50 (7, 9), 126.25 (8), 120.48 (3′), 113.89 (2′), 57.50 (2), 48.53 (2″), 32.60 (3″, 7″), 25.62 (5″), 24.85 (4″, 6″). Anal. ($C_{21}H_{23}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-D-phenylglycine Cyclohexanemethylamide (4m).** The analytically pure sample was obtained by recrystallization once from ether/petroleum ether and once from cyclohexane: yield 0.658 g (42%); mp 108−111 °C; IR (KBr, ν/cm^{-1}) 3427, 3304, 3090, 2923, 2850, 1744, 1654, 1557, 1513; ^{13}C NMR (DMSO-d_6) δ 168.07 (1), 147.37 (4), 145.01 (1′), 137.59 (5), 130.63 (6′), 129.68 (5′), 127.95 (6, 10), 126.59 (7, 9), 126.05 (4′), 125.18 (8), 119.41 (3′), 112.82 (2′), 56.66 (2), 44.56 (2″), 36.80 (3″), 29.63 (4″, 8″), 25.39 (6″), 24.79 (5″,7″). Anal. ($C_{22}H_{25}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-D-phenylglycine Piperidineamide (4n).** The analytically pure sample was obtained by triturating with ether: yield 0.625 g (43%); mp 120−122 °C; IR (KBr, ν/cm^{-1}) 3354, 2941, 2854, 1738, 1627, 1478, 1450; ^{13}C NMR (DMSO-d_6) δ 166.85 (1), 148.00 (4), 146.04 (1′), 137.58 (5), 131.60 (6′), 130.77 (5′), 129.39 (6, 10), 128.67 (4′), 127.72 (7, 9), 126.23 (8), 120.49 (3′), 113.82 (2′), 55.25 (2), 46.35 (2″), 43.58 (6″), 25.60 (3″, 5″), 24.18 (4″). Anal. ($C_{20}H_{21}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-D-phenylglycine Diphenylmethylamide (4o).** The analytically pure sample was obtained by triturating with ether and recrystallization from a cyclohexane/dichloromethane mixture: yield 0.923 g (50%); mp 89−93 °C; IR (KBr, ν/cm^{-1}) 3397, 3294, 2924, 1740, 1653, 1538, 1494; ^{13}C NMR (DMSO-d_6) δ 167.99 (1), 147.95 (4), 145.44 (1′), 141.63 (3″, 9″), 137.62 (5), 131.08 (6′), 130.14 (5′), 128.44 (6, 10), 128.37 (5″, 7″, 11″, 13″), 128.21 (7, 9), 128.05 (4″, 8″), 127.51 (10″, 14″), 127.17 (6″), 126.94 (12″), 126.80 (4′), 125.64 (8), 119.86 (3′), 113.26 (2′), 57.06 (2″), 56.22 (2). Anal. ($C_{28}H_{23}N_5O_2$) C, H, N.

***N*-(1-Benzotriazolecarbonyl)-D-phenylglycine Cyclopentylamide (4p).** The analytically pure sample was obtained by triturating with ether and recrystallization from a cyclohexane/dichloromethane/acetone mixture: yield 0.836 g (58%); mp 144−146 °C; IR (KBr, ν/cm^{-1}) 3408, 3322, 2927, 2867, 1730, 1646, 1544, 1492; ^{13}C NMR (DMSO-d_6) δ 167.88 (1), 147.82 (4), 145.46 (1′), 137.99 (5), 131.07 (6′), 130.13 (5′), 128.45 (6, 10), 127.88 (4′), 126.88 (7, 9), 125.63 (8), 119.87 (3′), 113.27 (2′), 56.90 (2), 50.67 (2″), 31.97 (3″, 6″), 23.34 (4″, 5″). Anal. $C_{20}H_{21}N_5O_2$ C, H, N.

Amino Acid Derivatives of Hydroxyurea (5a−o). General Procedure. To a solution of *N*-(1-benzotriazolecarbonyl)amino acid amide (**4**) (1 mmol) in toluene (25 mL) was slowly added a solution of hydroxylamine (2 mmol) in methanol (5 mL). The reaction mixture was stirred at room temperature from 2−10 days. New portions of hydroxylamine (2 mmol each) were added after 24 and 48 h. The precipitated BtH·hydroxylamine salt was filtered off and the mother liquor was evaporated in a vacuum.

^{1}H NMR spectral data for amino acid derivatives of hydroxyurea **5a**−**o** are given in the Supporting Information (Table 7).

***N*-Cyclopentyl-2-(*N*′-hydroxyureido)-L-3-methylbutanamide (*N*′-Hydroxycarbamoyl-L-valine cyclopentylamide) (5a).** Crude product was purified by column chromatography: yield 0.046 g (19%); mp > 250 °C; IR (KBr, ν/cm^{-1}) 3419, 3295, 2962, 1641, 1564, 1548; ^{13}C NMR (DMSO-d_6) δ 170.43 (1), 160.64 (4), 57.05 (2), 50.19 (2″), 32.29 (3″), 31.84 (6″), 31.58 (5), 23.29 and 23.26 (4″, 5″), 18.99 (6), 17.93 (7). Anal. ($C_{11}H_{21}N_3O_3$) C, H, N.

***N*-Cyclohexyl-2-(*N*′-hydroxyureido)-L-3-methylbutanamide (*N*′-Hydroxycarbamoyl-L-valine cyclohexylamide) (5b).** The analytically pure sample was obtained by triturating with ether: yield 0.064 g (25%); mp 153−155 °C; IR (KBr, ν/cm^{-1}) 3420, 3283, 2935, 2856, 1654, 1560, 1541; ^{13}C NMR (DMSO-d_6) δ 169.96 (1), 160.64 (4), 57.07 (2), 47.32 (2″), 32.37 and 32.09 (3″, 7″), 31.56 (5), 25.09 (5″), 24.40 and 24.34 (4″, 6″), 18.99 (6), 17.89 (7).

***N*-Cyclopentyl-2-(*N*′-hydroxyureido)-L-4-methylpentanamide (*N*′-Hydroxycarbamoyl-L-leucine cyclopentylamide) (5c).** The analytically pure sample was obtained by triturating with ether: yield 0.144 g (56%); mp 132−136 °C; ^{13}C NMR (DMSO-d_6) δ 171.73 (1), 160.48 (4), 50.88 (2), 50.11 (2″), 42.26 (5), 32.14 and 31.94 (3″, 6″), 24.15 (6), 23.35 (4″, 5″), 22.91 (7), 21.87 (8).

***N*-Cyclohexyl-2-(*N*′-hydroxyureido)-L-4-methylpentanamide (*N*′-Hydroxycarbamoyl-L-leucine cyclohexylamide) (5d).** The pure product was obtained by column chromatography and by triturating with petroleum ether: yield 0.027 g (10%); mp 154−157 °C; IR (KBr, ν/cm^{-1}) 3409, 3286, 2935, 2856, 1659, 1644, 1554, 1532.

***N*-Cyclohexanemethyl-2-(*N*′-hydroxyureido)-L-4-methylpentanamide (*N*′-Hydroxycarbamoyl-L-leucine cyclohexanemethylamide) (5e).** Crude product was purified by column chromatography: yield 0.068 g (24%); mp 168−169 °C; IR (KBr, ν/cm^{-1}) 3396, 3277, 2928, 2853, 1646, 1558, 1534; ^{13}C NMR (CD_3OD) δ 175.49 (1), 163.67 (4), 53.45 (2), 46.87 (2″), 43.22 (3″), 39.28 (5), 32.07 (3″, 6″), 27.71 (5″), 27.16 84″), 26.14 (5), 23.58 (6), 22.34 (7). Anal. ($C_{14}H_{27}N_3O_3$) C, H, N.

***N*-Diphenylmethyl-2-(*N*′-hydroxyureido)-L-4-methylpentanamide (*N*′-Hydroxycarbamoyl-L-leucine diphenylmethylamide) (5f).** The analytically pure sample was obtained by triturating with acetone: yield 0.171 g (48%); mp 203−205 °C; IR (KBr, ν/cm^{-1}) 3370, 3295, 2962, 2874, 1640, 1601, 1574, 1533; ^{13}C NMR (DMSO-d_6) δ 171.77 (1), 160.59 (4), 142.24 and 142.19 (3″, 9″), 128.31 (5″, 7″), 128.24 (11″, 13″), 127.30 (4″, 8″), 127.05 (10″, 14″), 126.89 (6″, 12″), 55.77 (2), 51.07 (2″), 41.91 (5), 24.16 (6), 22.94 (7), 21.75 (8). Anal. ($C_{20}H_{25}N_3O_3$) C, H, N.

***N*-Cyclopentyl-2-(*N*′-hydroxyureido)-L-3-phenylpropanamide (*N*′-Hydroxycarbamoyl-L-phenylalanine cyclopentylamide) (5g).** The analytically pure sample was obtained by triturating with acetone: yield 0.010 g (6%); mp 164−166 °C. IR (KBr, ν/cm^{-1}) 3431, 3329, 3292, 2957, 2928, 2857, 1658, 1540.

***N*-Cyclohexylamide-2-(*N*′-hydroxyureido)-L-3-phenylpropanamide (*N*′-Hydroxycarbamoyl-L-phenylalanine cyclohexylamide) (5h).** The analytically pure sample was

obtained by triturating with ether: yield 0.104 g (34%); mp 111−120 °C; IR (KBr, ν/cm^{-1}) 3422, 3307, 2933, 2855, 1654, 1560, 1541; ^{13}C NMR (DMSO-d_6) δ 169.94 (1), 160.31 (4), 137.45 (6), 129.20 (7, 11), 127.89 (8, 10), 126.11 (9), 53.45 (2), 47.33 (2″), 38.87 (5), 32.14 (3″, 7″), 25.09 (5″), 24.39 (4″, 6″).

***N*-Cyclohexanemethyl-2-(*N*′-hydroxyureido)-L-3-phenylpropanamide (*N*′-Hydroxycarbamoyl-L-phenylalanine cyclohexanemethylamide) (5i).** Sodium carbonate solution (w = 10%, 20 mL) was added to the reaction mixture. Precipitated product was filtered off and worked up with ethyl acetate. Product **5i** was isolated as the sodium salt from the organic extract: yield 0.051 g (15%); mp 142−146 °C. ^{13}C NMR (DMSO-d_6) δ 171.02 (1), 160.36 (4), 137.54 (6), 129.11 (7, 11), 127.89 (8, 10), 126.10 (9), 53.69 (2), 44.71 (2″), 38.58 (5), 37.21 (3″), 30.21 (4″, 8″), 25.91 (6″), 25.32 (5″, 7″). Anal. ($C_{17}H_{24}N_3O_3$-Na) C, H, N.

***N*-Pyrrolidinyl-2-(*N*′-hydroxyureido)-L-3-phenylpropanamide (*N*′-Hydroxycarbamoyl-L-phenylalanine pyrrolidineamide) (5j).** Crude product was purified by column chromatography: yield 0.053 g (19%); mp 176−177 °C; IR (KBr, ν/cm^{-1}) 3422, 3172, 2955, 2876, 1642, 1622, 1534; ^{13}C NMR (DMSO-d_6) δ 169.37 (1), 160.29 (4), 137.21 (6), 129.21 (7, 11), 128.03 (8, 10), 126.35 (9), 51.84 (2), 44.58 and 45.36 (2″, 5″), 38.12 (5), 25.43 (4″), 23.52 (3″). Anal. ($C_{14}H_{19}N_3O_3$) C, H, N.

***N*-Diphenylmethyl-2-(*N*′-hydroxyureido)-L-3-phenylpropanamide (*N*′-Hydroxycarbamoyl-L-phenylalanine diphenylmethylamide) (5k).** The analytically pure sample was obtained by triturating with ether: yield 0.148 g (38%); mp 162−170 °C; IR (KBr, ν/cm^{-1}) 3422, 3298, 3062, 3028, 2924, 1654, 1637, 1560, 1540; ^{13}C NMR (DMSO-d_6) δ 170.65 (1), 160.44 (4), 142.05 and 142.18 (3″, 9″), 137.40 (6), 129.20−126.16 (7−11, 4″-8″, 10″-14″), 55.85 (2), 53.67 (2″), 38.44 (5). Anal. ($C_{23}H_{23}N_3O_3$) C, H, N.

***N*-Cyclohexyl-2-(*N*′-hydroxyureido)-D-2-phenyletanamide (*N*′-Hydroxycarbamoyl-D-phenylglycine cyclohexylamide) (5l).** The analytically pure sample was obtained by triturating with acetone: yield 0.119 g (41%); mp >250 °C; IR (KBr, ν/cm^{-1}) 3396, 3305, 2937, 2855, 1643, 1622, 1554, 1536; ^{13}C NMR (DMSO-d_6) δ 168.81 (1), 159.85 (4), 140.06 (5), 128.11 (6, 10), 127.18 (8), 126.26 (7, 9), 55.45 (2), 47.61 (2″), 32.10 and 31.95 (3″, 7″), 25.03 (5″), 24.33 and 24.21 (4″, 6″). Anal. ($C_{15}H_{21}N_3O_3$) C, H, N.

***N*-Cyclohexanemethyl-2-(*N*′-hydroxyureido)-D-2-phenyletanamide (*N*′-Hydroxycarbamoyl-D-phenylglycine cyclohexanemethylamide) (5m).** The analytically pure sample was obtained by triturating with acetone and additional recrystallization from an ethanol/water mixture: yield 0.25 g (8%); mp 142−144 °C. IR (KBr, ν/cm^{-1}) 3296, 2925, 2856, 1630, 1560. Anal. ($C_{16}H_{23}N_3O_3$) C, H, N.

***N*-Piperidyl-2-(*N*′-hydroxyureido)-D-2-phenyletanamide (*N*′-Hydroxycarbamoyl-D-phenylglycine piperidineamide) (5n).** The pure product was obtained by column chromatography and by triturating with methanol: yield 0.06 g (5%); mp 216−218 °C; IR (KBr, ν/cm^{-1}) 3410, 3245, 2924, 2854, 1633, 1536. Anal. ($C_{14}H_{19}N_3O_3$) C, H, N.

***N*-Diphenylmethyl-2-(*N*′-hydroxyureido)-D-2-phenyletanamide (*N*′-Hydroxycarbamoyl-D-phenylglycine diphenylmethylamide) (5o).** The analytically pure sample was obtained by triturating with ether: yield 0.229 g (61%); mp >250 °C; IR (KBr, ν/cm^{-1}) 3400, 3359, 3311, 1654, 1638, 1560, 1541; ^{13}C NMR (DMSO-d_6) δ 169.29 (1), 159.86 (4), 142.02 and 141.75 (3″, 9″), 139.67 (5), 128.35−126.49 (6−10, 4″-8″, 10″-14″), 55.98 (2), 55.50 (2″). Anal. ($C_{22}H_{21}N_3O_3$) C, H, N.

Hydantoins (6a−e,m,p). General Procedure. To a solution of *N*-(1-benzotriazolecarbonyl)amino acid amide (**4**) (1.5 mmol) in acetone (60 mL) was added a 5% solution of sodium carbonate (5 mL). The reaction mixture was stirred for 2 h at room temperature. Acetone was evaporated in a vacuum and the precipitated product **6** was filtered off, washed with water, and recrystallized from acetone and water.

^{1}H NMR spectral data for hydantoins **6a**−**e**,**m**,**p** are given in the Supporting Information (Table 8).

3-Cyclopentyl-5-isopropyl Hydantoin (6a):[17] yield 0.495 g (72%); mp 125−126 °C (lit.[17] mp 107−109 °C); IR (KBr, ν/cm^{-1}) 3321, 2960, 2873, 1764, 1710, 1681, 1430; ^{13}C NMR (DMSO-d_6) δ 173.53 (1), 157.02 (4), 60.53 (2), 50.12 (2″), 28.63 (3″), 28.41 (6″), 24.60 (4″, 5″), 18.33 (6), 15.44 (7). Anal. ($C_{11}H_{18}N_2O_2$) C, H, N.

3-Cyclohexyl-5-isopropyl Hydantoin (6b):[17] yield 0.245 g (73%); mp 94−95 °C (lit.[17] mp 150.0−151.5 °C); IR (KBr, ν/cm^{-1}) 3236, 2937, 3857, 1766, 1705, 1433; ^{13}C NMR (DMSO-d_6) δ 173.52 (1), 156.98 (4), 60.42 (2), 49.89 (2′), 29.69 (5), 29.00 (3″), 28.77 (7″), 25.28 (4″, 6″), 24.75 (5″), 18.34 (6), 15.35 (7). Anal. ($C_{12}H_{20}N_2O_2$) C, H, N.

3-Cyclopentyl-5-isobutyl Hydantoin (6c): yield 0.273 g (81%); mp 80−81 °C; IR (KBr, ν/cm^{-1}) 3241, 2929, 2856, 1760, 1710, 1437; ^{13}C NMR (DMSO-d_6) δ 174.51 (1), 156.59 (4), 54.12 (2), 50.17 (2″), 40.58 (5), 28.54 (3″), 28.51 (6″), 24.57 (4″,5″), 23.94 (6), 22.94 (7), 21.42 (8). Anal. ($C_{12}H_{20}N_2O_2$) C, H, N.

3-Cyclohexyl-5-isobutyl Hydantoin (6d):[17] yield 0.232 g (65%); mp 98−99 °C (lit.[17] mp 126−127 °C); IR (KBr, ν/cm^{-1}) 3243, 2938, 2855, 1768, 1708, 1435; ^{13}C NMR (DMSO-d_6) δ 174.51 (1), 156.56 (4), 54.07 (2), 49.87 (2″), 40.59 (5), 28.87 (3″), 28.83 (7″), 25.75 (4″, 6″), 24.75 (5″), 23.93 (6), 22.98 (7), 21.43 (8). Anal. ($C_{13}H_{22}N_2O_2$) C, H, N.

3-Cyclohexanemethyl-5-isobutyl Hydantoin (6e): yield 0.329 g (87%); mp 141−144 °C; IR (KBr, ν/cm^{-1}) 3294, 2926, 2852, 1769, 1711, 1459; ^{13}C NMR (DMSO-d_6) δ 174.83 (1), 156.95 (4), 54.59 (2), 43.41 (2″), 40.85 (5), 35.91 (3″), 30.11 (4″, 8″), 25.76 (6″), 25.04 (5″, 7″). Anal. ($C_{14}H_{24}N_2O_2$) C, H, N.

3-Cyclohexanemethyl-5-phenyl Hydantoin (6m): yield 0.351 g (86%); mp 141−147 °C; IR (KBr, ν/cm^{-1}) 3234, 2924, 2850, 1772, 1709, 1452; ^{13}C NMR (DMSO-d_6) δ 172.63 (1), 157.01 (4), 135.80 (5), 128.64 (6, 10), 128.26 (8), 126.65 (7, 9), 59.64 (2), 43.68 (2′), 35.91 (3″), 29.99 (4″, 8″), 25.35 (6″), 25.03 (5″, 7″). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

3-Cyclopentyl-5-phenyl Hydantoin (6p): yield 0.150 g (40%); mp 124−126 °C; IR (KBr, ν/cm^{-1}) 3237, 3097, 2954, 2871, 1766, 1701, 1432; ^{13}C NMR (DMSO-d_6) δ 172.33 (1), 156.66 (4), 153.90 (5), 128.63 (6, 10), 128.23 (8), 126.65 (7, 9), 59.15 (2), 50.50 (2″), 28.66 (3″), 28.63 (6″), 24.63 (4″,5″). Anal. ($C_{14}H_{22}N_2O_2$) C, H, N.

X-ray Crystal Structure Determination. A single crystal of compound **6e** (for crystal data, see the Supporting Information) suitable for X-ray single-crystal analysis was obtained at room temperature by slow evaporation from ethanol solution (96%). The intensities were collected at 100 K on a Oxford Diffraction Xcalibur 2 diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The data collection and reduction were carried out with the CrysAlis programs.[8] Programs used for structure solution, refinement, analysis, and drawing include SHELXS97,[9] SHELXL97,[10] and PARST95,[11] and PLATON.[12] Crystallographic data excluding structure factors for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-245009. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax, +44-(0)1223−336033; e-mail, deposit@ccdc.cam.ac.uk].

Biological Tests. Cytostatic Activity Assays. Antitumor activity against L1210 (murine leukemia), Molt4/C8, and CEM (human T-lymphocytes) cell lines were measured essentially as originally described for the mouse leukemia (L1210) cell lines.[13]

Cell Culturing. The HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW 620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), Hep-2 (laryngeal carcinoma), and WI 38 (diploid fibroblasts) cells were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg mL^{-1} streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

Proliferation Assays. The HeLa, MCF-7, SW 620, MiaPaCa-2, Hep-2, and WI 38 cells were inoculated onto a series of standard 96-well microtiter plates on day 0. Test agents were then added in 5- and 10-fold dilutions (10^{-8}−10^{-4} M) and

incubated for 72 h further. Working dilutions were freshly prepared on the day of testing. After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay,[14] which detects dehydrogenase activity in viable cells. The absorbency (OD, optical density) was measured on a microplate reader at 570 nm. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

$$\text{if } (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}}) \geq 0, \text{ then}$$

$$\text{PG} = 100 \times (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}})/(\text{mean OD}_{\text{ctrl}} - \text{mean OD}_{\text{tzero}})$$

$$\text{if } (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}}) < 0, \text{ then}$$

$$\text{PG} = 100 \times (\text{mean OD}_{\text{test}} - \text{mean OD}_{\text{tzero}})/\text{OD}_{\text{tzero}}$$

where

mean OD_{tzero} = the average of optical density measurements before exposure of cells to the test compound,

mean OD_{test} = the average of optical density measurements after the desired period of time, and

mean OD_{ctrl} = the average of optical density measurements after the desired period of time with no exposure of cells to the test compound.

Each test point was performed in quadruplicate in three individual experiments. The results were expressed as IC_{50}, the concentration necessary for 50% of inhibition. Each result is a mean value from three separate experiments. The IC_{50} values for each compound were calculated from dose−response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (i.e. 50%). If, however, for a given cell line all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g. PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign.

Antiviral Activity Assays. Antiviral activity against herpes simplex virus type 1 and 2, vaccinia virus, cytomegalovirus, varicella-zoster virus, vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, and Punta Toro virus was determined essentially as described previously.[15,16] The antiviral activity results were expressed as EC_{50}, the effective concentration required to afford 50% protection against viral cytopathogenicity or viral plaque formation.

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Supporting Information Available: ^{1}H NMR spectral data of **4a**−**p**, **5a**−**o**, and **6a**−**e**,**m**,**p** (Tables 6−8) and crystal data and crystal packing diagram of **6e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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