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RESEARCH ARTICLE

New 4-iminohydantoin sulfamide derivatives with antiviral and anticancer activity

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Abstract: A number of sulfamides were obtained by reaction interaction of (5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl chloride with anilines, benzylamines, Boc-protected piperazine, methylalylamine, and amino acids methyl esters with primary and secondary amino group. The antiviral and anticancer activity of new derivatives was evaluated. The most effective compounds against *Human cytomegalovirus* were sulfamides based on anisidine (**1b**), *N*-Boc-piperazine (**1h**), and the derivatives **1n, o** with fragments of nipecotic and azetidine-3-carboxylic acids, respectively. Anticancer activity was most significant for sulfamides based on *p*-methoxybenzylamine (compound **1d**), benzylmethylamine (compound **1f**), and allylmethylamine (compound **1g**).

Keywords: hydantoins; sulfamides; antiviral activity; anticancer activity.

Introduction

The hydantoin motif often appears in bioactive molecules of both natural and synthetic origin (Figure 1) [1-2]. This heterocyclic system, due to its low aromaticity, can be consider as a cyclic combination of an α-amino acid and urea with all the resultant consequences, such as the possibility of assembly with a variation of substituents [3], the relative ease of combinatorial libraries creation [4], the possibility of further modification, as well as bioavailability and environmental friendliness [5]. These factors make hydantoin derivatives very attractive objects for medicinal chemistry, and a brief overview of the achievements in this area over the past decade can be found in the work [6]. At the same time, the possibility disagreeable side effects of a number of hydantoin drugs cannot be ignored [7-9]. However, it is also obvious that the main reason for the toxicity of some derivatives is not the hydantoin fragment (for example, Allantoin exhibits almost no undesirable effects), but the effect of substituents. Consequently, the creation of new substances with a hydantoin fragment and

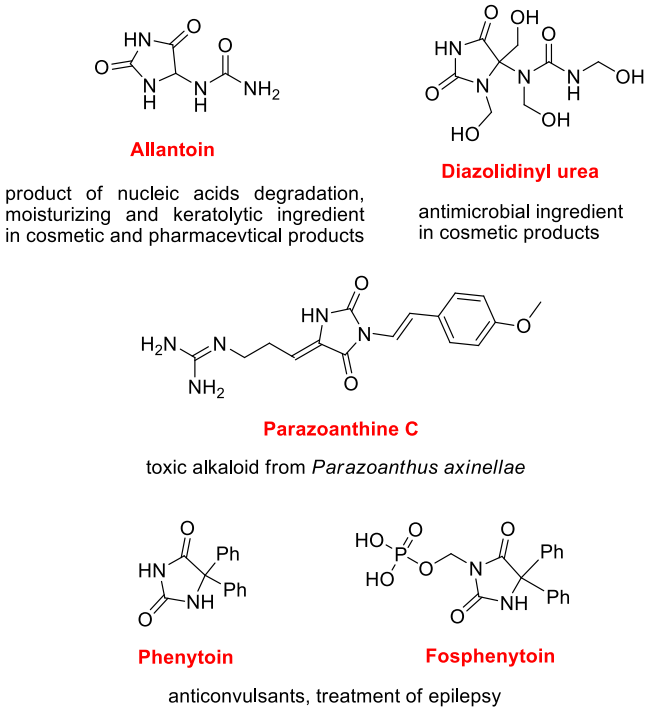

Figure 1. Practically used natural and synthetic hydantoin derivatives [1-2].

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the study of possible areas of their practical application is a crucial task.

Earlier, we established a convenient way to synthesize the novel sulfamides with a hydantoin fragment and found anticancer [10] and antiviral [11] activity for some of these compounds (Figure 2, general structure **1**). The results of studies of the anticancer activity of hydantoin sulfonamides were quite optimistic in terms of further prospects for this class of substances: for example, all of the first six synthesized derivatives were selected for single-dose assay, and five-dose assay were led for two compounds [10, 12]. Data on antiviral activity were less demonstrative [11], but also showed a significant dependence of the level of activity on the nature of the substituents R^1 and R^2.

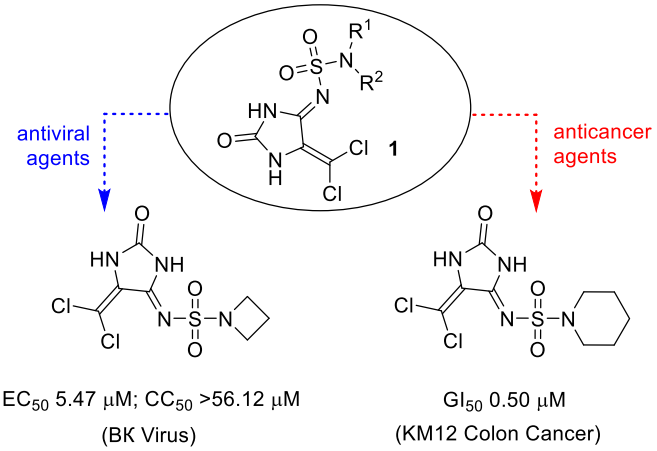


Figure 2. Bioactive (5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamides [10-12].

This has become a weighty reason for us to keep this research course; therefore, the aim of this work was to create new derivatives of general formula **1** (Figure 2), and study their antiviral and anticancer effects. This course is also supported by the data summarized in the review [13] concerning the anticancer and antiviral activity of sulfonamides: the varying the substituents can stimulate the displaying one or another type of activity.

Results and Discussion

Chemistry

The starting sulfamoyl chloride **2** is formed by the reaction of chlorosulfonylisocyanate (**3**) with 2-amino-3,3-dichloroacrylonitrile (ADAN, **4**) through the steps of acylation of the ADAN amino group with isocyanate, heterocyclization with the ADAN CN-group and the nitrogen atom of the newly formed urea, and, at last, recyclization [10]. Despite the presence of a several active functional fragments, compound **2** is relatively stable and suitable for long-term storage (at low temperature), therefore, it is a convenient "building block".

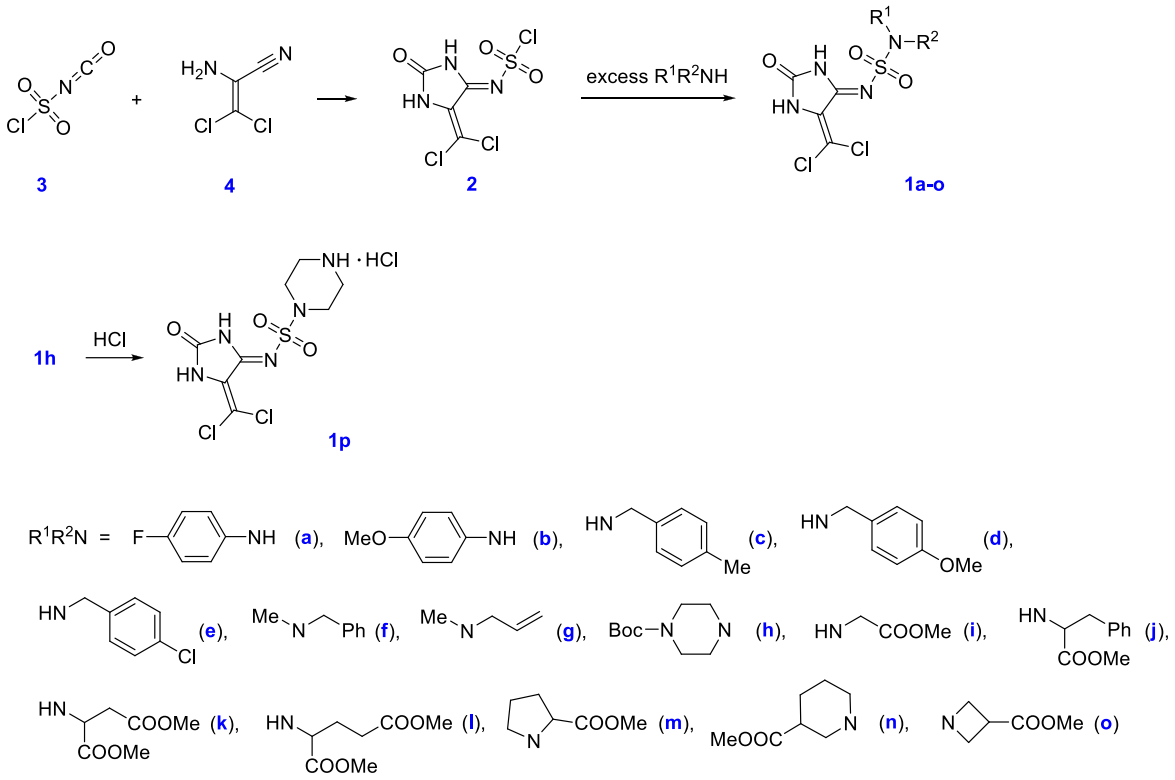
Compared with previous works, we have significantly expanded the list of amines that were involved in the interaction with sulfamoyl chloride **2** to obtain the target sulfamides **1a-o**. Substituted anilines, benzylamines, Boc-protected piperazine, methylalylamine, and amino acids methyl esters with primary and secondary amino group were used. The focus on a series of amino acid derivatives obtaining was due to previous results: a sulfamide of similar structure with a residue of ester nipecotic acid showed strong anticancer activity [10], and the corresponding acid exhibited antiviral action [11]. The formation of compounds **1** occurred under the action of 5-6 eq. amine on chloride **2** in THF solution, and the nature of the amine didn't have much effect on the yield of the product. Removal of Boc-protection of compound **1h** was applied in organic solvent with dry HCl.

Biological Assay

To establish antiviral activity *in vivo* against some strains of cytomegalovirus compounds **1b-h, k, l, n, o** were selected. Unfortunately, a number of investigated substances revealed low activity and insufficient chemotherapeutic index; namely *Human cytomegalovirus* was not suppressed by sulfamides **1c-g, k, l**. Compounds **1b, h** couldn't effectively suppress the growth of *H. cytomegalovirus* strain GDGr K_{17}, but proved to be quite effective against *H. cytomegalovirus* strain AD169 (see Table 1). The derivatives **1n, o** with a fragment of nipecotic and azetidine-3-carboxylic acids, respectively, also acted selectively only on certain species and strains of cytomegaloviruses and weren't active against the others ones. In particular the sulfamides **1n, o** weren't effective against *Guinea pig cytomegalovirus* (22122), *Murine cytomegalovirus* (Smith), but active against *H. cytomegalovirus* (see Table 1). The indexes of antiviral activity also depended on the detection method. For example, when determining the EC_{50} value of the compounds **1n, o** to *H. cytomegalovirus* (AD169) by quantitative polymerase chain reaction these substances were classified as inefficient, while when determining EC_{50} by the method of CellTiter-Glo the value were nearby to the comparison drugs (Table 1). Therefore, four substances showed worthy of interest antiviral activity; these data are shown in Table 1, where the EC_{50} – compound concentration that reduces viral replication by 50%, EC_{90} – concentration that reduces viral replication by 90%, CC_{50} – concentration that reduces cell viability by 50%, SI_{50} – CC_{50} / EC_{50}, SI_{90} – CC_{50} / EC_{90}. The EC_{50} value of the sulfamides **1b, h, n, o** were comparable to the Ganciclovir and Cidofovir. But they have an unremarkable chemotherapy index, especially SI_{90}

The possible anticancer effect was investigated not only for the sulfamides **1a-o** synthetized in this work, but also for the isopropylamine and tryptamine derivatives **1r**, **s**, described in the article [11]. As a result of the primary analysis, a number of biological screening substances were selected (see Table 2).

According to single-dose assay, not all of the studied derivatives had significant anticancer activity. The average percentage inhibition of cancer cell growth, the range of values, as well as the number of lines (from 60 studying ones) that experienced a growth inhibition of more than 50%, and the number of cell lines for which the test compo-



Scheme 1. Synthesis of new (5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamides.

unds were lethal are shown in Table 2. In the latter case, the record values of mortality rates are also reported.

Even in a such small list of compounds, the following tendency can be observed: sulfamides based on aliphatic and aromatic amines show, on average, low anticancer activity, and, on the other hand, the derivatives of benzylamines, allylmethylamine and tryptamine very effectively inhibit the growth of numerous cancer cell lines. It is noteworthy that two compounds – **1f** and **1g**, which were obtained from similar amines (benzylmethylamine and allylmethylamine, respectively), showed the highest anticancer activity against the same cancer cell lines (Table 2) with very similar values.

Table 1. The antiviral activity data of sulfamides **1b, h, n, o** against *H. cytomegalovirus*.*

Virus Strain	Compd	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
AD169	**Ganciclovir**	0.39	1.13	>150.00	>383	>133
	1b	0.21	>6.00	14.42	69	<2
	1h	0.95	>6.00	15.87	17	<3
	1n	0.15	>6.00	14.34	96	<2
	1o	0.19	>30.00	68.08	353	<2
GDGr K_{17}	**Ganciclovir**	13.44	>150.00	>150.00	>11	1
	Cidofovir	0.11	>30.00	117.81	1061	<4
	1n	0.13	>6.00	15.99	119	<3
	1o	0.54	>30.00	70.90	131	<2

* Cell Line: HFF; control assay: CellTiter-Glo (cytopathic effect/toxicity).

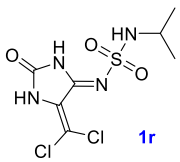
It is also interesting that the values of the activity of sulfamides **1f** and **1g** activity are in a plenty wide range. These compounds have shown very selective cytotoxicity against various cancer lines. As shown in Table 3, the growth inhibition rates of 8 melanoma lines were observed. It is easy to see that the compounds **1f, g** only slightly inhibit the growth of some lines, but a high percentage of lethality was recorded for LOX IMVI cells. Similar strong differences in the activity of compounds **1f, g** can be observed to different cell lines of colorectal, renal and ovarian cancers. But these substances were found to be highly effective against all studied lines of leukemia (Table 3) – from almost discontinuing of growth to significant lethality.

For substances **1d, f, g**, a five-dose assay of their anti-cancer activity were performed. In the Table 4 the average concentrations of GI_{50}, TGI, LC_{50} and the corresponding values for cancer lines that were inhibited most effectively in a single-dose experiment are shown (compare with Table 2; Melanoma LOX IMVI for compounds **1f, g** wasn't investigate), as well as values for the lines with the highest inhibition (marked in a color).

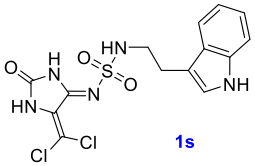
In average values, compound **1f** exhibits the highest anticancer activity, and the effect of sulfonamide **1g** is slightly less. Substance **1f** was able to inhibit growth by 50% at a concentration of less than 10^{-6} M (respectively, lg GI_{50} < -6) of 5 lines out of 59 tested in a five-dose experiment, including Colon Cancer KM12 (see Table 3), Leukemia RPMI-8226 and SR, Breast Cancer MCF7 and MDA-MB-468; and the substance **1g** in less than 10^{-6} M concentration inhibited the growth by 50% the same 5 lines.

Substance **1d** in less than micromolar concentrations inhibited by 50% the growth of 11 lines from 59, but TGI and LC_{50} values wasn't so conductive.

Table 2. The effect of compounds **1a-h, p-s** on the growth of cancer cells, determined by single-dose assay (C = 10^{-5} M); GP – Growth Percent, %; N_{50} – number of lines with GP <50%; N_0 – number of lines with GP <0%.



1r



1s

Compd (NCS code)	GP, Mean	GP, Range	N_{50}	N_0	The most significant inhibition, GP
1a (827223)	100.8	58.9	–	–	69.7 SNB-75 (CNS Cancer)
1b (827225)	93.8	108.7	4	–	15.4 MDA-MB-468 (Breast Cancer)
1c (827227)	52.9	121.6	29	3	-8.7 BT-549 (Breast Cancer) -3.0 SNB-75 (CNS Cancer) -1.1 HOP-92 (Non-Small Cell Lung Cancer)
1d (827226)	26.8	106.4	47	7	-23.2 SNB-75 (CNS Cancer) -22.9 SF-295 (CNS Cancer) -11.1 HL-60(TB) (Leukemia)
1e (827224)	98.7	82.7	1	–	44.1 MDA-MB-468 (Breast Cancer)
1f (828790)	36.0	201.2	30	13	-90.8 ACHN (Renal Cancer) -84.5 LOX IMVI (Melanoma) -81.7 OVCAR-3 (Ovarian Cancer)
1g (828791)	20.88	200.41	35	21	-96.7 ACHN (Renal Cancer) -88.3 LOX IMVI (Melanoma) -84.3 OVCAR-3 (Ovarian Cancer)
1h (827229)	83.9	176.1	7	1	-52.4 NCI-H522 (Non-Small Cell Lung Cancer)
1p (827228)	102.0	48.3	–	–	72.5 SNB-75 (CNS Cancer)
1r (812426)	95.5	102.6	1	–	19.7 SR (Leukemia)
1s (812428)	56.4	135.9	23	3	-3.3 MDA-MB-468 (Breast Cancer) -4.6 KM12 (Colon Cancer) -25.8 OVCAR-3 (Ovarian Cancer)

Conclusions

Thus, the diversity of the sulfamides obtained on the base of (5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl chloride was significantly supplemented by us, and the antiviral and anticancer activity of new derivatives was determined. It was found that the antiviral activity against *H. cytomegalovirus* is inherent for esters of nipecotic and azetidine-3-carboxylic acids **1n** and **1o**, respectively, as well as for anisidine **1b** and Boc-piperazine **1h** sulfamides, but, unfortunately, these compounds have a low chemotherapy index. Most of the tested compounds can effectively inhibit the growth of tumor cells, and strongest inhibition was observed for sulfamides based on *p*-methoxybenzylamine (compo-und **1d**), benzylmethylamine (compound **1f**), and allyl-methylamine (compound **1g**). The substances **1f, g** also have high selectivity for certain cancer cells lines. So, using various amines in the synthesis of *N*-[5-(dichloro-methylene)-2-oxoimidazolidin-4-ylidene]sulfami-des, it has been indeed possible to obtain compounds with either antiviral or anticancer activity.

Table 3. The effect of compounds **1f, g** on the growth of Melanoma and Leukemia cells.

	Growth Percent, %	
	1f (NSC 828790)	**1g** (NSC 828791)
Melanoma		
LOX IMVI	-84.5	-88.3
MALME-3M	-19.9	-69.4
MDA-MB-435	79.6	34.4
SK-MEL-2	87.8	90.0
SK-MEL-28	11.2	-83.6
SK-MEL-5	95.7	91.6
UACC-257	68.2	57.4
UACC-62	62.2	32.9
Leukemia		
CCRF-CEM	-12.9	-27.1
HL-60(TB)	-29.8	-51.7
K-562	1.1	-3.5
MOLT-4	10.3	-19.7
RPMI-8226	-1.1	5.2
SR	-0.4	-18.7

Table 4. The effect of compounds **1d, f, g** on the growth of cancer cells, determined by five-dose assay (the concentrations GI_{50}, TGI and LC_{50}*, mol/l, given as **lg**).

Compd (NCS code)	Cell line	Value of cancer cell lines' growth inhibition		
		lg GI_{50}	lg TGI	lg LC_{50}
1d (827226)	***Mean value***	-5.75	-4.46	-4.07
	SNB-75 (CNS Cancer)	-5.87	> -4.00	> -4.00
	SF-295 (CNS Cancer)	-5.54	> -4.00	> -4.00
	HL-60(TB) (Leukemia)	-6.12	-5.25	> -4.00
	MDA-MB-468 (Breast Cancer)	-6.74	-6.19	-4.11
1f (828790)	***Mean value***	-5.74	-5.26	-4.69
	ACHN (Renal Cancer)	-5.75	-5.50	-5.24
	OVCAR-3 (Ovarian Cancer)	-5.75	-5.47	-5.18
	KM12 (Colon Cancer)	-6.57	-6.13	-4.44
1g (828791)	***Mean value***	-5.66	-5.14	-4.61
	ACHN (Renal Cancer)	-5.75	-5.50	-5.24
	LOX IMVI (Melanoma)	-	-	-
	OVCAR-3 (Ovarian Cancer)	-5.72 -	5.42	-
	MCF7 (Breast Cancer)	-6.41	> -4.00	> -4.00
	RPMI-8226 (Leukemia)	-6.39	-5.01	> -4.00
	KM12 (Colon Cancer)	-6.36	-4.59	> -4.00

* GI_{50} (growth inhibitory activity) – concentration of the compound causing 50 % decrease in net cell growth; TGI (cytostatic activity) – concentration of the compound resulting in total growth inhibition; LC_{50} (cytotoxic activity) – concentration of the compound causing net 50 % loss of initial cells at the end of the incubation period of 48 h.

Experimental section

Chemistry

The solvents were purified according to the standard procedures. All materials were purchased from commercial sources and used without further purification. The success rate was calculated as the number of successful experiments divided by the total number of experiments. NMR spectra were recorded on Varian Union Plus spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in DMSO-d_6 solution. Chemical shifts are reported in ppm downfield from TMS as internal standards. Mass spectra were recorded on an LC-MS instrument with chemical ionization (CI). LC-MS data were acquired on an Agilent 1200 HPLC system equipped with DAD/ELSD/LCMS-6120 diode matrix and mass-selective detector. Melting points were measured on a MPA100 OptiMelt automated melting point system. Elemental analyses were performed at the Analytical Laboratory of the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the NAS of Ukraine, their results were found to be in good agreement (±0.4%) with the calculated values.

The method of sulfamoyl chloride **2** synthesis and general method of sulfamides **1a-o** synthesis from 1 g (3.59 mmol) sulfamoyl chloride **2** were given in publication [10]. The synthesis and the data of sulfamides **1r, s** see at [11].

*(Z)-N-[5-(Dichloromethylene)-2-oxoimidazolidin-4-yl-idene]-N′-(4-fluorophenyl)sulfamide (**1a**).*

Yield: 0.36 g, 28%; mp 220-221 °C. ^{1}H NMR δ 11.47 (br s, 1H, NH), 11.15 (br s, 1H, NH), 9.92 (s, 1H, SO_2NH), 7.05-7.28 (m, 4H, Ar). ^{13}C NMR δ 158.91 (d, J_{CF} 240.1 Hz, C-F), 152.2 (C=N or C=O), 151.1 (C=N or C=O), 134.4 (d, J_{CF} 1.6 Hz, NH-S), 129.7 (C=CCl_2), 122.85 × 2 (d, J_{CF} 8.2 Hz, CHCHCF), 115.56 × 2 (d, J_{CF} 22.5 Hz, CHCF), 107.3 (CCl_2). HPLC (CI) m/z (I_{rel}, %) 353.0 $[M+1]^+$ (100).

*(Z)-N-[5-(Dichloromethylene)-2-oxoimidazolidin-4-yl-idene]-N′-(4-methoxyphenyl)sulfamide (**1b**).*

Yield: 0.97 g, 74%; mp 195 °C (decomp.). ^{1}H NMR δ 11.38 (br s, 1H, NH), 11.13 (s, 1H, NH), 9.60 (s, 1H, SO_2NH), 7.13 (d, *J* 8.6 Hz, 2H, Ar), 6.87 (d, *J* 8.6 Hz, 2H, Ar), 3.70 (s, 3H, CH_3O). ^{13}C NMR δ 156.5 ($C_{Ar}O$), 152.2 (C=N or C=O), 150.9 (C=N or C=O), 130.6 (Ar), 129.7 (C=CCl_2), 123.8 × 2 (Ar), 114.1 × 2 (Ar), 107.2 (CCl_2), 55.2 (CH_3O). HPLC (CI) m/z (I_{rel}, %) 363.0 $[M-1]^-$ (100).

*(Z)-N-[5-(Dichloromethylene)-2-oxoimidazolidin-4-yl-idene]-N′-(4-methylbenzyl)sulfamide (**1c**).*

Yield: 0.91 g, 70%; mp 165-166 °C. ^{1}H NMR δ 11.02 (m, 2H, 2NH), 7.80 (t, *J* 6.6 Hz, 1H, $NHCH_2$), 7.19 (d, *J* 6.8 Hz 2H, Ar), 7.08 (d, *J* 6.8 Hz, 2H, Ar), 4.12 (d, *J* 6.6 Hz, 2H, $NHCH_2$), 2.26 (s, 3H, CH_3). ^{13}C NMR δ 152.2 (C=N or C=O), 150.2 (C=N or C=O), 136.3 (Ar), 134.4 (Ar), 129.5 (C=CCl_2), 128.5 × 2 (Ar), 127.8 × 2 (Ar), 106.9 (CCl_2), 46.3 (CH_2), 20.7 (CH_3). HPLC (CI) m/z (I_{rel}, %) 361.0 $[M-1]^-$ (100).

*(Z)-N-[5-(Dichloromethylene)-2-oxoimidazolidin-4-yl-idene]-N′-(4-methoxybenzyl)sulfamide (**1d**).*

Yield: 1.22 g, 90%; mp 190-191 °C. ^{1}H NMR δ 11.03 (br s, 2H, NH), 7.76 (t, *J* 5.6 Hz, 1H, $NHCH_2$), 7.22 (d, *J* 7.9 Hz, 2H, Ar), 6.83 (d, *J* 7.9 Hz, 2H, Ar), 4.09 (d, *J* 5.6 Hz, 2H, $NHCH_2$), 3.72 (s, 3H, CH_3O). ^{13}C NMR δ 158.5 (C_{Ar}–O), 152.2 (C=N or C=O), 150.1 (C=N or C=O), 129.6 (C=CCl_2), 129.4 (Ar), 129.2 × 2 (Ar), 113.4 × 2 (Ar), 106.9 (CCl_2), 55.1 (CH_3O), 46.0 (CH_2). HPLC (CI) m/z (I_{rel}, %) 379.0 $[M-1]^-$ (100).

*(Z)-N′-(4-Chlorobenzyl)-N-[5-(dichloromethylene)-2-oxo-imidazolidin-4-ylidene]sulfamide (**1e**).*

Yield: 1.13 g, 82%; mp 202-203 °C. ^{1}H NMR δ 11.12 (br s, 1H, NH), 11.06 (s, 1H, NH), 7.93 (t, *J* 5.7 Hz, 1H, $NHCH_2$), 7.29-7.41 (m, 4H, Ar), 4.16 (d, *J* 5.7 Hz, 2H,

$NHCH_2$). ^{13}C NMR δ 152.2 (C=N or C=O), 150.3 (C=N or ($C=CCl_2$), 128.0 × 2 (Ar), 107.0 (CCl_2), 45.7 (CH_2). HPLC (CI) m/z (I_{rel}, %) 383.0 $[M+1]^+$ (100).

*(Z)-N′-Benzyl-N-[5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene]-N′-methylsulfamide (**1f**).*

Yield: 1.15 g, 88%; mp 180-181 °C. ^1H NMR δ 11.41 (br s, 1H, NH), 11.13 (br s, 1H, NH), 7.22-7.46 (m, 5H, Ph), 4.19 (s, 2H, CH_2), 2.63 (s, 3H, CH_3). ^{13}C NMR δ 152.4 (C=N or C=O), 151.5 (C=N or C=O), 136.0 (Ph), 129.8 ($C=CCl_2$), 128.4 × 4 (Ph), 127.6 (Ph), 107.2 (CCl_2), 54.2 (CH_3N), 35.1. HPLC (CI) m/z (I_{rel}, %) 363.0 $[M+1]^+$ (100).

*(Z)-N′-Allyl-N-[5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)-N′-methylsulfamide (**1g**).*

Yield: 0.78 g, 69%; mp 102-103 °C. ^1H NMR δ 11.52 (br s, 1H, NH), 11.12 (br s, 1H, NH), 5.77-5.93 (m, 1H, –CH=), 5.28 (d, J_{trans} 17.1 Hz, 1H, $=CH_2$), 5.21 (d, J_{cis} 10.1 Hz, 1H, $=CH_2$), 3.66 (d, J 5.7 Hz, 2H, NCH_2), 2.67 (s, 3H, CH_3). ^{13}C NMR δ 152.4, 151.5, 132.8, 129.8, 118.9, 107.1 (CCl_2), 53.2, 34.8. HPLC (CI) m/z (I_{rel}, %) 313.0 $[M+1]^+$ (100).

*tert-Butyl (Z)-4-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)piperazine-1-carboxylate (**1h**).*

Yield: 1.02 g, 66%; mp 198-199 °C. ^1H NMR δ 11.64 (br s, 1H, NH), 11.13 (br s, 1H, NH), 3.37-3.50 (m, 4H, $N(CH_2)_2$), 2.96-3.09 (m, 4H, $N(CH_2)_2$), 1.39 (s, 9H, $C(CH_3)_3$). ^{13}C NMR δ 153.8 (CO_2), 152.5 (C=N or C=O), 152.3 (C=N or C=O), 130.0 ($C=CCl_2$), 107.1 (CCl_2), 79.3 (O–C), 46.1 × 4 (2 $N(CH_2)_2$), 28.0 × 3 ($C(CH_3)_3$). HPLC (CI) m/z (I_{rel}, %) 426.0 $[M-1]^-$ (100).

*Methyl (Z)-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)glycinate (**1i**).*

Yield: 0.83 g, 70%; mp 179-180 °C. ^1H NMR δ 11.16 (m, 2H, 2NH), 7.79 (t, J 5.8 Hz, 1H, $NHCH_2$), 3.84 (d, J 5.8 Hz, 2H, $NHCH_2$), 3.62 (s, 3H, CH_3O). ^{13}C NMR δ 169.7 (CO_2), 152.4 (C=N or C=O), 151.2 (C=N or C=O), 129.8 ($C=CCl_2$), 107.3 (CCl_2), 51.9 (CH_3O), 44.0 (CH_2). HPLC (CI) m/z (I_{rel}, %) 331.0 $[M+1]^+$ (100).

*Methyl (Z)-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)phenylalaninate (**1j**).*

Yield: 1.24 g, 82%; mp 174-175 °C. ^1H NMR δ 10.82-11.18 (m, 2H, 2NH), 7.96 (d, J 9.0 Hz, 1H, NHCH), 7.13-7.30 (m, 5H, Ph), 4.10-4.23 (m, 1H, NHCH,), 2.99 (dd, J_{hem} 13.6 Hz, J_{vic} 5.8 Hz, 1H, CH_2), 2.87 (dd, J_{hem} 13.6 Hz, J_{vic} 8.8 Hz, 1H, CH_2). ^{13}C NMR δ 171.6 (CO_2), 152.3 (C=N or C=O), 151.1 (C=N or C=O), 136.2 (Ph), 129.6 ($C=CCl_2$), 129.1 × 2 (Ph), 128.1 × 2 (Ph), 126.6 (Ph), 107.4 (CCl_2), 57.3 (NHCH), 51.9 (CH_3O), 38.0 (CH_2). HPLC (CI) m/z (I_{rel}, %) 421.0 $[M+1]^+$ (100).

*Dimethyl (Z)-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)aspartate (**1k**).*

Yield: 1.11 g, 77%; mp 168-169 °C. ^1H NMR δ 11.27 (br s, 1H, NH), 11.20 (br s, 1H, NH), 7.98 (d, J 7.9 Hz, 1H, CHNH), 4.23-4.38 (m, 1H, CHNH), 3.62 (s, 3H, CH_3O), C=O), 136.8 (Ar), 131.8 (Ar), 129.6 × 2 (Ar), 129.5 3.59 (s, 3H, CH_3O), 2.83 (d, J 4.9 Hz, 2H, $CHCH_2$). ^{13}C NMR δ 170.5 (CO_2), 170.1 (CO_2), 152.3 (C=N or C=O), 151.3 (C=N or C=O), 129.7 ($C=CCl_2$), 107.4 (CCl_2), 52.4 (CH_3O), 52.3 (CH_3O), 51.7 (CHNH). HPLC 36.8 (CH_2), (CI) m/z (I_{rel}, %) 403.0 $[M+1]^+$ (100).

*Dimethyl (Z)-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)glutamate (**1l**).*

Yield: 1.17 g, 78%; mp 175-176 °C. ^1H NMR δ 11.07-11.25 (m, 2H, 2NH), 7.97 (d, J 9.0 Hz, 1H, SO_2NH), 3.91-4.03 (m, 1H, CH), 3.61 (s, 3H, CH_3O), 3.58 (s, 3H, CH_3O), 2.34-2.46 (m, 2H, $CH_2CH_2CO_2Me$), 1.90-2.02 (m, 1H, $CHCH_2$), 1.75-1.88 (m, 1H, $CHCH_2$). ^{13}C NMR δ 172.4 (CO_2), 171.6 (CO_2), 152.2 (C=N or C=O), 151.0 (C=N or C=O), 129.7 ($C=CCl_2$), 107.3 (CCl_2), 54.8 (NHCH), 52.1 (CH_3O), 51.4 (CH_3O), 29.2 (CH_2), 27.2 (CH_2). HPLC (CI) m/z (I_{rel}, %) 415.0 $[M-1]^-$ (100).

*Methyl (Z)-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)prolinate (**1m**).*

Yield: 0.84 g, 63%; mp 142-143 °C. ^1H NMR δ 11.52 (br s, 1H, NH), 11.12 (br s, 1H, NH), 4.15-4.25 (m, 1H, NCH), 3.65 (s, 3H, CH_3O), 3.30 (m, 2H, NCH_2, with H_2O signal), 2.12-2.27 (m, 1H, H_{Pyr}), 1.78-1.98 (m, 3H, H_{Pyr}). ^{13}C NMR δ 172.3 (CO_2), 152.4 (C=N or C=O), 152.2 (C=N or C=O), 130.0 ($C=CCl_2$), 107.2 (CCl_2), 60.9 (NCH), 52.1 (CH_3O), 49.7 (NCH_2), 30.6 (CH_2), 24.5 (CH_2). HPLC (CI) m/z (I_{rel}, %) 371.2 $[M+1]^+$ (100).

*Methyl (Z)-1-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)piperidine-3-carboxylate (**1n**).*

Yield: 0.77 g, 56%; mp 151-152 °C. ^1H NMR δ 10.39-11.98 (br s, 2H, 2NH), 3.45-3.65 (m, 4H, CH_3O, H_{Pip}), 2.78-2.96 (m, 1H, H_{Pip}), 2.62-2.78 (m, 1H, H_{Pip}), 1.80-1.99 (m, 1H, H_{Pip}), 1.63-1.80 (m, 1H, H_{Pip}), 1.29-1.63 (m, 4H, H_{Pip}). ^{13}C NMR δ 173.0, 152.7, 152.1, 130.2, 107.6, 51.9, 48.2, 46.7, 26.0, 23.3. HPLC (CI) m/z (I_{rel}, %) 385.0 $[M+1]^+$ (100).

*Methyl (Z)-1-(N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl)azetidine-3-carboxylate (**1o**).*

Yield: 0.74 g, 58%; mp 211-212 °C. ^1H NMR δ 11.74 (br s, 1H, NH), 11.17 (br s, 1H, NH), 4.06-3.92 (m, 4H, $N(CH_2)_2$), 3.65 (s, 3H, OCH_3), 3.59-3.49 (m, 1H, $CHCO_2Me$). ^{13}C NMR δ 171.9 (CO_2Me), 152.9 (C=N or C=O), 152.4 (C=N or C=O), 130.0 ($C=CCl_2$), 107.5 (CCl_2), 53.2 (OCH_3), 52.1 × 2 ($N(CH_2)_2$), 30.8 ($CHCO_2Me$). HPLC (CI) m/z (I_{rel}, %) 355.0 $[M-1]^-$ (100).

*Hydrochloride of (Z)-N-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)piperazine-1-sulfonamide (**1p**).*

The substance **1h** (0.50 g, 1.17 mmol) was dissolved in 10 mL of dry CH_2Cl_2, and the 1.5 mL (5 eq.) of 4 M HCl in 1,4-dioxane was added. The reaction mixture was stirred for 8 h, than was evaporated, and the dry residue was triturated in 15 ml of MTBE, filtered and washed with MTBE (2 × 10 ml). Yield: 0.36 mg, 85%; mp 218-219 °C. ^1H NMR δ 11.94 (br s, 1H, NH), 11.19 (br s, 1H, NH), 9.50 (br s, 2H,

NH_2^+), 3.30-3.38 (m, 4H, $N(CH_2)_2$), 3.13-3.25 (m, 4H, $N(CH_2)_2$). ^{13}C NMR δ 152.6 (C=N or C=O), 152.3 (C=N or C=O), 129.6 ($C=CCl_2$), 108.2 (CCl_2), 43.4 × 2 ($N(CH_2)_2$), 41.7 × 2 ($N(CH_2)_2$). HPLC (CI) m/z (I_{rel}, %) 328.0 $[M+1]^+$ (100).

Biological Assay

The antiviral activity of synthesized compounds was tested in the Department of Pediatrics, University of Alabama, Birmingham; description of the technique see in [11].

The anticancer activity of synthesized compounds was tested according to the International Program of the National Institutes of Health – DTP (Developmental Therapeutic Program) of the National Cancer Institute (NCI, Bethesda, Maryland, USA) on 60 cancer cell lines [14]; a description of the technique is also given in [15].

Notes

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Disclaimer. This material should not be interpreted as representing the viewpoint of the U.S. National Institutes of Health, or the National Cancer Institute.

The authors declare that there is no conflict of interest.

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Нові сульфамідні похідні 4-іміногідантоїну з противірусною та протираковою активністю

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Резюме: Взаємодією (5-(дихлорометилен)-2-оксоімідазолідін-4-іліден)сульфамоїлхлориду з анілінами, бензиламінами, Boc-захищеним піперазином, метилаліламіном та метиловими естерами амінокислот з первинною та вторинною аміногрупами отримано ряд сульфамідів. Для встановлення противірусної активності *in vivo* щодо окремих штамів цитомегаловірусу сполук було відібрано 11 сполук. На жаль, низьку противірусну активність та хіміотерапевтичний індекс виявили похідні на основі *пара*-заміщених бензиламінів, бензилметил- та алілметиламіну, а також естерів аспарагінової та глутамінової кислоти. Сульфаміди, отримані за участю естерів ніпекотинової та азетидин-3-карбонової кислот, діяли селективно лише на певні види та штами цитомегаловірусів і не були активними щодо інших: зокрема, були неефективними проти цитомегаловірусу морської свинки (22122), цитомегаловірусу миші (Сміт), але активні щодо цитомегаловірусу людини. Показники противірусної активності також залежали від методу виявлення. Значення EC_{50} сульфамідів естерів ніпекотинової та азетидин-3-карбонової кислот, а також сульфамідів *пара*-анізидину та *N*-Boc-піперазину були близькі до показників препаратів порівняння Ганцикловіру та Цидофовіру, хоча досліджувані сполуки мали невисокі хіміотерапевтичні індекси, особливо SI_{90}. При аналізі протиракової активності синтезованих сульфамідів за результатами однодозових випробувань була встановлена висока активність похідних на основі заміщених бензиламінів, бензилметил- та алілметиламіну, *N*-Boc-піперазину та раніше синтезованого сульфаміду з фрагментом триптаміну. Найбільш перспективними у якості протиракових агентів виявились похідні бензилметил- та алілметиламіну, оскільки окрім активності в цілому вони виявляють високу селективність по відношенню до окремих ліній ракових клітин. У порівнянні із цими двома речовинами сульфамід на основі *пара*-метоксибензиламіну здатен до ефективного інгібування меншої кількості ліній ракових клітин; хоча середні показники EC_{50}, отримані внаслідок п'ятидозових випробувань, для усіх трьох похідних були близькі.

Ключові слова: гідантоїни; сульфаміди; противірусна активність; протипухлинна активність.