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Synthesis and anticancer activity of new substituted imidazolidinone sulfonamides

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A B S T R A C T

Obtained by the interaction of 2-amino-3,3-dichloroacrylonitrile and chlorosulphonyl isocyanate (*Z*)-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)sulfamoyl chloride reacts easily with excess of the aliphatic amine to form new (*Z*)-*N*-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)-*N'*-substituted sulfonamides. According to the National Cancer Institute (USA) examinations, two of the six synthesized sulfonamides showed a high anticancer activity.

1. Introduction

Sulfonamides are one of the oldest group of synthetic drugs. Investigations of the antibiotic properties of Prontosil (streptocide) made it possible to find of its activity source: metabolite Sulfanilamide (**Fig. 1**) which formed in organism through hydrolysis.[1]

This discovery initiated the era of drugs' directed synthesis and the study of the structure-activity relationship. Later it was shown that the list of bioactive sulfonamides is not limited to derivatives of benzenesulfonic acid, and among these substances not only effective antibiotics can be found. Even the simple functionalization of arylsulfonamides helped to create drugs that are effective in treating a wide range of diseases. A connection of the sulfonamide fragment to a variety of heterocyclic systems, either directly or through a linker, has significantly increased the number of sulfonamides suitable for use in different fields of medicine (**Fig. 1**).

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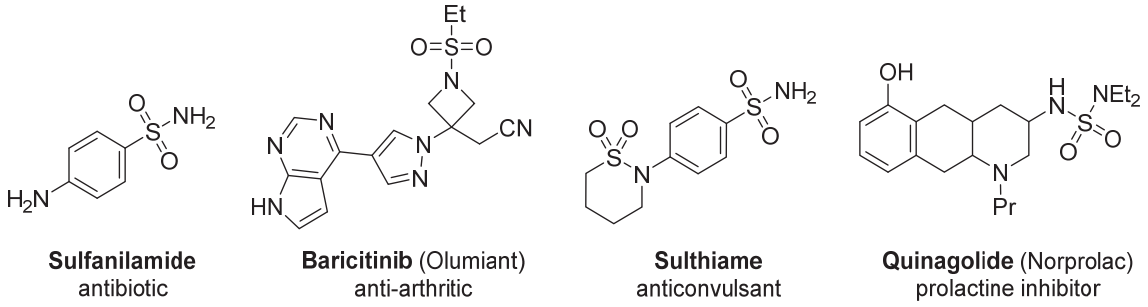


Sulfanilamide antibiotic

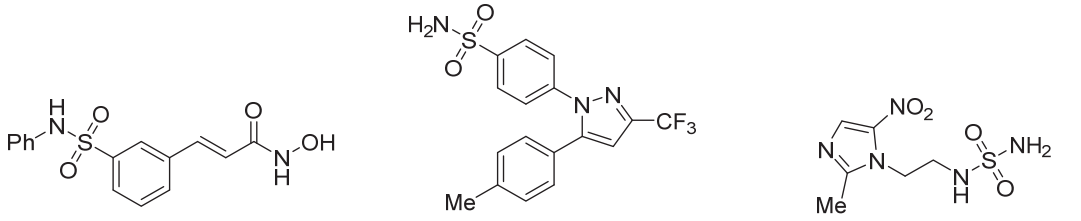
Baricitinib (Olumiant) anti-arthritic

Sulthiame anticonvulsant

Quinagolide (Norprolac) prolactine inhibitor

Fig. 1. Sulfonamide drugs

It is not surprising that researchers widely use sulfonamides also to solve one of the most burning problems of our time, namely the treatment of cancer. Now there are a number of sulfonamide drugs that can stop the growth of different types of malignant cells; and some of them can combine anticancer with other kinds of bioactivity, such as Celecoxib, an anti-inflammatory agent that caused an apoptosis and a decrease in angiogenesis of tumors and metastases (**Fig. 2**). However, the situation in this branch is still not flawless, so searching for new substances with anticancer activity will be vital task for a long time.



Belinostat (Beleodaq, PXD101) peripheral T-cell lymphoma treatment

Celecoxib nonsteroidal anti-inflammatory drug, reduces angiogenesis of tumors

DTP348 potential antineoplastic activity, CAIX inhibitor

Fig. 2. Sulfonamide drugs with anticancer activity

The efforts of our department in collaboration with National Cancer Institute (NCI) are aimed at finding new anticancer drugs among *N*-heterocycles, including structures with sulfonamide residues. It was found by us earlier that some oxazole[2,3] and thiazole[4] sulfonamide derivatives have the necessary level of anticancer activity. In the course of these investigations, we have paid attention to small sulfonamide type molecules with functionalized heterocyclic fragment. It should be noted that similar potential anti-cancer drugs have being actively studied now, e.g. the carbonic anhydrase IX (CAIX) inhibitor DTP348 (**Fig. 2**) is among them.[5]

This paper informs about the detection of high anticancer activity of the new heterocyclic derivatives with the sulfonamide fragment – (*Z*)-*N*-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)-*N′*-alkyl- or *N′,N′*-dialkylsulfonamides. These compounds were tested for their *in vitro* antitumor activity against a panel of 60 cancer cell lines at the National Cancer Institute, USA, within the framework of Developmental Therapeutic Program (http://dtp.cancer.gov).

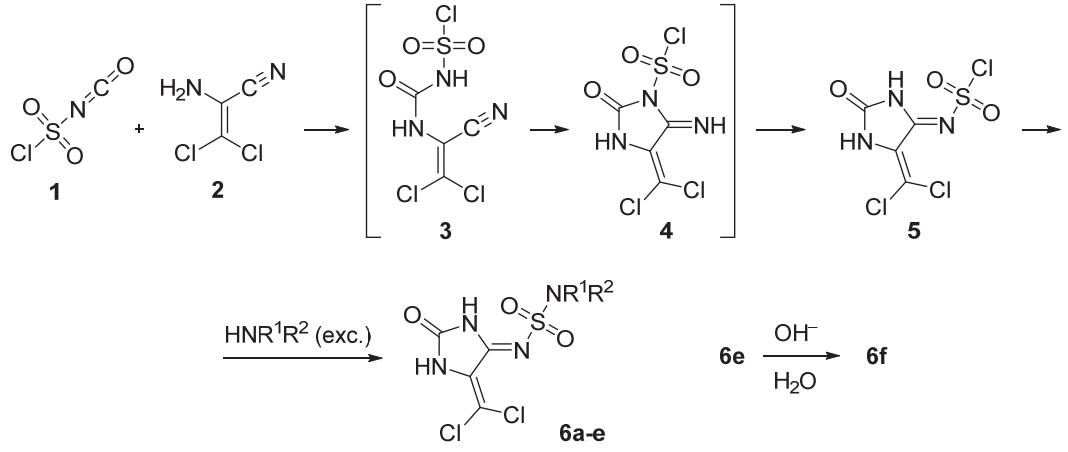
2. Results and Discussion

2.1. Synthesis and Structure Determination

It can be seen that the valid sulfonamide drugs contain in molecule various active functional groups or labile heterocycles (**Fig. 1, 2**); so why the preparation of such derivatives using the sulfochlorination stage involves some difficulties. Therefore, to synthesize new imidazolinone containing sulfonamides, we chose a different approach (**Scheme 1**), and formed this heterocycle system using a reagent already

containing the sulfochloride group – chlorosulfonyl isocyanate. Another component of the reaction was 2-amino-3,3-dichloroacrylonitrile[6] (ADAN), that previously was well recognized as a convenient precursor for the synthesis of functionalized heterocycles,[7,8] including reaction with tosyl isocyanate.[9]

In the first stage, compound **1**, being an extremely strong electrophile, acylates the amino group of ADAN **2**, although its low nucleophilicity. It caused heterocyclization involving a cyano group; and recyclization of the intermediate **4** gives the target sulfochloride **5**. The compound **5** easily reacted with aliphatic primary or secondary amines (excess), and sulfonamides **6a-e** were obtained; chemical structures, yeald and melting points are given at **Table 1**. Acid **6f** was prepared by hydrolysis of the ester **6e** (**Scheme 1**, **Table 1**). Structures of synthesized compounds were confirmed by the ^{1}H and ^{13}C NMR, IR, and LC-MS spectra (see experimental section).



Scheme 1. Synthesis of 2-Oxoimidazolidines **6a-f**

Table 1. Compounds **6a-f**

Entry	Compound	–NR^{1}R^2	NCI code NSC	Yield	mp, °C
1	**6a**	—NHMe		43	121-122
2	**6b**	—NHBn	NSC 802751	75	210-212 decomp.
3	**6c**	—N⟨ ⟩	NSC 802752	78	163-164
4	**6d**	—N⟨ ⟩O		82	240-243
5	**6e**	—N⟨ ⟩—COOEt	NSC 795241	86	166-167
6	**6f**	—N⟨ ⟩—COOH		84	250 decomp.

According to X-ray analysis of compound **6d**, C=N-bond is in *Z*-configuration with the N3-C5-N2-S1 torsion angle of -8.4(3)º (**Fig. 3**); that was predictable, because in such a structure steric hindrance is less.

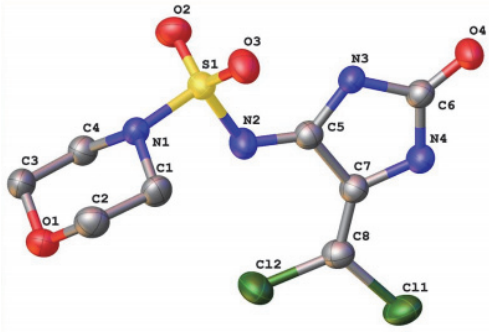
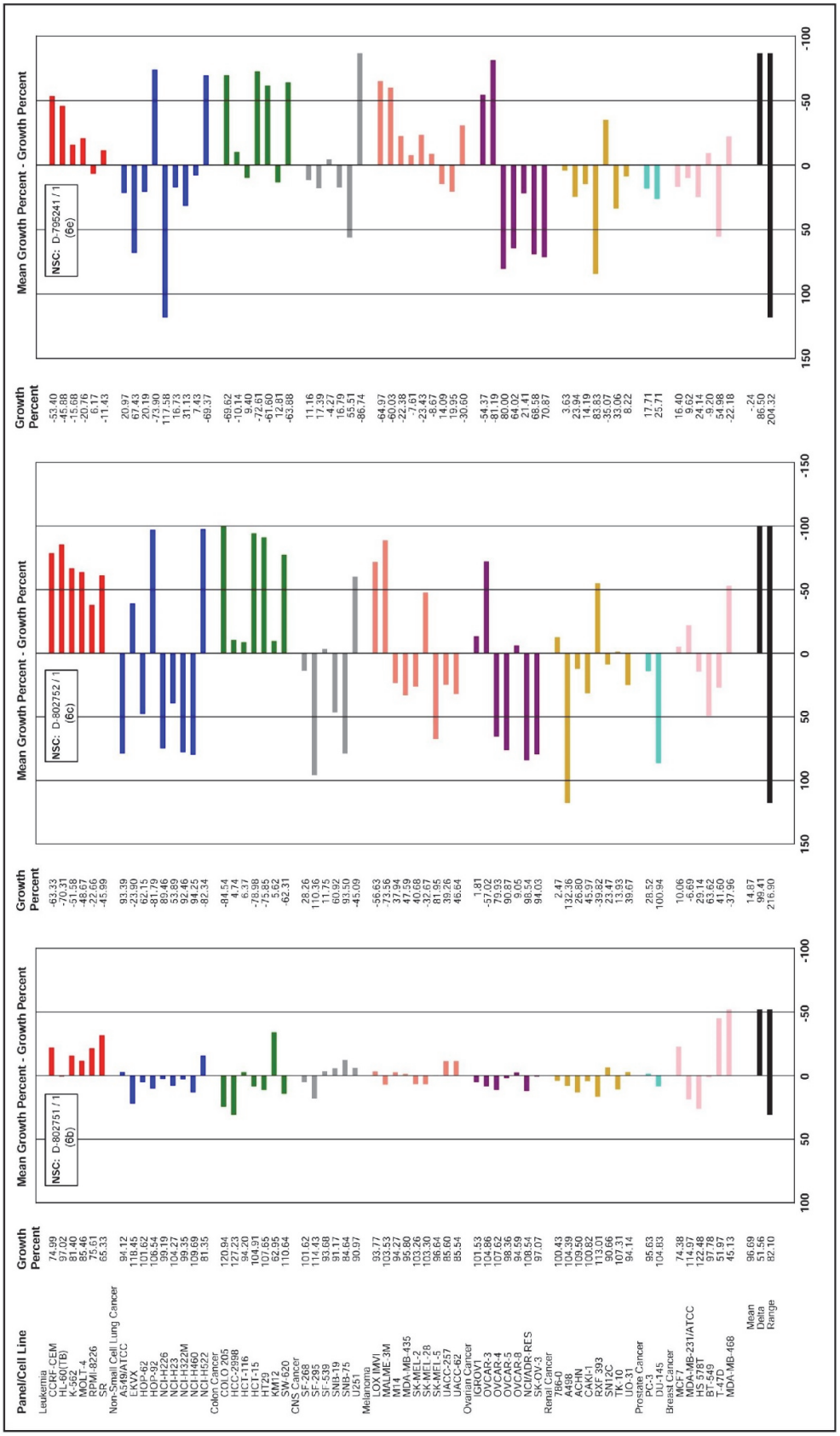


Fig. 3. Molecular Structure of **6d** (X-ray data)



Figs.4. One dose mean graphs of the cancer cells percent growth (compared to the untreated control cells) after treatment by compounds **6b** (NSC 802751), **6c** (NSC 802752), **6e** (NSC 795241) in 10^{-5} M concentration.

(Growth percent of 100 corresponds to growth seen in untreated cells. Growth percent of 0 indicates no net growth over the course of the assay (i.e., equal to the number of cells at time zero). Growth percent of -100 results when all cells are killed)

Table 2. Cytotoxic activities of **6c** (NSC 802752) against the NCI 60 human cancer cell lines

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 802752 / 1	Experiment ID : 1802NS34	Test Type : 08	Units : Molar
Report Date : February 27, 2018	Test Date : February 05, 2018	QNS :	MC :
COMI : SHA0000079	Stain Reagent : SRB Dual-Pass Related	SSPL : 0Y5P	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	1.142	3.509	3.499	3.508	3.475	0.642	0.591	100	100	99	-44	-48	2.19E-6	4.93E-6	> 1.00E-4
HL-60(TB)	0.201	1.615	1.660	1.622	1.402	0.111	0.148	103	100	85	-45	-26	1.86E-6	4.52E-6	> 1.00E-4
K-562	0.610	2.196	2.111	2.082	1.919	0.304	0.404	95	93	83	-50	-34	1.76E-6	4.19E-6	.
MOLT-4	0.672	2.722	2.658	2.712	2.593	0.322	0.382	97	100	94	-52	-43	1.99E-6	4.39E-6	.
RPMI-8226	0.721	2.421	2.486	2.443	1.270	0.582	0.580	104	101	32	-19	-20	5.53E-7	4.23E-6	> 1.00E-4
SR	0.374	1.779	1.624	1.667	1.376	0.189	0.230	89	92	71	-50	-39	1.50E-6	3.89E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.353	1.904	1.818	1.789	1.835	0.990	0.079	94	93	96	41	-78	6.84E-6	2.22E-5	5.85E-5
EKVX	0.573	2.072	1.980	1.982	1.974	0.217	0.292	94	94	93	-62	-49	1.90E-6	3.99E-6	.
HOP-62	0.670	2.077	2.008	1.973	2.038	0.641	0.042	95	93	97	-4	-94	2.92E-6	9.07E-6	3.24E-5
HOP-92	1.099	1.683	1.593	1.622	1.647	0.154	0.139	85	90	94	-86	-87	1.75E-6	3.33E-6	6.31E-6
NCI-H226	0.671	1.840	1.734	1.792	1.795	0.772	0.394	91	96	96	9	-41	3.37E-6	1.49E-5	> 1.00E-4
NCI-H23	0.556	2.056	2.051	2.120	2.024	0.322	0.338	100	104	98	-42	-39	2.20E-6	5.00E-6	> 1.00E-4
NCI-H322M	0.488	1.366	1.300	1.389	1.290	0.342	0.066	92	103	91	-30	-86	2.19E-6	5.66E-6	2.26E-5
NCI-H460	0.294	2.901	2.956	2.985	2.900	0.371	0.131	102	103	100	3	-56	3.27E-6	1.12E-5	8.02E-5
NCI-H522	0.695	2.051	1.880	1.881	1.784	0.202	0.204	87	87	80	-71	-71	1.59E-6	3.39E-6	7.26E-6
Colon Cancer															
COLO 205	0.385	1.414	1.420	1.450	1.449	0.107	0.104	101	103	103	-72	-73	2.01E-6	3.88E-6	7.46E-6
HCC-2998	1.529	3.524	3.525	3.532	3.515	0.931	0.247	100	100	100	-39	-84	2.28E-6	5.22E-6	1.75E-5
HCT-116	0.290	2.427	2.475	2.569	2.440	0.032	0.024	102	107	101	-89	-92	1.85E-6	3.39E-6	6.22E-6
HCT-15	0.196	1.527	1.496	1.468	1.380	0.076	0.078	98	96	89	-61	-60	1.82E-6	3.91E-6	8.42E-6
HT29	0.185	1.345	1.340	1.386	1.344	0.062	0.076	100	104	100	-66	-59	1.99E-6	3.98E-6	7.96E-6
KM12	0.413	2.425	2.530	2.590	0.913	0.435	0.067	105	108	25	1	-84	4.99E-7	1.03E-5	3.99E-5
SW-620	0.246	1.772	1.842	1.826	1.849	0.088	0.088	105	104	105	-64	-64	2.11E-6	4.17E-6	8.22E-6
CNS Cancer															
SF-268	0.668	2.157	2.061	2.245	2.079	0.524	0.126	94	106	95	-22	-81	2.43E-6	6.53E-6	3.00E-5
SF-295	0.535	2.112	2.048	2.056	2.104	1.006	0.025	96	96	99	30	-95	5.14E-6	1.73E-5	4.34E-5
SF-539	0.697	2.345	2.268	2.333	2.220	0.476	0.097	95	99	92	-32	-86	2.20E-6	5.55E-6	2.17E-5
SNB-19	0.454	1.887	1.861	1.862	1.938	0.278	0.011	98	98	104	-39	-98	2.38E-6	5.34E-6	1.55E-5
SNB-75	0.707	1.431	1.282	1.430	1.337	0.255	0.014	79	100	87	-64	-98	1.76E-6	3.77E-6	8.08E-6
U251	0.384	1.844	1.832	1.798	1.762	0.014	0.016	99	97	94	-96	-96	1.71E-6	3.12E-6	5.71E-6
Melanoma															
LOX IMVI	0.318	2.587	2.555	2.600	2.531	0.068	0.077	99	101	98	-79	-76	1.86E-6	3.58E-6	6.88E-6
MALME-3M	0.403	1.088	1.061	1.060	1.062	0.076	0.090	96	96	96	-81	-78	1.82E-6	3.49E-6	6.68E-6
M14	0.388	1.694	1.661	1.669	1.641	0.194	0.108	97	98	96	-50	-72	2.06E-6	4.54E-6	1.00E-5
MDA-MB-435	0.416	2.346	2.111	2.410	2.256	0.178	0.039	88	103	95	-57	-91	1.98E-6	4.21E-6	8.95E-6
SK-MEL-2	0.844	1.522	1.554	1.556	1.558	0.732	0.261	105	105	105	-13	-69	2.92E-6	7.72E-6	4.54E-5
SK-MEL-28	0.572	1.662	1.678	1.766	1.690	0.095	0.084	101	110	103	-83	-85	1.92E-6	3.56E-6	6.61E-6
UACC-257	1.058	2.337	2.266	2.219	2.228	0.887	0.113	94	91	91	-16	-89	2.43E-6	7.07E-6	2.90E-5
UACC-62	0.619	2.429	2.143	2.305	2.205	0.037	0.022	84	93	88	-94	-96	1.61E-6	3.04E-6	5.72E-6
Ovarian Cancer															
IGROV1	0.334	1.351	1.284	1.364	1.324	0.112	0.180	93	101	97	-66	-46	1.95E-6	3.93E-6	.
OVCAR-3	0.310	1.374	1.432	1.515	1.505	0.055	0.049	106	113	112	-82	-84	2.09E-6	3.78E-6	6.83E-6
OVCAR-4	0.727	1.863	1.978	2.008	1.808	0.138	0.303	110	113	95	-81	-58	1.80E-6	3.47E-6	6.66E-6
OVCAR-5	0.526	1.683	1.587	1.786	1.647	0.127	0.065	92	109	97	-76	-88	1.87E-6	3.64E-6	7.08E-6
OVCAR-8	0.411	1.852	1.874	1.849	1.809	0.208	0.186	102	100	97	-49	-55	2.10E-6	4.60E-6	1.30E-5
NCI/ADR-RES	0.438	1.824	1.808	1.887	1.843	0.631	0.368	99	105	101	14	-16	3.87E-6	2.92E-5	> 1.00E-4
SK-OV-3	1.054	2.155	2.167	2.158	2.131	1.981	0.009	101	100	98	84	-99	1.54E-5	2.88E-5	5.39E-5
Renal Cancer															
786-0	0.685	2.611	2.535	2.610	2.615	0.599	0.446	96	100	100	-13	-35	2.79E-6	7.74E-6	> 1.00E-4
A498	1.434	2.410	2.402	2.360	2.300	2.067	0.069	99	95	89	65	-95	1.24E-5	2.54E-5	5.22E-5
ACHN	0.270	1.290	1.362	1.435	1.400	0.009	0.008	107	114	111	-97	-97	1.96E-6	3.42E-6	5.95E-6
CAKI-1	0.650	1.987	1.997	1.869	1.942	0.055	0.036	101	91	97	-92	-94	1.77E-6	3.26E-6	6.01E-6
RXF 393	0.565	1.395	1.411	1.381	1.396	0.064	0.055	102	98	100	-89	-90	1.84E-6	3.39E-6	6.23E-6
SN12C	0.492	2.068	2.020	2.085	1.966	0.025	0.021	97	101	94	-95	-96	1.70E-6	3.14E-6	5.78E-6
TK-10	0.609	1.420	1.396	1.389	1.461	0.808	0.624	97	96	105	24	2	4.82E-6	> 1.00E-4	> 1.00E-4
UO-31	0.474	1.522	1.393	1.385	1.360	0.571	0.049	88	87	85	9	-90	2.87E-6	1.24E-5	3.97E-5
Prostate Cancer															
PC-3	0.513	2.178	2.176	2.118	2.102	0.542	0.066	100	96	95	2	-87	3.05E-6	1.05E-5	3.81E-5
DU-145	0.300	1.429	1.551	1.568	1.436	0.394	0.041	111	112	101	8	-87	3.53E-6	1.22E-5	4.12E-5
Breast Cancer															
MCF7	0.294	1.892	1.726	1.787	1.594	0.500	0.475	90	93	81	13	11	2.87E-6	> 1.00E-4	> 1.00E-4
MDA-MB-231/ATCC	0.495	1.599	1.643	1.647	1.601	0.063	0.049	104	104	100	-87	-90	1.85E-6	3.42E-6	6.33E-6
HS 578T	0.933	1.662	1.752	1.706	1.654	0.945	0.448	112	106	99	2	-52	3.18E-6	1.07E-5	9.18E-5
BT-549	0.938	2.039	2.033	2.031	2.017	0.631	0.020	100	99	98	-33	-98	2.33E-6	5.62E-6	1.84E-5
T-47D	0.839	1.750	1.665	1.647	1.599	1.206	0.760	91	89	83	40	-9	5.94E-6	6.45E-5	> 1.00E-4
MDA-MB-468	0.662	1.559	1.506	1.541	1.353	0.711	0.406	94	98	77	5	-39	2.39E-6	1.33E-5	> 1.00E-4

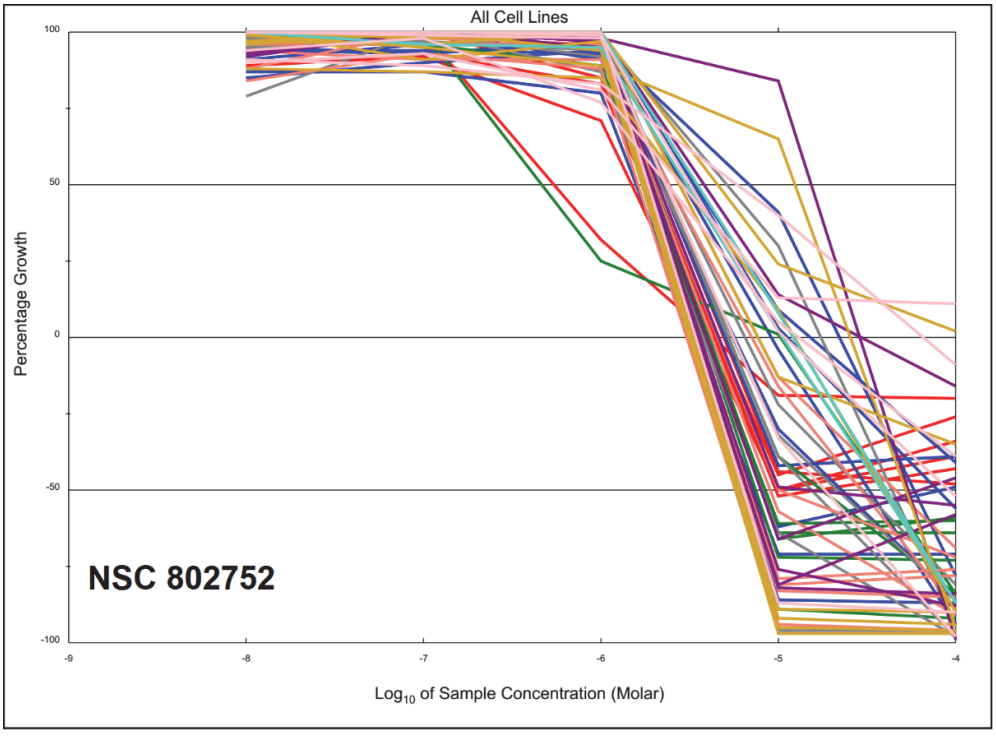
Table 3. Cytotoxic activities of **6e** (NSC 795241) against the NCI 60 human cancer cell lines

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 795241 / 1	Experiment ID : 1702NS56	Test Type : 08	Units : Molar
Report Date : February 27, 2018	Test Date : February 13, 2017	QNS :	MC :
COMI : SHA0000042	Stain Reagent : SRB Dual-Pass Related	SSPL : 0Y5P	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.504	2.357	2.184	2.325	1.634	0.197	0.201	91	98	61	-61	-60	1.23E-6	3.16E-6	8.14E-6
HL-60(TB)	0.668	2.272	2.092	2.393	1.957	0.363	0.358	89	108	80	-46	-46	1.74E-6	4.34E-6	> 1.00E-4
K-562	0.211	1.545	1.508	1.643	1.215	0.166	0.195	97	107	75	-22	-8	1.82E-6	5.99E-6	> 1.00E-4
MOLT-4	0.683	2.497	2.393	2.490	2.253	0.364	0.379	94	100	87	-47	-45	1.88E-6	4.46E-6	> 1.00E-4
RPMI-8226	0.610	2.044	2.136	1.935	0.753	0.611	0.415	106	92	10	.	-32	3.27E-7	1.01E-5	> 1.00E-4
SR	0.236	0.719	0.689	0.688	0.444	0.192	0.177	94	94	43	-19	-25	7.31E-7	4.96E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.570	2.232	2.223	2.255	2.410	0.933	0.191	99	101	111	22	-66	4.82E-6	1.77E-5	6.51E-5
EKVX	0.660	2.119	2.008	2.033	1.960	0.384	0.400	92	94	89	-42	-39	1.99E-6	4.79E-6	> 1.00E-4
HOP-62	0.685	1.497	1.394	1.457	1.086	0.863	0.145	87	95	49	22	-79	9.71E-7	1.65E-5	5.17E-5
HOP-92	1.070	1.658	1.623	1.623	1.565	0.230	0.281	94	94	84	-79	-74	1.62E-6	3.29E-6	6.68E-6
NCI-H226	0.980	2.434	2.387	2.357	2.321	0.978	0.541	97	95	92	.	-45	2.86E-6	9.94E-6	> 1.00E-4
NCI-H23	0.590	1.787	1.855	1.884	1.805	0.256	0.241	106	108	101	-57	-59	2.12E-6	4.38E-6	9.07E-6
NCI-H322M	0.868	2.038	1.972	1.920	1.833	1.019	0.116	94	90	82	13	-87	2.93E-6	1.35E-5	4.28E-5
NCI-H460	0.268	2.758	2.819	2.903	2.678	0.366	0.099	102	106	97	4	-63	3.19E-6	1.14E-5	6.35E-5
NCI-H522	1.072	2.341	2.364	2.350	2.482	0.376	0.396	102	101	111	-65	-63	2.22E-6	4.28E-6	8.22E-6
Colon Cancer															
COLO 205	0.429	1.233	1.231	1.309	1.312	0.093	0.105	100	109	110	-78	-76	2.08E-6	3.83E-6	7.06E-6
HCC-2998	0.490	1.733	1.758	1.835	1.883	0.067	0.083	102	108	112	-86	-83	2.05E-6	3.67E-6	6.55E-6
HCT-116	0.226	1.914	1.872	1.945	0.936	0.080	0.031	97	102	42	-65	-87	7.36E-7	2.48E-6	7.30E-6
HCT-15	0.466	2.748	2.609	2.726	2.622	0.108	0.149	94	99	94	-77	-68	1.82E-6	3.56E-6	6.97E-6
HT29	0.332	1.751	1.827	1.778	1.871	0.124	0.157	105	102	108	-63	-53	2.20E-6	4.30E-6	8.43E-6
KM12	0.427	2.259	2.258	1.922	0.493	0.455	0.149	100	82	4	2	-65	2.54E-7	1.05E-5	5.91E-5
SW-620	0.359	2.353	2.275	2.376	2.355	0.158	0.139	96	101	100	-56	-61	2.09E-6	4.37E-6	9.14E-6
CNS Cancer															
SF-268	0.687	2.146	2.043	2.097	1.774	0.611	0.257	93	97	75	-11	-63	1.93E-6	7.43E-6	5.68E-5
SF-295	0.827	2.686	2.502	2.621	2.521	1.134	0.054	90	97	91	16	-94	3.56E-6	1.41E-5	4.02E-5
SF-539	1.072	2.902	2.743	2.813	1.666	0.883	0.078	91	95	32	-18	-93	5.25E-7	4.44E-6	2.70E-5
SNB-19	0.424	1.474	1.469	1.504	1.261	0.285	0.039	100	103	80	-33	-91	1.84E-6	5.11E-6	1.98E-5
SNB-75	0.917	1.951	1.720	1.772	1.722	1.093	0.027	78	83	78	17	-97	2.86E-6	1.41E-5	3.86E-5
U251	0.476	1.911	1.991	1.976	1.950	0.061	0.080	106	105	103	-87	-83	1.90E-6	3.47E-6	6.37E-6
Melanoma															
LOX IMVI	0.263	1.655	1.644	1.664	0.562	0.107	0.145	99	101	21	-59	-45	4.36E-7	1.84E-6	.
MALME-3M	0.758	1.262	1.236	1.208	1.195	0.203	0.208	95	89	87	-73	-73	1.70E-6	3.48E-6	7.15E-6
M14	0.396	1.618	1.540	1.580	1.541	0.100	0.093	94	97	94	-75	-77	1.82E-6	3.60E-6	7.12E-6
MDA-MB-435	0.386	2.188	2.029	2.069	1.960	0.219	0.049	91	93	87	-43	-87	1.93E-6	4.66E-6	1.41E-5
SK-MEL-2	1.239	2.507	2.462	2.488	2.349	1.085	0.533	96	98	88	-12	-57	2.37E-6	7.50E-6	6.97E-5
SK-MEL-28	0.626	1.926	1.853	1.916	1.850	0.135	0.136	94	99	94	-79	-78	1.80E-6	3.51E-6	6.84E-6
SK-MEL-5	0.615	3.067	2.954	2.902	2.930	0.273	0.032	95	93	94	-56	-95	1.98E-6	4.26E-6	9.18E-6
UACC-257	1.416	2.605	2.542	2.583	2.475	1.211	0.367	95	98	89	-15	-74	2.38E-6	7.24E-6	3.94E-5
UACC-62	0.893	2.822	2.744	2.800	2.658	0.122	0.074	96	99	91	-86	-92	1.71E-6	3.27E-6	6.25E-6
Ovarian Cancer															
IGROV1	0.661	2.180	2.186	2.142	2.100	0.235	0.281	100	97	95	-65	-58	1.91E-6	3.93E-6	8.11E-6
OVCAR-3	0.432	1.612	1.603	1.580	1.569	0.043	0.040	99	97	96	-90	-91	1.77E-6	3.29E-6	6.09E-6
OVCAR-4	0.767	1.781	1.670	1.777	1.705	0.094	0.430	89	100	92	-88	-44	1.72E-6	3.26E-6	.
OVCAR-5	0.665	1.954	1.863	1.931	1.852	0.877	0.122	93	98	92	16	-82	3.60E-6	1.47E-5	4.76E-5
OVCAR-8	0.682	2.514	2.542	2.587	2.535	0.908	0.486	102	104	101	12	-29	3.76E-6	1.99E-5	> 1.00E-4
NCI/ADR-RES	0.579	2.043	2.128	2.154	2.055	0.892	0.502	106	108	101	21	-13	4.36E-6	4.14E-5	> 1.00E-4
SK-OV-3	0.895	1.952	1.879	1.933	1.930	1.422	0.013	93	98	98	50	-99	9.94E-6	2.17E-5	4.71E-5
Renal Cancer															
786-0	0.707	2.593	2.420	2.509	1.343	0.656	0.312	91	96	34	-7	-56	5.45E-7	6.64E-6	7.55E-5
A498	1.494	2.352	2.291	2.349	2.184	1.568	0.090	93	100	80	9	-94	2.65E-6	1.21E-5	3.72E-5
ACHN	0.430	1.930	1.876	1.882	1.936	0.033	0.029	96	97	100	-92	-93	1.83E-6	3.32E-6	6.03E-6
RXF 393	0.919	1.622	1.557	1.520	1.530	0.075	0.051	91	85	87	-92	-94	1.61E-6	3.06E-6	5.83E-6
SN12C	0.422	1.628	1.654	1.615	1.410	0.064	0.052	102	99	82	-85	-88	1.55E-6	3.10E-6	6.17E-6
TK-10	0.882	1.932	1.798	1.832	1.431	1.186	0.999	87	90	52	29	11	1.25E-6	> 1.00E-4	> 1.00E-4
UO-31	0.771	2.002	1.820	1.788	1.786	0.355	0.042	85	83	82	-54	-95	1.73E-6	4.02E-6	9.34E-6
Prostate Cancer															
PC-3	0.394	1.263	1.279	1.337	0.696	0.198	0.049	102	109	35	-50	-88	6.21E-7	2.58E-6	1.02E-5
DU-145	0.465	1.968	1.977	1.978	1.498	0.748	0.068	101	101	69	19	-85	2.37E-6	1.51E-5	4.57E-5
Breast Cancer															
MCF7	0.548	2.741	2.520	2.516	0.836	0.802	0.427	90	90	13	12	-22	3.30E-7	2.20E-5	> 1.00E-4
MDA-MB-231/ATCC	0.642	1.416	1.449	1.457	1.457	0.087	0.077	104	105	105	-87	-88	1.94E-6	3.54E-6	6.45E-6
HS 578T	1.159	2.213	2.154	2.216	2.158	1.667	1.032	94	100	95	48	-11	9.14E-6	6.53E-5	> 1.00E-4
BT-549	1.264	2.109	2.001	2.046	1.970	0.444	0.046	87	93	84	-65	-96	1.68E-6	3.65E-6	7.94E-6
T-47D	0.865	1.621	1.555	1.554	1.309	1.207	0.500	91	91	59	45	-42	4.41E-6	3.29E-5	> 1.00E-4
MDA-MB-468	0.732	1.357	1.308	1.325	0.848	0.442	0.243	92	95	18	-40	-67	3.87E-7	2.08E-6	2.40E-5

(a)



(b)

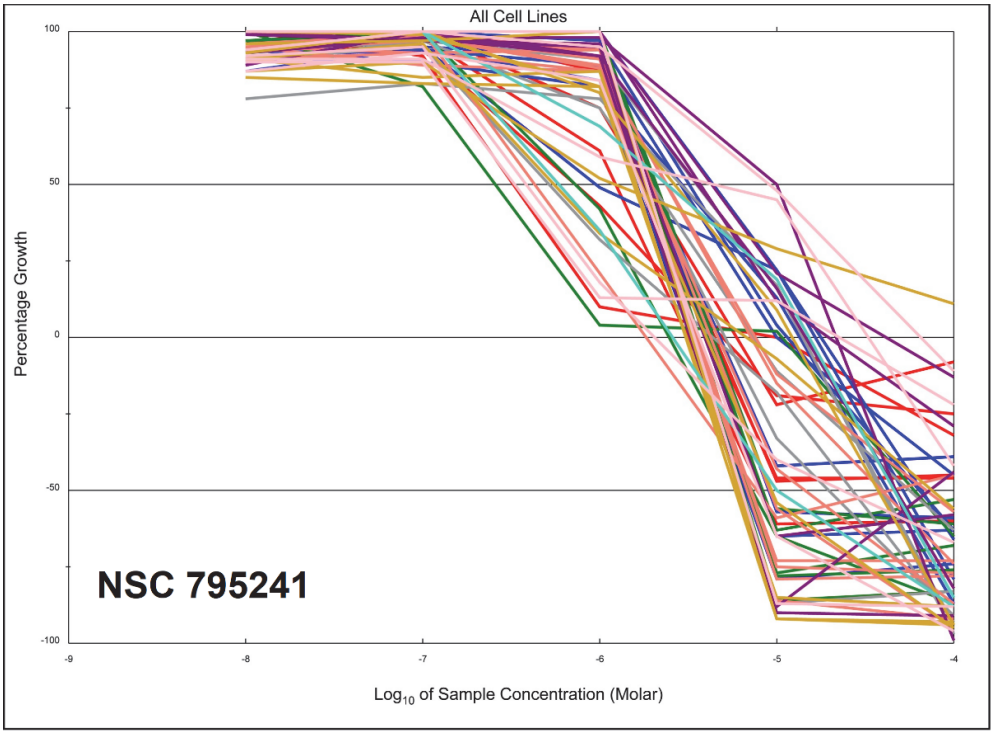


Fig. 5. Collective dose response curves of compound **6c** (NSC 802752, *a*) and **6e** (NSC 795241, *b*) for all NCI 60 cell lines of *in vitro* five dose assay

2.2. Biological Evaluation

2.2.1. Primary Single High Dose (10^{-5} M) against Full NCI 60 Cells Panel in Vitro Assay

Initial single dose (10^{-5} M) testing of primary choosing sulfonamides **6b** (NSC 802751), **6c** (NSC 802752), and **6e** (NSC 795241) against the 60 cell lines of NCI immediately made it possible to select the most promising objects – piperidine derivative **6c** and isonipecotate **6e**. On **Figs. 4** one dose mean graphs of the percent growth of the treated cells when compared to the untreated control cells for compounds **6b,c,e** is shown.

As we can see the most active compounds **6c,e** mainly have high selectivity to the cancer cell lines. For example, with the effect of substance **6e** in 10^{-5} M concentration on cultures that cause ovarian cancer, there was a more active growth of OVCAR-4 cells (up to 80 %). But for OVCAR-3 cells under the same conditions, lethality was 81 % (**Figures 4**). The effect of substance **6e** on various types of leukemia was more concerted: in five cell lines from six this sulfonamide caused death from 11 % (SR leukemia) to 53 % (leukemia CCRF-CEM) of the examined cells. The influence of substance **6c** on leukemia cells was also unidirectional, but stronger than for compound **6e**, and the death from 23 % (RPMI-8226) to 70 % (HL-60(TB)) was observed (**Figures 4**). An action of sulfonamide **6e** against the colon cancer lines expressed in the almost complete suppression of the growth of cancer cells, or in the destruction of their significant amount (**Figures 4**). And the compound **6c** was mainly lethal for colon cancer cells too.

Benzylamide **6b** didn't exhibit such a pronounced cytotoxicity, but some data is quite interest to use them subsequently. In despite of the summarily weak level of compound **6b** bioactivity, its exterminating of two lines of breast cancer (T-47D and MDA-MB-468) was unexpectedly notable (**Figs. 4**). Also it was remarkable the moderate but unequivocal predisposition of the amide **6b** to cause the death of almost all investigated leukemia cells lines. The presented facts allow expecting for the high selectivity of this substance and its analogues to various biological targets.

2.2.2. Five Doses Full NCI 60 Cell Panel Assay

The next step was to find the extrapolating parameters GI_{50}, TGI, and LC_{50} for substances **6c,e** (definition and method of calculation see below in experimental section). Control samples of 60 cancer cell lines were compared with the ones that treated by sulfonamides **6c,e** in five different concentration (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M).

By measuring of optical densities of cell medium percent growth was defined (see **Table 2,3**). These data are completely visualized on **Figures 5** and the most significant information is exposed in **Table 4**. High cytotoxical ability of substances **6c,e** is confirmed by the LC_{50} value, which in some cases was micromolar; e.g. for sulfonamide **6c** in action to Melanoma LOX IMVI and breast cancer MDA-MB-231/ATCC and for sulfonamide **6e** to MDA-MB-231/ATCC.

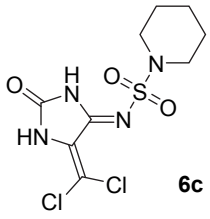
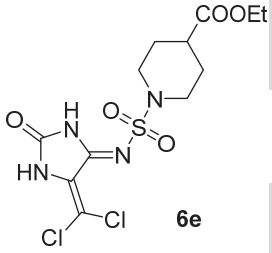
3. Conclusions

Thus, a convenient method for the synthesis of new (*Z*)-*N*-(5-(dichloromethylene)-2-oxoimidazolidin-4-ylidene)-*N*'-substituted sulfonamides with variation of amino compounds has been developed and high anticancer activity of two of the six derivatives has been set.

Acknowledgements

We would like to thank US Public Health Service and National Cancer Institute, USA, for in vitro evaluation of anticancer activity (providing the NCI-60 cell testing) within the framework of Developmental Therapeutic Program (http://dtp.cancer.gov), and *Enamine* Ltd for the material and technical support.

Table 4. *In vitro* five dose assay compound **6c** (NSC 802752) and **6e** (NSC 795241)

Compound, concentration (M)		Colon Canser KM12	Leukemia RPMI-8226	Melanoma LOX IMVI	Breast Cancer MDA-MB-231/ ATCC	Breast Cancer MDA-MB-468
 **6c**	**GI_{50}**	$4.99 \cdot 10^{-7}$	$5.53 \cdot 10^{-7}$	$1.86 \cdot 10^{-6}$	$1.85 \cdot 10^{-6}$	$2.39 \cdot 10^{-6}$
	TGI	$1.03 \cdot 10^{-5}$	$4.23 \cdot 10^{-6}$	$3.58 \cdot 10^{-6}$	$3.42 \cdot 10^{-6}$	$1.33 \cdot 10^{-5}$
	LC_{50}	$3.99 \cdot 10^{-5}$	$>1.10^{-4}$	$6.88 \cdot 10^{-6}$	$6.33 \cdot 10^{-6}$	$>1 \cdot 10^{-4}$
 **6e**	**GI_{50}**	$2.54 \cdot 10^{-7}$	$3.27 \cdot 10^{-7}$	$4.36 \cdot 10^{-7}$	$1.94 \cdot 10^{-6}$	$3.87 \cdot 10^{-7}$
	TGI	$1.05 \cdot 10^{-5}$	$1.01 \cdot 10^{-5}$	$1.84 \cdot 10^{-6}$	$3.54 \cdot 10^{-6}$	$2.08 \cdot 10^{-6}$
	LC_{50}	$5.91 \cdot 10^{-5}$	$>1.10^{-4}$	not determined	$6.45 \cdot 10^{-6}$	$2.40 \cdot 10^{-5}$

Disclaimer

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

4. Experimental

4.1. General Methods

All reagents and solvents were purchased from Aldrich and used as received. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded at Varian Unityplus 400 spectrometer in DMSO-d_6 solution with TMS as an internal standard. IR spectra were recorded on a Vertex 70 spectrometer from KBr pellets. The melting points were estimated on a Fisher-Johns instrument.

The chromatomass spectra were recorded on an Agilent 1100 Series high performance liquid chromatograph equipped with a diode matrix with an Agilent LC/MS mass selective detector allowing a fast switching the positive/negative ionization modes. The reaction progress was monitored by the TLC method on Silica gel 60 F_{254} Merck.

4.2. Synthetic Procedures and Spectral Data

*4.2.1. (Z)-(5-(Dichloromethylene)-2-oxoimidazolidin-4-ylidene)-sulfamoyl chloride (**5**).* Chlorosulphonyl isocyanate **1** (20.28 ml, 0.233 mol) was added dropwise with stirring to a solution of ADAN **2** (31.0 g, 0.233 mol) in absolute Et_2O (300 ml), and the mixture was stirred at 30-35 °C for 14 h. The resulting precipitate was collected by filtration and washed with Et_2O. Yield 85-90 %. Mp 120-125 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 11.25 (s, 1H, NH), 12.76 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 108.6, 139.9, 152.8, 159.4. IR (KBr), ν, cm^{-1}: 3311, 3178, 3073, 1766, 1654, 1596, 1389, 1363, 1323, 1139, 1034, 292, 822, 753, 581.

*4.2.2. Sulfonamides **6a-e** Synthesis*. To a solution of 6 eq (21.6 mmol) of corresponding amine in 50 ml of THF at 0-5 °C sulfamoyl chloride **5** (1 g, 3.6 mmol) was added with stirred in portions about 0.1 g. Reaction mixture was stirred at 20-25 °C for 6 h, then the solvent was evaporated in vacuo. To residue 10 ml of water was added and the mixture was acidified by diluted HCl. The precipitate that formed was filtered off, dried and recrystallized from ethanol.

*N-[(4Z)-5-(Dichloromethylidene)-2-oxoimidazolidin-4-ylidene]-N′-methylsulfuric diamide (**6a**)*. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.58 (s, 3H, CH_3), 7.17 (br. s, 1H, $\underline{NH}CH_3$), 11.09 (s, 1H, NH), 11.25 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 25.5, 107.2, 130.3, 150.9, 152.9. IR (KBr), ν, cm^{-1}: 3296, 3181, 3085, 2786, 1766, 1669, 1625, 1443, 1379, 1325, 1129, 923, 807, 757, 715, 621. LCMS, *m/z*: 273 $[M+1]^+$.

*N-[(4Z)-5-(Dichloromethylidene)-2-oxoimidazolidin-4-ylidene]-N′-benzylsulfuric diamide (**6b**)*. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 4.18 (d, *J*=5.6, 2H, $NH\underline{CH_2}$), 7.15-7.40 (m, 5H, Ph), 7.85 (t, *J*=5.6, 1H, $\underline{NH}CH_2$), 11.03 (s, 1H, NH), 11.08 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 46.4, 106.9, 127.1, 127.8×2, 128.1×2, 129.6, 137.6, 150.3, 152.2. IR (KBr), ν, cm^{-1}: 3298, 3224, 3065, 2864, 1766, 1668, 1624, 1440, 1390, 1324, 1136, 921, 809, 750, 694, 624. LCMS, *m/z*: 349 $[M+1]^+$.

*N-[(4Z)-5-(Dichloromethylidene)-2-oxoimidazolidin-4-ylidene]piperidine-1-sulfonamide (**6c**)*. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.40–1.75 (m, 6H, pip), 3.04 (br. s, 4H, pip), 10.8-11.4 (br. s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 20.1, 21.2, 23.2, 43.7, 47.0, 106.0, 130.1, 153.8×2. IR (KBr), ν, cm^{-1}: 3598, 3176, 3051, 2990, 2942, 2852, 2767, 1767, 1667, 1620, 1378, 1317, 1136, 1053, 930, 821, 732, 583.

*N-[(4Z)-5-(Dichloromethylidene)-2-oxoimidazolidin-4-ylidene]morpholine-4-sulfonamide (**6d**)*. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.03 (s, 4H, morph), 3.66 (s, 4H, morph), 11.15 (br. s, 1H, NH), 11.60 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 46.9, 65.6, 107.8, 130.4, 152.8, 152.9. IR (KBr), ν, cm^{-1}: 3175, 3090, 2866, 1765, 1667, 1615, 1454, 1394, 1326, 1150, 1074, 948, 917, 814, 749, 687, 606. LCMS, *m/z*: 329 $[M+1]^+$.

*Ethyl 1-{[(4Z)-5-(Dichloromethylidene)-2-oxoimidazolidin-4-ylidene]sulfamoyl}piperidine-4-carboxylate (**6e**)*. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.17 (t, *J*=7.0, 3H, $CH_2\underline{CH_3}$), 1.67 (q, *J*=10.5, 2H, pip), 1.89 (d, *J*=10.1, 2H, pip), 2.47 (m, 1H, pip), 2.77 (t, *J*=10.1, 2H, pip), 3.47 (d, *J*=12.2, 2H, pip), 4.06 (q, *J*=7.0, 2H, $\underline{CH_2}CH_3$), 11.17 (br. s, 1H, NH), 11.61 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.6, 27.1×3, 46.1×2, 60.5, 107.7, 130., 152.3, 152.9, 174.2. IR (KBr), ν, cm^{-1}: 3389, 3182, 3092, 2989, 1757, 1730, 1669, 1633, 1383, 1291, 1197, 1134, 1020, 925, 814, 751, 607. LCMS, *m/z*: 399 $[M+1]^+$.

*4.2.3. 1-{[(4Z)-5-(Dichloromethylidene)-2-oxoimidazolidin-4-ylidene]sulfamoyl}piperidine-4-carboxylic acid (**6f**)*. Ester **6e** (1 g, 2.5 mol) in KOH water solution (0.28 g, 5 mmol KOH in 5 ml of water) was heated with stirring up to boiling and solving. After cooling reaction mixture was acidified by diluted hydrochloric acid, and precipitate was filtered off, dried and recrystallized from ethanol. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.50-1.75 (m, 2H, pip), 1.87 (m, 2H, pip), 2.37 (br. s, 1H, pip), 2.75 (m, 2H, pip), 3.46 (m, 2H, pip), 11.04 (br. s, 1H, NH), 11.56 (br. s, 1H, NH), 12.30 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 26.7×3 , 45.7×2, 107.2, 129.8, 151.7, 152.4, 175.4. IR (KBr), ν, cm^{-1}: 3400-2800, 3191, 3070 (NH), 2951, 2930, 1750, 1663, 1619, 1415, 1350, 1327, 1286, 1203, 1136, 1042, 917, 814, 747, 659, 603. LCMS, *m/z*: 371 $[M+1]^+$.

4.3 X-Ray Analysis

The colourless crystals of sulfonamide **6d** ($C_8H_{10}O_4N_4Cl_2S$) are monoclinic. At 293 K a = 5.8274(2), b = 18.4851(5), c = 12.4109(3) Å, β = 102.555(3)°, V = 1304.92(7) Å^3, M_r = 329.16, Z = 4, space group $P2_1/c$, d_{calc} = 1.675 g/cm^3, μ(MoK_α) = 0.673 mm^{-1}, F(000) = 672. Intensities of 10648 reflections (3000 independent, R_{int}=0.033) were measured on the «Xcalibur-3» diffractometer (graphite monochromated MoK_α radiation, CCD detector, ω-scaning, $2\Theta_{max}$ = 55°). The structure was solved by direct method using SHELXTL package[10]. Positions of the hydrogen atoms were located from electron density difference maps and refined using "riding" model with $U_{iso} = 1.2U_{eq}$ of the carrier atom. Full-matrix least-squares refinement against F^2 in anisotropic approximation for non-hydrogen atoms using 3000 reflections was converged to $wR_2 = 0.085$ ($R_1 = 0.035$ for 2504 reflections with $F>4\sigma(F)$, S = 1.024). The final atomic coordinates, and crystallographic data for molecule **6d** have been deposited to the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition numbers CCDC 1894776).

4.4. In Vitro Anticancer Screening of the synthesized compounds

4.4.1. One Doses Full NCI 60 Cell Panel Assay. The newly synthesized compounds were submitted to National Cancer Institute NCI, Bethesda, Maryland, U.S.A., under the Developmental Therapeutic Program DTP. The cell line panel engaged a total of 60 different human tumor cell lines derived from nine cancer types, including lung, colon, melanoma, renal, ovarian, brain, leukemia, breast, and prostate. The target compounds **6a-f** were assigned with the NCI codes (see Table 1), respectively Primary *in vitro* one dose anticancer screening was initiated, in which the full NCI 60 panel lines were inoculated onto a series of standard 96-well microtiter plates on day 0 at 5000–40,00 cells/well in RPMI 1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine, and then preincubated in absence of drug at 37 °C, and 5 % CO_2 for 24 h. Test compounds were then added at one concentration of 10^{-5} M in all 60 cell lines, and incubated for a further 48 h at the same incubation conditions. Following this, the media were removed, the cells were fixed *in situ*, washed, and dried. The sulforhodamine B assay is used for cell density determination, based on the measurement of cellular protein content. After an incubation period, cell monolayers are fixed with 10 % (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye is removed by washing repeatedly with 1 % (vol/vol) acetic acid. The bound stain was resolubilized in 10 mM Tris base solution and measured spectrophotometrically on automated microplate readers for OD determination at 510 nm.

4.4.2. Five Doses Full NCI 60 Cell Panel Assay. All the 60 cell lines, representing nine cancer subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 and 10 μM) of the tested compounds. The outcomes were used to create $\log_{10}$ concentration *versus* percentage growth inhibition curves and three response parameters (GI_{50}, total growth inhibition (TGI) and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50 % decrease in net cell growth. The TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition. The LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50 % loss of initial cells at the end of the incubation period of 48 h. The three dose response parameters GI_{50}, TGI and LC_{50} were calculated for each experimental compound. Data calculations were made according to the method described by the NCI Development Therapeutics Program (https://dtp.cancer.gov/discovery_development/nci-60/default.htm).

The % growth curve is calculated as:
$[(T - T_0) / (C - T_0)] \times 100$,
where
T_0 is the cell count at day 0,
C is the vehicle control (without drug) cell count (the absorbance of the SRB of the control growth),

T is the cell count at the test concentration at day 3.

The GI_{50} and TGI value are determined as the drug concentration that results in a 50 % and 0 % growth at 48 hr drug exposure. Growth inhibition of 50 % (GI_{50}) is calculated from:
$[(T - T_0) / (C - T_0)] \times 100 = 50$.

The TGI is the concentration of test drug where:
$100 \times (T - T_0) / (C - T_0) = 0$.
Thus, the TGI signifies a cytostatic effect.

The LC_{50}, which signifies a cytotoxic effect, is calculated as:
$[(T - T_0) / T_0] \times 100 = -50$, when $T < T_0$.

References

1 Tréfouël J., Tréfouël T., Nitti F., & Bovet D. (**1935**) Activité du *p*-aminophénylsulfamide sur l'infection streptococcique expérimentale de la souris et du lapin. *C. R. Soc. Biol.*, *120*, 756-758.
2 Kachaeva M. V., Pilyo S. G., Demydchuk B. A., Prokopenko V. M., Zhirnov, V. V., & Brovarets, V. S. (**2018**) 4-Cyano-1,3-oxazole-5-sulfonamides as Novel Promising Anticancer Lead Compounds. *Intern. J. Current Res.*, *10*(5) 69410-69425.
3 Kachaeva M. V., Hodyna D. M., Semenyuta I. V., Pilyo S. G., Prokopenko V. M., Kovalishyn V. V., Metelytsia L. O., & Brovarets V. S. (**2018**) Design, synthesis and evaluation of novel sulfonamides as potential anticancer agents. *Comput. Biol. Chem.*, *74*, 294-303.
4 Zyabrev V. S., Babiy S. B, Turov K. V., Vasilenko O. M., Vinogradova T. K., & Brovarets, V. S. (**2015**) 2,4-Disulfonyl-5-cycloamino substituted thiazoles and their using as anticancer drugs. Patent 109165 UA (in Ukrainian).
5 Aspatwar A., Becker H. M., Parvathaneni N. K., Hammaren M., Svorjova A. Barker H., Supuran C. T., Dubois L., Lambin P., Parikka M., Parkkila S., & Winum J. Y. (**2018**) Nitroimidazole-based inhibitors DTP338 and DTP348 are safe for zebrafish embryos and efficiently inhibit the activity of human CA IX in Xenopus oocytes. *J. Enzyme Inhib. Med. Chem.*, *33*(1) 1064-1073.
6 Matsumura K., Saraie T., & Hashimoto N. (**1976**) Studies of Nitriles. VII. Synthesis and Properties of 2-Amino-3,3-dichloroacrylonitrile (ADAN). *Chem. Pharm. Bull.*, *24*(5) 912-923.
7 Matsumura K., Saraie T., & Hashimoto N. (**1976**) Studies of Nitriles. VIII. Reaction of N-Acyl Derivatives of 2-Amino-3,3-dichloroacrylonitrile (ADAN) with Amines. (1). A New Synthesis of 2-Substituted-5-(substituted amino)-oxazole-4-carbonitriles and -4-N-acylcarbocamides. *Chem. Pharm. Bull.*, *24*(5) 924-940.
8 Matsumura K., Saraie T., & Hashimoto N. (**1976**) Studies of Nitriles. XI. Preparation and chemistry of Schiff Bases of ADAN, 2-Amino-3,3-dichloroacrylonitrile. A highly Effective Conversion into 2-Substituted-4(5)-chloro-imidazole-5(4)-carbaldehydes. *Chem. Pharm. Bull.*, 24 (5) 960-969.
9 Kimura H., Yukitake H., Tajima Y., Suzuki H., Chikatsu T., Morimoto S., Funabashi Y., Omae H., Ito T., Yoneda Y., & Takizawa M. (**2010**) ITZ-1, a Client-Selective Hsp90 Inhibitor, Efficiently Induces Heat Shock Factor 1 Activation. *Chem. Biol*., 17 (1) 18-27.
10 Sheldrick G. (**2008**) A short history of SHELX. *Acta Cryst., Sect. A.,* 64 (Pt 1) 112-122.