Chemistry Research Journal, 2018, 3(5):81-93

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Synthesis, Characterization, and *In Vitro* Anticancer Evaluation of 7-(1,4-Diazepan)-substituted [1,3]oxazolo[4,5-*d*]pyrimidines

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Abstract A novel series of five 7-(1,4-diazepan)-substituted [1,3]oxazolo[4,5-*d*]pyrimidines have been synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, elemental analysis and chromato-mass-spectrometry. The anticancer activities of the all the newly synthesized compounds were valuated via single high dose (10 μM) against 60 cancer cell lines by the National Cancer Institute according to its own screening protocol. In the next phase, the compounds have been selected for five-dose assay. Among these compounds 7-(1,4-diazepan)-1-yl-5-(4-methylphenyl)-2-phenyl[1,3]oxazolo-[4,5-*d*]-pyrimidine and 7-(1,4-diazepan)-1-yl-2-(4-methylphenyl)-5-phenyl[1,3]oxazolo[4,5-*d*]pyrimidine displayed the most growth inhibitory (GI_{50} was in range of 0.9-1.9 μM), cytostatic (TGI – 2.1-3.6 μM) and cytotoxic (LC_{50} – 5.9-7.4 μM) activities against tested cancer subpanels. These results provided evidence that compound could be useful for developing new anticancer drugs.

Keywords [1,3]Oxazolo[4,5-*d*]pyrimidines,Anticancer activity,Growth inhibitory, Cytostaticactivity, Cytotoxic activity, Selectivity

Introduction

Cancer is the second leading cause of death after cardiovascular diseases worldwide [1]. The etiology of major cancers is still largely unknown and there is a need for more effective and less toxic chemotherapeutic agents.

1,4-Diazepine and 1,4-diazepane derivatives are the seven membered, nitrogen containing heterocyclic ring systems possessing a wide range of therapeutic application. These structures are used for the synthesis of various drug molecules with wide spread biological activities including antitumor action [2,3]. It is a coreelement in the structure of benzodiazepines that exhibited cytotoxic activities against tumor cells through binding DNA, cleaving DNA, cross-linking DNA, or depleting the endogenous ATP levels of the tumor cells [4].Oxazolo[4,5-*d*]pyrimidine derivatives are associated with diverse biological activities [5] including antitumor one [6,7].

Synthesis of fused molecules is an essential component in the search for new leads in anticancer drug design. In the current study, we have used fragment linking and structure based approaches for the design of diazepane substituted [1,3]oxazolo[4,5-*d*]pyrimidines. The synthesized compounds were screened for their anticancer activities against full NCI 60 cell line panel.

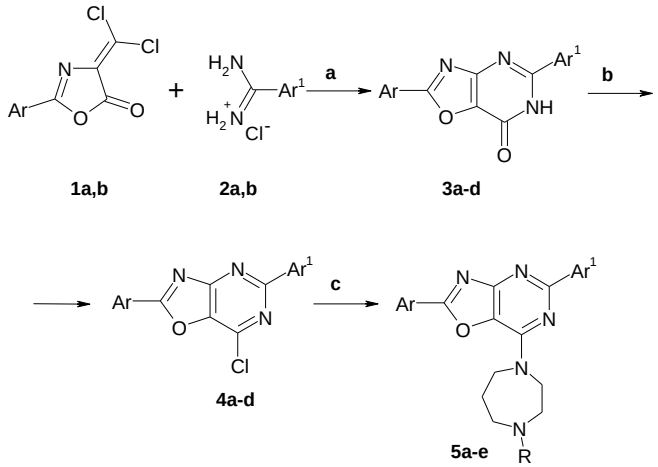
Results and Discussion

Chemistry

The synthesis of new 7-(1,4-diazepan)-substituted [1,3]oxazolo[4,5-*d*]pyrimidines**5a-e** depicted in Figure 1 was carried out by the route described previously [8]. Compounds **5a-e** are obtained by the sequence of reactions starting

from available 2-aryl-4-dichloromethylene-1,3-oxazol-5(4*H*)-ones **1a,b** [9]. Treating of **1a,b** with arylamidine hydrochlorides **2a,b** in the presence of triethylamine followed by heating with pyridine afforded the cyclocondensation products [1,3]oxazolo[4,5-*d*]pyrimidines**3a-d**. ^{1}H NMR of **3a-d** showed the presense of NH at 12.82-13.06 ppm.

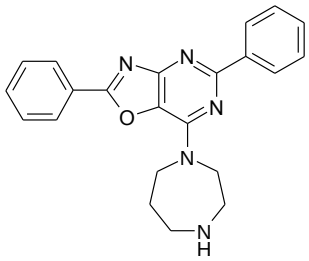
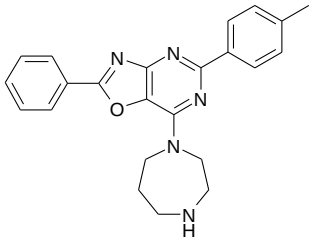
The reaction of compounds **3a-d** with trichlorophosphate in the presence *N,N*-dimethylaniline proceeded 2,5-diaryl-7-chloro-[1,3]oxazolo[4,5-*d*]pyrimidines**4a-d**. Compounds **4a-d** were converted into the corresponding 7-(1,4-diazepan)-substituted [1,3]oxazolo[4,5-*d*]pyrimidines**5a-e** (Table 1) by reaction with 1,4-diazepane or 1-methyl-1,4-diazepane. Structures of synthesized compounds were confirmed by the IR, ^{1}H and ^{13}C NMR, and LC-MS spectra. IR spectra of compounds **5a-d** showed the presence of NH and aryl absorption bands in the range 3362-2622 cm^{-1}.

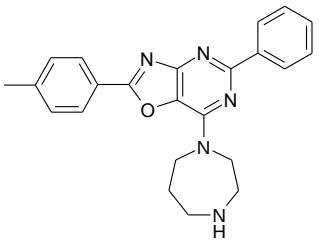
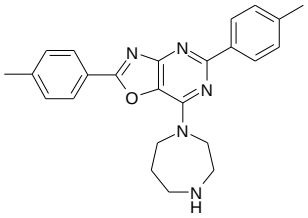
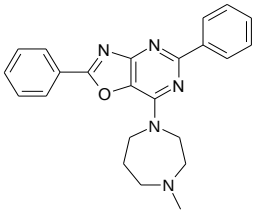


Ar = Ph (**1a**, **3a,b, 4a,b, 5a,b,e**), 4-MeC$_6$H$_4$ (**1b, 3c,d, 4c,d, 5c,d**);
Ar^1 = Ph (**2a, 3a,c, 4a,c, 5a,c,e**), 4-MeC$_6$H$_4$ (**2b, 3b,d, 4b,d, 5b,d**);
R = H (**5a-d**), Me (**5e**)

*Figure 1. Synthesis of 7-(1,4-diazepan)-substituted [1,3]-oxazolo[4,5-d]pyrimidines**5a-e**.Reagents and conditions: (a) TEA, THF, r.t., 72 h; Py, reflux, 10h; (b) $POCl_3$, Me_2NPh, reflux, 3 h; (c) 1,4-diazepane or 1-methyl-1,4-diazepane, TEA, dioxane, reflux, 6 h*

Table 1: Chemical structures of compounds5a-e

№	Mr	Chemical structure	Chemical name
5a	371.45		7-(1,4-diazepan-1-yl)-2,5-diphenyl-[1,3]oxazolo[4,5-*d*]pyrimidine
5b	385.47		7-(1,4-diazepan-1-yl)-5-(4-methylphenyl)-2-phenyl[1,3]-oxazolo[4,5-*d*]pyrimidine

5c	385.47		7-(1,4-diazepan-1-yl)-2-(4-methylphenyl)-5-phenyl[1,3]-oxazolo[4,5-*d*]pyrimidine
5d	401.47		7-(1,4-diazepan-1-yl)-2,5-bis(4-methylphenyl)[1,3]oxazolo[4,5-*d*]-pyrimidine
5e	385.47		7-(4-methyl-1,4-diazepan-1-yl)-2,5-diphenyl[1,3]oxazolo[4,5-*d*]-pyrimidine

Biological Evaluation

The tumor growth inhibition properties of the synthesized compounds were screened on human cancer cell lines at the NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI, for one dose anti-cancer assay. Results for each compound at a single dose concentration of 10 μM were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. The synthesized compounds showed a distinctive sensitivity against individual cell lines (Table 2).

Table 2: One dose human tumor cell lines anticancer screening data of synthesized compounds **5a-e**

Panel/cell line	**Growth percent in one-dose assay**				
	5a	**5b**	**5c**	**5d**	**5e**
			Leukemia		
CCRF-CEM	-21.47	-70.95	-75.75	-72.07	-25.18
HL-60(TB)	-62.17	-69.68	-69.81	-69.26	-62.47
K-562	-45.31	-63.56	-76.42	-76.03	-42.98
MOLT-4	-34.21	-73.19	-68.12	-66.04	-39.32
RPMI-8226	-19.40	-49.05	-55.00	-51.03	-27.25
SR	-0.07	-42.34	-49.71	-44.51	-2.79
			Non-Small Cell Lung Cancer		
A549/ATCC	-44.34	-87.85	-66.48	-62.34	-29.33
EKVX	58.24	38.24	39.00	115.55	101.07
HOP-62	9.82	-55.35	-27.07	71.83	-37.25
HOP-92	NT	-27.15	-33.51	60.92	NT
NCI-H226	103.22	93.14	88.91	100.53	90.76
NCI-H23	27.06	9.93	19.36	91.13	NT
NCI-H322M	NT	-70.44	-6.90	16.53	NT
NCI-H460	-50.29	-67.88	-57.71	-37.46	-58.72
NCI-H522	-64.06	-74.56	-69.62	64.96	-76.21

		Colon Cancer			
COLO 205	NT	-80.91	-86.92	-71.55	NT
HCC-2998	0.93	-92.11	-74.85	40.88	3.17
HCT-116	-92.61	-79.19	-71.40	27.49	-100.00
HCT-15	12.72	-85.07	-81.53	-72.91	17.63
HT29	-76.50	-82.81	-86.12	-87.13	-90.96
KM12	NT	-81.53	-92.34	11.30	NT
SW-620	-70.27	-73.27	-57.48	-49.67	-41.96
		CNS Cancer			
SF-268	-5.20	-42.87	-26.33	90.16	-43.93
SF-295	-56.30	-89.63	-87.28	108.38	-60.86
SF-539	-92.83	-91.38	-88.53	73.80	NT
SNB-19	36.98	8.75	25.94	58.24	35.96
SNB-75	-91.46	-67.33	-59.77	97.69	-96.76
U251	-78.83	-85.87	-84.94	-92.36	-82.91
		Melanoma			
LOX IMVI	-68.05	-91.55	-89.85	-79.32	-79.21
MALME-3M	-65.31	-92.36	-85.51	106.34	-64.74
M14	-79.07	-94.66	-93.86	-94.73	-93.50
MDA-MB-435	-44.52	-87.90	-83.11	70.82	-50.53
SK-MEL-2	NT	-67.21	-2.85	104.74	NT
SK-MEL-28	-64.75	-93.35	-89.26	89.84	-86.24
SK-MEL-5	16.44	-93.41	-57.29	88.11	58.71
UACC-257	90.66	-82.19	4.04	101.03	94.56
UACC-62	-84.33	-91.82	-81.36	92.04	74.33
		Ovarian Cancer			
IGROV1	8.78	-85.33	-73.84	12.23	14.80
OVCAR-3	-74.31	-85.66	-81.75	-67.67	-65.04
OVCAR-4	-58.21	-62.56	-52.28	2.20	2.83
OVCAR-5	47.27	-87.33	-32.36	78.54	-21.73
OVCAR-8	-56.81	-44.07	-48.44	-58.57	-52.87
NCI/ADR-RES	-13.54	-45.50	-29.78	16.35	-1.93
SK-OV-3	-13.38	95.46	79.88	98.06	91.21
		Renal Cancer			
786-0	NT	-89.79	-97.08	-96.16	
A498	53.65	93.38	98.38	100.93	51.38
ACHN	-79.81	-98.78	-98.87	-77.10	67.83
CAKI-1	90.69	-71.02	-9.04	107.96	85.25
RXF 393	-72.74	-74.29	-73.20	80.64	-85.90
SN12C	-1.76	-85.07	-87.89	59.07	-74.38
TK-10	-63.82	-95.87	-60.04	-1.53	NT
UO-31	-51.63	-99.04	-98.14	62.61	-51.06
		Prostate Cancer			
PC-3	28.60	-88.74	-88.86	-89.54	28.28
DU-145	NT	-15.18	-25.48	42.00	NT
		Breast Cancer			
MCF7	-32.05	-74.72	-78.43	-64.80	-28.75

MDA-MB-231/ATCC	-79.75	-91.70	-86.88	-78.77	-80.51
HS 578T	-31.06	-29.81	-18.08	110.44	76.30
BT-549	-25.50	81.18	18.04	90.20	81.53
T-47D	-22.91	-67.49	-44.99	-46.03	-57.62
MDA-MB-468	-70.7	-76.21	-67.54	5.40	-84.62
Mean	-27.82	-59.11	-50.27	15.71	-18.44
Range	196.05	194.50	197.25	211.71	201.07

NT – not tested

The compounds added at a concentration $1 \cdot 10^{-5}$ M and the culture incubated for 48 h. The number reported for the One-dose assay is growth relative to the no-drug control, and relative to the time zero number of cells. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). A value of 100 means no growth inhibition and one >100 growth stimulation. A value of 40 means 60% growth inhibition. A value of 0 means no net growth over the course of the experiment. A value of -40 would mean 40% lethality. A value of -100 means all cells are dead. NT: not tested

By investigating the variation in growth inhibition of the tested compounds over the full panel of cell lines, it was revealed that most of 7-(1,4-diazepan)-substituted [1,3]oxazolo[4,5-*d*]pyrimidines showed significant inhibition for the most cell lines used in one dose assay with exeption of compound **5d** (Table 2). Exceptions for individual compounds were EKVX, NCI-H226, UACC-257, A498 and CAKI-1 (**5a**), NCI-H226, SK-OV-3, A498 and BT-549 (**5b**), NCI-H226 and A498 (**5c**), EKVX, NCI-H226, SK MEL-5, UACC-257, UACC-62 (**5e**) cancer cell lines with growth inhibition less than 50%. Compound **5d** demonstrated slight growth inhibition of Non-Small Cell Lung Cancer, CNS Cancer, Melanoma and Renal Cancer subpanels (Table 2). Moreover, these compounds showed significant cytotoxicity against all tested cell lines of Leukemia subpanel, and compound **5c** induced lethality more than 60% of the tested cell lines.

All synthesized compounds satisfied the pre-determined threshold inhibition criteria of the NCI 60 One-Dose Screening were tested against the panels of 60 cancer cell lines of NCI. Table 3 represents the results of the five-dose assay for anticancer activity of these compounds against each cancer cell line.

Table 3: Parameter values of the anticancer activity (GI_{50}, TGI and LC_{50}) of the synthesized compounds **5a-e** against the NCI 60 human cancer cell lines (five-dose assay)

Cell Line	**Compound**														
	5a			**5b**			**5c**			**5d**			**5e**		
	GI_{50}	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**
Leukemia															
CCRF-CEM	NT	NT	NT	1.31	3.13	7.49	1.89	4.23	9.45	1.87	4.25	NT	1.96	6.25	>100
HL-60(TB)	2.16	4.01	7.45	1.35	3.17	7.42	0.73	2.49	7.51	0.18	0.37	0.77	2.19	4.12	7.77
K-562	1.09	4.10	52.9	0.99	2.97	NT	0.44	1.86	6.95	0.95	2.68	7.35	0.38	1.77	16.8
MOLT-4	1.83	3.63	7.16	1.79	3.58	7.16	1.44	3.15	6.90	1.77	3.59	7.31	2.49	5.25	37.6
RPMI-8226	NT	NT	NT	NT	NT	NT	1.96	4.19	NT	1.89	4.22	9.45	NT	NT	NT
SR	0.34	1.09	NT	0.24	0.62	4.31	0.22	0.55	3.93	0.21	0.58	4.33	2.29	6.96	>100
Non-Small Cell Lung Cancer															
A549/ATCC	1.82	3.50	6.76	1.71	3.12	5.69	1.65	3.06	5.67	1.72	3.13	5.69	1.71	NT	NT
EKVX	1.48	3.09	6.44	1.81	3.33	6.14	1.75	3.29	6.18	4.09	>100	>100	1.59	3.23	6.57
HOP-62	2.08	3.57	6.14	1.84	3.36	6.13	1.81	3.36	6.24	8.78	24.3	62.3	1.69	3.24	6.20
HOP-92	NT	NT	NT	NT	NT	8.65	1.44	3.01	6.27	1.01	2.30	5.21	1.22	2.56	5.39

NCI-H226	1.91	3.48	6.33	1.82	3.97	7.06	1.87	3.86	7.98	1.97	4.37	NT	1.88	3.53	6.62
NCI-H23	1.76	3.62	7.46	1.95	3.71	5.56	1.89	3.80	7.66	19.9	88.2	>100	1.64	3.36	6.90
NCI-H322M	NT	NT	NT	1.71	3.08	6.63	1.81	3.20	5.68	1.69	3.06	5.53	1.80	3.52	6.91
NCI-H460	1.82	3.44	6.52	1.85	3.50	6.28	1.86	3.53	6.69	1.89	3.43	6.20	1.90	3.76	NT
NCI-H522	1.97	3.91	7.78	1.72	3.29	NT	1.73	3.39	6.64	1.75	3.51	7.03	NT	NT	NT
Colon Cancer															
COLO 205	1.84	3.23	5.68	1.89	3.50	6.50	1.96	3.66	6.81	1.89	3.55	6.67	1.63	3.06	5.73
HCC-2998	1.87	3.40	6.20	1.82	3.27	5.89	2.04	4.17	8.50	32.3	>100	>100	1.78	3.50	6.88
HCT-116	1.44	2.75	5.27	0.28	0.78	NT	1.31	2.65	5.36	1.57	2.92	5.40	1.51	2.90	5.55
HCT-15	1.72	3.34	6.52	1.40	2.77	5.46	1.58	3.23	6.60	1.49	2.96	5.88	1.91	NT	NT
HT29	2.00	4.16	8.68	1.69	3.15	5.88	1.60	3.08	5.95	1.49	2.85	5.46	1.89	NT	NT
KM12	1.83	3.44	6.43	1.89	3.45	6.28	1.77	3.41	6.60	1.72	3.20	5.96	1.86	3.36	6.07
SW-620	1.37	2.76	5.58	1.68	3.21	6.14	1.55	3.17	6.50	1.66	3.20	6.14	1.63	3.30	6.68
CNS Cancer															
SF-268	1.86	3.77	7.62	1.83	3.66	7.33	1.74	3.63	7.58	1.77	3.48	6.84	1.80	3.43	6.55
SF-295	1.78	3.38	6.43	1.93	3.43	6.08	1.77	3.33	6.24	1.86	3.54	6.74	1.83	3.56	6.92
SF-539	1.84	3.33	6.02	1.79	3.26	5.93	1.82	3.31	6.02	1.76	3.29	6.14	1.76	3.30	NT
SNB-19	1.83	4.33	11.1	1.82	3.45	6.55	1.63	3.11	5.95	1.77	3.25	5.97	3.04	14.0	63.9
SNB-75	1.26	2.54	5.10	1.41	2.72	5.28	1.36	2.68	5.28	1.66	3.02	5.50	1.24	2.58	5.35
U251	NT	NT	NT	1.78	3.17	5.67	1.54	2.91	5.47	1.76	3.18	5.71	NT	NT	NT
Melanoma															
LOX IMVI	1.65	3.24	6.35	1.71	3.20	5.98	1.76	3.29	6.16	1.86	3.46	6.43	1.60	3.41	NT
MALME-3M	1.87	3.58	6.85	1.93	3.48	6.27	1.85	3.38	6.17	1.98	3.61	6.58	2.03	3.64	6.54
M14	1.97	3.58	6.51	1.76	3.29	6.14	1.96	3.69	6.93	1.85	3.55	6.80	1.96	3.64	NT
MDA-MB-435	1.73	3.20	5.93	1.77	3.19	5.75	1.93	3.42	6.08	1.78	3.17	5.63	1.85	3.63	NT
SK-MEL-2	2.29	4.49	8.80	2.03	3.77	6.99	2.11	3.84	7.01	21.3	45.4	96.9	3.18	7.82	>100
SK-MEL-28	1.67	3.09	5.72	1.85	3.39	6.22	1.84	3.28	5.87	1.93	3.51	6.42	1.70	3.21	6.06
SK-MEL-5	1.67	3.07	5.65	1.60	3.03	5.74	NT	NT	NT	NT	NT	NT	1.62	2.99	5.52
UACC-257	1.76	3.36	6.42	1.96	3.48	6.16	1.72	3.26	6.20	19.8	41.1	85.0	1.77	3.46	NT
UACC-62	1.78	3.24	5.90	1.71	3.30	6.36	1.68	3.14	5.89	1.67	3.08	5.67	1.72	3.27	6.21
Ovarian Cancer															
IGROV1	2.08	4.03	7.81	1.67	3.24	6.27	1.74	3.39	6.63	1.62	3.19	6.29	2.09	3.98	7.60
OVCAR-3	1.96	3.55	6.43	1.83	3.37	6.24	1.84	3.31	5.99	1.73	3.10	5.57	1.81	3.32	6.09
OVCAR-4	1.63	3.03	5.61	1.82	3.55	6.96	1.59	3.09	6.01	2.11	4.67	11.5	1.66	3.19	6.16
OVCAR-5	1.88	3.39	6.12	1.90	3.38	6.01	1.89	3.37	5.99	1.75	3.17	5.75	2.03	3.69	6.70
OVCAR-8	2.20	4.80	13.0	2.09	4.38	NT	2.06	4.10	8.20	2.11	4.22	8.42	1.92	4.69	>100
NCI/ADR-RES	2.17	5.16	23.6	2.06	4.07	NT	2.17	4.71	12.1	1.96	3.74	7.16	2.14	5.07	>100
SK-OV-3	1.90	3.31	5.75	1.89	3.31	5.78	1.81	3.27	5.89	17.8	31.6	56.3	1.91	3.39	6.02

Renal Cancer															
786-0	1.81	3.33	6.14	1.43	2.81	5.52	1.52	2.97	5.80	1.76	3.25	6.01	1.88	3.53	6.62
A498	1.23	2.57	5.39	1.66	3.16	5.98	1.53	3.13	6.39	15.8	31.7	63.8	1.42	2.90	5.92
ACHN	1.99	3.50	6.16	1.79	3.22	5.80	1.73	3.12	5.64	1.75	3.13	5.60	1.65	3.12	NT
CAKI-1	1.70	3.29	6.38	1.65	3.03	5.56	1.73	3.22	5.98	1.56	3.02	5.83	1.50	3.20	NT
RXF 393	1.62	3.04	5.72	1.75	3.29	6.16	1.69	3.32	6.52	1.65	3.14	6.00	1.62	3.03	5.69
SN12C	1.78	3.36	6.34	1.69	3.21	6.09	1.56	3.06	6.02	1.61	3.06	5.84	1.62	3.15	6.11
TK-10	2.00	3.53	6.23	1.80	3.20	5.70	1.73	3.14	5.71	2.08	3.96	7.53	2.08	3.67	6.47
UO-31	1.57	3.05	5.92	1.49	2.85	5.45	1.55	2.96	5.64	1.38	2.68	5.17	1.49	2.90	5.64
Prostate Cancer															
PC-3				1.57	3.00	5.72	1.69	3.17	5.95	1.51	2.91	5.63	2.14	5.40	>100
DU-145	1.90	3.46	6.32	1.89	3.40	6.12	1.82	3.30	5.99	1.89	3.30	5.74	1.83	3.37	6.22
Breast Cancer															
MCF7	1.41	2.83	5.69	1.59	3.44	7.45	1.48	3.50	8.29	1.85	3.88	8.14	1.55	3.04	5.95
MDA-MB-231/ATCC	1.56	2.97	5.65	1.77	3.27	6.06	1.81	3.32	6.10	1.69	3.11	5.73	1.50	3.02	6.08
HS 578T	1.86	4.13	9.16	1.94	4.22	9.21	1.83	4.02	8.85	12.6	31.9	80.9	1.63	3.88	NT
BT-549	1.73	3.18	5.86	1.62	3.14	NT	1.63	3.19	6.25	16.3	35.0	75.2	1.73	3.18	5.83
T-47D	1.69	3.48	7.15	2.02	4.00	7.94	1.72	3.50	7.10	1.94	4.17	8.96	2.00	3.96	NT
MDA-MB-468	1.48	2.87	5.54	1.55	3.24	6.75	1.59	3.35	7.04	1.96	4.39	NT	1.69	3.08	5.59

Compound **5a** showed GI_{50} values ranging from 0.3 (Leukemia SR cell line) to 2.3 μM (Melanoma SK-MEL-2 cell line), TGI – from 1.1 (Leukemia SR cell line) to 5.2 μM (Ovarian Cancer NCI/ADR-RES cell line), and LC_{50} – from 5.1 (CNS Cancer SNB cell line) to 52.9 μM (Leukemia K-562 cell line).

Compound **5b** showed GI_{50} values ranging from 0.2 (Leukemia SR cell line) to 2.1 μM (Ovarian Cancer OVCAR-8 and NCI/ADR RES cell lines), TGI – from 0.8 (Leukemia SR cell line) to 4.4 μM (Ovarian Cancer OVCAR-8 cell line), and LC_{50} – from 4.3 (Leukemia SR cell line) to 9.2 μM (Breast Cancer HS 578T cell line).

Compound **5c** showed GI_{50} values ranging from 0.2 (Leukemia HL-60(TB) and SR cell lines) to 2.2 μM (Ovarian Cancer NCI/ADR-RES cell line), TGI – from 0.6 (Leukemia SR cell line) to 4.7 μM (Ovarian Cancer NCI/ADR-RES cell line), and LC_{50} – from 3.9 (Leukemia SR cell line) to 12.1 μM (Ovarian Cancer NCI/ADR-RES cell line), with the exception of Leukemia CCRF CEM cell line (>100 μM).

Compound **5d** showed GI_{50} values ranging from 0.2 (Leukemia SR cell line) to 32.3 μM (Colon Cancer HCC-2998 cell line), TGI – from 0.4 (Leukemia HL-60(TB) cell line) to 88.2 μM (Lung Cancer NCI-H23 cell line), and LC_{50} – from 0.8 (Leukemia HL 60(TB) cell line) to 96.9 μM (Melanoma SK-MEL-2 cell line). LC_{50} of this compound for cancer cell lines of HCC-2998 (colon), EKVX and NCI-H23 (lung) exceeded 100 μM. TGI for EKVX (lung) and HCC-2998 (colon) cell lines was also more than 100 μM.

Compound **5e** showed GI_{50} values ranging from 0.4 (Leukemia HL-60(TB) cell line) to 3.2 μM (Melanoma SK-MEL-2 cell line). Level of TGI was changed from 1.8 (Leukemia HL-60(TB) cell line) to 14.0 μM (CNS Cancer SNB-19 cell line). Value of LC_{50} was changed from 5.4 (CNS Cancer SNB-75 and Lung Cancer HOP-92 cell lines) to 63.9 (CNS Cancer SNB-19 cell line) with the exception of cancer lines with GI_{50}> 100 μM.

The order of decreasing antitumor activity of tested compounds (GI_{50}, TGI and LC_{50}) is: **5b** ≈ **5c**>**5a** ≈ **5e**>**5d**. Compounds **5b** and **5c** exhibited the highest activity towards all tested cancer subpanels with the most selectivity to Leukemia subpanel (Table 4).

Table 4: Antitumor activity of compounds 5a-e against the particular cancer subpanels: median growth inhibitory (GI_{50}, μM), total growth inhibitory (TGI, μM), median lethal (LC_{50}, μM), and selectivity index of anticancer activity of compounds

Indices	Leukemia	Non-Small Cell Lung Cancer	Colon Cancer	CNS Cancer	Mela-noma	Ovarian Cancer	Renal Cancer	Prostate Cancer	Breast Cancer	MG-MID
Compound **5a**										
GI_{50}	1.10	1.82	1.70	1.70	1.82	1.96	1.70	1.91	1.62	1.70
SI_{GI50}	1.6	0.9	1.0	1.0	0.9	0.9	1.0	0.9	1.1	
TGI	2.82	3.47	3.24	3.39	3.39	3.80	3.16	3.47	3.24	3.39
SI_{TGI}	1.2	1.0	1.1	1.0	1.0	0.9	1.1	1.0	1.1	
LC_{50}	14.13	6.61	6.31	7.08	6.46	8.51	6.03	6.31	6.31	6.92
SI_{LC50}	0.5	1.1	1.1	1.0	1.1	0.8	1.2	1.1	1.1	
Compound **5b**										
GI_{50}	0.87	1.78	1.32	1.74	1.82	1.91	1.66	1.74	1.74	1.62
SI_{GI50}	1.9	0.9	1.2	0.9	0.9	0.9	1.0	0.9	0.9	
TGI	2.14	3.39	2.63	3.24	3.31	3.63	3.09	3.24	3.55	3.09
SI_{TGI}	1.4	0.9	1.2	1.0	0.9	0.9	1.0	1.0	0.9	
LC_{50}	6.03	6.46	6.03	6.03	6.17	6.17	5.75	5.89	7.41	6.17
SI_{LC50}	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.1	0.8	
Compound **5c**										
GI_{50}	0.85	1.74	1.66	1.62	1.82	1.86	1.62	1.74	1.66	1.62
SI_{GI50}	1.9	0.9	1.0	1.0	0.9	0.9	1.0	0.9	1.0	
TGI	2.30	3.39	3.31	3.16	3.39	3.55	3.09	3.24	3.47	3.24
SI_{TGI}	1.4	1.0	1.0	1.0	1.0	0.9	1.1	1.0	0.9	
LC_{50}	6.76	6.46	6.61	6.03	6.31	7.08	5.89	5.89	7.24	6.46
SI_{LC50}	1.0	1.0	1.0	1.1	1.0	0.9	1.1	1.1	0.9	
Compound **5d**										
GI_{50}	0.78	2.88	2.51	1.78	3.34	2.57	2.24	1.70	3.63	2.29
SI_{GI50}	2.9	0.8	0.9	1.29	0.7	0.9	1.0	1.4	0.6	
TGI	1.82	8.51	5.13	3.31	6.46	4.90	4.27	3.09	7.94	4.90
SI_{TGI}	2.7	0.6	1.0	1.5	0.8	1.0	1.2	1.6	0.6	
LC_{50}	4.47	16.22	8.91	6.17	12.30	9.77	7.94	5.62	19.06	9.55
SI_{LC50}	2.1	0.6	1.1	1.6	0.8	1.0	1.2	1.7	0.5	
Compound **5e**										
GI_{50}	1.55	1.66	1.74	1.82	1.91	1.95	1.66	1.95	1.66	1.74
SI_{GI50}	1.1	1.1	1.0	1.0	0.9	0.9	1.1	0.9	1.1	

TGI	4.47	3.31	3.24	4.27	3.72	3.80	3.16	4.27	3.31	3.63
SI_{TGI}	0.8	1.1	1.1	0.9	1.0	0.9	1.2	0.9	1.1	
LC_{50}	34.67	6.46	6.17	11.22	10.71	14.13	6.03	24.55	5.89	10.23
SI_{LC50}	0.3	1.6	1.7	0.9	1.0	0.7	1.7	0.4	1.7	

The present human tumor cell line *in vitro* screen provides preliminary data of anticancer activity of new compounds. This method is susceptible to false-positive and false-negative results. Cell culture studies are of limited usefulness because they do not reflect the heterogeneity of clinical malignant disease. It is clear that factors other than the chemosensitivity of tumor cells *in vitro* significantly influence the efficiency of chemotherapy *in vivo* (e.g., tumor microenvironment [10], lacking of specific targeting ability [11], bacterial presence in the body [12]). Such factors are not represented by the *in vitro* cell line screening assay. It is appreciated that this assay was designed only to select compounds for a secondary, more comprehensive, *in vivo* testing.

Experimental

Chemistry

All the chemicals and solvents used for the synthesis work acquired from commercial sources, were of analytical grade, and used without further purification. Melting points were measured on a Fisher-Johns apparatus. IR spectra were recorded on a Vertex-70 spectrometer from KBr pellets. ^{1}H NMR spectra were recorded on a Varian VXR-300 spectrometer (300 MHz), Varian Mercury 400 (400 MHz) or BrukerAvance DRX 500 (500 MHz) spectrometers in DMSO-d_6 or $CF_3C(O)OD$. ^{13}C NMR spectra were obtained on a BrukerAvance DRX 500 (150 MHz) spectrometer in DMSO-d_6 or $CF_3C(O)OD$. LC-MS analysis was performed on an Agilent 1200 Series system equipped with a diode array and a G6130A mass-spectrometer (atmospheric pressure electrospray ionization). Combustion elemental analysis was made in the Institute of Bioorganic Chemistry and Petrochemistry analytical laboratory.

General procedure for the synthesis of 2,5-diaryl[1,3]oxazolo[4,5-*d*]pyrimidin-7(6*H*)-ones 3a-d

To a solution of 1,3-oxazol-5(4*H*)-one **1a,b** [9] (40 mmol) in dry THF (100 mL) amidine hydrochloride **2** (40 mmol) was added followed by Et_3N (5.74 mL, 41 mmol). The mixture was stirred at r.t. for 72 h. The precipitate formed was filtered off, washed with H_2O, dried, dissolved in pyridine (60 ml) and refluxed for 10 h. The solvent was removed in vacuo. The residue was treated with H_2O, filtered off, dried, and recrystallized from DMF.

2,5-Diphenyl[1,3]oxazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3a) has been described in the literature [8].

5-(4-Methylphenyl)-2-phenyl[1,3]oxazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3b)

Color: Light yellow solid; Yield 84%; m.p.: 327-328°C; IR (KBr, cm^{-1}): 3344-2745 (NH, ArCH), 1693 (C=O), 1537, 1516, 1485, 1339, 918, 825, 776, 716, 685; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 12.82 (br s, 1H, NH), 8.22-8.07 (m, 4H, ArH), 7.73-7.66 (m, 3H, ArH), 7.40 (d, J = 7.0 Hz, 2H, ArH), 2.40 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm) 163.9, 157.9, 155.0, 151.3, 140.8, 131.8, 128.6, 128.5, 128.2, 127.1, 126.7, 126.6, 20.2; MS, m/z: 304 $[M+1]^+$. Anal. Calcd.for $C_{24}H_{25}N_5O$: C, 72.16; H, 6.31; N, 17.53. Found: 72.14; H, 6.28; N, 17.63%.

2-(4-Methylphenyl)-5-phenyl[1,3]oxazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3c)

Color: Light yellow solid; Yield 82%; m.p. 343-345 °C; IR (KBr, cm^{-1}): 3280-2600 (NH, ArCH), 1691 (C=O), 1541, 1493, 1339, 923, 822, 771, 742, 686; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 13.05 (br s, 1H, NH), 8.12-8.04 (m, 4H, ArH), 7.58-7.55 (m, 3H, ArH), 7.44-7.42 (d, J = 7.0 Hz, 2H,ArH), 2.40 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm) 165.6, 159.2, 156.4, 152.7, 143.7, 132.6, 132.2, 132.1, 130.5, 129.2, 128.5, 128.0, 123.4, 21.7; MS, m/z: 304 $[M+1]^+$. Anal. calcd. for $C_{18}H_{13}N_3O_2$: C, 71.28; H, 4.32; N, 13.85. Found: C, 71.25; H, 4.33; N, 13.77%.

2,5-Bis(4-methylphenyl)[1,3]oxazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3d)

Color: Light yellow solid; Yield 80%; m.p. 349-350°C; IR (KBr, cm^{-1}): 3271-2653 (NH, ArCH), 1692 (C=O), 1537, 1491, 1337, 919, 824, 776, 734, 690; 1H NMR (DMSO-d_6, 500 MHz) δ (ppm) 12.97 (s, 1H, NH), 8.07 (d, J = 8.0

Hz, 2H, ArH), 8.03 (d, *J* = 8.0 Hz, 2H, ArH), 7.45 (d, *J* = 7.5 Hz, 2H, ArH), 7.36 (d, *J* = 7.5 Hz, 2H, ArH), 2.42 (s, 3H, CH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 171.8, 159.1, 151.3, 151.0, 150.9, 149.1, 131.5, 131.0, 130.6, 129.1, 128.4, 120.3, 119.5, 20.4; MS, m/z: 318 $[M+1]^+$. Anal.calcd. for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.88; H, 4.74; N, 13.30%.

General procedure for the synthesis of 2,5-diaryl-7-chloro-[1,3]oxazolo[4,5-*d*]pyrimidines 4a-d

A mixture of compound 3a-d (10 mmol), $POCl_3$ (30 mL), and Me_2NPh (2.42 g, 20 mmol) was refluxed for 3 h. After evaporation of $POCl_3$ excess the residue was recrystallized from 1,4-dioxane.

7-Chloro-2,5-diphenyl[1,3]oxazolo[4,5-*d*]pyrimidine (4a) has been described in the literature [8].

7-Chloro-5-(4-methylphenyl)-2-phenyl[1,3]oxazolo[4,5-*d*]pyrimidine (4b)

Color: White solid; Yield 86%; m.p. 237-239°C; IR (KBr, cm^{-1}): 1601, 1540, 1482, 1374, 1325, 1046, 987, 784, 710, 690; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.30-8.23 (m, 4H, ArH), 7.80-7.25 (m, 5H, ArH), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 174.9, 156.4, 156.3, 149.3, 149.0, 140.5, 137.7, 131.0, 130.5, 130.1, 129.1, 125.0, 122.1, 20.3; MS, m/z: 322 $[M+1]^+$. Anal.calcd. for $C_{18}H_{12}ClN_3O$: C, 67.19; H, 3.76; Cl, 11.02; N, 13.06. Found: C, 67.15; H, 3.77; Cl, 11.10; N, 13.02%.

7-Chloro-2-(4-methylphenyl)-5-phenyl[1,3]oxazolo[4,5-*d*]pyrimidine (4c)

Color: White solid; Yield 84%; m.p. 197-199°C; IR (KBr, cm^{-1}): 1608, 1544, 1497, 1373, 1319, 1049, 984, 771, 693; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.43-8.20 (m, 4H, ArH), 7.54-7.47 (m, 5H, ArH), 2.48 (s, 3H, CH_3); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 175.6, 156.8, 156.1, 151.6, 148.8, 140.8, 136.0, 131.0, 130.8, 130.2, 129.0, 128.0, 119.1, 20.8; MS, m/z: 322 $[M+1]^+$. Anal.calcd. for $C_{18}H_{12}ClN_3$: C, 67.19; H, 3.76; Cl, 11.02; N, 13.06. Found: C, 67.14; H, 3.78; Cl, 11.08; N, 13.00%.

7-Chloro-2,5-bis(4-methylphenyl)[1,3]oxazolo[4,5-*d*]pyrimidine (4d)

Color: White solid; Yield 81%; m.p. 288-290°C; IR (KBr, cm^{-1}): 1598, 1541, 1372, 1318, 1049, 990, 787, 727; ^{1}H NMR ($CF_3C(O)OD$, 500 MHz) δ (ppm) 8.72 (d, *J* = 30.0 Hz, 4H, ArH), 7.96 (d, *J* = 17.5 Hz, 4H, ArH), 2.96 (s, 3H, CH_3), 2.93 (s, 3H, CH_3); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 175.3, 156.5, 156.1, 151.5, 149.1, 148.9, 140.4, 131.0, 130.7, 129.0, 125.0, 119.0, 20.8, 20.3; MS, m/z: 336 $[M+1]^+$. Anal.calcd. for $C_{19}H_{14}ClN_3O$: C, 67.96; H, 4.20; Cl, 10.56; N, 12.51. Found: C, 67.93; H, 4.19; Cl, 10.61; N, 12.45%.

General procedure for the synthesis of 7-(1,4-diazepan)-substituted [1,3]oxazolo[4,5-d]pyrimidines 5a-e

A mixture of compound **4** (2 mmol), appropriate 1,4-diazepane derivative (2 mmol), and Et_3N (0.28 ml, 2 mmol) in dioxane (15 ml) was refluxed for 6 h. After removal of the solvent, the residue was triturated with water, filtered off, dried, and recrystallized from DMF/MeCN (1:3).

7-(1,4-Diazepan-1-yl)-2,5-diphenyl[1,3]oxazolo[4,5-*d*]pyrimidine (5a)

Color: White solid; Yield 78%; m.p. 213-215°C; IR (KBr, cm^{-1}): 3330-2770 (NH, ArCH), 1622, 1549, 1480, 1449, 1370, 1133, 1021, 928, 769, 696, 662; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.37 (d, *J* = 6.4 Hz, 2H, ArH), 8.20 (d, *J*= 6.8 Hz, 2H, ArH), 7.70-7.63 (m, 3H, ArH), 7.49-7.48 (m, 3H, ArH), 4.05 4.01 (m, 4H, CH_2(1,4-diazepan)) 3.02 (s, 2H, CH_2(1,4- 2diazepan)), 2.76 (t, *J*=4.4 Hz, 2H, CH_2(1,4-diazepan)), 1.91 (t, *J* = 4.4 Hz, 2H, CH_2(1,4-diazepan)) (NH proton has not been observed); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 170.8, 155.7, 151.9, 150.0, 136.4, 135.3, 130.1, 130.0, 128.9, 128.6, 128.1, 128.0, 122.3, 49.4, 46.9, 46.4, 44.2 25.0; MS, m/z: 372 $[M+1]^+$. Anal. calcd. for $C_{22}H_{21}N_5O$: C, 71.14; H, 5.70; N, 18.85. Found: C, 71.10; H, 5.67; N, 18.92%.

7-(1,4-Diazepan-1-yl)-5-(4-methylphenyl)-2-phenyl[1,3]oxazolo[4,5-*d*]pyrimidine (5b)

Color: White solid; Yield 72%; m.p. 240-243°C; IR (KBr, cm^{-1}): 3362-2640 (NH, ArCH), 1621, 1546, 1369, 1290, 1135, 1060, 1020, 920, 781, 689; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.25 (d, *J* = 7.6 Hz, 2H, ArH), 8.19 (d, *J* = 6.4 Hz, 2H, ArH), 7.63 (d, *J* = 8.8 Hz, 2H, ArH), 7.27 (d, *J* = 7.6 Hz, 2H, ArH), 4.07-4.03 (m, 4H, CH_2(piperazine)), 3.05 (s, 2H, CH_2(piperazine)), 2.80 (s, 2H, CH_2(piperazine)), 2.37 (s, 3H, CH_3), 1.94 (s, 2H, CH_2(piperazine)) (NH proton has not been observed); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 170.6, 155.7, 151.7, 149.9, 147.9, 136.3, 130.7, 130.0, 128.8, 128.1, 128.0, 125.7, 122.3, 49.3, 46.9, 46.4, 44.1, 25.0, 20.2; MS, m/z: 386 $[M+1]^+$. Anal. calcd. for $C_{23}H_{23}N_5O$: C, 71.67; H, 6.01; N, 18.17. Found: C, 71.61; H, 5.98; N, 18.24%.

7-(1,4-Diazepan-1-yl)-2-(4-methylphenyl)-5-phenyl[1,3]oxazolo-[4,5-*d*]pyrimidine (5c)

Color: Light yellow solid; Yield 73%; m.p. 251-254°C; IR (KBr, cm^{-1}): 3342-2622 (NH, ArCH), 1613, 1550, 1378, 1268, 1143, 1069, 771, 702; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.39-8.37 (m, 2H, ArH), 8.11 (d, *J* = 7.6 Hz, 2H, ArH), 7.49-7.45 (m, 5H, ArH), 4.11-4.04 (m, 4H, CH_2(piperazine)), 3.06-3.04 (m, 2H, CH_2(piperazine)), 2.80-2.78 (m, 2H, CH_2(piperazine)), 2.44 (s, 3H, CH_3), 2.30 (br s, 1H, NH), 1.96-1.93 (s, 2H, CH_2(piperazine)); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 171.1, 155.6, 151.9, 149.8, 149.6, 135.2, 130.9, 130.0, 129.0, 128.7, 127.9, 119.3, 119.2, 49.4, 47.4, 47.0, 46.9, 46.5, 46.4, 46.2, 44.2, 25.0, 24.2, 20.6; MS, m/z: 386 $[M+1]^+$. Anal. calcd. for $C_{23}H_{23}N_5O$: C, 71.67; H, 6.01; N, 18.17; Found: C, 71.64; H, 5.99; N, 18.10%.

7-(1,4-Diazepan-1-yl)-2,5-bis(4-methylphenyl)[1,3]oxazolo[4,5-*d*]pyrimidine (5d)

Color: White solid; Yield 75%; m.p. 244-247°C; IR (KBr, cm^{-1}): 3339-2742 (NH, ArCH), 1616, 1574, 1550, 1488, 1455, 1390, 1368, 1328, 1204, 1082, 1014, 927, 781, 728; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.25 (d, *J* = 6.8 Hz, 2H, ArH), 8.07 (d, *J* = 7.6 Hz, 2H, ArH), 7.43 (d, *J* = 7.6 Hz, 2H, ArH), 7.23 (d, *J* = 7.2 Hz, 2H, ArH), 4.03 (d, *J* = 16.0 Hz, 4H, CH_2(piperazine)), 3.03 (bs, 2H, CH_2(piperazine)), 2.78 (br s, 2H, CH_2(piperazine)), 2.42 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 1.92 (bs, 2H, CH_2(piperazine)) (NH proton has not been observed); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 170.9, 155.6, 151.8, 149.8, 149.5, 147.9, 130.9, 130.8, 129.0, 128.0, 127.7, 125.7, 119.3, 49.3, 47.4, 47.0, 46.9, 46.5, 46.4, 46.1, 44.1, 25.0, 24.2, 20.5, 20.2; MS, m/z: 400 $[M+1]^+$. Anal.calcd. for $C_{24}H_{25}N_5O$: C, 72.16; H, 6.31; N, 17.53. Found: C, 72.13; H, 6.30; N, 17.62%.

7-(4-Methyl-1,4-diazepan-1-yl)-2,5-diphenyl[1,3]oxazolo[4,5-*d*]pyrimidine (5e)

Color: White solid; Yield 74%; m.p. 171°C; IR (KBr, cm^{-1}): 3105-2635 (ArCH), 1618, 1546, 1374, 1307, 1065, 1023, 770, 697; ^{1}H NMR ($CF_3C(O)OD$, 400 MHz) δ (ppm) 8.62-8.60 (m, 4H, ArH), 8.23-8.17 (m, 2H, ArH), 8.08 (s, 4H, ArH), 5.53-5.29 (m, 1H, CH_2(piperazine)), 5.18-5.02 (m, 3H, CH_2(piperazine)), 4.81-4.70 (m, 1H, CH_2(piperazine)), 4.60-4.35 (m, 1H, CH_2(piperazine)), 4.05-3.94 (m, 2H, CH_2(piperazine)), 3.57 (s, 3H, CH_3), 3.18-3.02 (m, 2H, CH_2(piperazine)); ^{13}C NMR ($CF_3C(O)OD$, 125 MHz) δ (ppm) 170.9, 155.7, 152.0, 150.0, 135.4, 135.3, 130.1, 130.0, 129.0, 128.6, 128.2, 128.0, 122.3, 56.9, 48.9, 45.0, 43.1, 24.2; MS, m/z: 386 $[M+1]^+$. Anal. calcd. for $C_{23}H_{23}N_5O$: C, 71.67; H, 6.01; N, 18.17. Found: C, 71.65; H, 5.99; N, 18.24%.

Biological Tests

One Doses Full NCI 60 Cell Panel Assay

Primary in vitro one dose anticancer screening was initiated by cell inoculating of each 60 panel lines into a series of standard 96-well microtiter plates at 5000–40000 cells/well in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L glutamine (day 0), and then preincubated in absence of drug at 37 °C and 5% CO_2 for 24 h. Test compounds were then added into the plates at one concentration of 10^{-5} M (day 1) followed to incubation for a further 48 h at the same conditions. Then the media were removed, the cells were fixed in situ, washed, and dried (day 3). The sulforhodamine B assay was used for cell density determination, based on the measurement of cellular protein content. After an incubation period, cell monolayers were fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye was removed by washing repeatedly with 1% (vol/vol) acetic acid. The bound stain was resolubilized in 10 mMTris base solution and measured spectrophotometrically on automated microplate readers for OD determination at 510 nm.

Five Doses Full NCI 60 Cell Panel Assay

Cells of all 60 lines, representing nine cancer subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 and 100 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}, 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/NIHDevelopment 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 values are determined as the drug concentrations result in a 50% and 0% growth at 48 h 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 $T < T_0$.

Selectivity index (SI) of the compounds is calculated as:

SI = MIDp/MIDsp,

whereMIDp – the average sensitivity of all cell lines towards the test agent,

MIDsp – the average sensitivity of all cell lines of a particular subpanel towards the test agent.

Conclusions

The novel series of 7-(1,4-diazepan)-substituted [1,3]oxazolo[4,5-*d*]pyrimidines have been synthesized in good yields and displayed high anticancer activity. All tested compounds demonstrated the anticancer activity against cancer cell lines at micromolar concentrations. Compounds **5b,c** having the highest activity among them can be used as the potent lead compounds for further research and anticancer drug discovery.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

We would like to thank US Public Health Service and National Cancer Institute, Bethesda, MD, 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.

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.

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