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Synthesis and cytotoxic activity of novel 4-amino-5-cyano-2-sulfonylpyrimidines

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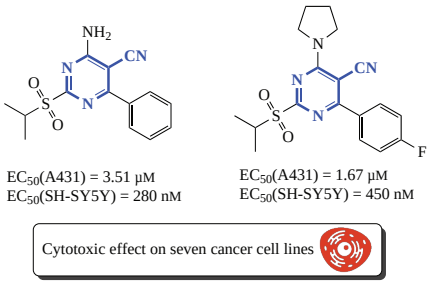
Novel 4-amino-5-cyano-2-sulfonylpyrimidines were prepared based on three-component cyclization between isothiouronium salts, benzaldehydes and malononitrile, followed by oxidation of the sulfide moiety with Oxone. The cytotoxic activity of the synthesized compounds, as well as the induction of apoptosis, inhibition of the cell cycle and proliferation tests were performed on selected cancer cell lines A431, A549, A375, HCT 116, MCF7, LNCap and SH-SY5Y.



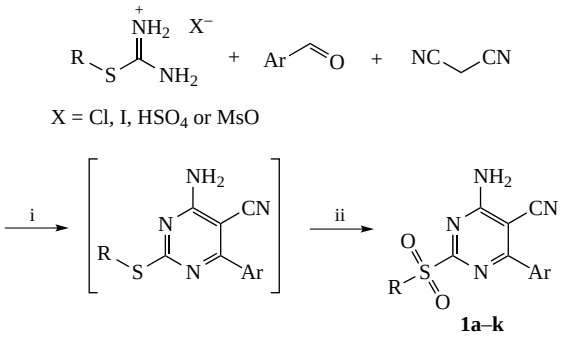
Keywords: heterocyclization, pyrimidines, isothiouronic salts, nitriles, sulfones, cytotoxic activity, cancer cell lines.

The development of new anticancer agents with various mechanisms of cytotoxic effect is an important task of modern medicinal chemistry. Creation of low molecular weight inhibitors of kinases,[1] inducers of apoptosis,[2] antiproliferative agents[3–9] should be noted. Heterocyclic pyrimidine-based scaffolds have shown special results in this respect.[10–13] However, data on 5-cyanopyrimidine derivatives are scanty although some of them manifested antibacterial and antifungal[14] as well as immunosuppressive[15] activities. On the basis of the 5-cyanopyrimidine scaffold, inhibitors PI3Kδ,[16] p38α MAP kinase,[17] Erk5,[18] VEGFR-2[19] and LSD1[20] were obtained.

Here, we report the synthesis of two series of cytotoxic 5-cyanopyrimidine derivatives (type I and II). Compounds of type I (Scheme 1) were assembled by the three-component reaction between aromatic aldehyde, isothiouronic salt and malononitrile. Further oxidation of the sulfide moiety in the Oxone/DMF/water system gave sulfones **1a**–**k**. It should be noted that the reported[9] oxidation procedures for pyrimidine sulfides turned out to be ineffective in our case and led to sulfone/sulfoxide mixtures.

The preparation of type II pyrimidines **2a**–**i** comprised the application of ethyl cyanoacetate and required the introduction of additional stages (Scheme 2). After the pyrimidine core was assembled, the cycle NH-amide function was converted into chloroimidoyl one on treatment with $POCl_3$. Chlorine atom in thus obtained 4-chloro-5-cyanopyrimidines was replaced by pyrrolidine residue. The final oxidation afforded pyrrolidino sulfones **2a**–**i**.

The structures of new compounds **1a**–**k** and **2a**–**i** were confirmed by NMR spectroscopy. As the pyrimidine moieties were fully substituted, the ^{1}H NMR spectra were deprived of their signals. Meanwhile, the ^{13}C NMR spectra contained peaks for the quaternary pyrimidine carbon atoms (see Online Supplementary Materials).

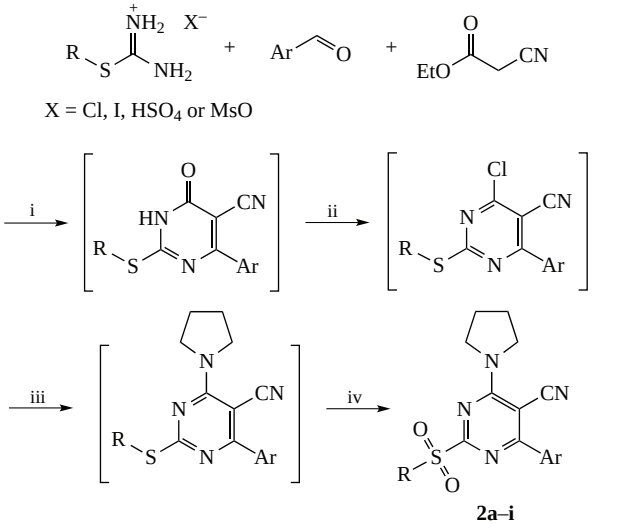


a R = Me, Ar = Ph, 73%
b R = Et, Ar = Ph, 74%
c R = Pr^i, Ar = Ph, 89%
d R = Bn, Ar = Ph, 91%
e R = Me, Ar = 4-FC_6H_4, 80%
f R = Me, Ar = 3-FC_6H_4, 73%
g R = Me, Ar = 2-FC_6H_4, 87%
h R = Pr, Ar = 4-FC_6H_4, 86%
i R = Pr, Ar = 3-FC_6H_4, 68%
j R = Pr^i, Ar = 4-FC_6H_4, 85%
k R = Me, Ar = 4-$MeOC_6H_4$, 66%

Scheme 1 *Reagents and conditions*: i, K_2CO_3, EtOH, reflux, 12 h; ii, Oxone, DMF/H_2O (5 : 1), 50 °C, 2–4 h.

The synthesized 5-cyanopyrimidine derivatives **1**, **2** were evaluated for cytotoxic activities by MTT assay against human lung adenocarcinoma cell line A549, human epidermoid carcinoma cell line A431, human melanoma cell line A375, human colorectal cancer cell line HCT-116, human breast cancer cell line MCF-7, human prostate cancer cell line LNCaP, and human neuroblastoma cell line SH-SY5Y (Table 1).

The synthesized compounds exhibited stronger anticancer activities against A431 and SH-SY5Y cell lines. Based on cell viability data we selected compounds **1c** and **2c** from each library for further study. As the compounds manifested cell cytotoxic activities against A431, A549, and MCF-7 cell lines, we selected some of them for further tests. To study the



a R = Pr^i, Ar = Ph, 55%
b R = Bn, Ar = Ph, 85%
c R = Pr^i, Ar = 4-FC_6H_4, 77%
d R = Pr^i, Ar = 3-FC_6H_4, 56%
e R = Me, Ar = 4-FC_6H_4, 78%
f R = Me, Ar = 3-FC_6H_4, 85%
g R = Me, Ar = 2-FC_6H_4, 98%
h R = Et, Ar = Ph, 73%
i R = Pr, Ar = 4-FC_6H_4, 70%

Scheme 2 *Reagents and conditions*: i, K_2CO_3, EtOH, reflux, 8 h; ii, $POCl_3$ (10 equiv.), 1,4-dioxane, reflux, 7–12 h; iii, pyrrolidine (2 equiv.), Pr^{i}OH, reflux, 5–8 h; iv, Oxone, DMF/H_2O (5 : 1), 50 °C, 2–4 h.

antiproliferative activity against A431 and A549 cell lines, we performed clonogenic cell survival assay with selected compounds (see Online Supplementary Materials, Figure S1).

Remarkably, compounds **1c** and **2c** exhibited similar inhibition of colony formation in A549 and A431 cells contrary to other selected compounds. Also, compounds **1c** and **2c** inhibited the migration of A431 and A549 cells (see Online Supplementary Materials, Figure S4). To elucidate the mechanisms responsible for antiproliferative activity of compounds **1c** and **2c**, we investigated inhibition of cell cycle and apoptosis induction. Apoptosis was quantified by double staining of cells treated with **1c** or **2c** for 24 h with Annexin-V-FITC and PI (Figure S2) to distinguish healthy cells from apoptotic cells and necrotic cells. Using the apoptosis assay, we demonstrated increase in apoptotic and necrotic cells in A431 cells following the treatment with **1c** and **2c**. Incubation with **1c** led to an increase of early, late apoptotic and necrotic cells and to reduction of viable cells in A431 cell lines. The percentage of total apoptotic cells (early and late apoptotic cells) in A431 increased from 5.60% (control) to 45.09% (**4c**) and 18.41% (**6c**), respectively (Figure S3). No significant increase in apoptotic and necrotic cells was detected in A549 cells.

After treating A431 and A549 cells with compounds **1c** and **2c** at different concentrations (EC_{50} and EC_{80}) for 24 h, cells were fixed and stained with PI for flow cytometry. Compound **1c** arrested the cell cycle at G2/M phase in a concentration-dependent manner, accompanied with the substantial decrease of cells at G0/G1 phase at EC_{80} concentration (see Figure S3).

To determine the possible mechanisms of the antiproliferative and cytotoxic action of derivatives **1c** and **2c**, we performed the determination of the EGF-induced expression of EGFR and its downstream signal molecules such as Akt, pAkt, ERK1/2 and pERK1/2 by In-Cell ELISA assay (ThermoFisher) (Figure S5). Component **1c** caused a decrease in the expression of the phosphorylated forms of the tyrosine kinase receptor, EGFR and the intracellular signal molecules pAkt and pERK1/2.

To sum up, compound **1c** exhibits inhibitory activity against tumor cells of various origins, which suggests the presence of inhibitory activity against other targets other than EGFR, and is the subject of our further studies.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.09.017.

Table 1 Cytotoxicity of the synthesized compounds against human cancer cell lines.

Compound	EC_{50}/μM						
	A431	A549	A375	HCT116	MCF-7	LNCaP	SH-SY5Y
Etoposide	1.63±0.15	139.54±7.05	0.69±0.04	1.73±0.21	10.91±2.1	14.4±3.23	0.75±0.04
1a	9.46±0.39	37.64±0.89	24.86±1.06	14.66±0.77	24.81±1.52	16.44±1.59	1.83±0.32
1b	16.60±1.13	26.28±1.61	16.48±0.22	8.99±0.19	17.55±1.29	13.96±1.09	0.79±0.25
1c	3.51±0.24	25.93±1.41	16.83±0.44	11.02±0.60	16.47±0.84	15.07±0.86	**0.28±0.14**
1d	24.85±1.19	31.86±0.94	18.47±0.68	10.73±0.40	19.56±1.14	12.08±1.15	1.41±0.35
1e	9.82±0.33	40.38±1.03	25.63±0.24	13.63±0.26	30.98±1.33	19.59±1.10	1.72±0.34
1f	16.44±0.67	46.56±2.58	35.05±0.43	16.65±0.39	34.94±1.75	22.52±1.66	3.00±0.50
1g	29.57±1.17	59.06±1.59	61.25±1.08	23.86±0.37	49.25±2.18	30.06±2.44	5.43±0.65
1h	4.63±0.21	29.80±1.47	18.58±0.52	10.49±0.45	17.49±0.75	11.99±1.74	1.02±0.22
1i	5.69±0.29	28.96±2.58	19.80±0.65	10.60±0.49	18.70±0.79	12.68±2.04	1.19±0.28
1j	3.67±0.25	32.90±1.96	16.43±0.51	10.13±0.56	14.97±0.52	12.76±1.11	0.66±0.32
1k	7.25±0.35	35.42±1.39	20.02±0.71	10.01±0.49	16.90±0.80	**7.68±0.97**	**0.28±0.15**
2a	3.75±0.71	23.07±1.77	13.80±0.49	6.23±0.26	17.28±0.97	12.42±0.35	0.64±0.51
2b	**2.55±0.22**	22.33±1.96	**8.65±0.30**	6.41±0.71	17.04±1.07	**7.68±0.51**	1.94±0.543
2c	**1.67±0.13**	**16.82±0.81**	**5.31±0.14**	**3.41±0.11**	**7.37±0.70**	**5.43±0.28**	0.45±0.10
2d	**3.34±0.26**	21.51±1.79	11.77±0.34	6.56±0.21	12.29±0.44	8.79±0.21	0.84±0.36
2e	4.72±0.44	23.37±0.91	14.57±0.36	5.06±0.26	10.14±1.38	9.24±0.24	0.85±0.23
2f	3.36±0.52	**21.94±1.08**	12.12±0.31	5.95±0.40	**9.82±1.10**	8.81±027	1.25±0.23
2g	3.92±0.64	27.18±2.08	11.45±0.89	6.14±0.45	13.19±0.65	8.67±0.54	0.78±0.43
2h	5.09±0.31	**19.06±0.88**	**10.47±0.34**	**4.51±0.26**	**9.97±0.64**	7.87±0.34	**0.19±0.11**
2i	4.60±0.54	23.11±1.07	15.01±0.32	**5.93±0.27**	10.28±1.22	9.03±0.20	0.97±0.18

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