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Synthesis of new diarylamides with pyrimidinyl pyridine scaffold and evaluation of their anti-proliferative effect on cancer cell lines

Ahmed Z. Abdelazem [a,b,d,*], Mohammad M. Al-Sanea [a,b,e], Hyun-Mee Park [c], So Ha Lee [a,b]

[a] Chemical Kinomics Research Center, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea
[b] Department of Biological Chemistry, University of Science and Technology, 113 Gwahangno, Yuseong-gu, Daejeon 305-333, Republic of Korea
[c] Advanced Analysis Center, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea
[d] Biotechnology & Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Egypt
[e] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Modern University for Technology & Information, Cairo, Egypt

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ABSTRACT

A new series of diarylamides, having a pyrimidinyl pyridine scaffold, was designed and synthesized. The target compounds were synthesized in three steps. A selected group from the target compounds was tested over a panel of 60 cancer cell lines at a single dose concentration of 10 μM, and the most active compound, **5j**, was further tested in a five-dose testing mode to determine its IC_{50} value over the 60 cell lines. In single-dose testing mode, compound **5j** showed the highest growth inhibition against the NCI-60 cancer cell lines, while other tested compounds showed a weak to moderate inhibitory activity against a range of different cancer cell lines. In five-dose testing mode, compound **5j** showed strong inhibitory activity in micro molar range against many cancer cell lines. Its major activity was against melanoma cancer cell lines. Therefore, compound **5j** is a promising hit compound targeting this severe form of cancer.

Cancer is a group of diseases characterized by abnormalities in cellular growth, proliferation, and survival pathways, resulting in uncontrolled expansion of cancer cells and tumor formation. Collectively, these diseases represent one of the most pressing challenging health problems in the 21st century.[1] The development of cancer is a complex multi-step process in which malignant cells gradually change as a result of a series of mutations.[2] There is still an urgent need for searching for and developing of more potential anticancer agents with minimal side effects.

Melanoma is a type of skin cancer which is formed from melanocytes (pigment-containing cells in the skin). Incidence of melanoma has tripled in the last 40 years.[3] Melanoma is less common than other skin cancers. However, it is much more dangerous if it is not found in the early stages. It causes the majority (75%) of deaths related to skin cancer.[4]

Diarylamides have been highlighted as potential antiproliferative agents against a variety of cancer cell lines.[5–13] Imatinib (Gleevec®) (Fig. 1) is an example of anticancer diarylamide that has been approved by the U.S. Food and Drug Administration (FDA) for treatment of chronic myeloid leukemia (CML) with diminished side effects.[14]

In a previous study by our group, we used the pyrimidinyl pyridine moiety as a part of the chemical scaffold of some potent antiproliferative compounds.[15] A noteworthy observation is that the diarylamide and the pyrimidinyl pyridine moieties were combined in the structure of Imatinib.

Encouraged by the interesting antiproliferative activity of the diarylamide derivatives, and as a continuation of our previous study about the pyrimidinyl pyridine scaffold,[15] we designed and synthesized a series of new diarylamides containing a pyrimidinyl pyridine scaffold (Fig. 1). A selected group (8 compounds) from the target compounds was tested over a panel of 60 cancer cell lines at a single dose concentration of 10 μM, and the most active compound **5j** was further tested in a five-dose testing mode to determine its IC_{50} value over the 60 cell lines.

A simple synthetic strategy was used to obtain the target compounds **5a**–**j** as illustrated in Scheme 1.

Treatment of 2-amino-4,6-dichloropyrimidine (**1**) with the amine **2a** or **2b** led to nucleophilic displacement of chloro group by aliphatic amine group. Two regioisomers resulted from this step, the major one was the 4-amino substituent, **3a** or **3b**, as it was proved using 2D NOESY, and also it coincides with literature data.[16] The pure isomers **3a** and **3b** were subjected to Suzuki coupling with 3-pyridine boronic acid to give compounds **4a** and **4b**,

* Corresponding author at: Chemical Kinomics Research Center, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea. Tel.: +82 02 958 6795.
E-mail address: aznagi2003@gmail.com (A.Z. Abdelazem).

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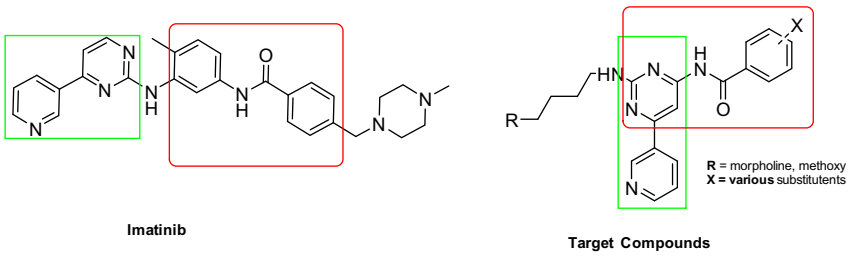
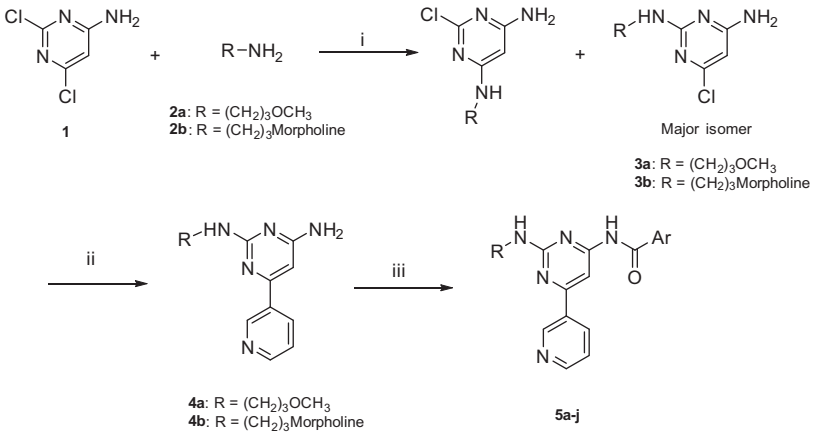


Figure 1. Structures of Imatinib and target compounds with highlight of the common structural features.



Scheme 1. Reaction conditions and reagents: (i) *n*-butanol, TEA, 90 °C, 12 h; (ii) 3-pyridineboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O (4:1), 12 h; (iii) ArCOOH, EDCI, THF, DMAP, 0 °C–rt, 24 h.

respectively. The nucleophilic substitution of the 4-chloro group in the first step was proved by two pieces of evidences. First, the 2D NOESY spectrum of compound **3a**, as a representative, did not show any coupling interaction between the secondary amine proton and the aromatic proton. Second, in the 2D NOESY spectrum of compound **4a**, as a representative, there is cross interaction between the aromatic proton of the pyrimidine ring and two aromatic protons from the pyridine ring (Fig. 2).

In the final step, through amide coupling, compounds **4a** and **4b** were coupled to five different substituted benzoic acid derivatives to give the target compounds **5a–j**. Structures of the target compounds are illustrated in Table 1.

The structure of the target compounds were submitted to the NCI, Bethesda, Maryland, USA, and eight compounds out of ten were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their

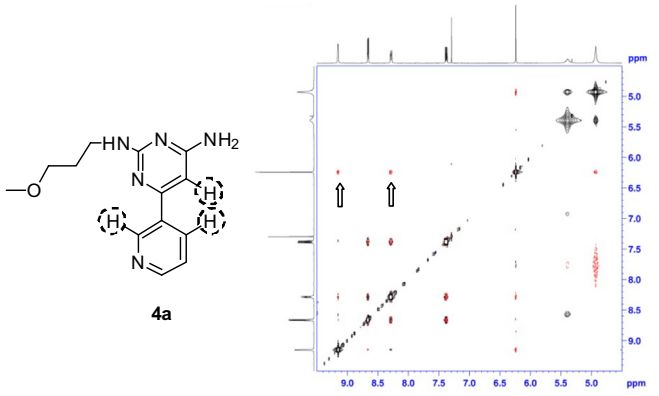
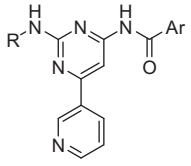


Figure 2. 2D-NOESY NMR spectrum of compound **4a**.

Table 1
Structures of the target compounds and mean% inhibition



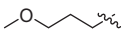
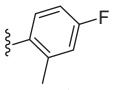
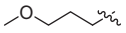
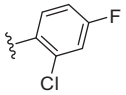
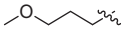
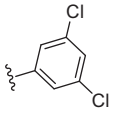
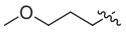
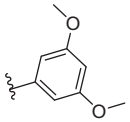
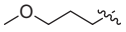
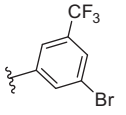
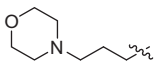
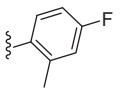
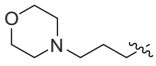
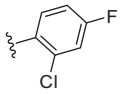
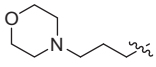
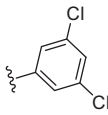
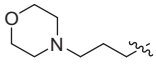
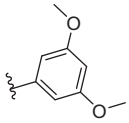
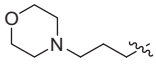
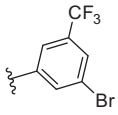
Compd No.	R	Ar	Mean% inhibition
5a			0.78
5b			2.78
5c			Not tested
5d			8.06
5e			11.33

Table 1 (*continued*)

Compd No.	R	Ar	Mean% inhibition
5f			1.46
5g			3.57
5h			Not tested
5i			8.88
5j			57.16

antiproliferative activity. The selected compounds were tested for their in vitro antiproliferative activity against tumor cells in a full panel of 60 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast).[17] The compounds were tested at a single-dose concentration of 10 μM. The mean inhibition percentages of all of the tested compounds over the full panel of cell lines are illustrated in Table 1 and the figures showing the inhibitions exerted by each compound over the full cell lines panel are showed in the Supplementary data. Compound **5j** was the most active against the tested cell lines; its mean% inhibition is far higher than other tested compounds. It showed growth inhibition value of more than 50% in 19 cancer lines, and lethal effect in 7 cancer lines (Fig. 3). Compounds **5a**, **5b**, **5d**, **5e**, **5f**, **5g** and **5i** showed weak to moderate growth inhibition against a range of cancer cell lines (Table 2). Certain cell lines were commonly sensitive to many of the tested compounds. The cell lines HOP-92, UO-31, T-47D, SK-MEL5, MDA-MB-231/ATCC and A498 were sensitive to three or more of the tested compounds. We tried to find a common genetic mutation in these cell lines in the light of a research about genetic mutation in NCI cell line set,[18] but the only common genetic mutation was CDKN2A, which is a common mutation in the majority of NCI-60 cancer cell lines and isn't specific to the commonly sensitive cell lines. Therefore, there might be another common genetic variation, that is, not elaborated yet.

Table 2
% inhibition of compounds **5a**, **5b**, **5d**, **5e**, **5f**, **5g** and **5i** against most sensitive cancer cell lines

Compd No.	Cell line	Cancer type	% inhibition at 10 μM
5a	HOP-92	Non-small cell lung cancer	32.97
	NCI-H522	Non-small cell lung cancer	21.40
5b	A498	Renal cancer	45.92
	UO-31	Renal cancer	21.50
5d	RPMI-8226	Leukemia	30.65
	HOP-92	Non-small cell lung cancer	47.41
	NCI-H522	Non-small cell lung cancer	23.53
	SK-MEL-2	Melanoma	21.20
	SK-MEL-5	Melanoma	27.60
	SNB-75	CNS cancer	26.88
	OVCAR-8	Ovarian cancer	20.70
	UO-31	Renal cancer	43.26
	MDA-MB-231/ ATCC	Breast cancer	30.87
	T-74D	Breast cancer	36.18
5e	K-562	Leukemia	26.67
	RPMI-8226	Leukemia	34.44
	HOP-92	Non-small cell lung cancer	42.06
	SK-MEL-5	Melanoma	29.51
	A498	Renal cancer	23.77
	UO-31	Renal cancer	39.45
	PC-3	Prostate cancer	29.38
	MDA-MB-231/ ATCC	Breast cancer	24.20
	T-74D	Breast cancer	34.98
5f	NCI-H226	Non-small cell lung cancer	22.36
	A498	Non-small cell lung cancer	22.81
5g	UO-31	Renal cancer	22.97
5i	K-562	Leukemia	25.87
	SR	Leukemia	42.09
	NCI-H226	Non-small cell lung cancer	32.33
	SK-MEL-5	Melanoma	31.69
	UO-31	Renal cancer	46.44
	MDA-MB-231/ ATCC	Breast cancer	20.44
	T-74D	Breast cancer	27.18

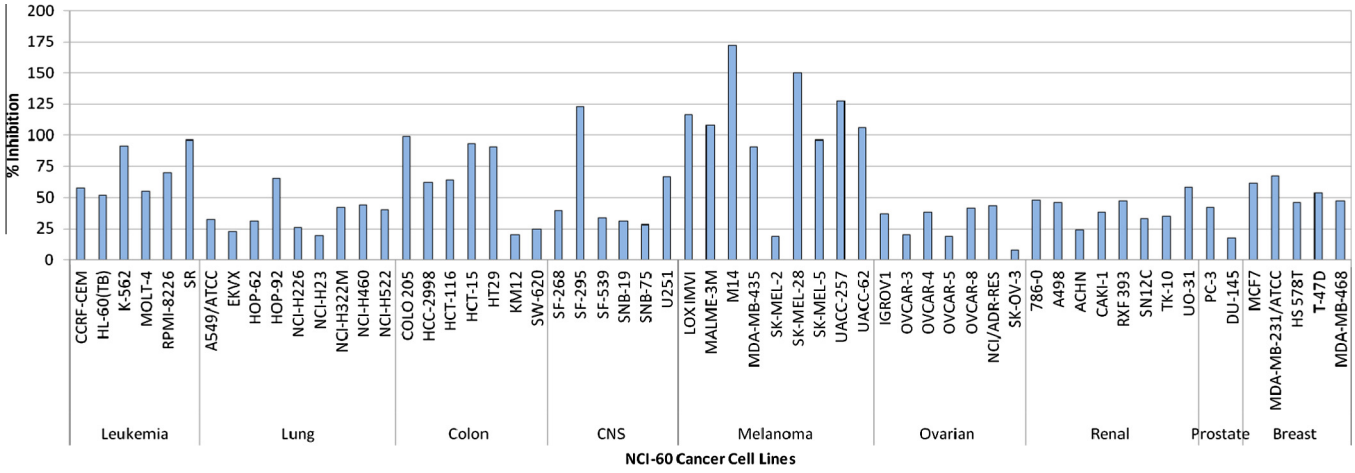


Figure 3. % inhibition expressed by compound **5j** at a single-dose concentration of 10 μM over the NCI-60 cancer cell lines.

Table 3
IC_{50} values (μM) of compound **5j** over the most sensitive cell lines

Cancer cell lines		IC_{50} (μM)
Lung	HOP-92	1.97
	NCI-H522	1.95
Colon	COLO 205	1.77
	HCC-2998	1.77
	HCT-15	1.92
CNS	SF-295	1.94
	SF-539	1.67
	SNB-75	1.52
Melanoma	LOX IMVI	1.62
	MALME-3M	1.76
	M14	1.67
	MDA-MB-435	1.66
	SK-MEL-28	1.73
	SK-MEL-5	1.62
	UACC-62	1.94
Renal	786-0	1.55
	RXF 393	1.80
	UO-31	1.40
Breast	MDA-MB-231/ATCC	1.79

Compound **5j** has the same pyrimidinyl pyridine core moiety as other compounds in this series. In addition to the common central moiety, compound **5j** has a 3-morpholinopropylamino as polar chain, and a 3-bromo-5-(trifluoromethyl)phenyl terminal ring. A notable observation is that the difference between compound **5e** and compound **5j** is only in the polar chain, but the activity of compound **5j** is far higher than compound **5e**. Therefore, it seems that the compound should be considered as a whole rather than its individual components.

Regarding the terminal aromatic ring substitution, the order of activity is, trifluoromethyl, bromo-substitution > dimethoxy-substitution > dihalo-substitution > monohalo-substitution. But the most promising finding from biological results was the high potency of compound **5j** against many cell lines of different cancer types; specially melanoma cell lines (Fig. 3). Compound **5j** showed lethal effect in six melanoma cell lines (LOX IMVI, MALME-3M, M14, SK-MEL-28, UACC-257, UACC-62) which indicates an apoptotic or cytotoxic phenotype, and it also showed high inhibition percentages of 96.22 and 90.45 against SK-MEL-5 and MDA-MB-435 melanoma cell lines, respectively, which indicates a cytostatic phenotype. A noteworthy observation is that all melanoma cell lines upon which compound **5j** showed cytotoxic effect carry B-Raf mutation.[18] Therefore, **5j** might be B-Raf inhibitor. But **5j** also showed a cytotoxic effect upon SF-268 cell line, which doesn't carry B-Raf mutation. Therefore, **5j** might have multiple possible targets.

Compound **5j** was selected by NCI for further testing in a five-dose testing mode, in order to determine its IC_{50} values over the 60 cancer cell lines. Compound **5j** showed inhibitory activity in micro molar scale over all the nine cancer types, and the highest activity was against melanoma cell lines. The IC_{50} values of compound **5j** tested in five-dose mode over the most sensitive cell lines (below 2 μM) are summarized in Table 3. Therefore, from single dose and five-dose data, compound **5j** represents a highly promising hit candidate to develop new effective drugs against melanoma, this severe form of cancer.

In summary, a series of new diarylamides possessing a pyrimidinyl pyridine scaffold was synthesized based on the Imatinib structure and also on our previous literature study. The in vitro antiproliferative activities were screened over the NCI-60 cancer cell line panel of nine different cancer types. It was found that compound **5j** with the common central pyrimidinyl pyridine moiety, 3-morpholinopropylamino as polar chain, and 3-bromo-5-(trifluoromethyl)phenyl terminal ring, showed the highest inhibition percentages with great selectivity toward melanoma cell lines. In five-dose testing mode, compound **5j** showed the highest inhibitory activity against melanoma cell lines. Therefore, due to its high activity toward melanoma cell lines, compound **5j** can be considered as a promising lead for the development of potent anticancer agents for treatment of melanoma.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.01.014.

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