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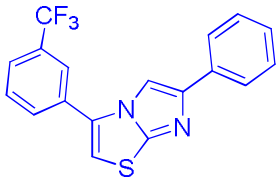

4j

Antiproliferative activity of compound **4j**

IC_{50} = **6.5** µM (HeLa)
IC_{50} = **8.9** µM (A549)
IC_{50} = **10.9** µM (MDA-MB-231)
IC_{50} = **17.4** µM (THP1)

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ABSTRACT

A series of new 3,6-diphenylimidazo[2,1-*b*]thiazole derivatives (**4a-l**) are synthesized and evaluated for their anticancer activity. Some of the synthesized compounds have shown potent anti-proliferative activity against HeLa, MDA-MB-231, A549 and THP1 human cancer cell lines. Among the active compounds, 3-(3-trifluoromethylphenyl)-6-phenylimidazo[2,1-*b*]thiazole (**4j**) has caused significant cytotoxicity in HeLa cells, with IC_{50} as low as 6.5 µM. Compound **4j** has induced caspase-3 and caspase-8 activation, leading to an apoptotic cell death. FACS analysis has revealed that compound **4j** arrests cells in G0/G1 phase. The presence of 3-(3-trifluoromethylphenyl)- or 3-(3-chlorophenyl)- substituent, in that order, on the 6-phenylimidazo[2,1-*b*]thiazole impacts more positively than other aryl-substituents, on the anti-proliferative properties of these compounds.

Thiazole scaffold is found in many natural and synthetic compounds with numerous applications in medicinal chemistry. Many thiazole derivatives have been reported in the drug development for the treatment of allergies,[1] inflammation,[2] hypertension,[3] bacterial infection,[4] schizophrenia,[5] hypnotics,[6] HIV infections[7] and thrombosis.[8] Sulfathiazol (antimicrobial drug), bleomycine and tiazofurin (antineoplastic drug) are some of the thiazole containing drugss. It has been found that 2-amino-4-phenylthiazole derivatives have shown anesthetic activity[9]. Some thiazole analogs have also exhibited antimicrobial[10,11], antitumor,[12,13] analgesic,[10,14] anti-inflammatory and antipyretic[10] properties.

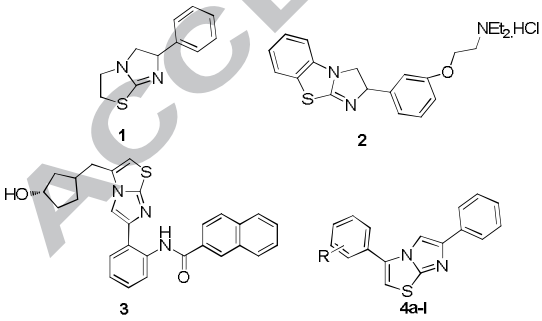
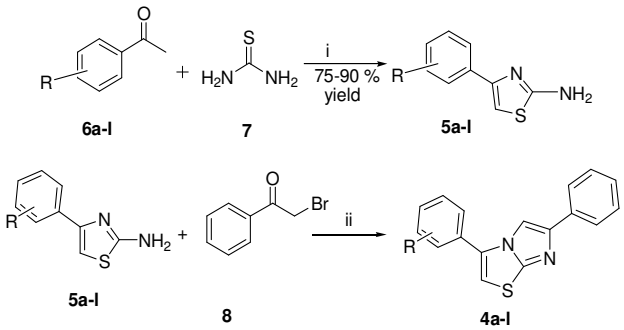


Fig 1. Representative structures of biologically active imidazo[2,1-*b*]thiazole derivatives.

Imidazo[2,1-*b*]thiazole derivatives are reported to possess anticancer activity against different types of human cancer cell lines.[15,16] The known immunomodulator and antihelminthic agent levamisole (**1**) and the antitumor agent YM-201627 (**2**, Fig 1) are some of the orally active imidazo[2,1-*b*]thiazole derivatives.[17] Human SIRT1 is an NAD^+-dependent deacetylase protein that plays a role in cell death/survival, senescence and endocrine signaling.[18] N-(2-(3-(3-hydroxycyclopentylmethyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)-2-naphthamide (**3**, Fig 1) has been reported as a SIRT1 activator.[19] Recently some thiazole compounds and imidazo[2,1-*b*]benzothiazole derivatives have been reported to exhibit anticancer activity.[20,21] These facts have inspired us to synthesize new 3,6-diphenylimidazo[2,1-*b*]thiazole derivatives for evaluating their anticancer potential.



Reagents and reaction conditions: i). I_2, 90 °C, 12 h;
ii). Ethanol, MW, 180 W, 5-6 min;

Scheme 1. Synthesis of 3,6-diarylimidazo[2,1-*b*]thiazoles (**4a-l**).

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Representative structures of biologically active imidazo[2,1-*b*]thiazole derivatives along with the target molecules (**4**) are shown in Fig 1. The 3,6-diphenylimidazo[2,1-*b*]thiazole derivatives (**4a-l**) are synthesized by the condensation of the individual *4-aryl-1,3-thiazol-2-amine* **(5a-l)** and *2-bromo-1-phenylethanone* (**8**) in refluxing ethanol for 12 h under N_2 atmosphere. Microwave irradiation of an ethanolic solution of *4-aryl-1,3-thiazol-2-amine* **(5a-l)** and *2-bromo-1-phenylethanone* (**8**) in a pressure tube at 100 ^oC has also resulted in condensation-cyclization reaction leading to the formation of respective 3,6-diphenylimidazo[2,1-*b*]thiazole (**4a-l**) as a solid product in reasonable yield, albeit in just 5-6 min. The *4-aryl-1,3-thiazol-2-amines* **5a-l** are obtained in 75-90 % yield, as per the procedure reported for compounds **5a-i** by us earlier.[22] These transformations are depicted in Scheme 1. The imidazo[2,1-*b*]thiazole derivatives **4a** and **4c-l** reported here are synthesized for the first time. 3-(4-Chlorophenyl)-6-phenylimidazo[2,1-*b*]thiazole (**4b**) has been reported earlier.[23] However, no spectral data is available for this compound.

The imidazo[2,1-*b*]thiazole derivatives (**4a-l**) synthesized in this study are characterized by ^1H NMR, ^{13}C NMR, ESI-MS, ESI-HRMS and IR spectroscopic data. In the IR spectra, the cyclic imine (C=N) stretching absorption band is seen around 1460-1478 cm^{-1}. The ^1H NMR signal due to the lone hydrogen atom present on thiazole ring has appeared as a singlet between δ 6.64-6.90 ppm, while the singlet signal due to the lone hydrogen on the imidazole moiety is seen in the range of δ 7.57-7.94 ppm, overlapping with multiplet signals of the aromatic hydrogens. Similarly, the proton decoupled ^{13}C NMR spectra of imidazo[2,1-*b*]thiazole compouds **4a-l** contained two singlet signals due to the two CH-carbons of imidazo[2,1-*b*]thiazole skeleton appearing at δ 106.7-108.5 (C-2) and 107.6-111.0 (C-5) ppm, respectively. The signals due to the remaining three quaternary carbons of imidazo[2,1-*b*]thiazole skeleton are seen at δ 128.7-130.7 (C-3), 144.9-148.8 (C-6), and 147.7-150.3 (C-8) ppm. In the case of 3-(2/4-fluorophenyl)-6-phenylimidazo[2,1-*b*]thiazoles (**4a** and **4f**), the signal due to the aromatic carbon attached to fluorine appeared as a doublet at δ163.3 ($^1J_{(C-F)}$ = 250.2 Hz) and 159.6 ($^1J_{(C-F)}$ = 251.4 Hz) ppm respectively. The spectral data is provided as part of the supplementary information.

The 3-aryl-6-phenylimidazo[2,1-*b*]thiazoles **4a-l** are evaluated for their anti-proliferative activity against a panel of four different human cancer cell lines namely, cervical (HeLa), breast (MDA-MB-231), lung (A549) and leukemic cancer cell lines (THP1) using the MTT assay.[24] The IC_{50} for each individual compound with respect to the four human cancer cell lines are calculated and the results are summarized in Table 1. These values represent the concentration at which 50% decrease in cell growth is observed after 48 h incubation in presence of the drug. They are compared with control cells treated with DMSO and positive control doxorubicin under similar conditions. Some of the synthesized compounds (**4a-l**) have exhibited promising anti-proliferative activity against HeLa (cervical), MDA-MB-231 (breast), A549 (lung), and THP1 (leukemia) human cancer cell lines. The substituent effect on their antiproliferative activity has been observed. The 3-aryl-6-phenylimidazo[2,1-*b*]thiazole derivatives containing 3-(3-chlorophenyl)- (**4e**) and the lipophilic 3-trifluoromethyl- (**4j**) moiety in the 3-aryl-substituent have shown significant antiproliferative activity against all the four human cancer cell lines tested (Table 1).

It is interesting to note that 6-phenylimidazo[2,1-*b*]thiazoles having lipophilic group in the form of 3-(4-tolyl)- (**4h**), 3-(2-anisyl)- (**4i**), 3-(3-trifluoromethylphenyl)- (**4j**), 3-(4-trifluoromethylphenyl)- (**4k**) and 3-(4-ethylphenyl)- (**4l**) substituent have shown significant antiproliferative activity against HeLa and moderate activity against A549 human cancer cell lines. The 3-(3-substituted-phenyl)-6-phenylimidazo[2,1-*b*]thiazoles (**4e**, **4h** and **4j**) have exhibited better activity than their analogs containing 3-(4-or 2-substituted-phenyl)- moiety. The 3-(4-halophenyl)- (**4a-c**) or 3-(2-(halo/methyl/methoxy)phenyl)- (**4d**, **4f**, **4g** and **4i**) substituted 6-phenylimidazo[2,1-*b*]thiazoles have possessed moderate activity. The 3-(3-trifluorophenyl)-6-phenylimidazo[2,1-*b*]thiazole (**4j**) has shown the best antiproliferative activity at a concentration range of 6-17.5 µM in different cell lines tested. As compound **4j** has caused significant cytotoxicity at lower concentration (6.5±0.56 µM) which coincided with the doxorubicin-induced cytotoxicity (6.0±0.7 µM) in HeLa cells, we have carried out further experiments in these cells. To understand the cell cycle phase specific alterations induced by compound **4j**, fluorescence activated cell sorting (FACS) analysis is also performed.[24]

Table 1

IC_{50} values for compounds (**4a-l**) against different human cancer cell lines

Comp	R	IC_{50} (µM)			
		HeLa[a]	A549[b]	MDA-MB-231[c]	THP1[d]
4a	4-F	20.2±3.06	51±3.67	19.7±0.71	27.7±0.3
4b	4-Cl	25.9±2.47	15.9±0.4	21±0.76	25±0.8
4c	4-Br	28.3±1.43	15.5±0.11	21.1±0.48	NA
4d	2-Cl	31.6±3.47	48.6±4.62	27.4±0.21	55.5±0.3
4e	3-Cl	14.0±3.06	15.0±3.1	12.8±1.5	21.7±1.2
4f	2-F	NA	NA	39.1±0.3	78.9±0.2
4g	2-Me	36.9±1.69	NA	25.8±0.23	55.5±0.3
4h	3-Me	13.7± 1.02	40.2± 4.03	NA	25±2.4
4i	2-OMe	9.5±1.09	39.0±2.9	25.3±0.18	50±1.03
4j	3-CF_3	6.5±0.56	8.9±0.46	10.9±0.44	17.4±1.34
4k	4-CF_3	8.5±2.43	21.5±0.2	NA	71.4±0.8
4l	4-Et	11.3±1.53	44.8±1.21	NA	68.2±0.5
Doxo[e]	-	6.0±0.7	3.4±0.9	2.0±1.0	3.5±0.8

[a]HeLa = Human cervical carcinoma cells; [b]A549 = Human lung adenocarcinoma epithelial cells; [c]MDA-MB-231 = Human breast carcinoma cells; [d]THP1 = Human leukemic cells; NA = denotes activity >100 µM; [e]Doxorubicin is used as a positive control; The IC_{50} values represent the required concentration (µM) of test compound to inhibit tumor cell proliferation by 50%; The data presented here is obtained from three independent experiments and standard deviation (S.D.) values are derived.

Table 2

Cell cycle distribution of compound **4j**

Compound	Concn (µM)	Cell cycle distribution (%)			
		Sub G1	G0/G1	S	G2/M
Control	0	1.38	35.73	18.18	44.71
4j	6.5	17.28	51.54	12.75	18.43
4j	10	19.0	54.78	8.33	17.89

Cell cycle analysis has been performed to explore the basis for observed antiproliferative properties of 3-(3-trifluoromethylphenyl)-6-phenylimidazo[2,1-*b*]thiazole (**4j**) at 6.5

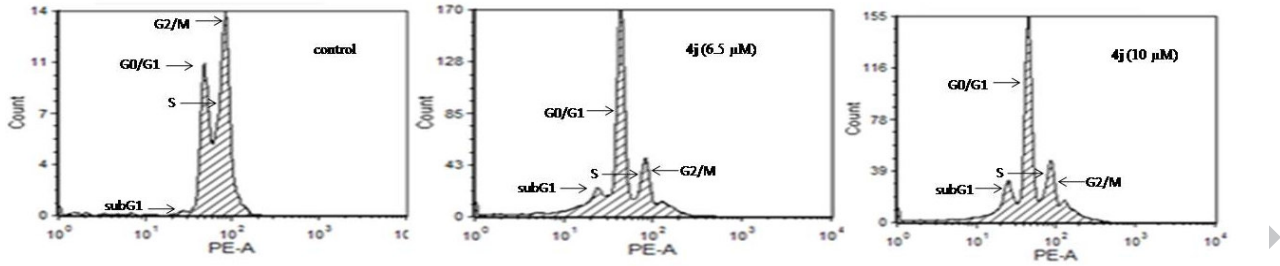

Fig 2. The effect of compound **4j** on cell cycle of HeLa (FACS analysis).[@]

@ HeLa cells are treated with compound **4j** (at 6.5 and 10 µM concentration) for a period of 24 h before FACS analysis is performed. Control = untreated cells.

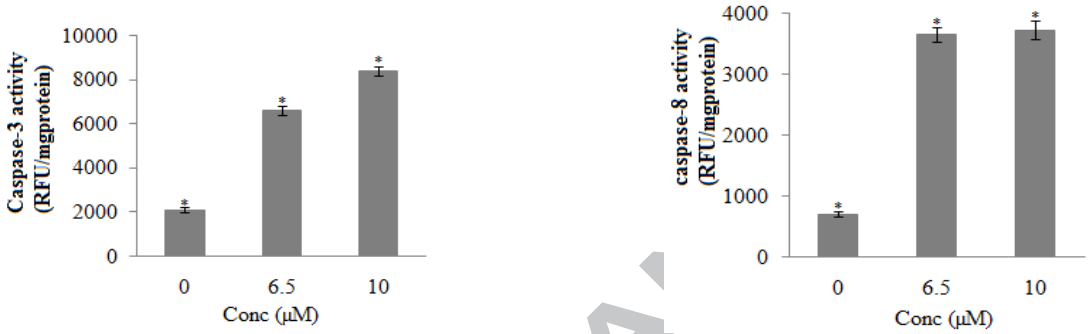

Fig 3. Effect of compound **4j** on caspase-3 and caspase-8 activation in HeLa cells.[§]

§ HeLa cells are treated with compound **4j** at indicated concentrations for a period of 24 h and caspase-3/-8 activities are measured as described in the supplementary information. *, $p < 0.05$ compared to no treatment.

µM (IC_{50}) and 10 µM concentrations in HeLa cells treated for a period of 24 h. It is observed that 51.54 % and 54.78% of cells are accumulated in G0/G1 phase with 6.5 µM and 10 µM of compound **4j** respectively, as compared to 35.73 % in control cells. Thereby, suggesting that compound **4j** arrests cell cycle events in G0/G1 phase (Table 2 & Fig 2). At the same time, it is observed that compound **4j** has caused increase in the cell population in sub-G1 phase (apoptosis) from 1.38 % in control cells to 17.28 % and 19 % at 6.5 µM and 10 µM concentration, respectively (Table 2 & Fig 2). This result shows that compound **4j** induces an apoptotic cell death in HeLa cells.

Apoptosis is an important process of cell death during which programmed cell extinction occurs to maintain harmony in multicellular organisms. To find out whether the 3-(3-trifluoromethylphenyl)-6-phenylimidazo[2,1-*b*]thiazole (**4j**) induces cell death in HeLa cells by apoptosis, we have measured caspase-3 and caspase-8 like activities in cells treated at concentrations of 6.5 and 10 µM for a period of 24 h.[25] It is found that **4j** has significantly increased caspase-3 and caspase-8 activities (Fig 3), suggesting that it induces HeLa cell death by apoptosis.

Thus, a series of new 3,6-diphenylimidazo[2,1-*b*]thiazole derivatives synthesized in this study are evaluated for their cytotoxic activity with reasonable success. Majority of these compounds have shown significant cytotoxic potential against the cervical cancer (HeLa) and lung cancer (A549) cell lines, while they are moderately active against human breast cancer (MDA-MB-231) and leukemic (THP1) cells. The effect of the presence of various substituents on 3-phenyl-ring on the antiproliferative

activity of 3,6-diphenylimidazo[2,1-*b*]thiazole derivatives is observed. The FACS analysis and measurement of caspase-3 and caspase-8 activities to study the basis of antiproliferative activity of the most active compound in HeLa cells has indicated that compound **4j** causes accumulation of cells in G0/G1 phase, thereby inducing apoptotic cell death in human cervical carcinoma cell line at 6.5 μM concentration.

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