

# Regioselective Synthesis of Triazolo[3,4-*e*]purine Derivatives and their Anti-Cancer Activity against NCI-60 Cell Lines

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## Abstract

**Introduction:** Due to the vast medicinal importance of purine nucleoside, a hybrid molecule of triazole with purine ring might explode a lead molecule in the pharma sector and based on the last decade's studies suggested that the nitrogen-rich molecules possess a wide range of medicinal importance.

**Aim:** Due to the vast application of purine nucleoside itself in the field of cancer research, we synthesized triazolo[3,4-*e*]purines and screened them for their anti-cancer study against NCI-60 cell lines by the protocol used by NIH.

**Materials and methods:** The targeted molecules, 4-chloro-5a,6-dihydro-8-substitutedphenyl-1*H*-[1,2,4]triazolo[3,4-*e*]purine derivatives (4a-4h) were synthesized in a two-step procedure by nucleophilic substitution (SN) at C-2 chlorine followed by formation of the triazole ring by acid-catalyzed reaction in the polar protic solvent.

**Results:** It was observed that the regioselective approach followed in C-2 chlorine replacement instead of C-6 chlorine during SN reaction. One-dose response of selected three molecules (4a, 4b, and 4c) showed that 4b (K-562: 64.47 μM & SR: 63.38 μM; mean GI<sub>50</sub>: 99.09 μM) was found to be more potent than 4a and 4c.

**Conclusions:** We have described in this study the general synthetic method for triazolo[3,4-*e*]purines as an innovative class of potential anticancer agents. The dose-response curve in the sense of mean GI<sub>50</sub> for three compounds across all 60 cell lines, 4b can be served as lead after necessary modification.

## Keywords

in vitro anticancer activity, purines, triazolopurines

## INTRODUCTION

In the field of synthetic organic chemistry and medicinal chemistry, nitrogen-based heterocyclic compounds attract more attention of researchers due to their extensive biologically active properties.<sup>1</sup> Among the group of such molecules, purine nucleoside has been found to be more potent

than any other nitrogen-rich scaffolds in the field of cancer therapy.<sup>2</sup> Derivatives of purines are used in current drug discovery system in the area of treatment of cancer in most cases.<sup>3</sup> In 1953, 6-mercaptopurine was found to be the first purine analogue having cancer activity and was approved by the FDA. A number of structures possessing the purine nucleus have been reported to show diverse biological acti-

vities (purinethol and fludarabine, leukemia; aciclovir, herpes infection; abacavir, HIV/AIDS; allopurinol, decrease uric acid levels in the blood).<sup>5</sup> Purine derivatives directly affect tumor cell growth by acting as active components in the biochemical processes in cancer and important in host and tumor interaction process.<sup>6-8</sup> The drug used in cancer therapy generally disturb the synthesis of DNA or snoop its function by one or other ways to kill or inhibit the cancer cell growth.<sup>9</sup> After concerning the potency of such active nucleoside, it is not surprising that the researcher focused on to study chemistry, synthesis or biochemical processes of purines in human host.<sup>10</sup>

Simultaneously, one more nitrogen annoying molecules, i.e. triazoles are an equally important type of heterocyclic compounds responsible for the supreme pharmacological effect.<sup>11</sup> Literature shows the diversity of both triazoles and purine compounds in the area of pharmacology due to their properties as anticancer<sup>12</sup>, antileukemic<sup>13-15</sup>, anti-HIV-1<sup>16,17</sup>, antiviral<sup>18,19</sup>, antitubercular<sup>20,21</sup>, antimicrobial agents<sup>22-24</sup>, acid base indicator<sup>25</sup>, and dyes<sup>26</sup>.

In addition, triazolo purine compounds act as a model for metal-ligand interaction observed in biological systems.<sup>27-30</sup> So, keeping the importance of these compounds in mind, we have taken up an extensive project to synthesize a new series of 1,2,4-triazoles of purine base analogues with the primary aim to evaluate their cytotoxic potency. In this work, we have reported the synthesis and anticancer screening of 4-chloro-5a,6-dihydro-8-phenyl-1H-[1,2,4]triazolo[3,4-*e*]purine derivatives.

## MATERIALS AND METHODS

All the chemicals and reagents were received from sigma-Aldrich and Merck. Silica gel plate G60 F254 (Merck) was used in thin layer chromatography to monitor the completion of the reaction. Visualization was made under UV light (254 nm and 365 nm). Infrared spectra of the compounds were recorded on IR Affinity-1S spectrophotometer (Shimadzu). <sup>1</sup>H (400 MHz) and <sup>13</sup>C (101.1 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in DMSO-*d*<sub>6</sub>. Mass spectrometer GCMS-QP 2010 (Shimadzu) was used to resolute the mass spectra of compounds and rotary evaporator was used for drying the compounds.

### Synthetic part

#### *General procedure for the synthesis of 1-(6-chloro-9H-purin-2-yl)hydrazine (2) (Step I)*

In a 100 ml conical flask 2, 6-dichloro-9H-purine (**1**) (5.2 mmol, 1.0 g) and hydrazine hydride (5.2 mmol, 0.168 g, 1 eq.) were added and stirred at room temperature for 1 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, diethyl ether (DEE) was added into the reaction mixture.

The isolated product was filtered, washed with DEE and dried. The obtained product used directly for the next step. Confirmation of the intermediate hydrazide of purine (**2**) was carried out using <sup>1</sup>H NMR spectrum of compound.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δppm: 12.23 (s, 1H, Imidazole ring -NH proton), 8.21 (s, 1H, Imidazole ring methine proton), 8.13 (s, 1H, Hydrazide substituent -NH protons), 4.58-4.56 (Two broad peak, 2H, Hydrazide substituent -NH<sub>2</sub> proton).

#### *General procedure for the synthesis of 4-chloro-5a,6-dihydro-8-substitutedphenyl-1H-[1,2,4]triazolo[3,4-*e*]purine derivatives (4a-4h) (Step II)*

To produce a series of 4-chloro-5a,6-dihydro-8-substitutedphenyl-1H-[1,2,4]triazolo[3,4-*e*]purine derivatives (4a-4h), 1-(6-chloro-9H-purin-2-yl)hydrazine (0.005 mmol) was reacted with different aromatic aldehydes (0.005 mmol) (**Fig. 1**) in presence of catalytic amount of concentrated hydrochloric acid and ethanol as a solvent. The reaction mixture was stirred at room temperature for 2 h. The reaction was monitored by thin layer chromatography and after completion of the reaction; the reaction mixture was poured onto crushed ice and stirred at room temperature for 1 h. The isolated product was filtered out and was purified by crystallization in ethanol.

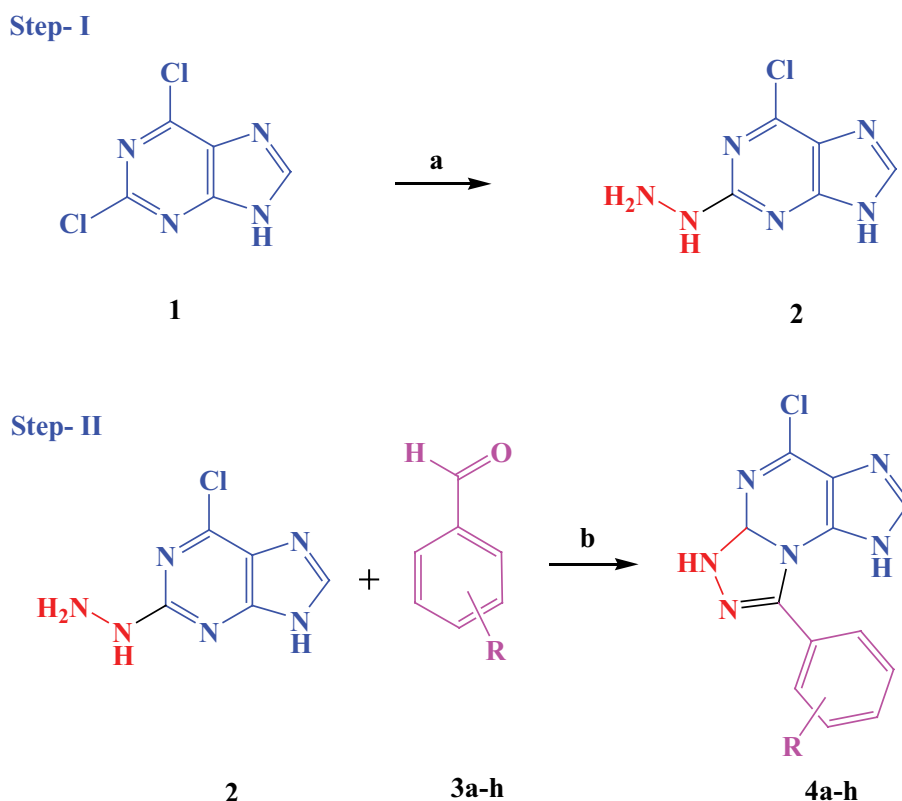
### Data analysis

#### *4-Chloro-8-(4-chlorophenyl)-5a,6-dihydro-1H-[1,2,4]triazolo[3,4-*e*]purine (4a)*

Yield: 90%; mp: 182°C; IR (cm<sup>-1</sup>): 3230.00 (-NH Stretching), 3120.82 (=C-H Stretching), 2856.58 (-C-H Stretching), 1620.21 (-NH Bending), 1595.13, 1489.05 1467.83 (aromatic ring skeleton), 1317.38 (-C-N Bending: aryl), 825.53 (*p*-disubstituted aromatic ring), 769.60 (-C-Cl Stretching); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δppm: 12.42 (s, 1H), 8.74 (s, 1H), 8.27 (s, 1H), 7.97-7.95 (d, *J* = 8 Hz, 2H), 7.55-7.53 (d, *J* = 8 Hz, 2H); <sup>13</sup>C NMR (101.1 MHz, DMSO-*d*<sub>6</sub>) δ: 154.70, 153.74, 150.46, 146.90, 143.59, 134.75, 131.90, 129.56, 126.63, 107.92, 47.89, 47.38; MS (m/z): 307.14.

#### *4-Chloro-5a,6-dihydro-8-(4-methoxyphenyl)-1H-[1,2,4]triazolo[3,4-*e*]purine (4b)*

Yield: 75%; mp: 162°C; IR (cm<sup>-1</sup>): 3210.00 (-NH Stretching), 3105.02 (=C-H Stretching), 2842.72 (-C-H Stretching), 1614.42 (-NH Bending), 1510.26 (aromatic ring skeleton), 1371.39 (C-N Bending: aryl), 1141.86 (-C-O Stretching), 823.60 (*p*-disubstituted aromatic ring), 785.03 (C-Cl Stretching); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 12.05 (s, 1H), 8.41 (s, 1H), 8.20 (s, 1H), 7.87-7.86 (d, *J* = 8 Hz, 2H), 7.04-7.01 (d, *J* = 12 Hz, 2H); <sup>13</sup>C NMR (101.1 MHz, DMSO-*d*<sub>6</sub>) δ: 160.72, 152.14, 150.46, 146.09, 145.37, 129.12, 126.24, 114.13, 55.26; HRMS (ESI) [M+H]<sup>+</sup> C<sub>13</sub>H<sub>11</sub>ClN<sub>6</sub>O calcd 303.0683, obtained 303.0466.



**Reaction & conditions:** (a)  $\text{NH}_2\text{NH}_2$ , Room Temperature, (b) Ethanol, Dil. HCl, 2.0 h

**Figure 1.** Reaction scheme: route of the synthesis for compounds 4a-4h.

**4-Chloro-8-(2-chlorophenyl)-5a,6-dihydro-1H-[1,2,4]triazolo[3,4-*e*]purine (4c)**

Yield: 72%; mp: 178°C; IR ( $\text{cm}^{-1}$ ): 3312.05 (–NH Stretching), 3123.09 (=C–H Stretching), 2900.84 (–C–H Stretching), 1623.13 (–NH Bending), 1585.49, 1465.90, 1435.04 (aromatic ring skeleton), 1396.46 (C–N Bending: aryl), 842.89 (*p*-disubstituted aromatic ring), 759.95 (C–Cl Stretching);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 11.27 (Broad-s, 2H), 9.01–8.97 (d,  $J = 8\text{Hz}$ , 1H), 8.74–8.72 (s, 1H), 8.40 (s, 1H), 8.15 (s, 1H), 7.53–7.47, 7(t, 2H);  $^{13}\text{C}$  NMR (101.1 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 158.27, 153.27, 150.51, 144.02, 143.31, 134.63, 133.07, 131.55, 130.60, 130.12, 129.69, 128.12, 127.40; MS ( $m/z$ ): 307.14.

**8-(3-Bromophenyl)-4-chloro-5a,6-dihydro-1H-[1,2,4]triazolo[3,4-*e*]purine (4d)**

Yield: 78%; mp: 173°C; IR ( $\text{cm}^{-1}$ ): 3201.96 (–NH Stretching), 3119.14 (=C–H Stretching), 2896.05 (–C–H Stretching), 1624.26 (–NH Bending), 1589.34, 1487.83, 1398.38 (aromatic ring skeleton), 1367.53 (C–N Bending: aryl), 852.54 (*p*-disubstituted aromatic ring), 792.24 (C–Cl Stretching), 601.79 (–C–Br Stretching);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 12.54 (s, 1H), 8.90 (s, 1H), 8.28 (s, 1H), 8.15–7.91 (d,  $J = 6.72\text{ Hz}$ , 1H), 7.65–7.63 (q, 1H), 7.45–7.41 (t, 1H);  $^{13}\text{C}$  NMR (101.1 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 152.96, 150.56,

145.68, 135.78, 132.67, 130.75, 129.60, 126.93, 122.19; HRMS (ESI)  $[\text{M}+\text{H}]^+$   $\text{C}_{12}\text{H}_8\text{BrClN}_6$  calcd 352.5891, obtained 352.9420.

**4-Chloro-8-(4-fluorophenyl)-5a,6-dihydro-1H-[1,2,4]triazolo[3,4-*e*]purine (4e)**

Yield: 86%; mp: 206°C; IR ( $\text{cm}^{-1}$ ): 3400.12 (–NH Stretching), 3150.19 (=C–H Stretching), 2859.08 (–C–H Stretching), 1620.03 (–NH Bending), 1510.26, 1470.26, 1369.46 (aromatic ring skeleton), 1315.45 (C–N Bending: aryl), 1111.00 (–C–F Bending), 877.61 (*p*-disubstituted aromatic ring), 785.03 (C–Cl Stretching), 601.79 (–C–F Stretching);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 12.33–12.03 (Distorted d, 2H), 8.74 (s, 3H), 7.51–7.50 (d, 1H), 7.35–7.28 (q, 2H);  $^{13}\text{C}$  NMR (101.1 MHz,  $\text{DMSO-d}_6$ )  $\delta$ : 162.07, 161.76, 159.28, 151.94, 149.94, 146.45, 138.58, 131.79, 127.26, 124.67, 121.07, 115.81, 108.35; HRMS (ESI)  $[\text{M}+\text{H}]^+$   $\text{C}_{12}\text{H}_8\text{FCIN}_6$  calcd 291.0483, obtained 291.0273.

**8-(4-Bromophenyl)-4-chloro-5a,6-dihydro-1H-[1,2,4]triazolo[3,4-*e*]purine (4f)**

Yield: 76%; mp: 232°C; IR ( $\text{cm}^{-1}$ ): 3298.19 (–NH Stretching), 3120.26 (=C–H Stretching), 2888.26 (–C–H Stretching), 1622.06 (–NH Bending), 1589.00, 1480.00, 1408.04 (aromatic ring skeleton), 1375.25 (C–N Bending: aryl), 875.68 (*p*-disubstituted aromatic ring), 785.03 (C–Cl Stret-

ching), 688.59 (–C–Br Stretching);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 12.24 (s, 1H), 8.44 (s, 1H), 8.22 (s, 1H), 7.86 (s, 2H), 7.67–7.65 (d,  $J$  = 8 Hz, 2H);  $^{13}\text{C}$  NMR (101.1 MHz, DMSO- $d_6$ )  $\delta$ : 152.06, 150.44, 144.84, 132.93, 131.97, 130.16, 123.11; HRMS (ESI)  $[\text{M}+\text{H}]^+$   $\text{C}_{12}\text{H}_8\text{BrClN}_6$  calcd 352.9662, obtained 352.9420.

#### 4-(4-Chloro-5a,6-dihydro-1H-[1,2,4]triazolo[3,4-*e*]purin-8-yl)phenol (4g)

Yield: 90%; mp: 164°C; IR ( $\text{cm}^{-1}$ ): 3400.56 (–OH Stretching), 3233.20 (–NH Stretching), 3112.89 (=C–H Stretching), 2856.26 (–C–H Stretching), 1618.21 (–NH Bending), 1590.18, 1469.47, 1434.32 (aromatic ring skeleton), 1368.29 (C–N Bending: aryl), 1141.86 (–C–O Stretching), 1026.13 (–C–O Stretching), 827.36 (*p*-disubstituted aromatic ring), 766.22 (C–Cl Stretching);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 12.02 (s, 1H), 10.01 (s, 1H), 8.45 (s, 1H), 8.14 (s, 1H), 7.77–7.75 (d,  $J$  = 8 Hz, 2H), 6.87–6.85 (d,  $J$  = 8 Hz, 2H);  $^{13}\text{C}$  NMR (101.1 MHz, DMSO- $d_6$ )  $\delta$ : 159.40, 152.27, 150.41, 146.73, 129.34, 124.58, 115.55; HRMS (ESI)  $[\text{M}+\text{H}]^+$   $\text{C}_{12}\text{H}_9\text{ClN}_6\text{O}$  calcd 289.0526, obtained 289.0347.

#### 4-Chloro-5a,6-dihydro-8-*p*-tolyl-1H-[1,2,4]triazolo[3,4-*e*]purine (4h)

Yield: 91%; mp: 156°C; IR ( $\text{cm}^{-1}$ ): 3322.06 (–NH Stretching), 3112.55 (=C–H Stretching), 2906.58 (–C–H Stretching), 1629.59 (–NH Bending), 1589.40, 1469.10, 1425.49 (aromatic ring skeleton), 1390.49 (C–N Bending: aryl), 846.58 (*p*-disubstituted aromatic ring), 770.58 (C–Cl Stretching);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 12.33 (s, 1H), 8.75–8.67 (d, 1H), 8.25 (s, 1H), 7.82–7.75 (t,  $J$  = 16.8 Hz, 2H),

7.32–7.14 (t,  $J$  = 9.2 Hz, 2H), 7.13 (s, 1H);  $^{13}\text{C}$  NMR (101.1 MHz, DMSO- $d_6$ )  $\delta$ : 161.23, 152.82, 150.48, 147.14, 140.00, 136.66, 129.48, 128.28, 127.63, 125.82; MS ( $m/z$ ): 286.07.

## Methodology for anti-cancer screening

The anticancer screening was performed at the National Cancer Institute (NCI), Bethesda, Maryland, USA. The compounds were evaluated in 60 cell lines in a one-dose primary anticancer assay subsequent to the NCI preclinical antitumor drug discovery screen.<sup>31</sup> Human tumor cell lines of the cancer screening panel were grown according to the specific protocol they follow and reported in literature.<sup>31,33</sup> For drug addition, an experimental drug was solubilized in dimethyl sulfoxide. The solubilized drug was applied on tumor cell lines (according to the standard procedure they followed).<sup>32</sup> Single dose response parameter was calculated by selected formula  $[(\text{Ti}-\text{Tz})/(\text{C}-\text{Tz})]\times 100 = 50$ . Net loss of cells was calculated from  $[(\text{Ti}-\text{Tz})/\text{Tz}]\times 100 = -50$ .<sup>33</sup>

## RESULTS AND DISCUSSIONS

### Synthetic part

The novel series of triazolo[3,4-*e*]purine derivatives were obtained by the sequence of reactions shown in Fig. 1. The substituents for the synthesis of target molecule with their % of yield and substituents effect on its cancer activity are summarised in Fig. 2.

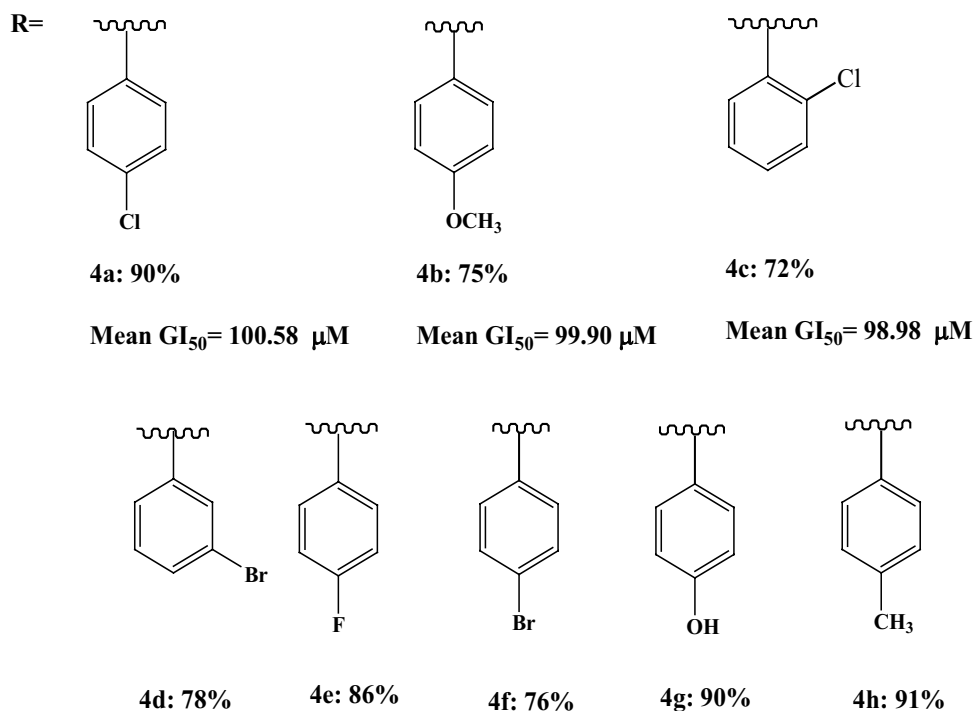


Figure 2. Used substituents, yields and their mean GI<sub>50</sub> values.

Nitrogen-rich purine hydrazide (2) was synthesized by coupling of 2,6-dichloropurine (1) with hydrazine hydrate (40%). Purine hydrazide intermediate (2) on reaction with substituted phenyl aldehyde in the presence of the catalytic amount of hydrochloric acid gave cyclized product 4a-4h at C-2 position having good yield. Optimizations carried out were by without acid catalyst and by varying solvents but no major progress of the reaction was obtained even by using heating conditions.

### Spectroscopic data analysis

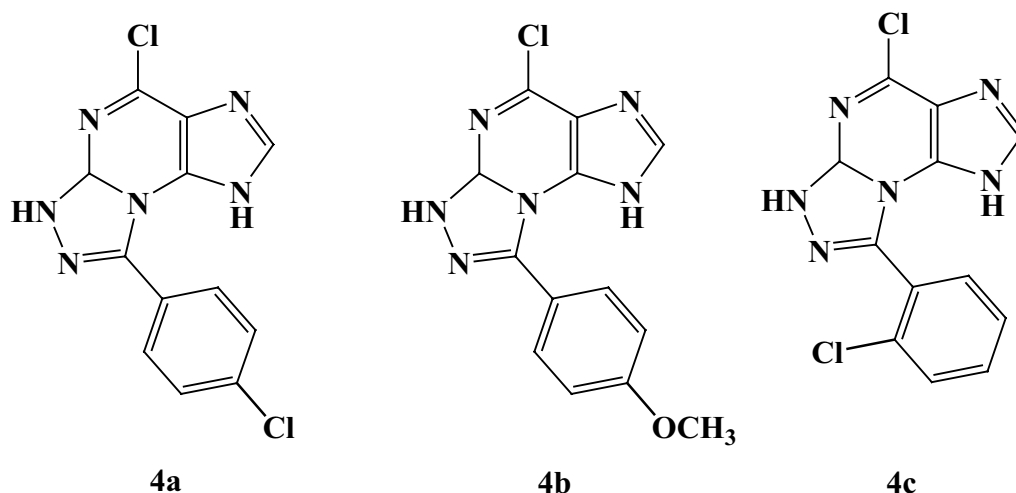
The structures of the synthesized compounds were confirmed by IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and mass spectral data.  $^1\text{H}$  NMR spectra revealed a singlet at  $\delta = 12$  ppm ( $^1\text{H}$ ) assigned to  $-\text{NH}$  of triazole aromatic ring, in addition to that, the presence of two more singlets in upfield region 3.0-3.8  $\delta$ ppm (purine five-membered ring  $-\text{NH}$  proton), 2.8-2.3  $\delta$ ppm (methine proton of purine five-membered ring) indicating the existence of this compound in the hydrazide form rather than the azo form. In compound 4c, 4f and 4g, one broad hump was observed at 10-11  $\delta$ ppm and 4-5  $\delta$ ppm due to tautomeric  $-\text{NH}$  proton in the five membered purine ring (see supplementary material). IR spectra of the synthesized compounds show a very weak peak at 3300 - 3150  $\text{cm}^{-1}$  due to tautomerisation of  $-\text{NH}$  stretching frequency. IR spectra of all newly synthesized compounds show a strong and sharp peak in the region of 1280-1230  $\text{cm}^{-1}$  (C-N stretching) which indicates the presence of aromatic amines.

### Anti-cancer results

After the screening, out of seven compounds, three compounds (4a, 4b, and 4c) were selected and evaluated for in vitro anticancer activity against NCI-60 cell-lines in nine different panels consisting of the leukemia cell line, the non-small lung cancer cell line (NSCLC), the colon cancer cell lines, CNS cancer cell-lines, melanoma cancer cell lines, the ovarian cancer cell lines, the renal cancer cell lines, the prostate cancer cell lines and the breast cancer cell-line. For all these cell line activities they follow the method given in literature.<sup>31,34</sup> Each cell lines have more than three sub-panels. Compounds 4a and 4b showed promising activity as compared to that of 4c. Compound 4a reduced the growth NSCLC (NCI-H522) and renal cancer cell line (A498) to near about 73%. The cell line growth data of compounds 4a, 4b, and 4c are given in **Table 1**.

Compound 4b showed maximum activity and gave better results in leukemia cancer cell lines (leukemia K-562 sub panel and leukemia SR sub panel cell lines;  $\text{GI}_{50}$  values: 64.47% and 63.38% respectively). In one response parameter,  $\text{GI}_{50}$  value was calculated for each used NCI-60 cell lines which correspond to the compound concentration causing 50% decreases in net cell growth.<sup>35,36</sup> Compound 4a showed promising  $\text{GI}_{50}$  value (75%) against non-small cell lung cancer cell-line (NCI-H522) and maximum active compound 4b also showed promising  $\text{GI}_{50}$  value (75%) against NSCLC (NCI-H522). These compounds have shown growth inhibition against the cancer cell line men-

**Table 1.** Growth percentages of the four cell line panel in the primary anticancer screen of the selected compounds (4a, 4b, 4c)



Comp. no.	NSC ID	Sample concentration	Growth percentages (In limit: < 85%)				
			Lung NCI-H522	Lung A549	Renal A498	Leukemia K-562	Leukemia SR
4a	D-783035	1.00E-05 Molar	73.17	81.39	73.10	-	-
4b	D-785530	1.00E-05 Molar	75.73	-	-	64.47	63.38
4c	D-785531	1.00E-05 Molar	80.96	-	-	-	-

tioned above probably due to the presence of electron-donating influence of its substituents.<sup>35</sup> On getting the final result of the activity, we were surprised for the compound 4c because it does not show any remarkable values but the mean GI<sub>50</sub> value (98.98%) was excellent than the 4a (100.58%) and 4b (99.09%). The GI<sub>50</sub> and full panel mean-graph midpoint (MG-MID) values respectively, are given in Table 2.

**Table 2.** Median growth inhibitory concentrations (GI<sub>50</sub>, μM) of *in vitro* subpanel tumor cell lines, and selectivity ratios of compound 4b toward the nine tumor cell lines

Subpanel tumor cell-lines <sup>a</sup>	GI <sub>50</sub> (μM)	Selectivity ratio
I	83.10	1.19
II	95.35	1.03
III	100.11	0.98
IV	97.97	1.01
V	103.05	0.96
VI	105.16	0.94
VII	99.19	0.99
VIII	100.10	0.98
IX	103.74	0.95
Full panel MG-MID	99.09 <sup>b</sup>	-

<sup>a</sup> I: leukemia; II: non-small cell lung cancer; III: colon cancer; IV: CNS cancer; V: melanoma; VI: ovarian cancer; VII: renal cancer; VIII: prostate cancer; IX: breast cancer; <sup>b</sup> GI<sub>50</sub> (1 M) full panel mean-graph mid-point (MG-MID) = The average sensitivity of all cell lines toward the test agent.

## Mechanism of action

Purine and pyrimidines are necessary components for the synthesis of DNA and among them about 14 antimetabolites were approved by FDA as anticancer precursors. Generally, purine derivatives act as an anticancer agent via membrane transporter.<sup>36</sup> After entering the cell, it is converted into genetic material nucleotides by enzymes. These nucleotides then inhibit one or more enzymes on the basis of wrong selection of nucleotides which are essential for the next step of DNA synthesis.<sup>37</sup> It is known that tumour cells have several mutations that might lead to defective apoptosis-inducing pathways, and this is probably at the basis of the initial expansion of the population of neoplastic cells. On the basis of the above discussion, it was realised that mismatching of DNA (genetic material) lead to mutagenesis in host cell which may result in the anticancer property of purine scaffolds. As per the literature survey, we haven't found any mutation rate of purine nucleoside and/or purine-triazole adducts.<sup>38,39</sup> We have synthesized fused triazolopurines derivatives which possess free -NH at the same

position which may act in the same way as depicted above. The substitution present on the phenyl ring is also responsible for the incorporation of DNA and RNA functionalities. Methoxy group present at 4<sup>th</sup> position has around to bind over there and is only possible via -NH of triazole which shows the highest activity for inhibition of DNA synthesis.

## CONCLUSIONS

Thus, we have the proficient general synthetic method for 4-chloro-5a,6-dihydro-8-substitutedphenyl-1*H*-[1,2,4]triazolo[3,4-*e*]purine as an innovative class of potential anticancer agents. The triazolopurines were obtained by cyclisation of the 1-(6-chloro-9*H*-purin-2-yl)hydrazine and substituted phenyl aldehyde by using concentrated hydrochloric acid as a dehydrating agent. NCI-60 cell-lines screened compounds have shown comparable activity according to its spatial arrangements. An overall study shows that the compounds 4a is promising active against renal cancer cell line (A498: GI<sub>50</sub> 73.10) NSCLC (NCI-522: GI<sub>50</sub> 73.17), compounds 4b shows the highest activity against leukemia cancer cell-line (SR: GI<sub>50</sub> 63.38) and NSCLC (NCI-522: GI<sub>50</sub> 75.73).

## Conflict of Interest

Authors declare that there is no conflict of interest.

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# Региоселективный синтез производных триазоло [3,4-е] пурина и их противораковая активность в отношении клеточных линий NCI-60

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## Резюме

**Введение:** Из-за широкой лекарственной ценности пуринового нуклеозида гибридная молекула триазоло с пуриновым кольцом может быть ведущим соединением в фармацевтической промышленности на основе исследований последнего десятилетия, которые доказывают, что молекулы, богатые азотом, имеют широкий спектр лекарственной ценности.

**Цель:** В связи с широким использованием самого пуринового нуклеозида в области рака, мы синтезировали триазоло [3,4-*e*] пурины и изучили их противораковые свойства в отношении клеточных линий NCI-60 в соответствии с протоколом, используемым Национальными институтами здравоохранения.

**Материалы и методы:** Производные целевых молекул (4а-4h) 4-хлор-5а, 6-дигидро-8-замещенного фенил-1Н- [1,2,4] триазоло [3,4-*e*] пурина были синтезированы двухшаговой процедурой путём нуклеофильного замещения (НЗ) хлора С-2 с последующим образованием триазольного кольца с помощью реакции, катализируемой кислотой, в полярном протонном растворителе.

**Результаты:** Было обнаружено, что региоселективный подход следует за заменой хлора С-2 вместо хлора С-6 во время реакции НЗ. Ответ на однократную дозу выбранных трёх молекул (4а, 4b и 4с) показал, что 4b (К-562: 64.47 мкМ и SR: 63.38 мкМ; средний GI<sub>50</sub>: 99.09 мкМ) оказался более эффективным, чем 4а и 4с.

**Заключение:** В этом исследовании мы описали общий метод синтеза триазоло [3,4-*e*] пуринов как инновационный класс потенциальных противораковых агентов. Кривая доза-ответ как средний GI<sub>50</sub> для трёх соединений по всем 60 клеточным линиям показала, что 4b может служить ведущим лекарственным средством после необходимых модификаций.

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## Ключевые слова

противоопухолевая активность in vitro, пурины, триазолопурины

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