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Synthesis and bioevaluation of 6-chloropyridazin-3-yl hydrazones and 6-chloro-3-substituted-[1,2,4]triazolo[4,3-*b*]pyridazines as cytotoxic agents

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ABSTRACT

An efficient synthesis of a series of 6-chloro-3-substituted-[1,2,4]triazolo[4,3-*b*]pyridazines is described *via* intramolecular oxidative cyclization of various 6-chloropyridazin-3-yl hydrazones with iodobenzene diacetate. The structures of the newly synthesized compounds were assigned on the basis of elemental analysis, IR, NMR (^{1}H and ^{13}C) and mass spectral data. All the thirty three compounds **3a-q** and **4b-q** synthesized in the present study were evaluated for their *in vitro* cytotoxic activities against two Acute Lymphoblastic Leukemia (ALL) cell lines named, SB-ALL and NALM-6, and a human breast adenocarcinoma cell lines (MCF-7). The results revealed that triazoles **4** exhibit better cytotoxicity than their hydrazone precursors **3**. Among triazoles, compounds **4f**, **4j** and **4q** exhibited potent cytotoxic activity against SB-ALL and NALM-6 with IC_{50} values in the range of ~1.64–5.66 μM and ~1.14–3.7 μM, respectively, compared with doxorubicin (IC_{50} = 0.167 μM, SB-ALL). Compounds **4f, 4j** and **4q** were subjected to apoptosis assay after 48 h treatment and these compounds induced apoptosis of NALM-6 cells *via* caspase 3/7 activation. Results revealed that compound **4q** represents potential promising lead.

1. Introduction

The 1,2,4-triazole nucleus, an important five-membered heterocyclic scaffold, is found in large number of marketed drugs such as Vorozole, Letrozole and Anastrozole which are potent and selective non-steroidal inhibitors of cytochrome P450 aromatase and used in treatment of hormone receptor-positive breast cancer (Fig. 1) [1,2]. Recently our group reported a series of 1,2,4-triazolo[4,3-*a*]quinoxalines exhibiting promising DNA photocleaving activity [3]. Pyridazine nucleus play a key role in drug discovery as it can improve the physicochemical profile of drug candidates by increasing their water solubility. Pyridazine ring has been known to be present in several natural products (antifungal antibiotic Pyridazomycin and meroterpenoid Azamerone) and drugs (nonsteroidal anti-inflammatory drug such as Emorfazone, phosphodiesterase inhibitors Amipizone, Pimobendan, Zardaverine, Milrinone, Imazodan, and cardiotonic drug Indolidan) [4]. 1,2,4-Triazolo[4,3-*b*]pyridazine derivatives, in particular, possess extensive therapeutic properties like anxiolytic [5], anticonvulsant [6], antimicrobial properties [7,8], antituberculostatic [8], simultaneous inhibitor of α5 subunit-containing GABAA receptors (GABAARs) and enhances α7 neuronal nicotinic-acetylcholine receptors (nAChRs) [9] besides being used as various enzyme inhibitors such as Leucine rich repeat kinase 2 (LRRK2) [10], phosphodiesterase (PDE4) [11]. Meticulously, (*S*)-6-(1-(6-(1-methyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[4,3-*b*]pyridazin-3-yl)ethyl)-quinoline (**I**) has been reported as potent and highly selective c-MET inhibitors (Fig. 1) having an important role in tumor invasive growth and metastasis [12]. 4-(2-(6-Methyl-[1,2,4]triazolo[4,3-*b*]pyridazin-8-ylamino)ethyl)-phenol (**II**) and (*R*)-3-(2,5-dimethoxyphenyl)-6-(4-methoxy-3-(tetrahydrofuran-3-yloxy)phenyl)-[1,2,4]triazolo[4,3-*b*]pyridazine (**III**) have been established as highly potent tankyrases (TNKSs) [13] and PDE4A inhibitors [11], respectively. 3,6-Diaryl-[1,2,4]triazolo[4,3-*b*]pyridazines (**IV**) displayed the highly active antiproliferative activity against SGC-7901, A549 and HT-1080 cell lines with IC_{50} values of in the range 0.008–0.014 M, respectively and effectively inhibited tubulin polymerization [14].

A number of synthetic methods have been developed for the synthesis of [1,2,4]triazolo[4,3-*b*]pyridazine derivatives which involve the oxidation of hydrazones with various reagents such as lead tetraacetate [15], bromine [15], nitrobenzene [16], copper dichloride [17], mixture of Me_4NBr and oxone® [7] etc, reaction of hydrazinopyridazine with aroyl chlorides [8]/ethyl orthoformate or ethyl orthoacetate [5,16,18], reaction of 3-chloropyridazines with acylhydrazines or

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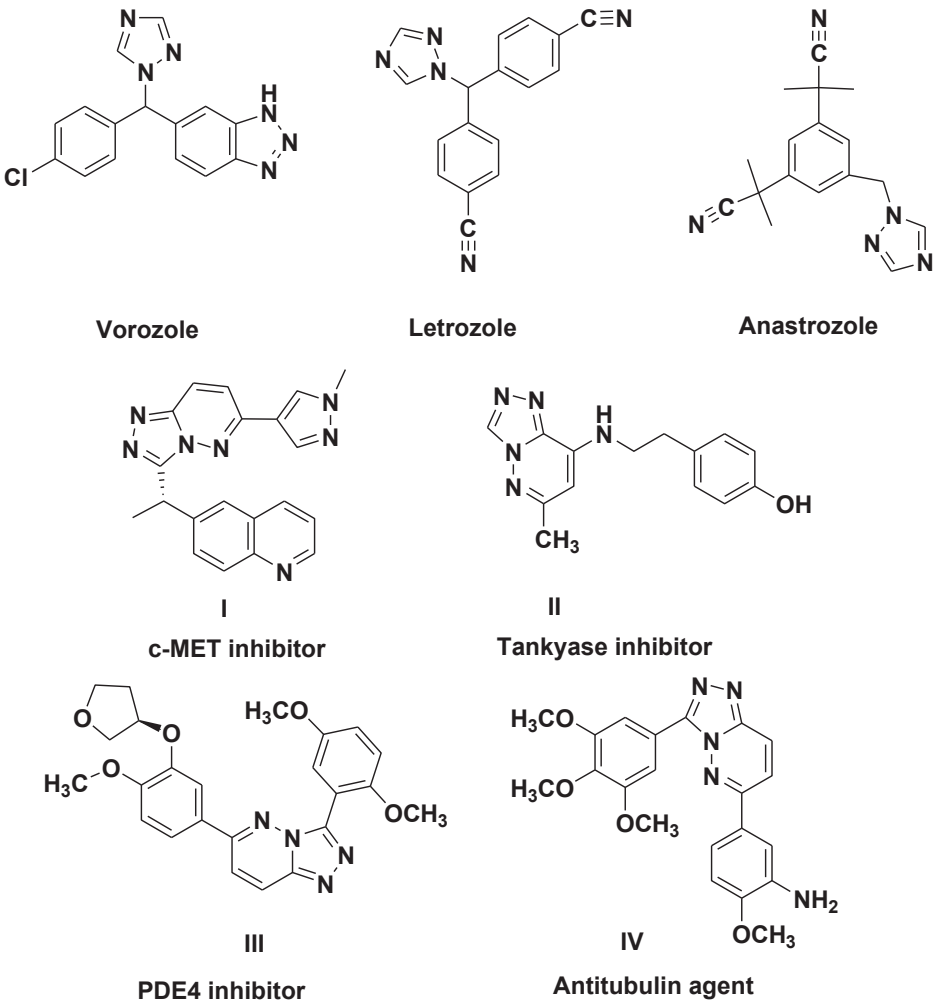


Fig. 1. Commercial anticancer drugs containing 1,2,4-triazole moiety and biologically active [1,2,4]triazolo[4,3-*b*]pyridazines.

formic hydrazide in *n*-butanol under reflux for 10–20 h [5,6]. Unfortunately, most of these methods suffer from various disadvantages such as hazardous materials, poor yields, longer reaction time, elevated temperatures and tedious work-up procedures. Therefore, a more efficient and mild synthesis of [1,2,4]triazolo[4,3-*b*]pyridazines is desirable. Utility of iodobenzene diacetate (IBD) in oxidative transformation is a valuable strategy for greener synthesis because of its easy availability, mild reaction condition and ease of handling.

Keeping in view the significance, and in continuation of our ongoing research work on hypervalent iodine reagents in organic transformations [19–22], we herein report the greener synthesis of a series of 6-chloro-3-substituted-[1,2,4]triazolo[4,3-*b*]pyridazines from hydrazone precursors using IBD and evaluate *in vitro* anticancer activity of hydrazones and triazoles against three different human cancer cell lines.

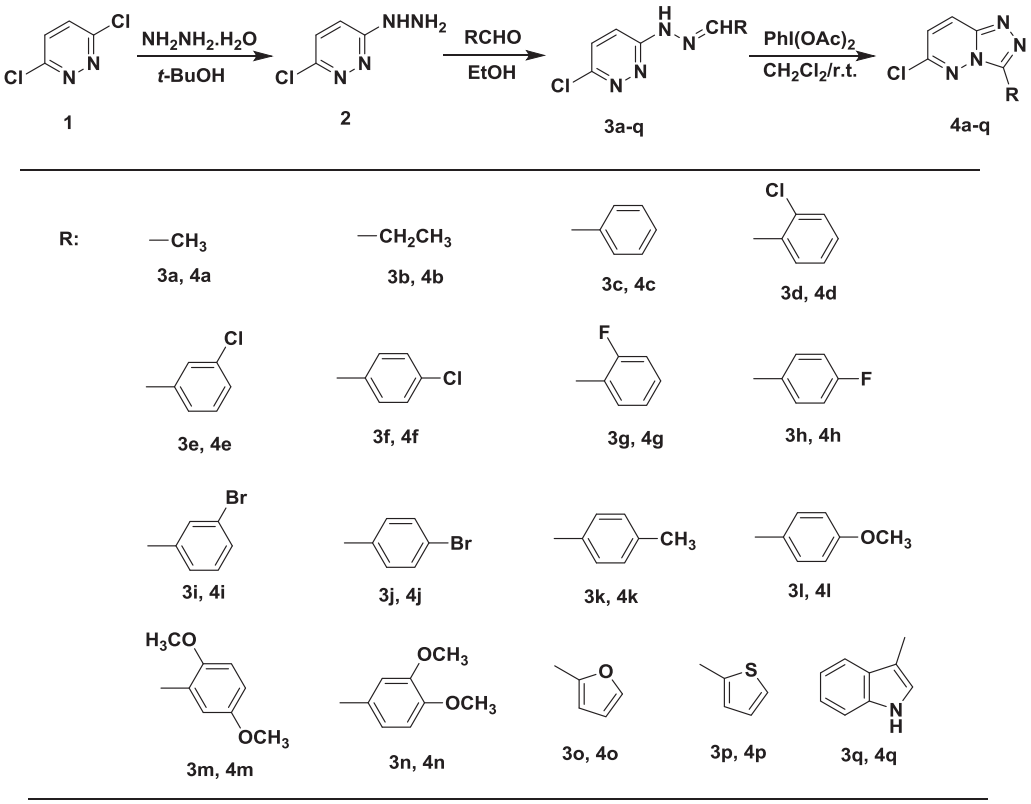
2. Results and discussion

2.1. Chemistry

Synthetic pathway to [1,2,4]triazolo-[4,3-*b*]-pyridazines is depicted in Scheme 1. Initially, 6-chloro-3-hydrazinopyridazine **2** was obtained by refluxing of 3,6-dichloropyridazine **1** with hydrazine hydrate in *tert*-BuOH. Compound **2** on reaction with equimolar amount of acetaldehyde in refluxing ethanol afforded the key intermediate, ethylidenehydrazino-6-chloropyridazine **3a**. The intramolecular oxidative cyclization of **3a** with 1.1 equivalents IBD in dichloromethane at room temperature provided the desired compound 6-chloro-3-methyl-[1,2,4]triazolo[4,3-*b*]pyridazine **4a** in good yield (80%). The structure and purity of **4a** was established by TLC and its spectral (IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analytical data. Encouraged by the success of the reaction, different aldehydes having electron donating group, electron withdrawing group, disubtituted aryl and heteroaryl groups were condensed with **2** and subsequent oxidation with IBD under identical conditions led to the formation of desired triazolo[4,3-*b*]pyridazines **4b-q** in 81–91% yields. The reaction has a broad scope in terms of substitution diversity and is scalable to gram level.

Additionally, we have attempted cyclization of 3-benzylidenehydrazino-6-chloropyridazine (**3c**) with chloramine-T, another eco-friendly and cheap oxidising reagent known for such type of oxidative cyclization. Contrary to our expectations, the reaction did not proceed to furnish the desired product 6-chloro-3-phenyl-[1,2,4]triazolo-[4,3-*b*]-pyridazine (**4c**) even after refluxing **3c** in ethanol for 15 h which was confirmed by running co-TLC with synthesized compound. Compound **4c** exhibits a fluorescent spot under long UV (365 nm) using TLC visualizer which was absent when the oxidative reaction was conducted with chloramine-T.

IR spectra of compounds **3** exhibited one characteristic absorption band in the range of 3024–3217 cm^{-1} for N—H stretching. ^{1}H NMR spectra of **3** displayed two characteristic singlets at δ 7.52–8.67 and 9.82–11.83 ppm assignable to methine proton (N=CH) and N—H protons, respectively, a pair of doublets of one proton intensity each for H-4 and H-5 of pyridazine ring at δ 7.00–7.82 and 7.48–7.92 ppm, respectively, having the coupling constant $^{3}J = \sim 9.36$ Hz. The characterization of products **4** were based upon comparison of their spectral



Scheme 1. Synthesis of 6-chloropyridazin-3-yl hydrazones **3a-q** and their oxidation to [1,2,4]triazolo[4,3-*b*]pyridazines **4a-q**.

data (IR and ^{1}H NMR) with those of intermediate hydrazones **3**. IR spectra of **4** were found to be transparent in the region of NH stretch and bend, thus confirming the oxidation of **3** into **4**. Further confirmation was established by ^{1}H NMR spectra of **4** where the disappearance of signals was observed at δ 7.52–8.67 and 9.82–11.83 ppm for aldehydic H and NH, respectively. Further ^{1}H NMR spectra of **4** exhibited a pair of doublets for H-4 and H-5 of pyridazine ring at δ 7.55–8.53 and 6.88–7.53 ppm, respectively having the coupling constant $^3J = \sim 9.6$ Hz. It is noteworthy that in compounds **4** the downfield shift of proton at position-4 of pyridazine ring in comparison with compound **3** may be attributed to the lone pair effect of the nitrogen of the triazole ring on the H-4. Known products were identified by comparison of their mp with those reported in literature [15,17].

2.2. In vitro cytotoxic evaluation and structure–activity relationship

Cytotoxic activity of all the synthesized compounds (**3a-q** and **4b-q**) were evaluated *in vitro* employing three human cancer cell lines namely SB-ALL, NALM-6 and MCF-7 by using the well-established MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] cell proliferation assay [23,24]. The three cell lines are efficient *in vitro* models for their cytotoxic evaluation of different types of chemotherapeutic agents such as DNA minor groove binders [25], protein kinase inhibitors [26] and caspases inhibitors [27,28] among others. Doxorubicin was used as standard drug.

When all the synthesized compounds were initially screened at 10 μM concentration against these three cancer cell lines, compounds **4f**, **4j** and **4q** showed significant cytotoxic effects comparable to commercial drug doxorubicin against the tested cancer cell lines (Tables 1 and 2).

As evident from results summarized in Table 1, hydrazone derivatives **3** showed poor cytotoxicity against these cell lines. Only two hydrazones **3m** and **3n** exhibited less than 50% cell survival against NALM-6 cell line. In contrast, most triazoles **4** show moderate to high cytotoxicity against different human cancer cell lines (Table 2). It is also evident that triazole derivatives **4f**, **4j** and **4q**, having *p*-chlorophenyl, *p*-bromophenyl and 3-indolyl group, respectively, at position-3 of triazole ring are the most potent amongst the tested compounds. Interestingly, except for MCF-7 cell line, it was noticed that substitution pattern on phenyl residue at position-3 of triazole ring has little influence on cytotoxicity of these triazoles. For triazole derivative **4f** which is substituted at *para*-position of phenyl ring with chloro group leads to an increase in cytotoxicity against SB-ALL and NALM-6 as compared to *ortho* and *meta*-substituted phenyl derivatives **4d** and **4e**, respectively. Similarly incorporation of *para*-substituent on the phenyl ring *viz.* Br (**4h**) and F (**4j**) increases the cytotoxicity as compared to *ortho* derivative **4 g** and *meta* derivative **4i**. No cytotoxicity was observed for compounds **4m** and **4n** having disubstitution of phenyl ring. It is also interesting to note that substitution of position-3 of triazole by a heteroaryl groups have led to the different effects on the cytotoxicity, as the sharp increase of cell viability against three cancer lines in case of compound **4o** (furan-2′-yl) and **4p** (thiophen-2′-yl). In contrast, compound **4q** with indol-3′-yl substituent in the triazole core was the most cytotoxic against all human cancer cell lines therefore indicating that indole group at position-3 of triazole ring plays a crucial role in imparting cytotoxicity to the molecule. For instance, at 10 μM concentration, compound **4q** shows only 14.8% cell survival against SB-ALL, 14.2% against NALM-6 and 31.0% against MCF-7 cell lines, respectively comparable to commercial drug doxorubicin with percentage cell survival 11.2%, 16.8% and 19.0%.

Compounds **4f**, **4j** and **4q** with less than 50% cell survival were chosen as lead compounds and further investigated for their IC_{50} values (Table 3). Compounds **4f** and **4j** were moderately active against MCF-7 cancer cell line with an IC_{50} value of 21.2 and 11.6 μM, respectively, but **4f** and **4j** were highly active against SB-ALL and NALM-6 with IC_{50} values in the range of 1.23–5.66 μM. Compound **4q** exhibited excellent cytotoxicity against SB-ALL (IC_{50} = 1.64 μM), NALM-6 (IC_{50} = 1.14 μM), and MCF-7 cancer (IC_{50} = 3.55 μM) cell lines.

Table 1
Percentage cell survival of compounds **3a-q** at 10 μM concentration using MTT assay against three cancer cell lines.

Tested Compd.	R	% Cell Survival ± S.D.[a]		
		SB-ALL	NALM-6	MCF-7
Cells		100 ± 7.6	100 ± 10.6	100 ± 14.5
3a	$-CH_3$	80.3 ± 23.4	93.0 ± 9.0	87.4 ± 16.3
3b	$-CH_2CH_3$	76.5 ± 5.6	92.5 ± 8.0	81.2 ± 18.7
3c	$-C_6H_5$	93.2 ± 14.4	111.2 ± 10.6	83.2 ± 20.8
3d	-*o*-ClC_6H_4	64.8 ± 23.1	51.9 ± 4.9	75.3 ± 17.4
3e	-*m*-ClC_6H_4	89.8 ± 21.9	117.7 ± 16.1	76.4 ± 17.0
3f	-*p*-ClC_6H_4	71.9 ± 5.9	62.9 ± 11.6	83.6 ± 40.7
3 g	-*o*-FC_6H_4	70.5 ± 12.1	68.6 ± 22.9	66.7 ± 16.8
3h	-*p*-FC_6H_4	72.2 ± 15.5	58.4 ± 7.9	74.7 ± 23.5
3i	-*m*-BrC_6H_4	74.3 ± 10.2	63.2 ± 11.4	65.9 ± 7.6
3j	-*p*-BrC_6H_4	79.0 ± 13.5	72.1 ± 15.5	62.9 ± 21.0
3k	-*p*-$CH_3C_6H_4$	73.3 ± 11.0	55.3 ± 15.5	61.7 ± 10.6
3l	-*p*-$OCH_3C_6H_4$	61.7 ± 12.7	53.0 ± 8.6	69.8 ± 14.1
3m	−2,5-di$(OCH_3)C_6H_3$	63.4 ± 8.2	46.5 ± 3.9	74.5 ± 12.9
3n	−3,4-di$(OCH_3)C_6H_3$	61.1 ± 7.2	45.2 ± 3.6	75.5 ± 19.8
3o	−2′-furyl	97.0 ± 21.4	101.8 ± 14.8	86.3 ± 24.9
3p	−2′-thienyl	80.7 ± 21.8	82.7 ± 12.3	81.8 ± 11.4
3q	−3′-indolyl	76.0 ± 9.2	104.4 ± 13.4	71.4 ± 17.9
Doxorubicin		11.2 ± 3.8	16.8 ± 7.6	19.0 ± 3.0

[a] The activity data represents mean values ± SD of experiments conducted in triplicates at three independent times.

Compounds **4f**, **4j** and **4q** were 2–9 fold more selective for SB-ALL and NALM-6 cell lines compared to MCF-7 cells. Results of cytotoxicity shows that compound **4q** could serve as potent cytotoxic agent by further derivatization.

To gain into deeper insight to mode of action for cytotoxic activity, compounds **4f**, **4j** and **4q** were analyzed for their ability to cause apoptosis (Fig. 2). Caspase 3/7 activation is one of the biochemical hallmarks of apoptotic cell death. Camptothecin was used a positive control.

As shown in Fig. 2, compounds **4f**, **4j** and **4q** caused ~ 3 to 3.5 fold increase in fluorescence respectively, compared to control indicative of caspase 3/7 activation. This data suggests that these compounds kill cancer cells *via* apoptosis induction.

Table 3
IC_{50} values of compounds **4f**, **4j** and **4q** against three cancer cell lines.

Compound	IC_{50}[a] value (in μM) against cancer cell line		
	SB-ALL	Nalm-6	MCF-7
4f	5.66 ± 2.6	3.7 ± 1.6	21.2 ± 3.6
4j	1.87 ± 0.56	1.23 ± 0.26	11.6 ± 2.32
4q	1.64 ± 0.74	1.14 ± 0.24	3.55 ± 0.54
Doxorubicin	0.167 ± 0.032	0.32 ± 0.08	0.48 ± 0.12

[a] IC_{50} = half maximal concentration represents the concentration of drug able to inhibit by 50% the *in vitro* growth. Each value represents mean ± SD of three experiments.

3. Conclusion

In summary, a series of triazolo[4,3-*b*]pyridazines **4a-q** have been synthesized by an efficient and ecofriendly route utilizing iodobenzene diacetate as an oxidant. Compounds **4f**, **4j** and **4q** were found to have significant cytotoxic potency against three cancer cell lines (SB-ALL, NALM-6 and MCF-7) and induced apoptosis of NALM-6 cells *via* caspase 3/7 activation. Preliminary results indicate that the varying substitution patterns at 3-position of triazole ring could have an effect on the

Table 2
Percentage cell survival of compounds **4b-q** at 10 μM concentration using MTT assay against three cancer cell lines.

Tested Compd.	R	% Cell Survival ± S.D.[a]		
		SB-ALL	NALM-6	MCF-7
Cells		100 ± 7.6	100 ± 10.6	100 ± 14.5
4b	$-CH_2CH_3$	77.3 ± 12.5	101.8 ± 13.7	70.7 ± 20.2
4c	$-C_6H_5$	68.4 ± 11.9	96.0 ± 8.7	74.7 ± 15.6
4d	-*o*-ClC_6H_4	72.8 ± 10.9	87.8 ± 13.5	81.4 ± 14.6
4e	-*m*-ClC_6H_4	72.5 ± 13.6	103.7 ± 13.1	78.4 ± 12.5
4f	-*p*-ClC_6H_4	37.8 ± 15.3	32.4 ± 24.1	91.2 ± 17.2
4g	-*o*-FC_6H_4	82.2 ± 11.9	104.6 ± 14.0	75.9 ± 16.1
4h	-*p*-FC_6H_4	60.2 ± 15.3	41.7 ± 7.1	79.3 ± 11.1
4i	-*m*-BrC_6H_4	68.3 ± 8.3	73.0 ± 12.9	65.7 ± 20.1
4j	-*p*-BrC_6H_4	23.8 ± 17.7	22.0 ± 6.7	77.3 ± 14.9
4k	-*p*-$CH_3C_6H_4$	48.4 ± 22.7	96.9 ± 10.5	89.2 ± 33.2
4l	-*p*-$OCH_3C_6H_4$	87.8 ± 24.8	100.3 ± 6.8	79.4 ± 14.1
4m	−2,5-di$(OCH_3)C_6H_3$	82.3 ± 23.3	103.4 ± 10.6	78.7 ± 21.5
4n	−3,4-di$(OCH_3)C_6H_3$	65.7 ± 17.1	94.4 ± 10.9	70.7 ± 32.9
4o	−2′-furyl	74.9 ± 5.0	97.1 ± 11.9	63.8 ± 25.0
4p	−2′-thienyl	42.1 ± 15.9	74.8 ± 21.6	75.8 ± 16.5
4q	−3′-indolyl	14.8 ± 4.4	14.2 ± 6.4	31.0 ± 25.8
Doxorubicin		11.2 ± 3.8	16.8 ± 7.6	19.0 ± 3.0

[a] The activity data represents mean values ± SD of experiments conducted in triplicates at three independent times.

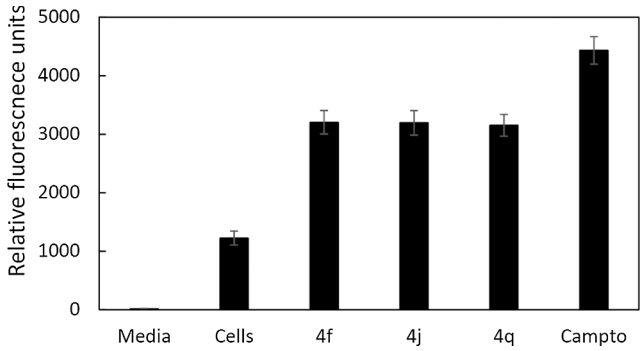


Fig. 2. Caspase activation in NALM-6 cells treated with **4f**, **4j** and **4q** (Data represents mean values ± SD of experiments conducted in duplicates at two independent times).

cytotoxic activity and compound **4q** has the potential to be promising lead.

4. Experimental section

4.1. General chemistry

Melting points were determined in digital melting point apparatus MEPA. IR spectra were recorded on a Buck Scientific IR M−500 spectrophotometer in KBr pellets (ν_{max} in cm^{-1}). Analytical TLC was performed using Merck Kieselgel 60 F254 silica gel plates. Visualisation was performed under UV light using Ultra Violet Flourescence Inspection Cabinet (Perfit, India). ^1H and ^{13}C NMR spectra for analytical purpose were recorded in $CDCl_3$ and DMSO-d_6 on a Bruker instrument; chemical shifts are expressed in δ-scale downfield from TMS as an internal standard. Elemental analyses were performed at Sophisticated Analytical Instrument Facility, Panjab University, Chandigarh, India.

6-Chloro-3-hydrazinopyridazine (**2**) was synthesized according to the literature procedure [29,30].

4.2. Synthesis of 6-chloro-3-ethylidenehydrazinopyridazine (3a)

To an ethanolic solution (15 ml) of 3-chloro-6-hydrazinopyridazine **(2)** (0.36 g, 2.5 mmol) was added acetaldehyde (0.11 g, 2.5 mmol), and the reaction mixture was refluxed for 30 min. During refluxing a solid separated out. The solvent was evaporated in vacuo and the reaction mixture cooled to room temperature. The obtained solid was filtered off, washed with cold ethanol and recrystallized with ethanol to afford **3a**.

Yield 78%; mp > 315 °C. IR (KBr, cm^{-1}): 3202 (−NH str). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.01 (s, 3H, $-CH_3$), 7.28–7.30 (d, 1H, J = 9.36 Hz, pyridazine-4H), 7.50–7.52 (d, 1H, J = 9.36 Hz, pyridazine-5H), 7.54 (s, 1H, methine-H), 10.51 (bs, 1H, −NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.18, 116.29, 140.89, 143.65, 147.22, 159.16. Ms: m/z = 170.04 $[M]^+$, 172.04 $[M+2]^+$, (3:1). Anal. Calcd for $C_6H_7ClN_4$: C, 42.24; H, 4.14, N, 32.84; Found: C, 42.44; H, 4.19, N, 32.94.

Similarly, other compounds (**3b-q)** are synthesized by following the above procedure using appropriate aldehydes.

4.2.1. 6-Chloro-3-propylidenehydrazinopyridazine (3b)

Yield 72%; mp 144.5 °C. IR (KBr, cm^{-1}): 3209 (−NH str). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.13–1.17 (t, 3H, J = 7.52 Hz, $-CH_3$), 2.33–2.37 (q, 2H, J = 7.52 Hz, $-CH_2$), 7.29–7.31 (d, 1H, J = 9.32 Hz, pyridazine-4H), 7.48–7.51 (d, 1H, J = 9.32 Hz, pyridazine-5H), 7.52 (s, 1H, methine-H), 9.82 (bs, 1H, −NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.53, 25.85, 116.18, 129.89, 147.14, 148.27, 159.00. Ms: m/z = 184.05 $[M]^+$, 186.05 $[M+2]^+$, (3:1). Anal. Calcd for $C_7H_9ClN_4$: C, 45.54; H, 4.91, N, 30.35; Found: C, 45.50; H, 4.90; N, 30.33.

4.2.2. 3-Benzylidenehydrazino-6-chloropyridazine (3c)

Yield 77%; mp 258.5 °C; Lit. mp [15] 260.5 °C. ^{13}C NMR (100 MHz, DMSO): 115.45, 126.10, 128.36, 128.78, 129.41, 134.61, 141.66, 147.19, 158.72.

4.2.3. 3-(2′-Chlorobenzylidenehydrazino)-6-chloropyridazine (3d)

Yield 74%; mp 225.5 °C; IR (KBr, cm^{-1}): 3194 (−NH str). ^1H NMR (400 MHz, DMSO) δ: 7.32–7.35 (m, 2H, Ph-4′, 5′-H), 7.40–7.42 (m, 1H, Ph-3′-H), 7.53–7.56 (d, 1H, J = 9.36 Hz, pyridazine-4H), 7.65–7.67 (d, 1H, J = 9.36 Hz, pyridazine-5H), 8.01–8.04 (m, 1H, Ph-6′-H), 8.51 (s, 1H, methine-H), 11.83 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO): δ 116.04, 126.48, 127.45, 128.16, 129.82, 130.11, 131.87, 132.16, 137.64, 147.81, 158.69. Ms: m/z = 266.01 $[M]^+$, 268.10 $[M+2]^+$, 270.03 $[M+4]^+$, (9:6:1). Anal. Calcd for $C_{11}H_8Cl_2N_4$: C, 49.46; H, 3.02; N, 20.98. Found: C, 49.24; H, 3.41, N, 20.84.

4.2.4. 3-(3′-Chlorobenzylidenehydrazino)-6-chloropyridazine (3e)

Yield 76%; mp 224 °C. IR (KBr, cm^{-1}): 3194 (−NH str). ^1H NMR (400 MHz, DMSO) δ: 7.48–7.55 (m, 3H, Ph-4′, 5′, 6′-H), 7.60 (m, 1H, Ph-2′-H), 7.80–7.82 (d, 1H, J = 9.6 Hz, pyridazine-4H), 7.90–7.92 (d, 1H, J = 9.6 Hz, pyridazine-5H), 8.67 (s, 1H, methine-H), 11.83 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 116.50, 127.41, 128.52, 130.30, 130.85, 131.20, 134.41, 135.61, 143.20, 145.50, 160.21. Ms: m/z = 266.01 $[M]^+$, 268.11 $[M+2]^+$, 270.01 $[M+4]^+$, (9:6:1). Anal. Calcd for $C_{11}H_8Cl_2N_4$: C, 49.46; H, 3.02; N, 20.98. Found: C, 49.83; H, 3.33, N, 20.58.

4.2.5. 3-(4′-Chlorobenzylidenehydrazino)-6-chloropyridazine (3f)

Yield 70%; mp 260 °C; Lit. mp [15] 295–296 °C.

4.2.6. 6-Chloro-3-(2′-fluorobenzylidenehydrazino)pyridazine (3 g)

Yield 76%; mp 240 °C. IR (KBr, cm^{-1}): 3027 (−NH str). ^1H NMR (400 MHz, DMSO) δ: 7.14–7.21 (m, 2H, Ph-3′, 5′-H), 7.34–7.39 (m, 1H, Ph-4′-H), 7.55–7.57 (d, 1H, J = 9.36 Hz, pyridazine-4H), 7.64–7.66 (d, 1H, J = 9.36 Hz, pyridazine-5H), 7.93–7.97 (t, 1H, J = 7.44 Hz, Ph-6′-H), 8.33 (s, 1H, methine-H), 11.78 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 115.78, 122.17, 124.76, 125.99, 126.02, 130.87, 130.95, 134.39, 147.70, 158.73, 161.32. Ms: m/z = 250.00 $[M]^+$, 252.00 $[M+2]^+$, (3:1). Anal. Calcd for $C_{11}H_8ClFN_4$: C, 52.71; H, 3.22, N, 22.35. Found: C, 52.31; H, 3.50; N, 22.58.

4.2.7. 6-Chloro-3-(4′-fluorobenzylidenehydrazino)pyridazine (3h)

Yield 72%; mp 256 °C. IR (KBr, cm^{-1}): 3024 (−NH str). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.17–7.21 (t, 2H, J = 8.8 Hz, Ph-3′, 5′-H), 7.57–7.59 (d, 1H, J = 9.4 Hz, pyridazine-4H), 7.63–7.66 (d, 1H, J = 9.32 Hz, pyridazine-5H), 7.71–7.75 (m, 2H, Ph-2′, 6′-H), 8.11 (s, 1H, methine-H), 11.68 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 115.64, 116.16, 128.38, 130.00, 131.28, 132.59, 147.38, 158.87, 159.63, 160.46. Ms: m/z = 250.01 $[M]^+$, 252.00 $[M+2]^+$, (3:1). Anal. Calcd for $C_{11}H_8ClFN_4$: C, 52.71; H, 3.22, N, 22.35. Found: C, 52.63; H, 3.61; N, 22.02.

4.2.8. 3-(3′-Bromobenzylidenehydrazino)-6-chloropyridazine (3i)

Yield 83%; mp 195 °C. IR (KBr, cm^{-1}): 3024 (−NH str). ^1H NMR (400 MHz, DMSO) δ: 7.32–7.36 (t, 1H, J = 7.84 Hz, Ph-5′-H), 7.48–7.51 (m, 1H, Ph-4′-H), 7.57–7.60 (d, 1H, J = 9.4 Hz, pyridazine-4H), 7.62–7.64 (d, 1H, J = 7.76 Hz, Ph-6′-H), 7.68–7.70 (d, 1H, J = 9.4 Hz, pyridazine-5H), 7.88–7.89 (t, 1H, J = 1.68 Hz, Ph-2′-H), 8.07 (s, 1H, methine-H), 11.83 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 116.12, 122.22, 125.62, 128.26, 130.04, 130.84, 131.61, 137.17, 140.03, 147.67, 158.78. Anal. Calcd for $C_{11}H_8BrClN_4$: C, 42.41; H, 2.59; N, 17.98. Found: C, 42.33; H, 2.77, N, 17.81.

4.2.9. 3-(4′-Bromobenzylidenehydrazino)-6-chloropyridazine (3j)

Yield 82%; mp 215 °C. IR (KBr, cm^{-1}): 3217 (−NH str). ^1H NMR (400 MHz, DMSO) δ: 7.56–7.58 (d, 2H, J = 8.52 Hz, Ph-2′, 6′-H),

7.60–7.62 (d, 1H, J = 9.4 Hz, pyridazine-4H), 7.65–7.67 (d, 1H, J = 9.36 Hz, pyridazine-5H), 7.68–7.70 (d, 2H, J = 8.52 Hz, Ph-3′, 5′-H), 8.08 (s, 1H, methine-H), 11.75 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 115.93, 122.20, 128.22, 130.05, 131.68, 133.98, 140.58, 147.57, 158.79. Anal. Calcd for $C_{11}H_8BrClN_4$: C, 42.41; H, 2.59; N, 17.98. Found: C, 42.27; H, 2.36, N, 18.19.

4.2.10. 6-Chloro-3-(4′-methylbenzylidenehydrazino)pyridazine ***(3k)***

Yield 78%; mp 212 °C. IR (KBr, cm^{-1}): 3209 (−NH str). ^{1}H NMR (400 MHz, DMSO) δ: 2.34 (s, 3H, Ph-4′-CH_3), 7.18–7.20 (d, 2H, J = 7.48 Hz, Ph-3′, 5′-H), 7.51–7.53 (d, 1H, J = 9.2 Hz, pyridazine-4H), 7.54–7.56 (d, 2H, J = 7.48 Hz, Ph-2′, 6′-H), 7.60–7.63 (d, 1H, J = 9.2 Hz, pyridazine-5H), 8.08 (s, 1H, methine-H), 11.53 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 20.95, 115.77, 126.33, 129.34, 129.95, 131.95, 138.86, 142.01, 147.18, 158.88. Ms: m/z = 246.01 $[M]^+$, 248.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_{12}H_{11}ClN_4$: C, 58.42; H, 4.49; N, 22.71. Found: C, 58.74; H, 4.86, N, 22.63.

4.2.11. 6-Chloro-3-(4′-methoxybenzylidenehydrazino)pyridazine ***(3l)***

Yield 74%; mp 207 °C. IR (KBr, cm^{-1}): 3204 (−NH str). ^{1}H NMR (400 MHz, DMSO) δ: 3.83 (s, 3H, Ph-4′-OCH_3), 6.94–6.96 (d, 2H, J = 8.72 Hz, Ph-3′, 5′-H), 7.00–7.02 (d, 1H, J = 9.36 Hz, pyridazine-4H), 7.59–7.60 (d, 1H, J = 9.36 Hz, pyridazine-5H), 7.78–7.80 (d, 2H, J = 8.72 Hz, Ph-2′, 6′-H), 8.59 (s, 1H, methine-H), 11.81 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 55.21, 114.22, 115.65, 127.28, 127.88, 141.91, 146.97, 158.90, 160.14. Ms: m/z = 262.01 $[M]^+$, 264.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_{12}H_{11}ClN_4O$: C, 54.87; H, 4.22, N, 21.33. Found: C, 54.36; H, 4.56; N, 21.69.

4.2.12. 6-Chloro-3-(2′,5′-dimethoxybenzylidenehydrazino)pyridazine ***(3m)***

Yield 74%; mp 229 °C. IR (KBr, cm^{-1}): 3016 (−NH str). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.76 (s, 3H, Ph-5′-OCH_3), 3.81 (s, 3H, Ph-2′-OCH_3), 6.78–6.85 (m, 2H, Ph-3′, 4′-H), 7.28–7.31 (d, 1H, J = 9.32 Hz, pyridazine-4H), 7.39–7.40 (d, 1H, J = 2.76 Hz, Ph-6′-H), 7.57–7.59 (d, 1H, J = 9.28 Hz, pyridazine-5H), 8.39 (s, 1H, methine-H), 10.11 (bs, 1H, −NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.00, 55.10, 115.22, 116.30, 116.85, 117.81, 120.31, 132.00, 142.33, 146.32, 152.30, 158.36. 158.58; Ms: m/z = 292.01 $[M]^+$, 294.11 $[M+2]^+$, (3:1). Anal. Calcd for $C_{13}H_{13}ClN_4O_2$: C, 53.34; H, 4.48; N, 19.14. Found: C, 53.87; H, 4.20, N, 18.93.

4.2.13. 6-Chloro-3-(3′,4′-dimethoxybenzylidenehydrazino)pyridazine ***(3n)***

Yield 78%; mp 234 °C; Lit. mp [17] 235–237 °C.

4.2.14. 6-Chloro-3-(furan-2′-ylmethylenehydrazino)pyridazine ***(3o)***

Yield 81%; mp 196.5 °C. IR (KBr, cm^{-1}) 3132 (−NH str). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.49–6.50 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 1.8 Hz, furan-4′-H), 6.69–6.70 (d, 1H, $J_{3',4'}$ = 3.4 Hz, furan-3′-H), 7.37–7.39 (d, 1H, J = 9.36 Hz, pyridazine-4H), 7.51–7.52 (d, 1H, $J_{4',5'}$ = 1.6 Hz, furan-5′-H), 7.65–7.68 (d, 1H, J = 9.36 Hz, pyridazine-5H), 8.11 (s, 1H, methine-H), 11.02 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 110.71, 111.52, 115.51, 129.34, 132.16, 143.24; 147.17, 149.74, 158.27. Ms: m/z = 222.06 $[M]^+$, 224.00 $[M+2]^+$, (3:1). Anal. Calcd for $C_9H_7ClN_4O$: C, 48.55; H, 3.17, N, 25.17. Found: C, 48.18; H, 3.63, N, 25.55.

4.2.15. 6-Chloro-3-(thiophen-2′-ylmethylenehydrazino)pyridazine ***(3p)***

Yield 78%; mp > 315 °C. IR (KBr, cm^{-1}): 3024 (−NH str). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.82–6.84 (m, 1H, thiophene-4′-H), 7.20–7.22 (d, 1H, J = 9.8 Hz, pyridazine-4H), 7.34–7.35 (m, 1H, thiophene-3′-H), 7.41–7.42 (m, 1H, thiophene-5′-H), 7.52–7.54 (d, 1H, J = 9.8 Hz, pyridazine-5H), 8.14 (s, 1H, methine-H), 11.10 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 115.12, 115.84, 121.00, 124.52, 130.65, 132.56, 140.12, 146.23, 150.12. Ms: m/z = 238.00 $[M]^+$, 240.02 $[M+2]^+$, (3:1). Anal. Calcd for $C_9H_7ClN_4S$: C, 45.29; H, 2.96; N, 23.47. Found: C, 45.73; H, 2.85; N, 23.13.

4.2.16. 6-Chloro-3-(indole-3′-ylmethylenehydrazino)pyridazine ***(3q)***

Yield 75%; mp 235 °C. IR (KBr, cm^{-1}): 3094 (−NH str). ^{1}H NMR (400 MHz, DMSO) δ: 7.12–7.20 (m, 2H, indole-5′, 6′-H), 7.41–7.43 (d, 1H, J = 7.4 Hz, indole-7′-H), 7.53–7.55 (d, 1H, J = 9.4 Hz, pyridazine-4H), 7.57–7.59 (d, 1H, J = 9.4 Hz, pyridazine-5H), 7.62–7.63 (d, 1H, J = 2.68 Hz, indole-2′-H), 8.17–8.19 (t, 1H, J = 7.16 Hz, indole-4′-H), 8.34 (s, 1H, methine-H), 11.23 (bs, 1H, −NH), 11.38 (bs, 1H, −NH). ^{13}C NMR (100 MHz, DMSO) δ: 111.63, 111.94, 114.90, 120.18, 121.50, 122.31, 123.97, 128.54, 129.37, 136.98, 139.81, 145.99, 158.81. Anal. Calcd for $C_{13}H_{10}ClN_5$: C, 57.47; H, 3.71, N, 25.78. Found: C, 57.19; H, 3.99, N, 25.41.

4.3. Synthesis of 6-chloro-3-methyl-[1,2,4]triazolo[4,3-b]pyridazine ***(4a)***

To a solution of 6-chloropyridazin-3-yl hydrazone **(3a)** (0.34 g, 2 mmol) in dichloromethane (25 ml), IBD (0.70 g, 2.2 mmol) was added in small portions and the reaction mixture was stirred for 2–3 h or until the completion of reaction as monitored by TLC. Excess solvent was distilled off in vacuo, and the residual mass was triturated with petroleum ether to remove the excess of iodobenzene and a solid product separated out, which was recrystallized from aqueous ethanol to afford **4a**.

Yield 80%; mp 75.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.74 (s, 3H, $-CH_3$), 7.01–7.03 (d, 1H, J = 9.64 Hz, pyridazine-5H), 7.97–7.99 (d, 1H, J = 9.6 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 9.70, 121.77, 126.30, 142.65, 147.28, 149.16. Ms: m/z = 168.02 $[M]^+$, 170.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_6H_5ClN_4$: C, 42.75; H, 2.99, N, 33.23 Found: C, 42.33; H, 3.19, N, 33.38.

Similarly, other compounds (**4b-q**) are synthesized by using the above procedure.

4.3.1. 6-Chloro-3-ethyl-[1,2,4]triazolo[4,3-b]pyridazine ***(4b)***

Yield 82%; mp 93 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48–1.53 (t, 3H, J = 7.5 Hz, $-CH_3$), 3.17–3.24 (q, 2H, J = 7.5 Hz, $-CH_2$), 7.08–7.11 (d, 2H, J = 9.6 Hz, pyridazine-5H), 8.04–8.07 (d, 1H, J = 9.6 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 10.44, 17.16, 121.85, 126.57, 142.44, 148.49, 150.34. Ms: m/z = 182.00 $[M]^+$, 184.01 $[M+2]^+$, (3:1); Anal. Calcd for $C_7H_7ClN_4$: C, 46.04; H, 3.86, N, 30.68. Found: C, 46.37; H, 3.51, N, 30.88.

4.3.2. 6-Chloro-3-phenyl-[1,2,4]triazolo[4,3-b]pyridazine ***(4c)***

Yield 82%; mp 199.5 °C; Lit. mp [15] 200–201 °C.

4.3.3. 6-Chloro-3-(2′-chlorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4d)***

Yield 84%; mp 155 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.17–7.20 (d, 1H, J = 9.64 Hz, pyridazine-5H), 7.44–7.48 (dt, 1H, J = 7.46 Hz, J = 1.2 Hz, Ph-4′-H), 7.52–7.56 (dt, 1H, J = 7.42 Hz, J = 1.68 Hz, Ph-5′-H), 7.59–7.61 (d, 1H, J = 8.0 Hz, Ph-3′-H), 7.67–7.69 (dd, 1H, J = 7.58 Hz, J = 1.6 Hz, Ph-6′-H), 8.16–8.18 (d, 1H, J = 9.54 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 122.65, 124.76, 126.48, 126.96, 130.26, 132.16, 132.60, 134.81, 149.57. Ms: m/z = 264.08 $[M]^+$, 266.01 $[M+2]^+$, 268.00 $[M+4]^+$, (9:6:1). Anal. Calcd for $C_{11}H_6Cl_2N_4$: C, 49.84; H, 2.28, N, 21.13. Found: C, 49.55; H, 2.67, N, 21.01.

4.3.4. 6-Chloro-3-(3′-chlorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4e)***

Yield 85%; mp 201.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.17–7.20 (d, 1H, J = 9.6 Hz, pyridazine-5H), 7.06–7.36 (m, 3H, Ph-4′,5′,6′-H), 7.48–7.50 (m, 1H, Ph-2′H), 7.55–7.59 (d, 1H, J = 9.6 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 121.00, 123.24, 125.36, 126.52, 132.10, 132.19,

133.26, 134.52, 148.56. Ms: m/z = 264.00 $[M]^+$, 266.01 $[M+2]^+$, 268.02 $[M+4]^+$ (9:6:1). Anal. Calcd for $C_{11}H_6Cl_2N_4$: C, 49.84; H, 2.28, N, 21.13. Found: C, 49.49; H, 2.12, N, 21.36.

4.3.5. 6-Chloro-3-(4′-chlorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4f)***

Yield 81%; mp 191.5 °C; Lit. mp [15] 193–194 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, DMSO) δ: 7.50–7.53 (d, 1H, J = 9.64 Hz, pyridazine-5H), 7.63–7.67 (d, 2H, J = 8.6 Hz, Ph-3′, 5′-H), 8.35–8.38 (d, 2H, J = 8.5 Hz, Ph-2′, 6′-H), 8.51–8.53 (d, 1H, J = 9.68 Hz, pyridazine-4H).

4.3.6. 6-Chloro-3-(2′-fluorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4 g)***

Yield 83%; mp 160.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.18–7.21 (d, 1H, J = 9.6 Hz, pyridazine-5H), 7.28–7.37 (m, 2H, Ph-3′, 5′-H), 7.60 (m, 1H, Ph-4′-H), 7.89 (m, 1H, Ph-6′-H), 8.16–8.19 (d, 1H, J = 9.3 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 113.46, 116.43, 124.50, 126.46, 131.50, 132.83, 143.46, 145.56, 149.64, 159.22, 161.75. Ms: m/z = 248.00 $[M]^+$, 250.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_{11}H_6ClFN_4$: C, 53.14; H, 2.43, N, 22.53. Found: C, 53.43; H, 2.79, N, 22.84.

4.3.7. 6-Chloro-3-(4′-fluorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4h)***

Yield 86%; mp 192.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.16–7.18 (d, 1H, J = 9.48 Hz, pyridazine-5H), 7.24–7.29 (m, 2H, Ph-3′, 5′-H), 8.15–8.17 (d, 1H, J = 9.56 Hz, pyridazine-4H), 8.46–8.51 (m, 2H, Ph-2′, 6′-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 115.98, 116.20, 121.90, 126.73, 129.89, 147.36, 149.53, 162.91, 165.34. Ms: m/z = 248.06 $[M]^+$, 250.00 $[M+2]^+$, (3:1). Anal. Calcd for $C_{11}H_6ClFN_4$: C, 53.14; H, 2.43, N, 22.53. Found: C, 52.14; H, 2.49, N, 22.91.

4.3.8. 6-Chloro-3-(3′-bromophenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4i)***

Yield 90%; mp 179.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.17–7.20 (d, 1H, J = 9.6 Hz, pyridazine-5H), 7.42–7.47 (t, 1H, J = 7.8 Hz, Ph-5′-H), 7.65–7.68 (d, 1H, J = 8.1 Hz, Ph-4′-H), 8.16–8.19 (d, 1H, J = 9.6 Hz, pyridazine-4H); 8.43–8.45 (d, 1H, J = 7.8 Hz, Ph-6′-H), 8.65 (m, 1H, Ph-2′-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 122.19, 122.91, 126.06, 126.73, 127.38, 130.39, 133.64, 139.11, 149.74. Anal. Calcd for $C_{11}H_6BrClN_4$: C, 42.68; H, 1.95, N, 18.10. Found: C, 42.87; H, 2.09, N, 18.33.

4.3.9. 6-Chloro-3-(4′-bromophenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4j)***

Yield 91%; mp 171 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.16–7.18 (d, 1H, J = 9.6 Hz, pyridazine-5H), 7.69–7.72 (d, 2H, J = 8.64 Hz, Ph-2′, 6′-H), 8.15–8.17 (d, 1H, J = 9.6 Hz, pyridazine-4H), 8.35–8.38 (d, 2H, J = 8.6 Hz, Ph-3′, 5′-H). ^{13}C NMR (100 MHz, DMSO) δ: 122.05, 124.41, 125.23, 125.86, 126.73, 129.03, 129.95, 132.14, 149.65. Anal. Calcd for $C_{11}H_6BrClN_4$: C, 42.68; H, 1.95, N, 18.10. Found: C, 42.42; H, 1.63, N, 18.21.

4.3.10. 6-Chloro-3-(4′-methylphenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4k)***

Yield 83%; mp 253.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.45 (s, 3H, Ph-4′-CH_3), 7.12–7.14 (d, 1H, J = 9.6 Hz, pyridazine-5H), 7.37–7.39 (d, 2H, J = 8.08 Hz, Ph-3′, 5′-H), 8.12–8.14 (d, 1H, J = 9.6 Hz, pyridazine-4H), 8.33–8.35 (d, 2H, J = 8.28 Hz, Ph-2′, 6′-H). ^{13}C NMR (100 MHz, DMSO) δ: 22.68, 122.13, 122.67, 125.71, 127.00, 129.21, 133.43, 140.12, 143.14, 149.12. Ms: m/z = 244.01 $[M]^+$, 246.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_{12}H_9ClN_4$: C, 58.90; H, 3.71, N, 22.90. Found: C, 58.72; H, 3.66, N, 23.12.

4.3.11. 6-Chloro-3-(4′-methoxyphenyl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4l)***

Yield 88%; mp 215.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.92 (s, 3H, –OCH_3), 7.09–7.15 (m, 3H, Ph-3′, 5′-H, pyridazine-5H), 8.13–8.16 (d, 1H, J = 9.6 Hz, pyridazine-4H), 8.42–8.45 (d, 2H, J = 9.0 Hz, Ph-2′, 6′-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.42, 114.24, 117.97, 121.53, 126.63, 129.28, 143.37, 147.09, 149.18, 161.39. Ms: m/z = 260.01 $[M]^+$, 262.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_{12}H_9ClN_4O$: C, 55.29; H, 3.48, N, 21.49. Found: C, 55.46; H, 3.75, N, 21.66.

4.3.12. 6-Chloro-3-(2′,5′-dimethoxyphenyl)-[1,2,4]triazolo[4,3-b] pyridazine ***(4m)***

Yield 84%; mp 78 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.81 (s, 3H, Ph-5′-OCH_3), 3.82 (s, 3H, Ph-2′-OCH_3), 7.03–7.05 (m, 1H, Ph-3′-H) 7.10–7.12 (d, 1H, J = 9.72 Hz, pyridazine-5H), 7.14 (m, 1H, Ph-4′-H), 7.21–7.22 (m, 1H, J = 2.72 Hz, Ph-6′-H), 8.11–8.13 (d, 1H, J = 9.52 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 56.00, 56.50, 113.41, 114.86, 116.47, 118.43, 122.19, 126.36, 143.37, 147.91, 148.98, 152.59, 153.57. Ms: m/z = 290.00 $[M]^+$, 292.04 $[M+2]^+$, (3:1). Anal. Calcd for $C_{13}H_{11}ClN_4O_2$: C, 53.71; H, 3.81; N, 19.27. Found: C, 53.28; H, 4.09, N, 19.39.

4.3.13. 6-Chloro-3-(3′,4′-dimethoxyphenyl)-[1,2,4]triazolo[4,3-b] pyridazine ***(4n)***

Yield 84%; mp 230.5 °C; Lit. m.pt. [17] 235–237 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.98 (s, 3H, Ph-4′-OCH_3), 4.02 (s, 3H, Ph-3′-OCH_3), 7.04–7.07 (d, 1H, J = 8.4 Hz, Ph-5′-H), 7.11–7.15 (d, 1H, J = 9.6 Hz, pyridazine-5H), 8.03 (s, 1H, Ph-2′-H), 8.12–8.17 (m, 2H, Ph-6′-H, pyridazine-4H).

4.3.14. 6-Chloro-3-(furan-2′-yl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4o)***

Yield 83%; mp 212 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.61–6.62 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 1.8 Hz, furan-4′-H), 6.88–6.91 (d, 1H, J = 10.08 Hz, pyridazine-5H), 7.40–7.41 (dd, 1H, $J_{3',4'}$ = 3.32 Hz, $J_{3',5'}$ = 0.64 Hz, furan-3′-H), 7.65–7.66 (dd, 1H, $J_{4',5'}$ = 1.72 Hz, $J_{3',5'}$ = 0.68 Hz, furan-5′-H), 7.86–7.88 (d, 1H, J = 10.08 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 111.88, 113.24, 122.26, 126.54, 140.09, 141.95, 142.86, 144.86, 150.00. Ms: m/z = 220.03 $[M]^+$, 222.02 $[M+2]^+$, (3:1). Anal. Calcd for $C_9H_5ClN_4O$: C, 49.00; H, 2.28, N, 25.40. Found: C, 49.00; H, 2.28, N, 25.40.

4.3.15. 6-Chloro-3-(thiophen-2′-yl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4p)***

Yield 86%; mp 179.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (300 MHz, DMSO): 7.25–7.32 (m, 1H, thiophene-4′-H), 7.50–7.54 (d, 1H, J = 9.6 Hz, pyridazine-5H), 7.68–7.76 (m, 1H, thiophene-3′-H), 8.10–8.20 (m, 1H, thiophene-5′-H), 8.47–8.50 (d, 1H, J = 9.6 Hz, pyridazine-4H). ^{13}C NMR (100 MHz, DMSO) δ: 122.16, 126.00, 126.84, 127.71, 127.83, 128.59, 142.99, 143.76, 149.43. Ms: m/z = 236.02 $[M]^+$, 238.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_9H_5ClN_4S$: C, 45.67; H, 2.13, N, 23.67. Found: C, 45.24; H, 2.61, N, 23.89.

4.3.16. 6-Chloro-3-(1H-indole-3′-yl)-[1,2,4]triazolo[4,3-b]pyridazine ***(4q)***

Yield 82%; mp 173.5 °C. IR (KBr, cm^{-1}): transparent in the region of −NH str. ^{1}H NMR (400 MHz, DMSO) δ: 7.21–7.27 (m, 2H, indole-5′, 6′-H), 7.34–7.37 (d, 1H, J = 9.52 Hz, pyridazine-5H), 7.52–7.55 (m, 1H, indole-7′-H), 8.38–8.41 (d, 1H, J = 9.52 Hz, pyridazine-4H), 8.44–8.45 (d, 1H, J = 2.72 Hz, indole-2′-H), 8.51–8.53 (d, 1H, J = 7.24 Hz, indole-4′-H), 11.73 (s, 1H, −NH). Ms: m/z = 269.02 $[M]^+$, 271.01 $[M+2]^+$, (3:1). Anal. Calcd for $C_{13}H_8ClN_5$: C, 57.90; H, 2.99; N, 25.97. Found: C, 57.57; H, 3.12; N, 25.69.

4.4. Biological assays

4.4.1. Cytotoxic activity

4.4.1.1. Cell culture. NALM-6 and SB-ALL cell lines were maintained in

Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% fetal serum albumin (FBS) and 50 μg/ml of penicillin and streptomycin (pen/strep) at 37 °C with 5% of CO_2. MCF-7 cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 50 μg/ml of pen/strep at 37 °C with 5% of CO_2.

4.4.1.2. Cell viability assay. Cells were seeded in a 96-well plate at a density of 100,000 per mL and grown overnight. Cells were treated with various compounds at a final concentration of 10 μM and incubated for 48 h at 37 °C. Cell viability assay was performed using a MTT cell proliferation kit from ATCC (American Type Culture Collection) (#30–1010 K). After adding 10 μL MTT reagent to each well, cells were placed back in incubator for 4 hr at 37 °C. 100 μL of detergent (from kit) was added and absorbance data was collected at 570 nm using Biotek synergy 2 spectrophotometer. Data was calculated as percentage of cell survival using the following formula:

$$\% \text{ Cell survival} = (100/A_t * A_s)$$

where A_t and A_s are the absorbance of wells treated with test compounds and solvent control, respectively. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software "Prism 6.07".

4.4.1.3. Caspase 3/7 assay. 100 000 JURKAT cells were plated in a 24 well plate and treated with 1 μM of **4f**, **4j** and **4q** for 48 h, 100 μL sample was mixed with equal amount of substrate reagent and fluorescence was measured as an indicator of apoptosis (as per kit Promega G7790). Camptothecin was used as a positive control for inducing apoptotic cell death.

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Appendix A. Supplementary material

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