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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel 5-[(1,2,3-triazol-4-yl)methyl]-1-methyl-3*H*-pyridazino[4,5-*b*]indol-4-one derivatives by click reaction and exploration of their anticancer activity

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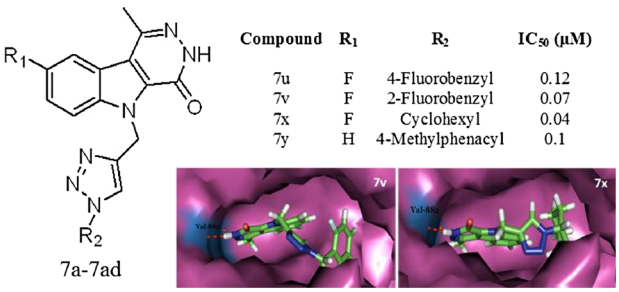
Abstract A series of pyridazino[4,5-*b*]indole derivatives containing alkyl-, benzyl- and phenacyl-substituted 1,2,3-triazolylmethyl units was synthesized using click chemistry approach. All 30 compounds of the series were screened in vitro against four cancer cell lines, viz. breast cancer cells MDA-MB-231 and MCF 7, human primary glioblastoma U-87 and human neuroblastoma IMR-32 cell lines. Most of the compounds exhibited potent cancer cell growth inhibition activity at very low micromolar concentrations. The IC_{50} value of compounds **7v** and **7x** against human neuroblastoma IMR-32 cell line is 0.07 and 0.04 μM, respectively. Among the tested compounds, ten compounds showed IC_{50} value less than 1 μM against MDA-MB-231 cells, whereas against IMR-32 cells, nine compounds and, against U-87 cells, six compounds showed similar inhibition activity. Further, these molecules exhibited prominent binding affinity and docking scores in the molecular simulation study with the target enzyme PI3 kinase.

Graphical Abstract This paper illustrates the synthesis of new fused indole–pyridazinone derivatives containing substituted 1,2,3-triazoles via click chemistry approach. Most of the compounds exhibited potent cancer cell growth inhibition activity at very low micromolar concentrations.



Keywords Pyridazino[4,5-*b*]indole · 1,2,3-Triazole · Click reaction · Breast cancer · Neuroblastoma · PI3 kinase

Introduction

The pyridazino[4,5-*b*]indole structural unit (Fig. 1) has extensive applications in medicinal chemistry because of its bio-isosterism with β-carboline and γ-carboline. The pharmacological significance of pyridazino[4,5-*b*]indole systems has been well explored in terms of antiarrhythmic (Lerch and Kaiser, 1982), antihypertensive (Monge *et al.*, 1991b), blood platelet aggregation and inotropics (Monge *et al.*, 1991a), thromboxane A_2 synthetase inhibitor (Monge *et al.*, 1987), serotonin antagonistic (Nantka-Namirski and Ozdowska, 1972), MAO inhibitor (Monge *et al.*, 1980), anxiolytic (Evanno *et al.*, 1999), antihistaminic and HIV-1 reverse transcriptase inhibitor (Font *et al.*, 1995) activities. The naturally occurring β-carboline

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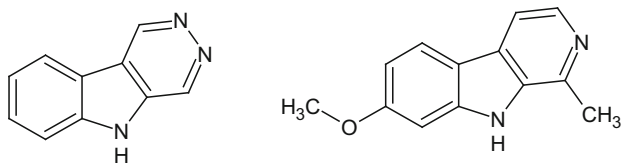


(a) (b)

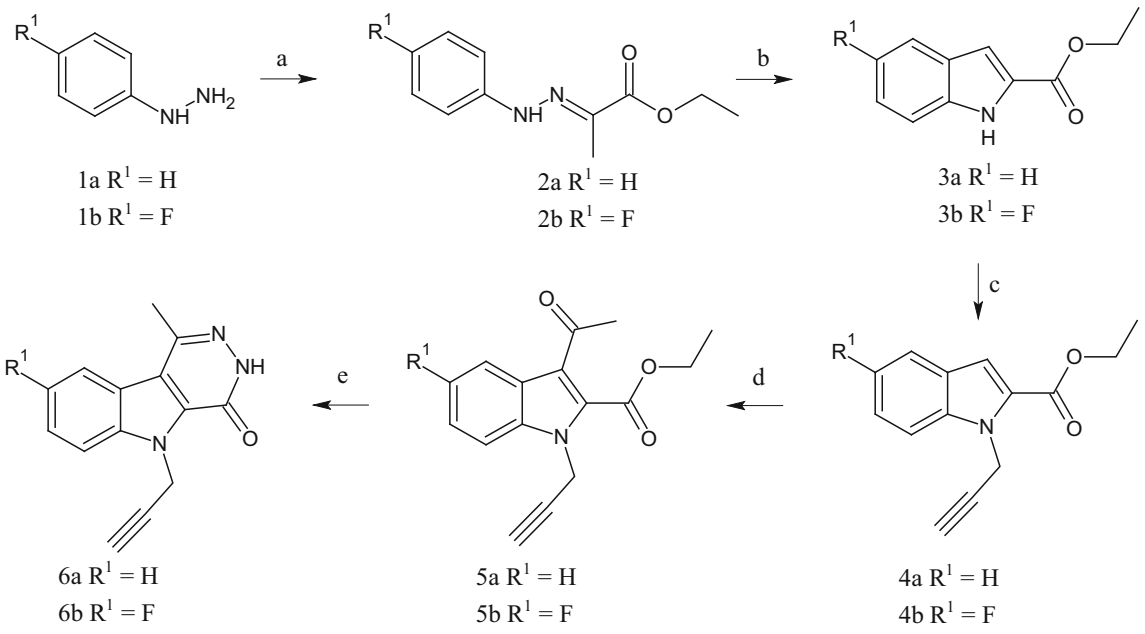
Fig. 1 Structures of pyridazino[4,5-*b*]indole (**a**) and harmine (**b**)

alkaloid, harmine (Fig. 1), inhibits the dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) activity, which is associated with the neurodegenerative diseases such as Down syndrome and the Alzheimer disease, by interacting with residues Leu241 (hinge region) and Lys188 in the ATP-binding pocket through two hydrogen bonds. The methoxy group and the nitrogen atom of the pyridine ring are involved in the formation of these two hydrogen bonds (Adayev *et al*., 2011; Ogawa *et al*., 2010). It is proposed that fused indole–pyridazinone system could be a proper molecular structure to provide an effective interaction with the hinge fraction of the target proteins. This is further supported by the fact that 5-benzylated 4-oxo-3,4-dihydro-5*H*-pyridazino[4,5-*b*]indoles are highly selective toward phosphatidylinositol-3-kinases (PI3Ks) (Bruel *et al*., 2012). On the other hand, 1,2,3-triazole and its derivatives find great importance in medicinal chemistry research due to their important biological actions in addition to their synthetic applications (Agalave *et al*., 2011; Thirumurugan *et al*., 2013). Triazole is a core structural moiety found in some important drugs like tazobactam, cefatrizine, carboxyamidotriazole and rufinamide. The most significant and current studies have discovered that 1,2,3-triazole derivatives exhibit a broad spectrum of pharmacological activity such as antimicrobial (Kushwaha *et al*., 2014), anti-inflammatory (Shafi *et al*., 2012), anticonvulsant (Ulloora *et al*., 2013), antituberculosis (Addla *et al*., 2014; Patpi *et al*., 2012; Yempala *et al*., 2014) and, in particular, anticancer (Akselsen *et al*., 2012; Howell *et al*., 2009; Pagliai *et al*., 2006) activity. Owing to the drug-like properties of 1,2,3-triazole derivatives and also based on the preliminary structure–activity information (Güven and Jones, 1993), we have planned to incorporate 1,2,3-triazole unit into the pyridazino[4,5-*b*]indole structure with an expectation that the amalgamation of these two active pharmacophores in a single molecular framework would enhance the efficacy of the hybrid molecule. With this background, we have synthesized a library of 30 indole-fused pyridazinone derivatives containing substituted 1,2,3-triazoles at the indole nitrogen via click chemistry approach and explored in vitro anticancer activity of these hybrid molecules.

Results and discussion

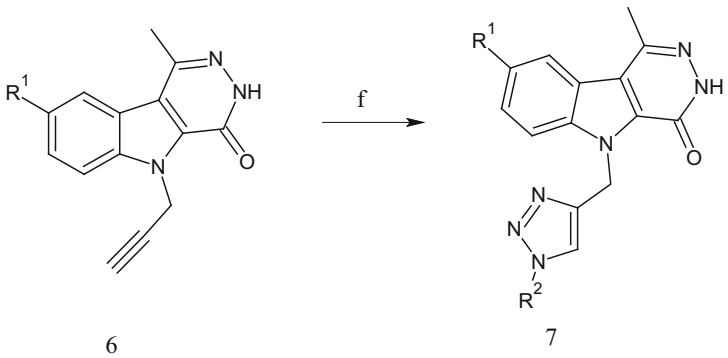
Chemistry

The basic indole moiety was built by precise and proficient three-step synthesis following Fischer indole synthetic protocol (Humphrey and Kuethe, 2006) as given in Scheme 1. The indole-2-carboxylic ester intermediate (**3**) was alkylated using propargyl bromide to get compound **4**. The intermediate **4** was then acylated at position 3 by following Friedel–Craft acylation protocol using $AlCl_3$ as the Lewis acid catalyst to get acyl derivative (**5**). The indole-fused pyridazinone ring system (**6**) was then constructed by refluxing intermediate **5** with hydrazine hydrate in ethanol. In all the steps, the products were isolated with excellent yield (>80 %). Finally, 1,2,3-triazole system was introduced by following the conventional click chemistry protocol with the propargylated intermediate (**6**) using various alkyl, benzyl and phenacyl azides (Scheme 2). We primarily focused on scrutinizing the variation in the activity of molecules by the incorporation of alkyl, benzyl and phenacyl substitutions with various electron-withdrawing and electron-donating groups on the triazole fragment of the hybrid structure. Hence, a library of 30 molecules was synthesized. The structural specifics of the synthesized compounds are depicted in Table 1. All the intermediate and target compounds were characterized using spectral techniques as well as by elemental analysis. The ^{1}H NMR spectrum of **4a** displayed singlet peaks at δ 5.79 and 2.64 ppm due to methylene (CH_2) and alkyne ($\equiv$CH) protons, respectively, of the propargyl group which confirms the successful execution of the N-propargylation reaction. The signals at δ 4.42 and 1.44 ppm in the spectrum correspond to methylene and methyl protons, respectively, of the ester group. Further, the spectrum showed multiplet signals in the region δ 7.01–7.71 ppm corresponding to aromatic protons. The acetylation of **4a** to give **5a** was evident by the ^{1}H NMR spectrum of **5a** which displayed a singlet peak at δ 2.61 ppm due to methyl protons of the acetyl group (–$COCH_3$) along with other characteristic signals. In the ^{13}C NMR spectrum, the carbonyl and methyl carbons of the acetyl group appeared at δ 171.2 and 30.2 ppm, respectively. The conversion of **5a to 6a** with the formation of indole-fused pyridazinone ring is confirmed by the appearance of a singlet, corresponding to ring –CONH proton, at δ 12.72 ppm in the ^{1}H NMR spectrum of **6a**. Also the signals due to the ester group disappeared in the spectrum which further confirms the formation of the pyridazinone ring. In the ^{1}H NMR spectra of the final compounds, the presence of a singlet peak at δ $\sim$6.0 ppm due to protons of methylene (which links the indole nitrogen and C-4 of the triazole ring) group



Scheme 1 Synthesis of pyridazino[4,5-*b*]indole scaffolds. Reagents and conditions: *a* ethyl pyruvate, cat. HOAc, EtOH, 80 °C 1 h; *b* PPA, Toluene, 100 °C, 5 h; *c* K_2CO_3, DMF, TBAB, propargyl bromide, RT, 6 h; *d* $AlCl_3$, acetyl chloride, DCM, reflux, 2 h; *e* NH_2NH_2, EtOH

Scheme 2 Synthesis of target compounds. *f* *t*-BuOH/H_2O (1:1), alkyl/benzyl/phenacyl bromides, NaN_3, 80 °C for 1 h, CuI (after temperature was brought back to RT), Al_2O_3, RT, O/N



and disappearance of signals due to the propargyl group confirm the successful completion of the click reaction and the formation of 1,2,3-triazole ring system in these molecules. Further, ^{13}C NMR and mass spectral analysis data are in agreement with the chemical structure of these target molecules. The ^{13}C spectrum of **7a** shows peaks at δ 54.1, 40.5 and 11.0 corresponding to the benzylic carbon, methylene carbon linked to indole nitrogen and the methyl group present in the pyridazinone ring, respectively, which confirms the structure of the product. The characterization data are given in the experimental part, and some representative spectra are given in the supporting information.

In vitro anticancer activity

All thirty compounds were subjected to in vitro cell proliferation assay study against a panel of four cancer cell lines, viz. breast cancer cells MDA-MB-231 and MCF7, human primary glioblastoma U-87 and human neuroblastoma IMR-32 cell lines, in order to investigate the cytotoxic nature of the molecules. The MTT assay, with doxorubicin as the standard drug, was used for the screening of compounds. The growth corresponding to the control group (with cells and medium alone) was considered as 100 % (or 0 % inhibition). The percentage inhibition with respect to the compound-treated cells was calculated relative to the control group. It was observed that most of the synthesized molecules are active against IMR-32 and U-87 cells at a test concentration of 10 μM (Fig. 2). A few molecules are potent enough to inhibit the growth close to 90 % against the tested cancer cell lines, in particular against U-87 cells. Compounds **7h**, **7k**, **7u**, **7v**, **7w** and **7aa** exhibited prominent antiproliferative activity (>80 % growth inhibition), and most of the other

Table 1 Structural parameters of target compounds

Compound	R^1	R^2	Mol. wt.	Hydrogen bond acceptors	Hydrogen bond donors	LogP
7a	H	Benzyl	370.40	4	1	2.62
7b	H	4-Methoxybenzyl	400.43	5	1	2.43
7c	H	4-(Trifluoromethyl)benzyl	438.40	4	1	3.48
7d	H	4-(Trifluoromethoxy)benzyl	454.40	5	1	4.09
7e	H	4-Cyanobenzyl	395.41	5	1	2.59
7f	H	4-Nitrobenzyl	415.40	5	2	2.21
7g	H	4-Fluorobenzyl	388.39	4	1	2.72
7h	H	2-Fluorobenzyl	388.39	4	1	2.72
7i	H	Cyclopentyl	348.39	4	1	1.96
7j	H	Cyclohexyl	362.42	4	1	2.37
7k	H	4-Methylphenacyl	412.44	5	1	2.23
7l	H	4-Methoxyphenacyl	428.44	6	1	1.62
7m	H	4-Nitrophenacyl	443.41	7	2	1.53
7n	H	4-Fluorophenacyl	416.40	5	1	1.90
7o	F	Benzyl	389.40	4	1	2.89
7p	F	4-Methoxybenzyl	418.42	5	1	2.98
7q	F	4-(Trifluoromethyl)benzyl	456.39	4	1	3.64
7r	F	4-(Trifluoromethoxy)benzyl	472.39	5	1	4.24
7s	F	4-Cyanobenzyl	413.40	5	1	2.75
7t	F	4-Nitrobenzyl	433.39	6	2	2.48
7u	F	4-Fluorobenzyl	406.38	4	1	2.88
7v	F	2-Fluorobenzyl	405.38	4	1	2.88
7w	F	Cyclopentyl	366.38	4	1	2.11
7x	F	Cyclohexyl	380.41	4	1	2.53
7y	F	4-Methylphenacyl	430.43	5	1	2.39
7z	F	4-Methoxyphenacyl	446.43	6	1	1.78
7aa	F	4-Nitrophenacyl	461.40	7	2	1.83
7ab	F	4-Fluorophenacyl	434.39	5	1	2.06
7ac	H	Isopropyl	323.36	4	1	1.81
7ad	F	Isopropyl	341.35	4	1	2.08

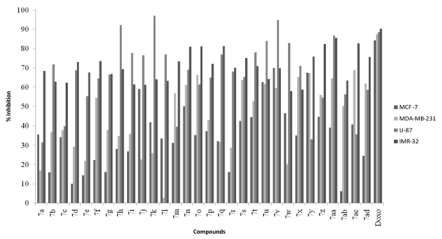


Fig. 2 Cell proliferation assay results of the compounds at a concentration of 10 μM against MCF-7, MDA-MB-231, U-87 and IMR-32 cells. Doxorubicin (Doxo) was used as the standard drug

compounds exhibited moderate activity (>50 % growth inhibition) against U-87 cells. U-87, derived from human malignant gliomas, is the most commonly investigated glioblastoma cell line. Glioblastoma is the most common malignant primary brain tumor in adults and is one of the most fatal of all cancers. Compounds **7n**, **7o**, **7q**, **7z**, **7aa** and **7ac** showed a significant growth inhibition activity (>80 %) against IMR-32 cell line. Interestingly, all other compounds of the series demonstrated moderate activity (nearly 60 % growth inhibition) against the same cell line. IMR-32 is associated with a childhood tumor of the nervous system that expresses remarkable clinical heterogeneity. Some of the test compounds, viz. **7j**, 7**n**, **7u**, **7v** and **7y,** possess promising antiproliferative activity (nearly 70 % growth inhibition) against breast cancer cells MCF-7, whereas compounds **7f**, **7m**, **7n**, **7o**, **7s**, **7t**, **7u**, **7v**, **7x**, **7y**, **7z**, **7aa**, **7ab**, **7ac** and **7ad** showed admirable activity against MDA-MB-231 cells. Further, most of the compounds of this series are non-toxic to non-cancerous HEK 293 cells (Fig. 3) which signifies the specificity of the

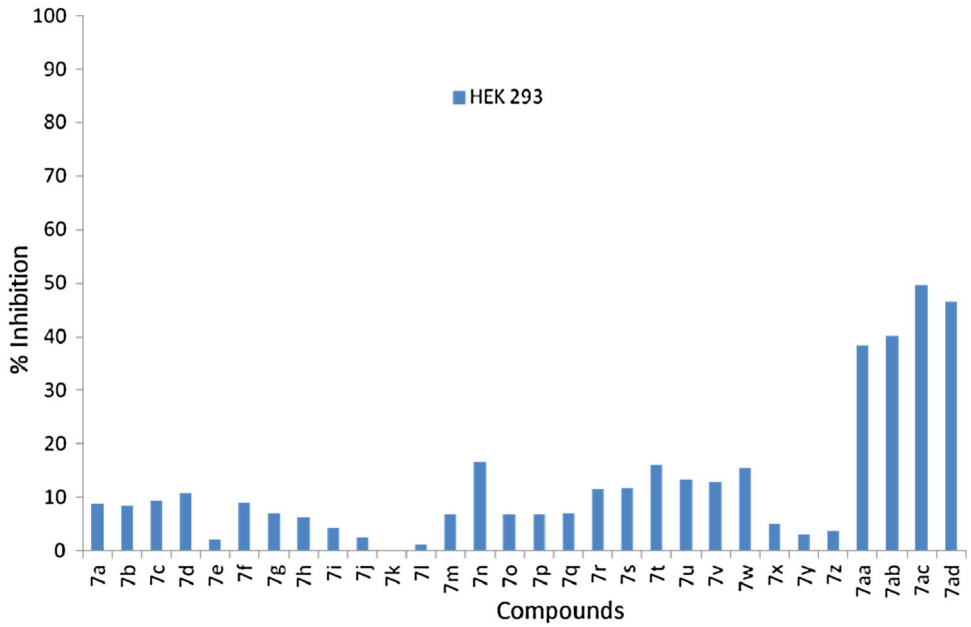


Fig. 3 Cell proliferation assay results of the tested compounds at a concentration of 10 μM against non-cancerous HEK 293 cells

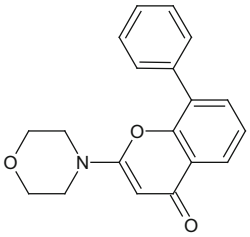
Table 2 IC_{50} value of the compounds

Compound	IC_{50} (μM)			
	MDA-MB-231	MCF-7	U-87	IMR-32
7f	0.550 ± 0.005	38.690 ± 0.105	1.293 ± 0.001	1.380 ± 0.001
7n	0.266 ± 0.013	14.060 ± 0.033	0.314 ± 0.008	0.853 ± 0.01
7o	0.493 ± 0.046	5.375 ± 0.007	1.417 ± 0.001	1.126 ± 0.002
7s	0.839 ± 0.011	13.490 ± 0.234	1.109 ± 0.009	0.289 ± 0.007
7t	1.177 ± 0.007	0.797 ± 0.023	0.283 ± 0.001	0.839 ± 0.012
7u	0.626 ± 0.026	3.997 ± 0.023	0.326 ± 0.002	0.120 ± 0.004
7v	0.325 ± 0.001	1.433 ± 0.004	0.217 ± 0.003	0.0726 ± 0.003
7x	0.478 ± 0.024	5.569 ± 0.007	0.303 ± 0.012	0.042 ± 0.007
7y	0.646 ± 0.004	4.751 ± 0.001	19.51 ± 0.101	0.100 ± 0.004
7aa	0.607 ± 0.003	0.501 ± 0.005	0.145 ± 0.006	0.504 ± 0.001
7ac	0.423 ± 0.012	17.230 ± 0.034	10.210 ± 0.010	0.116 ± 0.010
Doxorubicin	0.425 ± 0.035	0.743 ± 0.062	0.120 ± 0.015	0.080 ± 0.012

compounds toward cancerous cells. Only four compounds, viz. **7aa**, **7ab**, **7ac** and **7ad,** show toxic nature against the non-cancerous cell line in which **7ac** and **7ad** effect significantly the growth of normal cell (nearly 50 % growth inhibition). Both compounds contain an isopropyl group on the 1,2,3-triazole ring, and the presence of this group may be responsible for the observed toxicity.

The IC_{50} value was determined for compounds which have shown a significant inhibitory activity against all the tested cell lines, and the values are given in Table 2. From the data, it is evident that all compounds show prominent inhibition response against IMR-32 cells with very low IC_{50} values. Compound 5-[(1-cyclohexyl-1*H*-1,2,3-triazol-4-yl)methyl]-8-fluoro-1-methyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indol-4-one (**7x**) is the most potent among the tested molecules (IC_{50} = 0.04 μM). It was assumed that inhibition of the PI3K pathway may improve the ability of TRAIL (TNF-related apoptosis-inducing ligand) to induce apoptosis in neuroblastoma cells (Efron *et al.*, 2003). Compounds **7n**, **7t**, **7u**, **7v**, **7x** and **7aa** containing 4-fluoro phenacyl, 4-nitrobenzyl, 4-fluorobenzyl, 2-fluorobenzyl, cyclohexyl and 4-nitrophenacyl, respectively, exhibit good activity against U-87 cells with IC_{50} values less than 0.3 μM. The PI3K/Akt oncogenic pathway is also vital in

Fig. 4 The structure of 1E7V



glioblastomas. The malfunctioning of tumor suppressor gene phosphatase and tensin homolog (PTEN), a regulator of the PI3K pathway or activated PI3K/Akt pathway that impel increased proliferation, survival, neovascularization, glycolysis and invasion is reported in 70–80 % of malignant gliomas. Thus, PI3K inhibitors could be an attractive therapeutic target for treating malignant glioma. Compounds **7f**, **7n**, **7o**, 7**v**, **7x** and **7ac** demonstrated good activity against MDA-MB-231 and **7aa** against MCF-7 with IC_{50} value less than 0.5 μM. Hence, these molecules with excellent inhibition activity against the cancerous cells at very low micromolar concentrations are promising lead molecules for further biological investigation and to develop efficient antitumor drugs.

The phosphatidylinositol-3-kinases play an important role in controlling various characteristics of the malignant phenotypes, including proliferation, survival and apoptosis, adhesion and mobility, angiogenesis and cell size. The PI3K pathway is activated by the breakdown of PTEN gene and also by extension and mutation of the PIK3CA protein, which encodes the p110α PI3K isoform. The PI3K pathway is reported to be the most frequently activated pathway in periodic human tumors. It has been anticipated that mutation in one or more PI3K pathway components is responsible for up to 30 % of all human cancers. In order to study probable binding mode for all the compounds (**7a–7ad**) inside the ATP-binding site of PI3K, molecular modeling studies using the accessible crystal structure of p110 gamma isoform in complex with a known inhibitor 1E7V (PDB code, 1E7V) (Fig. 4) with a resolution of 2.4 Å (Walker *et al*., 2000) were carried out. The docking score, number of hydrogen bonds and details of the interacting amino acid for the target molecules are given in Table 3. The docking pose of the lead molecules and 1E7V with the enzyme is illustrated in Fig. 5.

Table 3 and Fig. 5 illustrate that almost all compounds show interaction with Val882, implying that the presence of pyridazine ring adjacent to Val882 contributes a favorable role in forming extra hydrogen bonds as compared to 1E7V crystal structure. In addition, the presence of fused indole and pyridazine ring system makes the head part of the compounds more rigid that makes the structures more favorable to fit into the pocket aligning adjacent to the

Table 3 Molecular docking details of target molecules and 1E7V

Compounds	Number of hydrogen bonds	Docking score	Amino acid interactions
7a	3	−8.8999	Val882 (3)
7b	3	−10.6532	Val882 (3)
7c	3	−9.5463	Val882 (3)
7d	2	−7.9752	Ala805, Val882
7e	4	−9.0334	Lys808, Val882 (3)
7f	4	−10.6405	Lys808, val882 (3)
7g	3	−10.5067	Val882 (3)
7h	3	−10.3951	val882 (3)
7i	3	−9.7354	Val882 (3)
7j	2	−9.3282	Val882 (2)
7k	3	−9.8436	Val882 (3)
7l	3	−11.2026	val882 (3)
7m	4	−9.8651	Val882 (2), Ala805
7n	3	−9.7615	Ser806, Val882 (2)
7o	3	−11.0338	Val882 (3)
7p	2	−6.5465	Lys833, Val822
7q	3	−11.3501	Val882(3)
7r	3	−10.9229	Val882 (3)
7s	4	−11.3099	Lys890, Val882 (3)
7t	4	−11.5770	Val882, Lys808
7u	3	−7.2421	Lys833, Asp964 (2)
7v	3	−10.9706	val882 (3)
7w	3	−10.7928	Val882 (3)
7x	2	−10.0281	Val882 (2)
7y	2	−9.2907	Ser806, Val882
7z	2	−10.1327	Ser806, Val882
7aa	2	−10.6458	Ser806, Val882
7ab	4	−10.3578	Ser806, Val882
7ac	3	−10.0428	Val882 (3)
7ad	3	−10.8599	Val882 (3)
1E7V	1	−7.6617	Val882

val882. The triazole extension attached to the indole ring acts as a tail freely orienting into the pocket. The pocket of this protein to which the ligand binds is quite deep enough and the amino acid interacting with the ligands is at the rim of the pocket (Val882, Ser806), which may not allow the ligands to escape, further making the ligands to fix firmly into the pocket.

Conclusions

We illustrate a simple, mild and efficient synthesis of pyridazino[4,5-*b*]indole ring systems containing alkyl-, benzyl- and phenacyl-substituted 1,2,3-triazolylmethyl

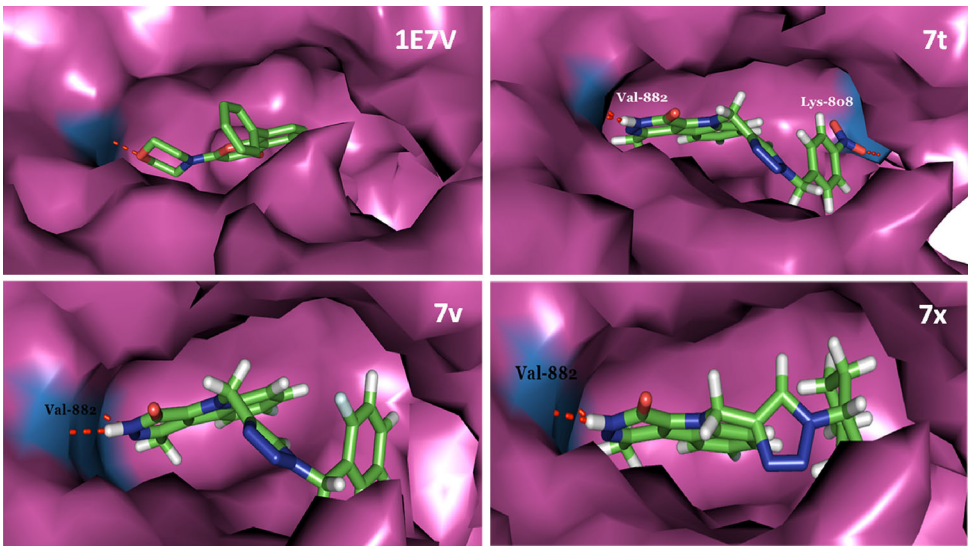


Fig. 5 Docking pose of the lead molecules and 1E7V

units using Fisher indole, Friedel–Craft acylation and click chemistry protocols. The preliminary antiproliferative examination of the molecules was carried out against four cancer cell lines, viz. breast cancer cells MDA-MB-231 and MCF-7, human primary glioblastoma U-87 cell line and human neuroblastoma IMR-32 cell line. Most of the compounds showed a significant activity against all the tested cell lines with IC_{50} values ranging in lower micromolar concentrations. Compound **7x** containing cyclohexyl substitution at the 1,2,3-triazole unit exhibited outstanding activity against three among four tested cancer cell lines with very low IC_{50} values, particularly against IMR-32 cell with an IC_{50} value of 0.04 μM. Compound **7v** containing 2-fluorobenzyl-substituted 1,2,3-triazole unit happens to be the next prominent active molecule which exhibited an IC_{50} value of 0.07 μM against IMR-32 cell line. However, it was observed that replacement of a 2-fluorobenzyl group with a 4-fluorobenzyl group reduces the activity comparatively. Among 4-cyanobenzyl, 4-nitrobenzyl and 4-fluoro benzyl substitutions on the triazole segment, 4-fluoro substitution was found to be important in enhancing the activity. It was also noted that molecules with a fluorine atom on the indole nucleus show improved activity when compared to their non-fluorine analogues. The molecular docking study was carried out for all target compounds inside the ATP-binding site of PI3K with the crystal structure of p 110 isoform with a known inhibitor 1E7V. The docking scores and the binding modes of the molecules with the receptor suggest that these compounds could be potential lead pharmacophores to inhibit the overexpression of PI3 kinases, as PI3K pathway is known to be the most frequently activated pathway in human tumors. Hence, these molecules, with a significant in vitro activity and docking score, are promising lead molecules to be considered for further in vivo biological evaluations.

Experimental

Materials and instruments

The chemicals and solvents used for the synthesis were procured from Sigma-Aldrich (Germany), Merck (India) and Spectrochem Chemicals Pvt. Ltd. All the solvents were distilled and dried before usage. The progress of the reactions was monitored by TLC using pre-coated aluminum sheets with 60 F_{254} silica gel (Merck KGaA). Silica gel with a mesh size of 230–400 was used as the stationary phase in column chromatography purification. The melting point of the synthesized compounds was recorded by a Stuart SMP3 melting point apparatus. The ^{1}H NMR spectra of the intermediates and final compounds were recorded using a Bruker 300 MHz/400 MHz NMR spectrometer using TMS as internal standard. The ^{13}C NMR spectra of the compounds were recorded with a 100-MHz spectrometer. The elemental analysis was done using a Thermo Electron Corporation EA-112 series C, H, N, S analyzer. The mass spectra were recorded using a Waters micromass Q-Tof microspectrometer. The LC–MS analysis was performed using an Agilent 1200 series mass spectrometer.

General procedure for the synthesis of ethyl 1-(prop-2-ynyl)-1H-indole-2-carboxylates (**4a and 4b**) To a solution of ethyl-1*H*-indole-2-carboxylate intermediate (**3a/3b**) in DMF, K_2CO_3 was added and the reaction mass was kept for stirring at RT for 15 min. Propargyl bromide (80 % solution in toluene) was then charged into the reaction mass followed by catalytic amount of n-tetrabutyl ammonium bromide, and the reaction mass was stirred at room temperature (RT) for 6 h. The reaction was monitored using TLC, and after the completion of the reaction, the reaction mass was added to cold water with stirring. The

product was extracted with ethyl acetate, and the organic layer was given thorough water wash followed by brine wash. The organic layer was then dried using Na_2SO_4 and concentrated to get the crude product. The crude product was then purified using column chromatography using ethylacetate/pet ether (1:1) as the eluent to get the pure product (**4a/4b**).

Ethyl 1-(prop-2-ynyl)-1H-indole-2-carboxylate (**4a**) The above-mentioned procedure was followed for compound **3a** (7 g, 37.04 mmol) in DMF (35 mL) using K_2CO_3 (12.8 g, 92.6 mmol) and propargyl bromide (44.44 mmol) to get the intermediate (**4a**) as red viscous liquid. Yield: 7.4 g (88 %); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.01–7.71 (5H, m, Ar–H), 5.79 (2H, s, N–CH_2), 4.42 (2H, q, $J = 7.4$ Hz, CO_2CH_2), 2.64 (1H, s, –C≡CH), 1.44 (3H, t, $J = 7.4$ Hz, $CO_2CH_2\underline{CH_3}$); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 164.2 (C, C=O), 149.5 (C, C-8), 145.3 (C, C-9), 138.4 (C, C-2), 125.6 (CH, C-5), 123.1 (CH, C-6), 120.5 (CH, C-4), 120.2 (CH, C-7), 110.9 (CH, C-3), 75.1 (C, C≡CH), 69.3 (CH, C≡CH), 61.2 (CH_2, OCH_2), 38.7 (CH_2, NCH_2), 10.9 (CH_3). Anal. calculated for $C_{14}H_{13}NO_2$; C, 73.99; H, 5.77; N, 6.16. Found: C, 74.28; H, 5.00; N, 6.22.

Ethyl 5-fluoro-1-(prop-2-ynyl)-1H-indole-2-carboxylate (**4b**) The above-mentioned procedure was followed for compound **3b** (7g, 33.82 mmol) in DMF (35 mL), using K_2CO_3 (11.67 g, 84.54 mmol) and propargyl bromide (40.58 mmol) to afford the intermediate (**4b**) as reddish brown viscous mass. Yield: 7 g (84 %); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 6.97–7.59 (4H, m, Ar–H), 5.80 (2H, s, N–CH_2), 4.41 (2H, q, $J = 7.4$ Hz, CO_2CH_2), 2.66 (1H, s, –C≡CH), 1.45 (3H, t, $J = 7.4$ Hz, $CO_2CH_2\underline{CH_3}$); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 163.6 (C, C=O), 159.0 (C, C-5), 147.6 (C, C-8), 143.4 (C, C-9), 139.6 (C, C-2), 124.8 (CH, C-6), 122.1 (CH, C-4), 118.9 (CH, C-7), 111.6 (CH, C-3), 74.8 (C, C≡CH), 65.3 (CH, C≡CH), 59.6 (CH_2, OCH_2), 38.5 (CH_2, NCH_2), 11.2 (CH_3). Anal. calculated for $C_{14}H_{12}FNO_2$; C, 68.56; H, 4.93; N, 5.71. Found: C, 68.29; H, 4.98; N, 5.62.

General procedure for the synthesis of ethyl 3-acetyl-1-(prop-2-ynyl)-1H-indole-2-carboxylates (**5a** and **5b**) Anhydrous $AlCl_3$ was taken in dry CH_2Cl_2 in a RB flask. The content of the flask was cooled to 0 °C, and calculated amount of acetyl chloride was added dropwise into the reaction mass. After the addition, the reaction mass was kept for stirring at 0 °C for about 15 min. A solution of intermediate (**4a/4b**) in CH_2Cl_2 was then charged dropwise into the reaction mass at the same temperature. After the addition, the reaction mass was refluxed at 40 °C for 2 h. The reaction was monitored using TLC. After the completion of the reaction, the reaction mass was poured into a mixture of ice-cold water and dichloromethane. The resulted mass was filtered through Celite, and the organic layer was separated. The organic layer was washed with water then with brine solution, dried using Na_2SO_4 and concentrated. The crude product was then purified using column chromatography using ethyl acetate/pet ether (1:1) as the eluent.

Ethyl-3-acetyl-1-(prop-2-ynyl)-1H-indole-2-carboxylate (**5a**) Intermediate **5a** was synthesized by carrying out the above-mentioned procedure for **4a** (6.5 g, 28.63 mmol) with $AlCl_3$ (4.2 g, 31.49 mmol), acetyl chloride (2.5 g, 31.49 mmol) to get the pure product as greenish yellow oily compound. Yield: 6.3 g (82 %); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.26–7.56 (4H, m, Ar–H), 5.76 (2H, s, N–CH_2), 4.46 (2H, q, $J = 7.8$ Hz, CO_2CH_2), 2.68 (1H, s, –C≡CH), 2.61 (3H, s, $COCH_3$), 1.44 (3H, t $J = 7.8$ Hz, $CO_2CH_2\underline{CH_3}$); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 171.2 (C, COMe), 162.3 (C, CO_2Et), 157.5 (C, C-3), 148.7 (C, C-8), 142.0 (C, C-9), 137.8 (C, C-2), 126.0 (CH, C-5), 123.6 (CH, C-6), 119.1 (CH, C-4), 109.6 (CH, C-7), 75.2 (C, C≡CH), 68.0 (CH, C≡CH), 60.6 (CH_2, OCH_2), 38.3 (CH_2, NCH_2), 30.2 (CH_3, $COCH_3$), 12.0 (CH_3); Anal. calculated for $C_{16}H_{15}NO_3$; C, 71.36; H, 5.61; N, 5.20. Found: C, 71.57; H, 5.69; N, 5.12.

Ethyl-3-acetyl-5-fluoro-1-(prop-2-ynyl)-1H-indole-2-carboxylate (**5b**) The acylated intermediate **5b** was synthesized by following the above procedure for intermediate **4b** (6 g, 24.46 mmol) with $AlCl_3$ (3.6 g, 26.91 mmol), acetyl chloride (2.1 g, 26.91 mmol) to obtain the pure product as yellow oily compound. Yield: 5.9 g (84 %); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.14–7.56 (3H, m, Ar–H), 5.78 (2H, s, N–CH_2), 4.41 (2H, q, $J = 7.8$ Hz, CO_2CH_2), 2.64 (1H, s, –C≡CH), 2.60 (3H, s, $COCH_3$), 1.42 (3H, t, $J = 7.8$ Hz, $CO_2CH_2\underline{CH_3}$); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.3 (C, COMe), 163.8 (C, CO_2Et), 159.6 (C, C-5), 157.1 (C, C-3), 146.9 (C, C-8), 142.1 (C, C-9), 138.1 (C, C-2), 125.1 (CH, C-6), 120.9 (CH, C-4), 110.1 (CH, C-7), 75.9 (C, C≡CH), 68.2 (CH, C≡CH), 61.6 (CH_2, OCH_2), 39.9 (CH_2, NCH_2), 30.0 (CH_3, $COCH_3$), 12.0 (CH_3); Anal. calculated for $C_{16}H_{14}FNO_3$; C, 66.89; H, 4.91; N, 4.88. Found: C, 66.75; H, 5.01; N, 4.92.

General procedure for the synthesis of 1-methyl-5-(prop-2-ynyl)-3H-pyridazino[4,5-b]indol-4(5H)-one (**6a** and **6b**) To a solution of intermediate **5a/5b** in ethanol, hydrazine hydrate was added and the reaction mass was heated to 80 °C for 6 h and kept for stirring at RT for 16 h. The reaction was monitored using TLC, and after the completion of the reaction, ethanol was evaporated under reduced pressure and the residue was poured into cold dilute HCl with stirring. The precipitated product was filtered, was given thorough water wash followed by diethyl ether wash and then dried in an vacuum oven to get the pure product.

1-Methyl-5-(prop-2-ynyl)-3H-pyridazino[4,5-b]indol-4(5H)-one (**6a**) The indole–pyridazinone-fused intermediate **6a** was synthesized by following the above-described procedure for **5a** (5 g, 18.57 mmol) using ethanol (25 mL) and hydrazine hydrate (37.13 mmol) to yield the product as white solid. Yield: 3.2 g (73 %); mp > 250 °C; **^{1}H NMR** (300 MHz, [D_6]DMSO): δ 12.72 (1H, s, NH), 7.44–8.17 (4H, m, Ar–H), 5.79 (2H, s, NCH_2), 2.72 (3H, s, Ar–CH_3), 2.66 (1H, s, C≡CH); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 158.1 (C, C=O), 147.8 (C, C-1), 145.9 (C, C-12), 131.2 (C, C-13), 130.1 (C, C-11), 125.6 (C, C-10), 121.5 (CH, C-8), 120.1 (CH, C-7), 119.4 (CH, C-9), 110.8 (CH, C-6), 76.2 (C, C≡CH), 69.8 (CH, C≡CH), 39.0 (CH_2, NCH_2), 22.1 (CH_3); Anal. calculated for $C_{14}H_{11}N_3O$; C, 70.87; H, 4.67; N, 17.71. Found: C, 71.12 H, 4.62; N, 17.79.

8-Fluoro-1-methyl-5-(prop-2-ynyl)-3H-pyridazino[4,5-b]indol-4(5H)-one (**6b**) Intermediate **6b** was synthesized by carrying out the above-illustrated procedure for **5b** (5 g, 17.40 mmol) using ethanol (25 mL) and hydrazine hydrate (34.81 mmol) to yield the product as white solid. Yield: 3.2 g (72 %); mp > 250 °C; **^{1}H NMR** (300 MHz, [D_6] DMSO): δ 12.74 (1H, s, NH), 7.39–8.11 (3H, m, Ar–H), 5.76 (2H, s, NCH_2), 2.70 (3H, s, Ar–CH_3), 2.68 (1H, s, C≡CH); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 157.6 (C, C=O), 156.8 (C, C-8), 149.8 (C, C-1), 145.1 (C, C-12), 132.7 (C, C-13), 131.9 (C, C-11), 124.2 (C, C-10), 121.0 (CH, C-7), 120.1 (CH, C-9), 111.2 (CH, C-6), 77.4 (C, C≡CH), 70.2 (CH, C≡CH), 38.5 (CH_2, NCH_2), 22.9 (CH_3); Anal. calculated for $C_{14}H_{10}FN_3O$; C, 65.88; H, 3.95; N, 16.46. Found: C, 65.99; H, 3.87; N, 16.51.

General procedure for the synthesis of final compounds (**7a–z, 7aa–ad**) A calculated amount of alkyl/benzyl/phenacyl bromide was taken in *tert*-butanol/H_2O (1:1) solvent system, to which calculated amount (1.1 equivalents) of NaN_3 was added and the reaction mass was stirred at 80 °C for about 1 h. After the formation of alkyl/benzyl/phenacyl azide, the temperature was brought back to RT. The propargylated pre-final intermediate, **6a/6b**, (1 equivalent), was then introduced to the reaction mass followed by the addition of CuI (catalytic amount) and a pinch of alumina (as support to the copper catalyst). The reaction mass was stirred at room temperature overnight. After the completion of the reaction, the solvent was evaporated under reduced pressure and the product was extracted with ethyl acetate. The copper residue present in the organic layer was removed by washing it with ammonia. The organic layer was then dried using Na_2SO_4 and concentrated under reduced pressure. The crude product was then purified by column chromatographic technique using petroleum ether/ethyl acetate (1:1) as the mobile phase.

The structural characterization data of the final compounds are listed below.

5-[(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7a**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.16–7.84 (10H, m, Ar–H), 6.00 (2H, s, NCH_2-Ar), 5.41 (2H, s, NCH_2-Ph), 2.63 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 162.9 (C, C=O), 159.9 (C, C-5 of triazole ring), 144.7, 139.1, 134.5, 129.0, 128.9, 128.0, 127.9, 127.7, 126.9, 125.1, 122.9, 122.0, 121.8, 121.2, 111.5, 107.1 (Ar–C), 54.1 (CH_2, CH_2Ph), 40.5 (CH_2, CH_2N<), 11.0 (CH_3); Anal. calculated for $C_{21}H_{18}N_6O$; C, 68.09; H, 4.90; N, 22.69. Found: C, 68.21; H, 4.83; N, 22.60.

5-{[1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl]methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7b**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.81–7.68 (9H, m, Ar–H), 6.14 (2H, s, NCH_2-Ar), 5.42 (2H, s, NCH_2-Ph), 3.77 (3H, s, OCH_3), 2.63 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.0 (C, C=O), 160.9 (C, PhOMe), 158.7, 138.6, 136.2, 129.9, 127.8, 126.3, 126.0, 124.5, 123.1, 121.9, 120.1, 119.8, 119.0, 113.6, 110.1, 107.4 (Ar–C), 60.3 (CH_3, OMe), 55.9 (CH_2, CH_2Ph), 41.2 (CH_2, CH_2N<), 11.0 (CH_3); Anal. calculated for $C_{22}H_{20}N_6O_2$; C, 65.99; H, 5.03; N, 20.99. Found: C, 65.90; H, 5.08; N, 21.14.

5-{{1-[4-(Trifluoromethyl)benzyl]-1H-1,2,3-triazol-4-yl}methyl}-1-methyl-3H-pyridazino [4,5-b]indol-4(5H)-one (**7c**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.17–7.83 (9H, m, Ar–H), 6.0 (2H, s, NCH_2-Ar), 5.47 (2H, s, NCH_2-Ph), 2.63 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.0 (C, C=O), 159.9, 145.1, 139.1, 138.5, 131.4, 130.7, 128.1, 126.9, 126.1 (Ar–C), 125.1 (C, CF_3), 123.1, 122.4, 122.0, 121.8, 121.2, 111.4, 109.2, 107.1 (Ar–C), 54.2 (CH_2, CH_2Ph), 41.0 (CH_2, CH_2N<), 10.7 (CH_3); Anal. calculated for $C_{22}H_{17}F_3N_6O$; C, 60.27; H, 3.91; N, 19.17. Found: C, 60.36; H, 3.88; N, 19.14.

5-{{1-[4-(Trifluoromethoxy)benzyl]-1H-1,2,3-triazol-4-yl}methyl}-1-methyl-3H-pyridazino [4,5-b]indol-4(5H)-one (**7d**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.16–7.88 (9H, m, Ar–H), 6.02 (2H, s, NCH_2-Ar), 5.43 (2H, s, NCH_2-Ph), 2.66 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 161.8 (C, C=O), 160.4 (C, $PhOCF_3$), 158.6, 145.2, 141.6, 136.1, 134.2, 133.6, 131.2, 131.4, 130.1, 129.2, 125.1, 124.4, 123.4, 122.1, 122.0 (Ar–C), 121.1 (C, OCF_3), 119.6 (Ar–C), 54.8 (CH_2, CH_2Ph), 40.6 (CH_2, CH_2N<), 10.9 (CH_3); **MS** (*m/z*) 455.1 $(M + H)^+$; Anal. calculated for $C_{22}H_{17}F_3N_6O_2$; C, 58.15; H, 3.77; N, 18.49. Found: C, 58.05; H, 3.80; N, 18.54.

4-{{4-[(1-Methyl-4-oxo-3,4-dihydropyridazino[4,5-b]indol-5-yl)methyl]-1H-1,2,3-triazol-1-yl}methyl}benzonitrile (**7e**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.17–7.99 (9H, m, Ar–H), 6.29 (2H, s, NCH_2-Ar), 5.32 (2H, s, NCH_2-Ph), 2.66 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 161.6 (C, C=O), 160.1, 146.7, 145.1, 139.2, 136.2, 132.1, 132.0, 130.1, 130.0, 127.6, 125.5, 124.9, 122.9, 121.8, 119.6 (Ar–C), 117.0 (C, CN), 112.0, 106.8 (Ar–C), 54.7 (CH_2, CH_2Ph), 40.2 (CH_2, CH_2N<), 10.8 (CH_3); Anal. calculated for $C_{22}H_{17}N_7O$; C, 66.82; H, 4.33; N, 24.80. Found: C, 66.96; H, 4.28; N, 24.83.

5-{[1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl]methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7f**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.20–8.21 (9H, m, Ar–H), 6.03 (2H, s, NCH_2-Ar), 5.55 (2H, s, NCH_2-Ph), 2.66 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 162.7 (C, C=O), 160.9, 158.7, 146.1, 142.7, 138.1, 133.1, 132.0, 131.0, 129.2, 128.5, 126.1, 124.6, 122.2, 122.0, 121.1, 111.6, 107.0 (Ar–C), 54.6 (CH_2, CH_2Ph), 40.2 (CH_2, CH_2N<), 10.6 (CH_3); Anal. calculated for $C_{21}H_{17}N_7O_3$; C, 60.72; H, 4.12; N, 23.60. Found: C, 60.96; H, 4.24; N, 23.75.

5-{[1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7g**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.97–7.84 (9H, m, Ar–H), 5.99 (2H, s, NCH_2-Ar), 5.36 (2H, s, NCH_2-Ph), 2.64 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 164.0 (C, C-F), 162.9 (C, C=O), 161.5, 159.9, 144.8, 139.1, 130.4, 129.9, 129.8, 126.9, 125.1, 122.8, 122.0, 121.2, 116.1, 115.9, 111.5, 107.0 (Ar–C), 53.3 (CH_2, CH_2Ph), 40.5 (CH_2, CH_2N<), 11.0 (CH_3); Anal. calculated for $C_{21}H_{17}FN_6O$; C, 64.94; H, 4.41; N, 21.64. Found: C, 64.80; H, 4.48; N, 21.71.

5-{[1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7h**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.03–7.84 (9H, m, Ar–H), 6.01 (2H, s, NCH_2-Ar), 5.47 (2H, s, NCH_2-Ph), 2.64 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 162.9 (C, C-F), 161.6 (C, C=O), 159.9, 159.1, 139.1, 130.8, 130.3, 126.9, 125.0, 124.8, 123.1, 122.1, 121.8, 121.2, 115.8, 115.6, 111.5, 107.1 (Ar–C), 47.6 (CH_2, CH_2Ph), 40.5 (CH_2, CH_2N<), 11.0 (CH_3); Anal. calculated for $C_{21}H_{17}FN_6O$; C, 64.94; H, 4.41; N, 21.64. Found: C, 65.13; H, 4.48; N, 21.70.

5-[(1-Cyclopentyl-1H-1,2,3-triazol-4-yl)methyl]-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7i**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.11–7.88 (5H, m, Ar–H), 5.99 (2H, s, NCH_2-Ar), 4.79–4.84 (1H, m, NCH<), 2.68 (3H, s, Ar–CH_3), 1.67–2.67 (8H, m, CH_2 of cyclopentyl); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.0 (C, C=O), 160.0, 144.0, 139.1, 126.9, 125.0, 122.1, 121.7, 121.4, 121.1, 111.6, 107.0 (Ar–C), 61.8 (CH, C-1 of cyclopentyl ring), 40.6 (CH_2, CH_2N<), 33.3 (CH_2, C-2/C-5 of cyclopentyl ring), 24.0 (CH_2, C-3/C-4 of cyclopentyl ring), 11.0 (CH_3); Anal. calculated for $C_{19}H_{20}N_6O$; C, 65.50; H, 5.79; N, 24.12. Found: C, 65.71; H, 5.71; N, 24.14.

5-[(1-Cyclohexyl-1H-1,2,3-triazol-4-yl)methyl]-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7j**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.17–7.89 (6H, m, Ar–H), 6.03 (2H, s, NCH_2-Ar), 4.76–4.85 (1H, m, NCH<), 2.68 (3H, s, Ar–CH_3), 1.86–2.23 (10H, m, CH_2 of cyclohexyl); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 162.9 (C, C=O), 160.0, 144.0, 139.1, 126.9, 125.0, 122.1, 121.7, 121.4, 121.1, 111.6, 107.0 (Ar–C), 61.8 (CH, C-1 of cyclohexyl ring), 40.6 (CH_2, CH_2N<), 33.3 (CH_2, C-2/C-6 of cyclohexyl ring), 28.5 (CH_2, C-4 of cyclohexyl ring), 24.0 (CH_2, C-3/C-5 of cyclohexyl ring), 11.0 (CH_3); Anal. calculated for $C_{20}H_{22}N_6O$; C, 66.28; H, 6.12; N, 23.19. Found: C, 66.40; H, 6.18; N, 23.08.

1-Methyl-5-{{1-[2-oxo-2-(p-tolyl)ethyl]triazol-4-yl}methyl}-3H-pyridazino[4,5-b]indol-4-one (**7k**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.14–8.01 (9H, m, Ar–H), 6.11 (2H, s, NCH_2-Ar), 5.75 (2H, s, NCH_2COPh), 2.66 (3H, s, Ar–CH_3), 2.45 (3H, s, CH_3Ph); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 187.2 (C, COPh), 165.6 (C, C=O), 161.9, 160.8, 159.8, 141.9, 135.7, 133.3, 130.2, 129.6, 124.4, 124.2, 123.0, 122.0, 121.2, 116.8, 116.1, 112.6, 109.4 (Ar–C) 52.8 (CH_2, CH_2COPh), 43.9 (CH_2, CH_2N<), 21.1 (CH_3, PhCH_3), 11.2 (CH_3); Anal. calculated for $C_{23}H_{20}N_6O_2$; C, 66.98; H, 4.89; N, 20.38. Found: C, 66.87; H, 4.93; N, 20.44.

5-{{1-[2-(4-Methoxyphenyl)-2-oxo-ethyl]triazol-4-yl}methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4-one (**7l**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.24–7.99 (10H, m, Ar–H), 6.18 (2H, s, NCH_2-Ar), 5.78 (2H, s, NCH_2COPh), 3.79 (3H, s, OCH_3), 2.65 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 185.2 (C, COPh), 163.1 (C, C=O), 160.82, 159.9, 159.0, 159.0, 140.9, 133.7, 132.1, 129.2, 124.9, 124.2, 123.0, 122.0, 121.2, 116.8, 116.1, 112.6, 109.4 (Ar–C), 58.2 (CH_3, OCH_3), 50.3 (CH_2, CH_2COPh), 40.5 (CH_2, CH_2N<), 10.9 (CH_3); **MS** (*m/z*) 429.1 $(M + H)^+$; Anal. calculated for $C_{23}H_{20}N_6O_2$; C, 66.98; H, 4.89; N, 20.38. Found: C, 67.13; H, 4.81; N, 20.49.

5-{{1-[2-(4-Nitrophenyl)-2-oxo-ethyl]triazol-4-yl}methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4-one (**7m**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.26–8.21 (10H, m, Ar–H), 6.19 (2H, s, NCH_2-Ar), 5.74 (2H, s, NCH_2COPh), 2.64 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 185.3 (C, COPh), 162.6 (C, C=O), 161.1, 160.7, 158.6, 144.3, 138.7, 133.1, 130.6, 125.7, 125.0, 125.0, 124.5, 123.0, 122.2, 118.1, 117.5, 114.1, 111.2 (Ar–C), 51.7 (CH_2, CH_2COPh), 41.7 (CH_2, CH_2N<), 10.8 (CH_3); **MS** (*m/z*) 444.1 $(M + H)^+$; Anal. calculated for $C_{22}H_{17}N_7O_4$; C, 59.59; H, 3.86; N, 22.11. Found: C, 59.67; H, 3.80; N, 22.17.

5-{{1-[2-(4-Fluorophenyl)-2-oxo-ethyl]triazol-4-yl}methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4-one (**7n**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.19–7.87 (10H, m, Ar–H), 6.25 (2H, s, NCH_2-Ar), 5.79 (2H, s, NCH_2COPh), 2.63 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 184.9 (C, COPh), 161.0 (C, C=O), 160.5, 159.7, 158.6, 148.7, 141.5, 139.3, 135.6, 128.5, 124.1, 123.0, 122.7, 122.0, 121.1, 120.3, 119.3, 116.7, 114.3 (Ar–C), 50.6 (CH_2, CH_2COPh), 40.9 (CH_2, $CH_2N<$), 10.4 (CH_3); Anal. calculated for $C_{22}H_{17}FN_6O_2$; C, 63.46; H, 4.11; N, 20.18. Found: C, 63.65; H, 4.02; N, 20.24.

5-[(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]-8-fluoro-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7o**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11–7.82 (10H, m, Ar–H), 6.11 (2H, s, NCH_2-Ar), 5.44 (2H, s, NCH_2-Ph), 2.64 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 161.0 (C, C=O), 160.8, 157.3, 145.9, 140.4, 136.1, 130.8, 129.2, 128.9, 127.5, 127.0, 126.6, 124.9, 122.8, 122.3, 121.6, 110.9, 108.3 (Ar–C), 54.8 (CH_2, CH_2Ph), 40.7 (CH_2, $CH_2N<$), 10.0 (CH_3); Anal. calculated for $C_{21}H_{17}FN_6O$; C, 64.94; H, 4.41; N, 21.64. Found: C, 65.10; H, 4.32; N, 21.60.

5-{[1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl]methyl}-8-fluoro-1-methyl-3H-pyridazino [4,5-b]indol-4(5H)-one (**7p**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.08–7.92 (9H, m, Ar–H), 5.95 (2H, s, NCH_2-Ar), 5.34 (2H, s, NCH_2-Ph), 3.77 (3H, s, OCH_3), 2.64 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.0 (C, C=O), 159.8, 159.6, 157.4, 144.3, 135.7, 129.5, 127.1, 127.0, 126.5, 123.4, 122.6, 114.4, 114.0, 113.7, 112.7, 106.6, 106.2 (Ar–C), 58.3 (CH_3, OMe), 53.7 (CH_2, CH_2Ph), 40.6 (CH_2, $CH_2N<$), 11.0 (CH_3). Anal. calculated for $C_{22}H_{19}FN_6O_2$; C, 63.15; H, 4.58; N, 20.08. Found: C, 63.29; H, 4.49; N, 20.11.

5-{{1-[4-(Trifluoromethyl)benzyl]-1H-1,2,3-triazol-4-yl}methyl}-8-fluoro-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7q**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.16–7.87 (8H, m, Ar–H), 5.99 (2H, s, NCH_2-Ar), 5.51 (2H, s, NCH_2-Ph), 2.65 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.1 (C, C=O), 159.8, 159.6, 157.4, 144.8, 138.4, 135.7, 128.1, 127.1, 127.0, 126.0 (Ar–C), 124.4 (C, CF_3), 123.3, 123.2, 114.1, 113.9, 112.6, 106.7, 106.0 (Ar–C), 53.4 (CH_2, CH_2Ph), 40.6 (CH_2, $CH_2N<$), 11.0 (CH_3); **MS** (*m/z*) 457.1 $(M + H)^+$; Anal. calculated for $C_{22}H_{16}F_4N_6O$; C, 57.90; H, 3.53; N, 18.41. Found: C, 57.79; H, 3.58; N, 18.47.

5-{{1-[4-(Trifluoromethoxy)benzyl]-1H-1,2,3-triazol-4-yl}methyl}-8-fluoro-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7r**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11–7.84 (8H, m, Ar–H), 5.97 (2H, s, NCH_2-Ar), 5.42 (2H, s, NCH_2-Ph), 2.64 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.1 (C, C=O), 159.8, 157.4, 149.4, 144.6, 135.7, 134.3, 133.2, 131.3, 130.9, 129.8, 128.8, 123.3, 125.6, 123.1, 121.6, 121.5 (Ar–C), 121.2 (C, OCF_3), 119.1, 53.2 (CH_2, CH_2Ph), 40.6 (CH_2, $CH_2N<$), 10.9 (CH_3); Anal. calculated for $C_{22}H_{16}F_4N_6O_2$; C, 55.94; H, 3.41; N, 17.79. Found: C, 55.82; H, 3.49; N, 17.83.

4-{{4-[(8-Fluoro-1-methyl-4-oxo-3,4-dihydropyridazino[4,5-b]indol-5-yl)methyl]-1H-1,2,3-triazol-1-yl}methyl}benzonitrile (**7s**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.13–7.87 (8H, m, Ar–H), 5.99 (2H, s, NCH_2-Ar), 5.51 (2H, s, NCH_2-Ph), 2.66 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.2 (C, C=O), 159.8, 157.4, 144.9, 139.6, 135.7, 132.8, 128.4, 127.1, 127.0, 123.4, 123.3 (Ar–C), 118.1 (C, CN), 114.2, 113.9, 112.7, 112.5, 106.7, 106.1 (Ar–C), 53.4 (CH_2, CH_2Ph), 40.5 (CH_2, $CH_2N<$), 11.0 (CH_3); **MS** (*m/z*) 414.1 $(M + H)^+$; Anal. calculated for $C_{22}H_{16}FN_7O$; C, 63.92; H, 3.90; N, 23.72. Found: C, 64.04; H, 3.94; N, 23.63.

5-{[1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl]methyl}-8-fluoro-1-methyl-3H-pyridazino[4,5-b] indol-4(5H)-one (**7t**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.13–8.21 (9H, m, Ar–H), 5.99 (2H, s, NCH_2-Ar), 5.55 (2H, s, NCH_2-Ph), 2.66 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 162.0 (C, C=O), 160.7, 159.6, 157.1, 145.1, 141.9, 138.1, 132.2, 131.6, 131.1, 130.9, 130.1, 127.6, 127.1, 125.6, 123.6, 120.6, 119.6 (Ar–C), 54.5 (CH_2, CH_2Ph), 40.1 (CH_2, $CH_2N<$), 10.6 (CH_3); Anal. calculated for $C_{21}H_{16}FN_7O_3$; C, 58.20; H, 3.72; N, 22.62. Found: C, 58.28; H, 3.69; N, 22.58.

5-{[1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]methyl}-8-fluoro-1-methyl-3H-pyridazino[4,5-b] indol-4(5H)-one (**7u**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11–7.94 (9H, m, Ar–H), 6.01 (2H, s, NCH_2-Ar), 5.50 (2H, s, NCH_2-Ph), 2.63 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.6 (C, C-F), 163.0 (C, C=O), 162.8, 160.6, 159.1, 146.6, 142.3, 132.8, 130.9, 130.1, 128.5, 126.6, 124.1, 123.2, 122.4, 122.0, 116.0, 112.6 (Ar–C), 52.5 (CH_2, CH_2Ph), 40.9 (CH_2, $CH_2N<$), 10.3 (CH_3); Anal. calculated for $C_{21}H_{16}F_2N_6O$; C, 62.06; H, 3.97; N, 20.68. Found: C, 62.19; H, 3.90; N, 20.62.

5-{[1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]methyl}-8-fluoro-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7v**)**^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11–7.94 (9H, m, Ar–H), 6.08 (2H, s, NCH_2-Ar), 5.56 (2H, s, NCH_2-Ph), 2.61 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.0 (C, C-F), 163.6 (C, C=O), 161.6, 161.1, 159.0, 140.2, 133.1, 132.8, 129.3, 128.4, 124.8, 124.0, 123.9, 122.7, 117.1, 116.3, 113.2, 109.8 (Ar–C), 47.7 (CH_2, CH_2Ph), 40.5 (CH_2, $CH_2N<$), 10.8 (CH_3); Anal. calculated for $C_{21}H_{16}F_2N_6O$; C, 62.06; H, 3.97; N, 20.68. Found: C, 62.18; H, 4.00; N, 20.60.

5-[(1-Cyclopentyl-1H-1,2,3-triazol-4-yl)methyl]-8-fluoro-1-methyl-3H-pyridazino[4,5-b] indol-4(5H)-one (**7w**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.08–7.79 (4H, m, Ar–H), 6.05 (2H, s, NCH_2-Ar), 4.81–4.86 (1H, m, NCH<), 2.66 (3H, s,

Ar–CH_3), 1.65–2.66 (8H, m, CH_2 of cyclopentyl); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 164.7 (C, C=O), 162.9, 161.2, 146.7, 144.1, 129.8, 124.9, 123.9, 122.1, 121.1, 113.8, 109.1 (Ar–C), 61.8 (CH, C-1 of cyclopentyl ring), 40.6 (CH_2, $CH_2N<$), 34.3 (CH_2, C-2/C-5 of cyclopentyl ring), 25.1 (CH_2, C-3/C-4 of cyclopentyl ring), 10.9 (CH_3); **MS** (m/z) 367.1 $(M + H)^+$; Anal. calculated for $C_{19}H_{19}FN_6O$; C, 62.28; H, 5.23; N, 22.94. Found: C, 62.37; H, 5.18; N, 22.89.

5-[(1-Cyclohexyl-1H-1,2,3-triazol-4-yl)methyl]-8-fluoro-1-methyl-3H-pyridazino[4,5-b] indol-4(5H)-one (**7x**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.09–7.81 (5H, m, Ar–H), 6.04 (2H, s, NCH_2-Ar), 4.79–4.85 (1H, m, NCH<), 2.66 (3H, s, Ar–CH_3), 1.86–2.23 (10H, m, CH_2 of cyclohexyl); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 164.6 (C, C=O), 162.8, 161.3, 145.9, 141.5, 128.3, 127.3, 123.6, 122.0, 122.0, 113.3, 109.7 (Ar–C), 60.8 (CH, C-1 of cyclohexyl ring), 40.8 (CH_2, $CH_2N<$), 33.4 (CH_2, C-2/C-6 of cyclohexyl ring), 28.6 (CH_2, C-4 of cyclohexyl ring), 24.9 (CH_2, C-3/C-5 of cyclohexyl ring), 10.7 (CH_3); Anal. calculated for $C_{20}H_{21}FN_6O$; C, 63.14; H, 5.56; N, 22.09. Found: C, 63.24; H, 5.52; N, 21.99.

8-Fluoro-1-methyl-5-{{1-[2-oxo-2-(p-tolyl)ethyl]triazol-4-yl}methyl}-3H-pyridazino[4,5-b] indol-4-one (**7y**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.12–8.01 (8H, m, Ar–H), 6.06 (2H, s, NCH_2-Ar), 5.74 (2H, s, NCH_2COPh), 2.64 (3H, s, Ar–CH_3), 2.45 (3H, s, CH_3Ph); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 184.1 (C, COPh), 163.0 (C, C=O), 161.1, 160.9, 155.6, 146.8, 142.2, 137.2, 135.7, 131.4, 127.5, 126.9, 124.5, 123.0, 121.0, 119.6, 118.3, 114.8, 112.8 (Ar–C), 52.7 (CH_2, CH_2COPh), 40.2 (CH_2, $CH_2N<$), 19.1 (CH_3, $PhCH_3$), 10.9 (CH_3); Anal. calculated for $C_{23}H_{19}FN_6O_2$; C, 64.18; H, 4.45; N, 19.52. Found: C, 64.29; H, 4.39; N, 19.47.

8-Fluoro-5-{{1-[2-(4-methoxyphenyl)-2-oxo-ethyl]triazol-4-yl}methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4-one (**7z**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.12–7.94 (9H, m, Ar–H), 6.09 (2H, s, NCH_2-Ar), 5.79 (2H, s, NCH_2COPh), 3.77 (3H, s, OCH_3), 2.64 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 184.1 (C, COPh), 164.6 (C, C=O), 162.8, 161.6, 160.7, 155.5, 142.4, 134.7, 133.9, 131.0, 126.7, 126.0, 125.9, 123.8, 121.1, 119.7, 118.3, 115.1, 112.3 (Ar–C), 57.7 (CH_3, OMe), 51.3 (CH_2, CH_2COPh), 40.9 (CH_2, $CH_2N<$), 10.9 (CH_3); Anal. calculated for $C_{23}H_{19}FN_6O_3$; C, 61.88; H, 4.29; N, 18.82. Found: C, 61.80; H, 4.34; N, 18.86.

8-Fluoro-5-{{1-[2-(4-nitrophenyl)-2-oxo-ethyl]triazol-4-yl}methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4-one (**7aa**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.17–7.97 (9H, m, Ar–H), 6.21 (2H, s, NCH_2-Ar), 5.76 (2H, s, NCH_2COPh), 2.66 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 185.2 (C, COPh), 163.3 (C, C=O), 162.9, 162.0, 160.6, 155.1 146.2, 140.6, 135.1, 133.1, 130.4, 126.6, 125.2, 124.0, 123.3, 120.1, 119.6, 117.0, 113.5 (Ar–C), 52.6 (CH_2, CH_2COPh), 40.1 (CH_2, $CH_2N<$), 11.0 (CH_3); Anal. calculated for $C_{22}H_{16}FN_7O_4$; C, 57.27; H, 3.50; N, 21.25. Found: C, 57.20; H, 3.57; N, 21.29.

8-Fluoro-5-{{1-[2-(4-fluorophenyl)-2-oxo-ethyl]triazol-4-yl}methyl}-1-methyl-3H-pyridazino[4,5-b]indol-4-one (**7ab**) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.15–7.91 (9H, m, Ar–H), 6.22 (2H, s, NCH_2-Ar), 5.77 (2H, s, NCH_2COPh), 2.61 (3H, s, Ar–CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 186.0 (C, COPh), 162.5 (C, C=O), 162.1, 160.9, 159.1, 158.6, 150.5, 145.5, 142.2, 140.2, 130.1, 128.9, 126.9, 125.5, 122.3, 121.7, 120.9, 120.7, 118.3 (Ar–C), 51.9 (CH_2, CH_2COPh), 41.6 (CH_2, $CH_2N<$), 10.7 (CH_3); Anal. calculated for $C_{22}H_{16}F_2N_6O_2$; C, 60.83; H, 3.71; N, 19.35. Found: C, 60.94; H, 3.67; N, 19.29.

5-[(1-Isopropyl-1H-1,2,3-triazol-4-yl)methyl]-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7ac**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.11–7.87 (5H, m, Ar–H), 5.80 (2H, s, NCH_2-Ar), 4.01–4.15 (1H, m, $NCH(Me)_2$), 2.63 (3H, s, Ar–CH_3), 1.58 (6H, d, CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.6 (C, C=O), 146.8, 145.3, 143.3, 133.9, 124.1, 123.1, 122.1, 121.1, 118.5, 118.1, 115.4 (Ar–C), 55.1 (CH, $CH(CH_3)_2$), 41.7 (CH_2, $CH_2N<$), 23.9 (2 CH_3, $CH(CH_3)_2$), 10.8 (CH_3); Anal. calculated for $C_{17}H_{18}N_6O$; C, 63.34; H, 5.63; N, 26.07. Found: C, 63.43; H, 5.67; N, 25.98.

8-Fluoro-5-[(1-isopropyl-1H-1,2,3-triazol-4-yl)methyl]-1-methyl-3H-pyridazino[4,5-b]indol-4(5H)-one (**7ad**) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.14–7.82 (4H, m, Ar–H), 5.77 (2H, s, NCH_2-Ar), 4.11–4.18 (1H, m, $NCH(Me)_2$), 2.66 (3H, s, Ar–CH_3), 1.61 (6H, d, CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.0 (C, C=O), 157.7, 148.1, 147.2, 145.8, 139.5, 128.6, 125.1, 122.0, 120.6, 117.34, 114.8 (Ar–C), 53.8 (CH, $CH(CH_3)_2$), 41.8 (CH_2, $CH_2N<$), 22.9 (2 CH_3, $CH(CH_3)_2$), 10.7 (CH_3); Anal. calculated for $C_{17}H_{17}FN_6O$; C, 59.99; H, 5.03; N, 24.69. Found: C, 60.11; H, 5.08; N, 24.60.

Cell proliferation assay study

Cell lines and culture conditions

Human breast cancer cells (MDA-MB 231), MCF-7, human primary glioblastoma cell line U-87 and human neuroblastoma IMR-32 cell line were procured from National Center for Cell Sciences, Pune, India. All cells were grown in RPMI-1640 supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM glutamine.

Cultures were maintained in a humidified atmosphere with 5 % CO_2 at 37 °C. The cells were subcultured twice each week, seeding at a density of about 2×10^3 cells/mL.

MTT assay

The cell viability was determined by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (5×10^3 cells/well) were seeded to 96-well culture plate and cultured with or without compounds at 10 μM concentration for 72 h in a final volume of 200 μL. After treatment, the medium was removed and 20 μl of MTT (5 mg/ml in PBS) was added to the fresh medium. After 2-h incubation at 37 °C, 100 μl of DMSO was added to each well and plates were agitated for 1 min. Absorbance was read at 570 nm on a multi-well plate reader (VICTOR3, PerkinEmler). Percent inhibition of proliferation was calculated as a fraction of control (without compound). The experiment was performed in triplicate.

Docking studies

The crystal structure of PI3 kinase alpha with inhibitor (pdb id: 1E7V) having resolution of 2.4 Å was retrieved from the Protein Data Bank, and it was prepared using protein preparation wizard (Maestro, v9.2, Schrodinger, LLC, New York). Initially formal charges and bond orders were added for heterogroups, and later hydrogens were added to the system. After removing water molecules in all structures, the structure was energy-minimized. The compounds that were synthesized were drawn and prepared using Ligprep (LigPrep v2.2, Schrodinger LLC, New York) with Epik (Epik v1.6, Schrodinger, LLC, New York) to expand protonation and tautomeric states at 7.0 ± 2.0 pH units. The conformational sampling was performed on all database molecules using the ConfGen search algorithm. The ConfGen with force field (OPLS_2005) was employed, and duplicate poses within 1.0 Å rmsd were eliminated to remove repeated conformers. The glide energy grid was generated, and it was defined by a rectangular box surrounding the crystal ligand in X-ray structure. The prepared ligands were then docked using Glide 5.0 module (Glide v5.0 Schrodinger, LLC, New York). The binding site created earlier was chosen, and XP (extra precision) docking was performed, which gives precise protein–ligand binding affinities.

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