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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and cytotoxicity studies of 3,5-diaryl *N*-acetyl pyrazoline—isatin hybrids

Manmohan Sharma · Sahil Sharma · Abhishek Buddhiraja · A. K. Saxena · Kunal Nepali · P. M. S. Bedi

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Abstract Numerous reports highlighting the cytotoxic effects of 3,5-diaryl *N*-acetyl-pyrazolines and isatin tempted us to synthesise conjugates of the functionalities via alkyl armed triazole tetheration. The hybrids were synthesized by click chemistry approach and were evaluated against a panel of cell lines i.e. viz HeLa (cervix cancer), CAKI-I (Renal cancer), PC-3 (Prostate cancer) and Miapaca-2 (pancreatic cancer). The hybrids were classified into right-handed and left-handed conjugates on the basis of the placement of the isatin ring. The length of the alkyl armed triazole linker was varied from 2 to 6. Structure activity relationship has also been presented. A preliminary cytotoxic assay was performed on the series of 3,5-diaryl *N*-acetyl-pyrazolines and only the potent 3,5-diaryl *N*-acetyl-pyrazolines were selected for their inclusion in the hybrid scaffold. Among the cell lines employed, HeLa cell line was the most sensitive towards the exposure of test compounds. Out of all the compounds evaluated, two right-handed conjugates MI-7b and MI-8b and two left-handed conjugates MI-4b, MI-6b displayed significant cytotoxic potential and exhibited an IC_{50} range from 1.3 to 3.5 μM against HeLa Cell line.

Keywords Molecular hybridization · Hybrids · Isatin · Cell lines · Cytotoxic

Introduction

Cancer, the uncontrolled, rapid and pathological proliferation of abnormal cells, is one of the most formidable afflictions in the world. (Fadeyi *et al.,* 2008; Rostom, 2006). Over the years, the design of cancer chemotherapy has become increasingly sophisticated. Yet, there is no cancer treatment that is 100 % effective against disseminated cancer. At present cancer therapy interfering with a single biological molecule or pathway has been successfully utilized (Sawyers, 2004; Petrelli and Giordano, 2008; Overington *et al.,* 2006). Still the problem of drug resistance and a general belief that agents modulating more than one target could have superior efficacy compared to single target drugs (Bode and Dong, 2009; Zhan and Liu, 2009) has initiated the search for molecules modulating multiple targets. Modulating multiple targets simultaneously can be achieved either by the combination of multiple drugs with different mechanisms or by single chemical entity that could modulate several targets of a multifactorial disease. (Morphy and Rankovic, 2005; Hopkins, 2008) The molecular hybridization (MH) is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-units, provides new hybrid architectures (Viegas-Junior *et al.,* 2007). With this background and promising results

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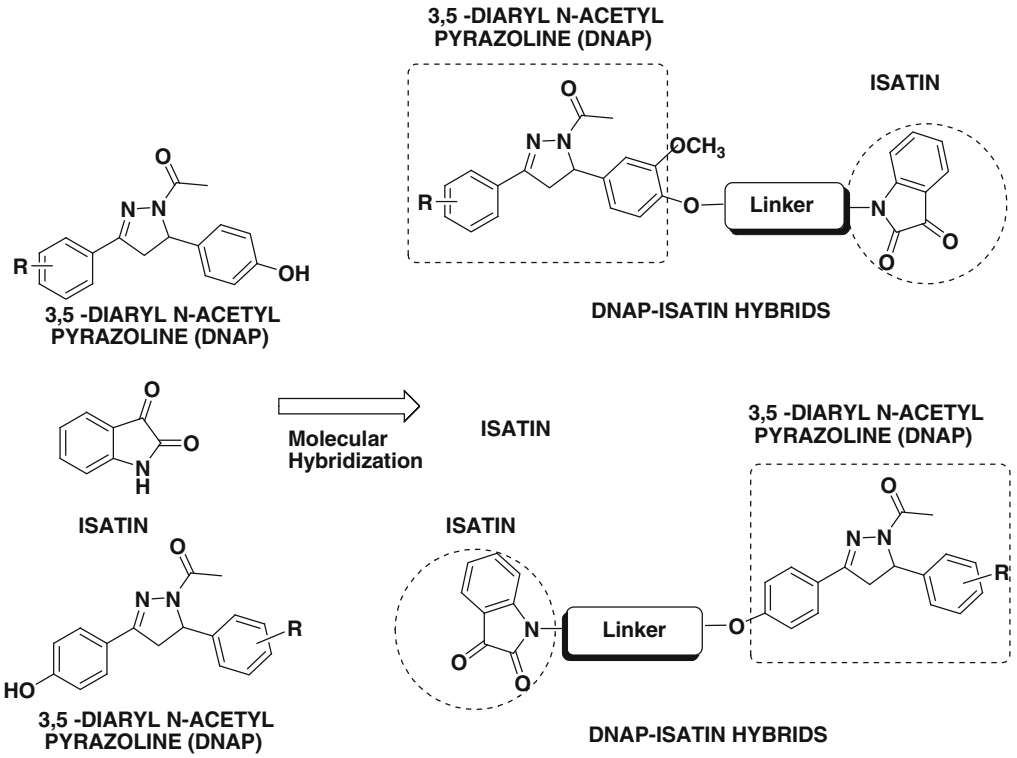


Fig. 1 1 DNAP-Isatin hybrids

achieved with hybrids structure-based design approach, hybrids of 3,5-diaryl-4,5-dihydro-pyrazolines and isatin were designed in view of the promising anticancer potential displayed by these functionalities.

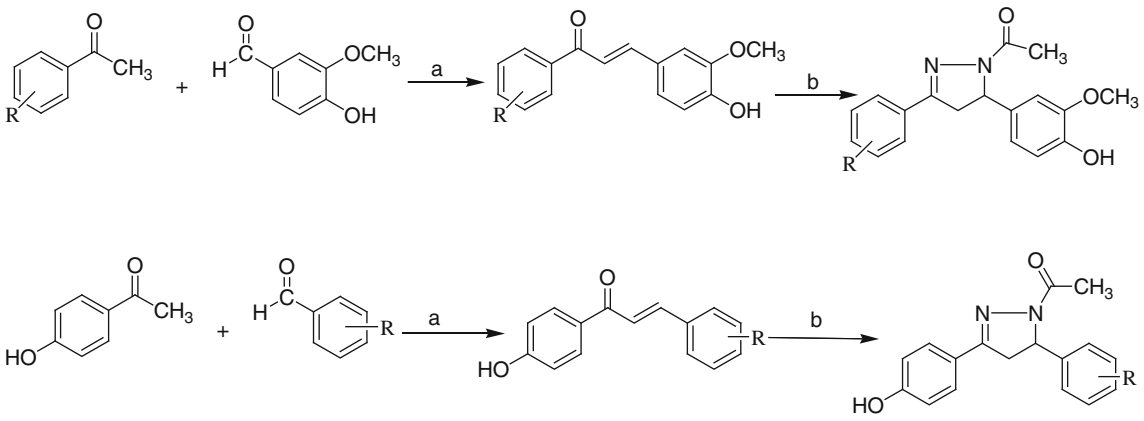
3,5-Diaryl-4,5-dihydro-pyrazolines have been well explored to be endowed with anticancer potential and inhibitory effects on various enzymes. Zhou *et al.,* (2013) recently reported 1-(5-(3,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone as potent tyrosinase inhibitor with IC_{50} value of 0.301 μM. Chloroquinolinyl-based pyrazoline derivatives (Insuasty *et al.,* 2013) displayed remarkable antitumor activity against cancer cell lines, with low GI_{50} values ranging from 0.13 to 0.99 μM. Manna *et al.,* (2005) reported some appropriate pyrazolines capable of inhibiting P-glycoprotein-mediated multidrug resistance by direct binding to a purified protein domain containing an ATP-binding site and a modulator interacting region. Ethiraj *et al.,* (2012) also reported pyrazolines derived from methoxy-substituted naphthyl chalcones with potent cytotoxicity against HCT15, A549 and HeLa, respectively. Thus, the numbers of reports on the anticancer profile of N-acetyl pyrazolines along with their synthetic accessibility and ease of synthesis led to their inclusion within the designed hybrids chemical architecture.

Number of recent reports on cell killing potential of Uracil-isatin conjugates (Kumar *et al.,* 2012), triazole tethered isatin conjugates (Singh *et al.,* 2012), isatin–benzothiazole analogues (Solomon *et al.,* 2009), isatin-4-pirazinylquinoline hybrids (Solomon *et al.,* 2010) and isatin-thiazolidinone hybrid (Ramshid *et al.,* 2010) clearly indicates the success of isatin-based molecular hybrids and justifies its inclusion in the conjugates designed by our research group.

With this background and in continuation of our ongoing research on 3,5-diaryl-4,5-dihydro-pyrazolines based conjugates (Singh *et al.,* 2013), hybrids of the two functionalities (Fig. 1) were synthesized and evaluated for cytotoxic activity against four human cancer cell lines viz HeLa (cervix cancer), CAKI-I (Renal cancer), PC-3 (Prostate cancer) and Miapaca-2 (pancreatic cancer). The selection of triazole for tethering 3,5-diaryl *N*-acetyl pyrazoline (DNAP) and isatin was attributed to its moderate dipole character, hydrogen bonding capability, rigidity and stability under in-vivo conditions. (Kamal *et al.,* 2008; Wang *et al.,* 2010; He *et al.,* 2010; Kolb and Sharpless, 2003).

Results and discussion

In our efforts to discover potent DNAP-ISATIN anticancer hybrids, we explored a series of DNAPs as cytotoxic agents for their inclusion in the hybrids chemical architecture. The DNAPs were synthesized as per Scheme 1.



Scheme 1 Reagents and conditions: **a** CH_3COOH, H_2SO_4, stirring, 48 h; **b** $NH_2NH_2.H_2O$, CH_3COOH, 2 h, reflux

The DNAPs were evaluated for their anticancer activity against four human cancer cell lines viz HeLa (cervix cancer), CAKI-I (Renal cancer), PC-3 (Prostate cancer) and Miapaca-2 (pancreatic cancer) using sulforhodamine B assay. Among the series, only the potent pyrazolines were selected for the synthesis of the target hybrids.

Table 1 clearly indicates that the *N*-acetyl pyrazolines displayed significant cytotoxicity against PC-3 and HeLa cell lines. Among the series, M-8 exhibited remarkable cytotoxicity against HeLa cell lines with an IC_{50} value of 5.1 μM, and M-6 was endowed with significant potential against PC-3 cell lines with an IC_{50} value of 8 μM. Overall, five pyrazolines M-3, 4, 6, 7 and 8 were selected for the inclusion in the hybrids chemical architecture. The pyrazolines lacking the methoxy groups and bearing halo groups and nitro groups displayed poor cytotoxic profile. Keeping in view, the results of the cytotoxic studies of pyrazolines, we hypothesized that incorporating the structural features of M-3, 4, 6, 7 and 8 would result in potent cytotoxic hybrids.

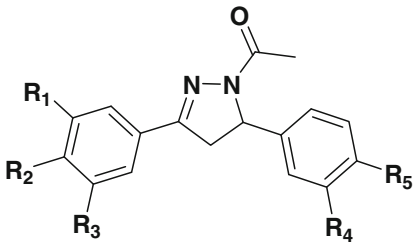
The potent pyrazolines were propargylated for condensation with isatin azides (Scheme 2).

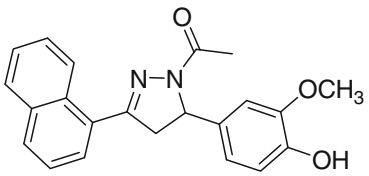
The target hybrids (MI) were synthesized by utilizing regioselective azide-alkyne cycloaddition reaction of the acetylenic DNAP and the isatin azides. Linker length was varied from 2 to 6. The hybrids were classified as right-handed and left-handed conjugates on the basis of the placement of isatin ring (Scheme 3).

The synthesized hybrids (MI) were evaluated for their anticancer activity against four human cancer cell lines viz HeLa (cervix cancer), CAKI-I (Renal cancer), PC-3 (Prostate cancer) and Miapaca-2 (pancreatic cancer). The results of the cytotoxicity studies are presented in Table 1. It was observed that incorporation of DNAP in the hybrid scaffold resulted in diminished cytotoxic effect against PC-3 cell lines, whereas the DNAP (M-3, 4, 6, 7, 8) displayed significant cytotoxicity against PC-3 cell line (IC_{50} value range of 5.3–11 μM). Among the cell lines employed, HeLa cell line was the most sensitive towards the exposure of test compounds. Out of all the compounds evaluated, two right-handed conjugates MI-7b and MI-8b and two left-handed conjugates MI-4b, MI-6b displayed significant cytotoxicity and exhibited an IC_{50} value ranging from 1.3 to 3.5 μM against HeLa cell line. The compounds having IC_{50} value more than 100 μM were considered inactive. The most active hybrid i.e. MI-6b possessed an IC_{50} value of 1.3 μM and displayed striking selectivity i.e. around 10 folds towards HeLa cell lines. MI-4b was the second most potent hybrids of the series against HeLa cell lines with an IC_{50} values of 1.7 μM. While most of the hybrids were inactive against other cell lines, the hybrids with a propyl chain were found to be endowed with moderate cytotoxicity against other cell lines. MI-4b inhibited CAKI-I, PC-3, Miapaca-2 cell lines with an IC_{50} value of 18, 37 and 67 μM. MI-6b inhibited CAKI-I, PC-3, Miapaca-2 cell lines with an IC_{50} value of 14, 31 and 35 μM. MI-8b inhibited CAKI-I, PC-3, Miapaca-2 cell lines with an IC_{50} value of 18, 27 and 38 μM (Table 2).

A closure look into the structure activity relationship for the inhibitory effects against HeLa cell lines indicated that the length of the linker connecting the two functionalities remarkably influences the cytotoxic activity. The cytotoxic potential of the hybrids significantly improved on increasing the length of the linker from $n = 2$ to $n = 3$ and it was drastically decreased on increasing the chain length i.e. $n > 3$. Thus, the optimum length of the linker is $n = 3$ as hybrids with propyl chain (MI-4b, MI-6b, MI-7b, MI-8b) exhibited potent cytotoxicity in addition to selectivity towards the HeLa cell lines. All the hybrids with linker length $n > 3$ were almost inactive with most of them displaying an IC_{50} value of >100 μM (mentioned as NA). Apart from the length of linker, the substituents enhancing the electron charge density at the periphery, i.e. placement of methoxy phenyl rings and naphthyl ring, were found to

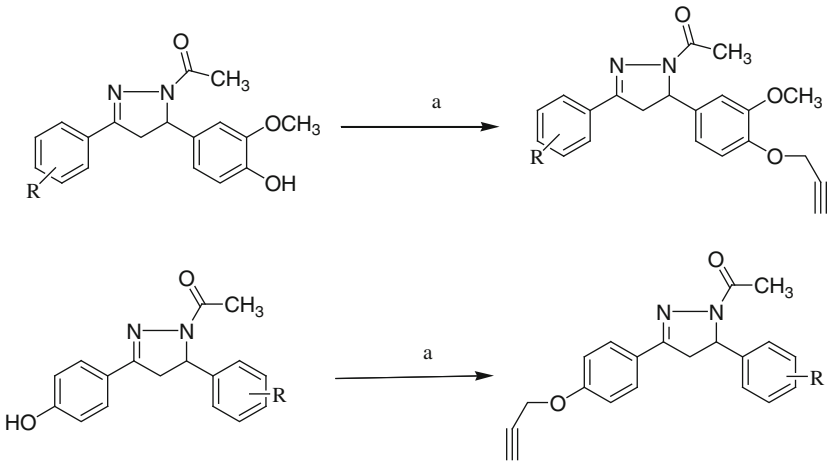
Table 1 IC_{50} values of 3,5-diaryl *N*-acetyl pyrazolines



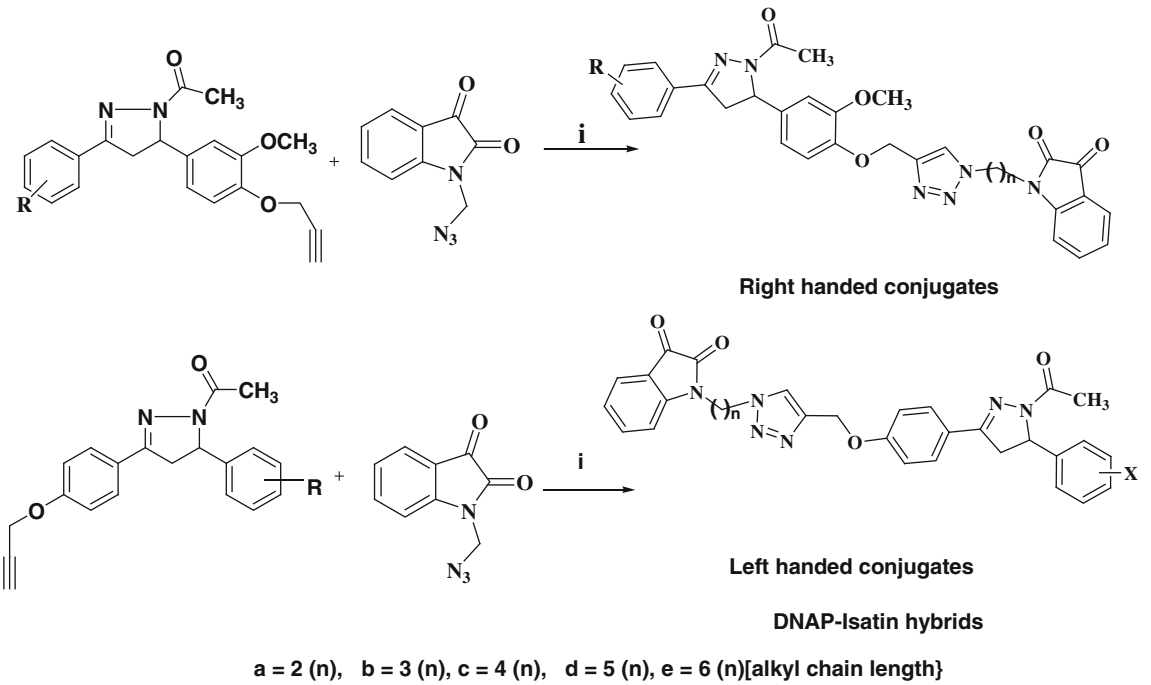
Code	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)			MiaPaca-2
						HeLa	CAKI-I	PC-3	
M-1	H	H	H	OCH_3	OH	65	NA	13	83
M-2	H	Cl	H	OCH_3	OH	74	NA	21	NA
M-3	H	OCH_3	H	OCH_3	OH	**21**	76	**11**	57
M-4	H	OH	H	H	OCH_3	**10**	53	**9**	42
M-5	H	NO_2	H	OCH_3	OH	97	NA	31	NA
M-6	H	OH	H	OCH_3	OCH_3	**7**	48	**8**	31
M-7						**8**	51	**10**	46
M-8	OCH_3	OCH_3	OCH_3	OCH_3	OH	**5.1**	21	**5.3**	32
M-9	H	OH	H	H	H	18.9	38.7	17	51
M-10	H	Br	H	OCH_3	OH	81	NA	29.3	NA

Bold values indicate potent compounds

Scheme 2 Reagents and conditions: **a** Propargyl bromide, K_2CO_3, DMF, stirring, 24 h



be beneficial in potentiating the cytotoxic activity. Among the right-handed conjugates, MI-8b possessing a trimethoxy phenyl ring and MI-7b possessing a naphthyl ring in place of trimethoxy phenyl ring were the two active hybrids. This further indicated that the naphthyl ring can be a surrogate for the trimethoxy phenyl ring in the hybrid scaffold; however, MI-8b was two folds more active than MI-7b. Among the left-hand conjugates, MI-4b and MI-6b were the potent hybrids. Both of them possessed methoxy substituent/s at the periphery of the hybrid structure.



Scheme 3 (i) $CuSO_4 \cdot 5H_2O$, Sodium Ascorbate, DMF, stirring, 2 h

Doubly substituting the phenyl ring with methoxy groups was found to be beneficial as MI-6b was found to be more active than MI-4b. As far as the competition between the right-hand and left-hand conjugates is concerned, the left-hand conjugates were found to be more toxic to the HeLa cells as compared to right-hand conjugates. Overall, the MI-4b and MI-6b are good hits among the hybrids as they are endowed with promising results and will be further investigated in detail.

Conclusion

Thus, in the present study, DNAP-Isatin bifunctional hybrids were synthesized by a cycloaddition reaction via click chemistry. The synthesized hybrids were evaluated for their anticancer activity against four human cancer cell lines viz HeLa (cervix cancer), CAKI-I (Renal cancer), PC-3 (Prostate cancer) and Miapaca-2 (pancreatic cancer) using sulforhodamine B assay. The most active hybrid i.e. MI-6b possessed an IC_{50} value of 1.3 μM (HeLa Cell line) and displayed striking selectivity i.e. around 10 folds towards HeLa cell lines. MI-4b was the second most potent hybrid of the series against HeLa cell lines with an IC_{50} values of 1.7 μM. Structure activity relationship revealed that both electronic effects as well as length of the linker remarkably influenced the cytotoxic activity of the hybrids. The chain length i.e. $n = 3$ and substitution of methoxy groups on the phenyl rings of *N*-acetyl pyrazoline functionality of the hybrids were observed as important structural prerequisites for the activity. Overall, the MI-4b and MI-6b are good hits among the hybrids as they are endowed with significant cytotoxicity. The potential of these hybrids to selectively kill the HeLa cells makes them promising candidates for detailed investigation. In future both of them will be utilized as leads for the synthesis of derivatives with diverse permutation and combination.

Experimental

The reagents were purchased from Sigma Aldrich, Merck, CDH, Loba chem., Spectro chem., India and used without further purification. All yields refer to isolated products after purification. Products were characterized by spectral techniques; ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance II 400 NMR Spectrometer and JEOL AL 300 NMR Spectrometer. The spectra were measured in DMSO-d_6 relative to TMS (0.00 ppm). Melting points were determined in open capillaries and were uncorrected.

Procedure for the synthesis of 1,3-Diarylpropenones:

Concentrated sulphuric acid (2.5 ml) was added slowly to the stirring solution of various substituted benzaldehydes (1 mmol) and acetophenones (1 mmol) in glacial acetic acid in a 500 ml round-bottom flask. The stirring was continued for 48 h keeping the temperature of reaction

Table 2 In-vitro cytotoxic activity of hybrids against human cancer cell lines

Sample	Cell line				
	HeLa		CAKI-I	PC-3	MiaPaca-2
	n	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
MI-3a	2	48	NA	83	NA
MI-4a	2	16.2	32	47	NA
MI-6a	2	13.31	38.4	45.6	51.2
MI-7a	2	NA	NA	69.5	88
MI-8a	2	18.21	29	42.2	59.5
MI-3b	3	31	NA	75	NA
MI-4b	***3***	**1.7**	18	37	67
MI-6b	***3***	**1.3**	14	31	35
MI-7b	***3***	**3.5**	21	NA	NA
MI-8b	***3***	**2.4**	18	27	38
MI-3c	4	NA	30	NA	NA
MI-4c	4	39.7	NA	NA	NA
MI-6c	4	36.2	NA	NA	NA
MI-7c	4	NA	73	NA	96
MI-8c	4	43	NA	32	NA
MI-3d	5	90	NA	90	NA
MI-4d	5	NA	NA	NA	94
MI-6d	5	75	NA	82	NA
MI-7d	5	91	NA	NA	78
MI-8d	5	95	NA	NA	NA
MI-3e	6	NA	87	78	NA
MI-4e	6	94	NA	NA	85
MI-6e	6	NA	88	78	NA
MI-7e	6	NA	NA	NA	77
MI-8e	6	93	NA	76	NA
Adriamycin		0.21	0.15	0.17	0.13

Bold values indicate potent compounds

mixture below 5 °C. The completion of the reaction was monitored by TLC. After the completion, the reaction mixture was poured over crushed ice and extracted thrice with ethylacetate. The organic layer was pooled and concentrated under reduced pressure. The concentrated residue was adsorbed on silica gel (60–120 mesh), and the desired product was isolated by column chromatography employing increasing % age of ethylacetate in hexane.

Procedure for the synthesis of 3, 5 diaryl N-Acetyl pyrazoline (DNAP)

A mixture of diarylpropenone (1 mmol) in acetic acid and hydrazine hydrate (1 mmol) was refluxed for 2 h. The mixture was then poured onto crushed ice to get crude pyrazole, which was purified by crystallization from methanol to afford the desired product.

Procedure for the synthesis of O-propargylated DNAP

To the stirred suspension of K_2CO_3 (1.5 mmol) in dry DMF, the pyrazole (1 mmol) was added. To the reaction mixture, propargyl bromide (1.5 mmol) was added. The reaction was kept on stirring at 0 °C under anhydrous condition for 24 h. After completion of reaction, as evident by TLC, the reaction mixture was poured onto crushed ice. Precipitates were then filtered out. Propargylated pyrazole was obtained in good yield.

Procedure for the synthesis of Isatin azide

A mixture of 1-(bromo alkyl) indoline (1 mmol) in DMF and sodium azide (1 mmol) was stirred for 2 h at room temperature. After completion of reaction, as monitored by TLC, reaction mixture was extracted with ethyl acetate. Ethyl acetate layer was evaporated to get the desired product in good yield.

Procedure for the synthesis of DNAP-Isatin hybrids

To the stirred solution, *O*-propargylated DNAP (1 mmol) and Isatin azide (1.5 mmol) in DMF, copper sulphate (0.055 mmol) and sodium ascorbate (0.143 mmol) were added in succession, at room temperature. On completion of reaction, as monitored by TLC, the reaction mixture was poured onto crushed ice. Precipitates were then filtered out. Desired product was obtained in good yield.

The physical data of characteristic compounds are provided below.

*1-(5-(4-((1-(2-(2,3-dioxoindolyl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-4,5-dihydro-3-(4-methoxyphenyl)pyrazol-1-yl)ethanone (**MI-3a**)* Yield: 71 %. mp 86–88 °C; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 7.68 (2H, d, J = 7.8. Hz), 6.93 (2H, d, J = 7.8 Hz), 6.51–6.90 (4H,m) 6.95–7.64 (4H, m), 5.52 (1H, bs), 5.17 (2H, s), 4.67 (2H, bs), 4.23 (2H, bs), 3.85 (3H, s), 3.81(3H, s), 3.69 (1H, bs), 3.11 (1H, bs), 2.40 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 184.2, 168.4, 158.5, 153.4, 150.5, 148.6, 147.5, 147.5,146.4,146.4, 138.4, 137.9, 135.4, 128. 4, 125.4, 124.5, 123.5, 118.4, 114.5, 110.5, 61.6, 56.2, 46.6, 42.8, 40.7, 21.56. MS: m/z = 594.1524. Anal. Calcd. for $C_{32}H_{30}N_6O_6$: C, 64.64; H, 5.09; N, 14.13; O, 16.14, Found C, 64.78; H, 5.01; N, 14.07.

*1-(3-(4-((1-(2-(2,3-dioxoindolyl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-4,5-dihydro-5-(4-methoxyphenyl)pyrazol-1-yl)ethanone (**MI-4a**)* Yield: 71 %. mp 87–92 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.29 (1H, s), 7.68 (2H, d, J = 8.2 Hz), 7.48 (2H, m), 7.03–7.09 (6H, m), 6.83 (2H, d, J = 6.6 Hz), 5.48 (1H, bs), 5.11 (2H, s), 4.

71 (2H, bs), 4.19 (2H, bs), 3.76 (3H, s), 3.68 (1H, dd, bs), 3.11 (1H, bs), 2.31 (3H, s). ^{13}C NMR (DMSO-d_6, 75 MHz, δ, TMS = 0): 184.1, 168.2, 158.3, 153.4, 150.2, 148.4, 147.3, 147.4, 146.4, 146.3, 138.4, 137.7, 135.6, 128.2, 125.1, 124.3, 123.2, 118.2, 114.1, 110.3, 99.9, 61.6, 56.2, 46.6, 42.8, 40.7, 21.5. MS: *m/z* = 565.1810 (M^+ +. Anal. Calcd. for $C_{31}H_{28}N_6O_5$: C, 65.95; H, 5.00; N, 14.89; O, 14.17, Found C, 65.73; H, 5.19; N, 14.73.

*1-(3-(4-((1-(2-(2,3-dioxoindolyl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-4,5-dihydro-5-(3,4-dimethoxyphenyl)pyrazol-1-yl)ethanone (**MI-6a**)* Yield: 75 %. mp 86–90 °C ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.07 (1H, s), 7.68 (2H, d, *J* = 7.8 Hz), 7.48 (1H, m), 7.00–7.05 (4H, m), 6.68–6.85 (4H, m), 5.48 (1H, dd, *J* = 4.2 and 11.4 Hz), 5.12 (2H, s), 4.71 (2H, bs), 4.13 (2H, bs), 3.77 (3H, s), 3.76 (3H, s), 3.70 (1H, dd, *J* = 11.4 and 17.7 Hz), 3.10 (1H, dd, *J* = 4.2 and 17.7 Hz), 2.32 (3H, s). ^{13}C NMR (DMSO-d_6, 75 MHz, δ, TMS = 0): 188.0, 172.3, 164.7, 163.2, 158.9, 155.3, 154.0, 153.1, 143.2, 140.1, 133.3, 129.6, 129.1, 128.4, 122.4, 122.3, 120.0, 117.7, 115.4, 114.8, 66.3, 64.2, 60.7, 60.6, 52.2, 47.7, 44.4, 42.1, 26.9. MS: *m/z* = 594.1524. Anal. Calcd. for $C_{32}H_{30}N_6O_6$: C, 64.64; H, 5.09; N, 14.13; O, 16.14, Found C, 64.93; H, 5.01; N, 13.99.

*1-(5-(4-((1-(2-(2,3-dioxoindolyl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)3-methoxyphenyl)-4,5-dihydro-3-(naphthalen-4-yl)pyrazol-1-yl)ethanone (**MI-7a**)* Yield: 78 %. mp 83–86 °C; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, TMS = 0): 8.08 (1H, d, *J* = 8.7 Hz), 7.89–7.94 (5H, m), 7.51–7.61 (4H, m), 6.70 (4H, m), 6.50 (1H, d, *J* = 8.1 Hz), 5.57 (1H, dd, *J* = 4.2 and 11.4 Hz), 5.16 (2H, s), 4.66 (2H, bs), 4.21 (2H, bs), 3.90 (3H, s), 3.88 (1H, dd, *J* = 11.4 and 17.7 Hz), 3.10 (1H, dd, *J* = 4.2 and 17.7 Hz), 2.41 (3H, s). MS *m/z*: 616 (M^+).^{13}C NMR ($CDCl_3$, 75 MHz, δ, TMS = 0): 182.3, 168.4, 158.5, 154.0, 149.9, 149.7, 146.8, 138.7, 135.7, 134.1, 133.0, 128.9, 128.5, 128.4, 127.8, 127.3, 127.3, 126.8, 125.6, 124.2, 123. 2, 117.6, 117.3, 114.3, 109.7, 109.4, 99.9, 59.8, 55.9, 47.9, 42.3, 40.6, 22.0. MS: *m/z* = 615.2354 (M^+ + 1) Anal. Calcd. for $C_{35}H_{30}N_6O_5$ C, 68.39; H, 4.92; N, 13.67; O, 13.02, Found C, 68.69; H, 4.74; N, 13.87.

*1-(5-(4-((1-(2-(2,3-dioxoindolyl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)-4,5-dihydro-3-(3,4,5-trimethoxyphenyl)pyrazol-1-yl)ethanone (**MI-8a**)* Yield: 74 %. mp 90–92 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.90 (1H, s), 7.10–7.47 (4H, m), 6.99 (2H, s), 6.66–6.76 (3H,m), 5.53(1H,dd, *J* = 4.2 and 11.6 Hz), 5.02 (2H, s), 4.72 (2H, bs), 4.20 (2H, bs), 3.89 (6H, s,), 3.80 (6H, s), 3.69 (1H, dd, *J* = 11.4 and 17.7 Hz), 3.10 (1H, dd, *J* = 4.2 and 17.7 Hz), 2.38 (3H, s). ^{13}C NMR (DMSO-d_6, 75 MHz, δ, TMS = 0): 183.2, 169.1, 158.2, 153.3, 150.1, 148.4, 147.4, 147.2, 146.4,146.1, 138.3, 137.7, 135.2, 128.2, 125.3, 124.1, 123.1, 118.1, 114.3, 110.1, 61.2, 56.2, 54.4, 46.9, 42.2, 40.2, 21.2. Anal. Calcd. for $C_{34}H_{34}N_6O_8$: C, 62.38; H, 5.23; N, 12.84; O, 19.55, Found C, 62.73; H, 5.09; N, 12.64.

The characterization data of rest of the compounds has been provided as supplementary file.

Pharmacological evaluation

Hala **(Cervix cancer)**, CAKI-I (**Renal cancer**), PC-3 (**Prostate cancer**) and MiaPaca-2 **(Pancreatic Cancer)** cell lines were procured from the National Cancer Institute, Frederick, USA. The cells were grown in RPMI-1640 medium supplemented with 10 % heat inactivated foetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine(0.3 mg/ml), pyruvic acid, 0.37 % $NaHCO_3$ and 50 µM of 2-mercaptoethanol at 37 °C in an atmosphere of 95 % air and 5 % CO_2 with 90 % humidity. Cells were cultured in culture medium in a 75 cm^2 tissue culture flask (BD Falcon). Cell density was always kept under 1×10^6/ml to avoid mutations of the cells. Passaging of the cells was done four times a week. Experiments were performed with cells cultivated not longer than twenty passages. Further passaging of immortalized cell lines is possible but the risk of genotypic changes increases. Cultivation and experimental incubation were performed in a cell incubator at 37 °C, 90 % air humidity and 5 % CO_2. Cells grown in logarithmic phase were treated with synthesized compounds dissolved in DMSO, while the untreated control cultures received only the vehicle (DMSO, <0.5 % v/v). The growth inhibitory effect of different samples towards the Hala, CAKI-I, PC-3 and MiaPaca-2 cells was measured by means of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide) assay. The cleavage and conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial dehydrogenase of living cells have been used to develop an assay system for measurement of cell proliferation (Kumar *et al.,* 2013).

Conflict of interest The authors declare no conflicts of interest.

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