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Original article

Synthesis and biological evaluation of 3,5-diaryl isoxazoline/isoxazole linked 2, 3-dihydroquinazolinone hybrids as anticancer agents

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ABSTRACT

A series of new 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydro quinazolinone hybrids with different linker architectures have been designed and synthesized. These compounds have been evaluated for their anticancer activity. One of the compounds **4c** amongst this series has shown promising anticancer activity. Further some detailed biological assays relating to the cell cycle aspects and tubulin depolymerization activity have been examined with a view to understand the mechanism of action of this conjugate.

1. Introduction

Cancer is a serious disease with a complex pathogenesis, which threats human life greatly. A number of chemotherapeutic drugs have been developed to treat cancer that includes DNA-alkylating agents and antimitotic agents. Many natural products such as taxol and vinca alkaloids are known for the effective antimitotic drugs. However, the complex synthesis, difficult formulation, lack of oral availability makes these drugs suboptimum for clinical treatment of cancer [1]. Hence, there is considerable interest in the design and development of novel molecules that inhibit tubulin polymerization. The design of hybrid drugs is one of the approaches to improve the efficiency of drugs.

Quinazolinone is a naturally occurring alkaloid and found in a variety of bioactive natural products and possess wide range of biological activities [2–7]. Among the various classes of quinazolinones, 2,3-dihydroquinazolinones form an important component of pharmacologically active compounds [8–11] as they are associated with inhibitory effects on tubulin polymerization and the anticancer activities of 2,3-dihydro-2-aryl-4-quinazolinones (DHPQZ) are well established [12,13].

Combretastatin A-4 (CA-4, Fig. 1), isolated from the bark of the South African tree *Combretum caffrum*, [14] has shown potent cytotoxicity against a variety of human cancer cell lines including multiple drug-resistant cancer cell lines [15]. A number of *cis*-restricted analogues of CA-4 have been prepared using five-membered heterocycles [16–18]. In a recent study, *cis*-restricted analogues of CA-4 (diaryl substituted isoxazoline/isoxazole derivatives) are reported to possess antitubulin activity [19,20].

In continuation to our efforts on the design of new anticancer agents, [21–24] we became interested in the development of some new hybrid molecules that comprises of two phamacophores in a single molecule with an intention to enhance the efficacy and bring synergy in hybrids. In this context, 3,5-diaryl isoxazoline/isoxazoles have been linked to quinazolinones through different alkane spacers and these new classes of hybrids have been evaluated for their anticancer activity.

2. Chemistry

The synthesis of 3,5-diaryl isoxazoline and isoxazole derivatives (**10a**,**b** and **15a**,**b**) [19,20] was carried out from aldehydes **6a**,**b** and **11** as the starting materials. Reaction of these aldehydes with

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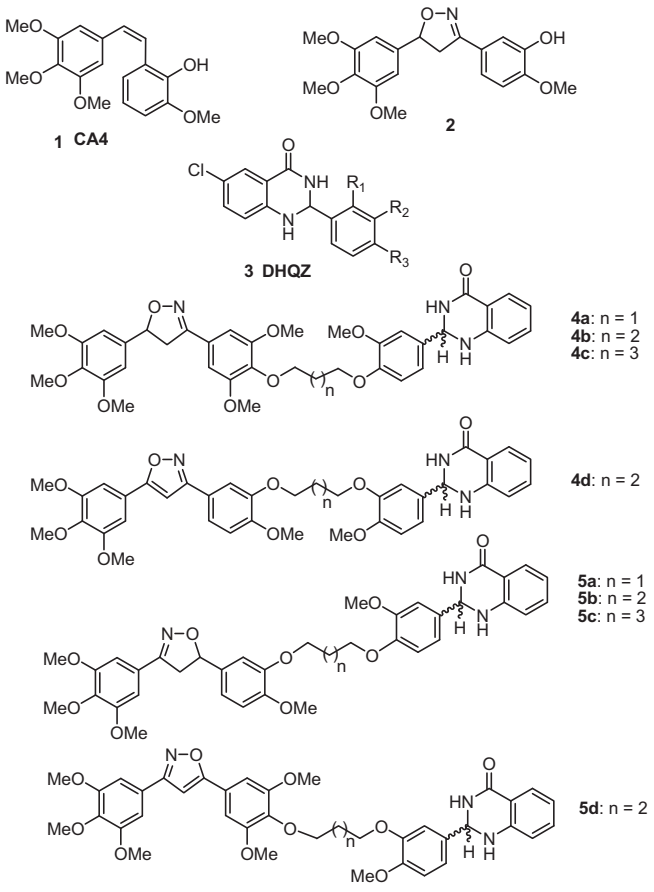


Fig. 1. Chemical structures of combretastatin (CA-4, **1**), isoxazoline (**2**), 2,3-dihydro quinazolinone (DHQZ, **3**), 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids (**4a**–**d** and **5a**–**d**).

hydroxylamine in a MeOH/H_2O (3:1) solution produced the corresponding oximes **7a**,**b** and **12** in high yields. Olefins **8a** and **13a** were obtained by the reaction of aldehydes **11** and **6b** respectively with methyltriphenyl phosphonium bromide, in the presence of sodium hydride. These olefins **8a** and **13a** were then coupled to oximes (**7a** and **12**) to provide the corresponding *tertiary* butyldimethyl silyl (TBDMS) protected isoxazolines (**9a** and **14a**). Similarly, TBDMS protected isoxazoles (**9b** and **14b**) were prepared by employing alkynes (**8b** and **13b**) and oximes (**7b** and **12**). Further, these compounds (**9a**,**b** and **14a**,**b**) upon deprotection with tetrabutylammonium fluoride gave the desired precursors (**10a**,**b** and **15a**,**b**) as shown in Scheme 1.

The synthesis of 2,3-dihydroquinazolinones is outlined in Scheme 2. Compounds **16a**,**b** were treated with benzamide in presence of *N*,*N*-dimethylacetamide (DMAC) to give compounds **17a**,**b** respectively. These upon etherification with dibromoalkanes using K_2CO_3 in DMF provided the 2,3-dihydroquinazolinone precursors (**18a**–**d**). The synthesis of hybrid compounds **4a**–**d** and **5a**–**d** was carried out from the compounds **18a**–**d** and 3,5-diaryl isoxazoline/isoxazole precursors (**10a**,**b** and **15a**,**b**) using K_2CO_3 in DMF as shown in Scheme 3.

3. Results and discussion

3.1. Evaluation of biological activity

3.1.1. In vitro cytotoxic activity

Among all the synthesized hybrid compounds, compound **4c** was evaluated in the anticancer screen for human disease-oriented tumor cell line developed at the National Cancer Institute (NCI), Bethesda. Initially compound **4c** was evaluated for a pre-test also called as one dose test consisting of sixty tumor cell lines. Compound **4c** reduced the growth of all sixty cell lines by 32% or more and subsequently passed on for evaluation in the main test, consisting of approximately 60 cell lines over a 5-log dose range (10^{-4}–10^{-8} M).

Within the main test the anticancer activity of a test compound is expressed by three different dose–response parameters for each of the 60 cell lines derived from nine different types of cancer, namely, leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast: GI_{50} (molar concentration required for half growth inhibition), TGI (molar concentration leading to total growth inhibition), and LC_{50} (molar concentration required for 50% cell death). Additionally, a mean graph midpoint (MG-MID) is calculated for each of the above-mentioned parameters, which displays an averaged activity parameter over all cell lines, as well as the Delta parameter that is the difference between the highest and the average values.

Table 1 represents a general overview on these main parameters that characterize cytostatic activity of the tested compound. Compound **4c** exhibited significant activity against RPMI-8226 (leukemia) and SK-MEL-5 (melanoma) cell lines with log GI_{50} values of −7.07 and −6.95, log TGI values of −5.64 and −6.49 and log LC_{50} values of > −4.00 and −6.03 respectively. Moreover, compound **4c** demonstrated significant activity with log GI_{50} values in the range of −4.22 to −7.07 against 51 cell lines out of 56 cell lines tested with it and the mean log GI_{50} value of this compound is −5.31. The promising anticancer activity exhibited by compound **4c** prompted us to evaluate the anticancer activity of the other analogues (**4a**, **4b**, **4d** and **5a**–**d**) in selected human cancer cell lines by using Sulforhodamine B (SRB) method. The compounds exhibiting $GI_{50} \leq 10^{-5}$ μM are considered to be active on the respective cell lines. Table 2 reveals that most of the compounds exhibit significant anticancer activity against MCF-7 and PC3 cell lines. The positive control compound adriamycin [25] demonstrated significant activity with the GI_{50} in the range from 0.13 to 0.19 μM. Compounds **5a**–**d** showed promising anticancer activity against A549 cell line with GI_{50} values in the range of 0.15–0.18 μM.

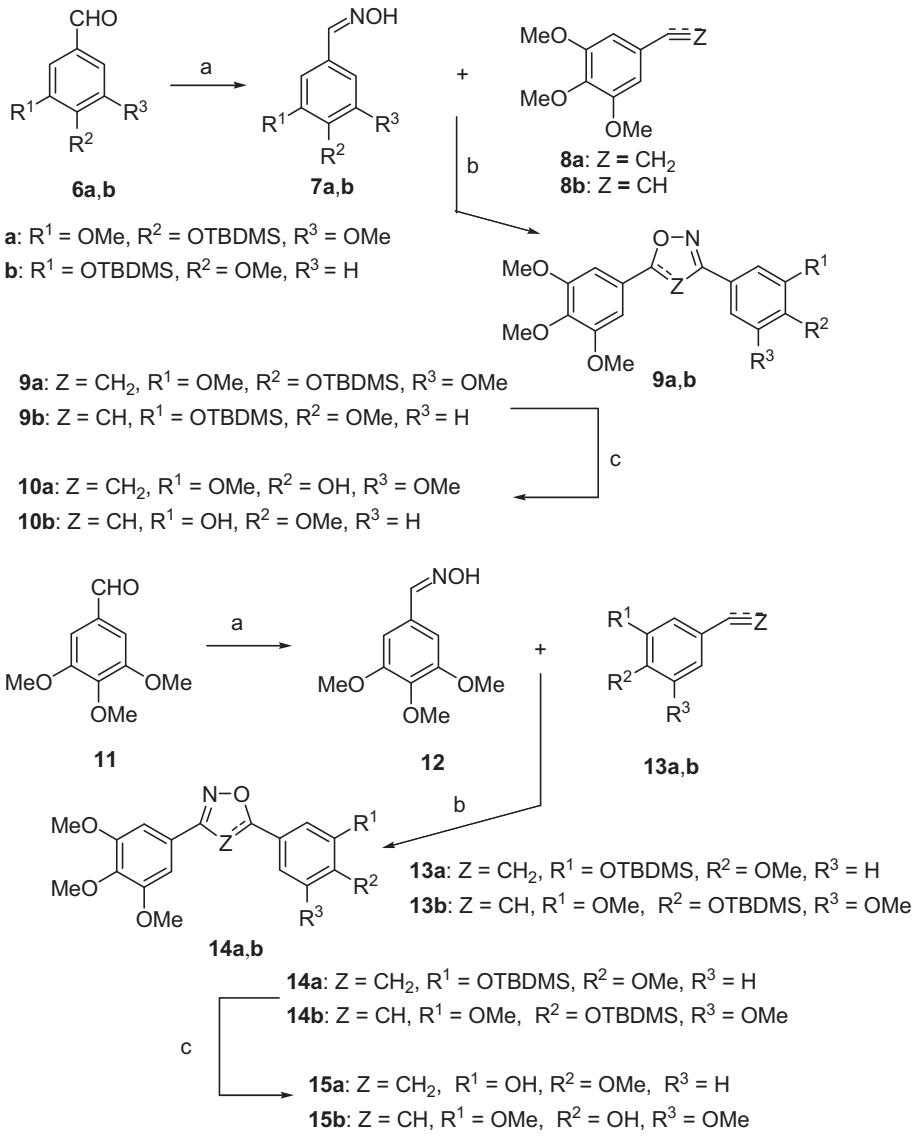
Further, MTT assay was also carried out for the most potent hybrid compound **4c**, along with the left side conjugate partner (**10a**) as well as the right side conjugate partner (**18c**) to verify that whether this hybrid compound shows better cytotoxicity than the conjugate partners. Interestingly, the hybrid compound **4c** showed enhanced cytotoxicity at 5 μM in comparison to the conjugate partners in MCF-7 cell lines. The results are shown in Fig. 2 and CA-4 was used as the positive control.

3.1.2. Effect of hybrid compound *4c* on cell cycle effects of MCF-7 cell line

The molecules exhibiting the effect on tubulin assembly could cause alteration of cell cycle parameters with preferential G2/M blockade. Hence, to investigate the mechanism underlying the antiproliferative effect of the most effective compound **4c**, the cell cycle distribution of MCF-7 cells was analyzed by flow cytometry. MCF-7 cells were treated with compounds **1**, **4c**, **10a** and **18c** at 5 μM concentration and the majority of control cells treated with DMSO showed 18.27% of cells in G2/M phase. Here the positive control CA-4 (**1**) showed 65.50% of cells in G2/M phase. Compound **4c** treated cells showed 43.90% of cells in G2/M phase. The left conjugate partner (**10a**) showed 34.36% of cells in G2/M phase, where as the right side conjugate partner (**18c**) had no effect and the results are shown in Fig. 3a,b.

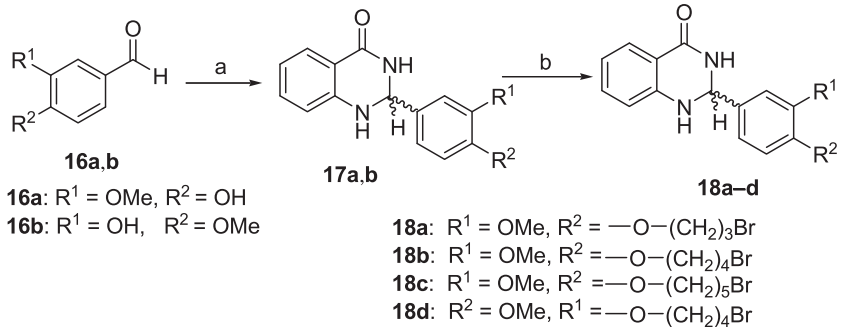
3.1.3. Effect of hybrid compound *4c* on the inhibition of tubulin polymerization activity

Generally, the inhibition of tubulin polymerization is associated with an arrest in cell cycle progression at the G2-M phase transition

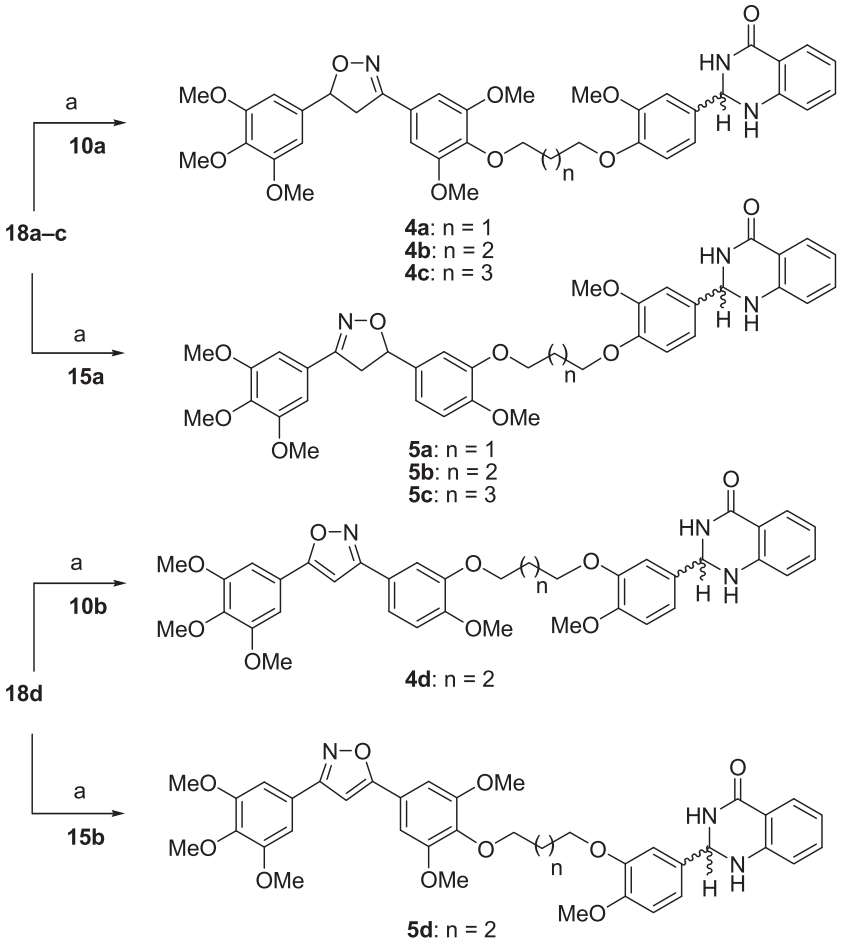


Scheme 1. Synthesis of 3,5-diaryl isoxazoline/isoxazole analogues (**10a**,**b** and **15a**,**b**). *Reagents and conditions*: a) $NH_2OH.HCl$, $NaHCO_3$, CH_3OH:H_2O (3:1), 0 °C, then rt, 6 h; b) 13% aq NaOCl, Et_3N, CH_2Cl_2, 0 °C, then rt, 24 h; c) TBAF, THF, rt, 2 h.

by interrupting mitotic spindle formation and chromosome segregation [26]. Further, from the previous studies it is known that both the conjugate partners have been reported to possess moderate inhibition of tubulin polymerization activity. Hence it was considered of interest to understand the underlying mechanism of anticancer activity of compound **4c** in relation to an interaction with microtubule system. Therefore, compound **4c** was evaluated for inhibitory effects on tubulin polymerization at 5 μM by taking nocodazole as the standard and DMSO as the control on the tubulin purified from MCF-7 cancerous cell lines. It is observed that compound **4c** inhibited the polymerization of tubulin (data not shown). With a view to further substantiate the observed effects of this hybrid (**4c**) on the inhibition



Scheme 2. Synthesis of 2,3-dihydroquinazolinone precursors (**18a**–**d**). *Reagents and conditions*: a) Benzamide, *p*-toulene sulfonicacid monohydrate, *N*,*N*-dimethylacetamide, rt, 2 h; b) $Br(CH_2)_nBr$, K_2CO_3, DMF, rt, 24 h.



Scheme 3. Synthesis of 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids (**4a–d** and **5a–d**). *Reagents and conditions*: a) K_2CO_3, DMF, rt, 24 h.

of tubulin polymerization, immunofluorescence and microscopy studies using anti-α-tubulin antibodies were carried out. In this study, untreated human breast cancer cells (MCF-7) showed the normal distribution of microtubules. However, cells treated with hybrid compound **4c** and CA-4 (**1**) showed the disrupted microtubule organization at 5 μM concentration, where as the standard compound nocodazole showed the disrupted microtubule organization even at 2.5 μM concentration as illustrated in Fig. 4.

Since several of the microtubule distributing agents are also known to induce apoptosis in cells, the cellular effects of this hybrid (**4c**) on the nuclear fragmentation were analyzed by DAPI staining. The results indicate that cells treated with **4c** as well as CA-4 (**1**) showed significant effect on nuclear fragmentation at a concentration of 5 μM as shown in Fig. 4. Nocodazole was used as the positive control that showed significant nuclear fragmentation even at a concentration of 2.5 μM.

3.1.4. *Effect of hybrid compound **4c** on cyclins and CDK1*

It is well known from the previous studies that the cell cycle progression is regulated by the expression of some cell cycle specific cyclins. Hence, to understand the mechanism underlying the G2/M cell cycle arrest in these compounds, we examined the effects of the hybrid compound (**4c**) on the expression of cyclin A and cyclin B1, which control cell cycle progression through the S and G2/M phases. MCF-7 cells were treated with hybrid **4c** as well as CA-4 (**1**) at 5 μM concentration for 24 h and Western blot analysis was carried out. Treatment with hybrid compound **4c** and CA-4 (**1**) resulted a decrease in cyclin A expression when compared to control.

From the previous studies it is known that cyclin B1 is over expressed in breast and cervical cancer cells [27]. Down regulation of cyclin B1 inhibited the proliferation of MCF-7 cells and is an effective and attractive as well as promising strategy for controlling breast cancer cell proliferation. Interestingly, a drastic reduction in the cyclin B1 expression was observed in case of compound **4c** as shown in Fig. 5.

It is well known that CDK1 is essential for cell cycle regulation and a major force driving cell proliferation. Cyclin B1, the regulatory subunit of CDK1, controls the activity of CDK1 as it associates with and thereby activates CDK1. Further, Among the CDKs that regulate cell cycle progression, CDK1and CDK2 kinases are activated primarily in association with cyclin A and B1 in the G2/M phase progression [28]. Hence, it is considered of interest to study the levels of CDK1 by treating MCF-7 cells with compound **4c** as well as CA-4 (**1**). As we observed a down regulation of cyclin B1, it is expected that there might be down regulation of CDK1. As expected, the expression of CDK1 level was reduced in comparison to control as shown in Fig. 5.

3.1.5. *Effect of hybrid compound **4c** on PARP*

Further, it is considered of interest to check some apoptotic proteins like PARP. The cleavage of PARP, a eukaryotic DNA-binding protein that specifically recognizes the double stranded breaks [29]. MCF-7 cells were treated with compounds **1** and **4c** at a concentration of 5 μM for 24 h and the level of cleaved PARP was observed. The results were shown in Fig. 6 and the expression level of cleaved PARP was increased in all cases, when compared to

Table 1
In vitro cytotoxicity of the compound **4c** on selected human cancer cell lines[a].

Cancer panel or cell line	Compound **4c**		
	log GI_{50}[b]	log TGI[c]	log LC_{50}[d]
Leukemia			
CCRF-CEM	−6.20	> −4.00	> −4.00
HL-60 (TB)	−4.95	> −4.00	> −4.00
K-562	−6.30	> −4.00	> −4.00
MOLT-4	−6.32	> −4.00	> −4.00
RPMI-8226	−7.07	−5.64	> −4.00
SR	−6.02	> −4.00	> −4.00
Non-small cell lung cancer			
A549/ATCC	−6.49	> −4.00	> −4.00
EKVX		> −4.00	> −4.00
HOP-62	−4.72	−4.09	> −4.00
HOP-92	−4.22	> −4.00	> −4.00
NCI-H226	−5.47	> −4.00	> −4.00
NCI-H23	−6.22	> −4.00	> −4.00
NCI-H322M	> −4.00	> −4.00	> −4.00
NCI-H460	−4.87	−4.22	> −4.00
NCI-H522	−4.97	−4.50	−4.03
Colon cancer			
COLO-205	−4.46	> −4.00	> −4.00
HCC-2998	−4.72	−4.28	> −4.00
HCT-116	−6.15	−4.39	> −4.00
HCT-15	−6.15	> −4.00	> −4.00
KM12	−5.26	> −4.00	> −4.00
SW-620	−4.99	> −4.00	> −4.00
Prostate cancer			
PC3	−4.54	> −4.00	> −4.00
DU-145	−4.65	> −4.00	> −4.00
CNS cancer			
SF-268	−4.67	> −4.00	> −4.00
SF-295	−6.40	> −4.00	> −4.00
SF-539	−4.75	−4.05	> −4.00
SNB-19	−4.78	> −4.00	> −4.00
SNB-75	−4.95	> −4.00	> −4.00
U251	−4.82	> −4.00	> −4.00
Melanoma			
LOX IMVI	−4.85	> −4.00	> −4.00
MALME-3M		> −4.00	> −4.00
M14	−4.68	> −4.00	> −4.00
MDA-MB-435	−6.38	> −4.00	> −4.00
SK-MEL-2	−4.45	> −4.00	> −4.00
SK-MEL-28	−5.28	> −4.00	> −4.00
SK-MEL-5	−6.95	−6.49	−6.03
UACC-62	−6.59	> −4.00	> −4.00
Ovarian cancer			
IGROV-1	−5.95	−4.30	> −4.00
OVCAR-3	−4.89	−4.20	> −4.00
OVCAR-4	−6.45	> −4.00	> −4.00
OVCAR-5	−4.44	> −4.00	> −4.00
OVCAR-8	−4.61	> −4.00	> −4.00
NCI-ADR/RES	−4.82	> −4.00	> −4.00
SK-OV-3	−4.60	> −4.00	> −4.00
Renal cancer			
786−0	−4.63	−4.21	> −4.00
A498	−4.71	> −4.00	> −4.00
ACHN	−6.49	> −4.00	> −4.00
CAKI-1	> −4.00	> −4.00	> −4.00
SN12C	−4.60	> −4.00	> −4.00
TK-10	> −4.00	> −4.00	> −4.00
UO-31	−4.97	> −4.00	> −4.00
Breast cancer			
MCF-7	−6.55	> −4.00	> −4.00
MDA-MB-231/ATCC	−4.83	> −4.00	> −4.00
HS 578T	−4.78	> −4.00	> −4.00
BT-549	−6.30	> −4.00	> −4.00
T-47D	−6.72	> −4.00	> −4.00
MG_MID[e]	−5.31	−4.11	−4.04
Delta[f]	1.76	2.38	1.99

[a] Data obtained from NCI in vitro anticancer activity cells screen.
[b] The log of the molar concentration that inhibits 50% net cell growth.
[c] The log of the molar concentration leading to total growth inhibition.
[d] The log of the molar concentration leading to 50% net cell death.

control cells, revealing the possible occurrence of DNA damage and apoptosis.

4. Conclusion

In conclusion, in the present study a series of new 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinones were synthesized and evaluated for their anticancer activity. Among the hybrid compounds prepared, compound **4c** exhibited significant anticancer activity against 18 human cancer cell lines with GI_{50} values less than 1 μM. The other compounds also exhibited potent anticancer activity against MCF-7 and PC3 cancer cell lines. Further, MTT assay revealed that compound **4c** is more effective than the conjugate partners. Flow cytometric data of these compounds showed increased cells in G2/M phase, which is suggestive of G2/M cell cycle arrest. Further, compound **4c** showed the disruption of microtubules as well as fragmentation of nuclei. Interestingly, the compound **4c** was also identified as an effective cyclin B1 inhibitor as well as CDK1 inhibitor and the data obtained suggests that these hybrid molecules could be considered as novel candidates for their further development as potential anticancer agents.

5. Experimental protocols

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded on Gemini Varian-VXR-unity (200 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI^+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Melting points were determined with an electro thermal melting point apparatus, and are uncorrected.

5.1. Synthesis of 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydro quinazolinone conjugates

5.1.1. Synthesis of 4-(tert-butyldimethylsilyloxy)-3,5-dimethoxy-benzaldehyde oxime (**7a**)

To a solution of hydroxylamine hydrochloride (12 mmol) dissolved in water (7 mL), $NaHCO_3$ (18 mmol) was added portion wise at 0 °C, and the mixture was stirred for 30 min at room temperature. The compound **6a** (2.96 g, 10 mmol), dissolved in methanol (5 mL), was then added to the solution, and stirring was continued for an additional 6 h. Methanol was evaporated in vacuo, and the residue extracted with diethyl ether. The organic extracts were washed with brine, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate/light petroleum (10%) as eluent and the compound **7a** was obtained as oil (1.8 g, 60%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.98 (s, 1H, Ar**H**), 6.74

[e] MG_MID is mean graph midpoint – arithmetical mean value for all tested cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.
[f] Delta – Difference in log GI_{50} value of the most sensitive cell lines and the MG_MID value. Delta is considered low if <1, moderate >1 and <3, high if >3.

Table 2
GI_{50} values[a] (in μM) for compounds **4a**,**b**, **4d**, **5a**–**d** in selected human cancer cell lines.

Compound	A549[b]	A2780[c]	PC3[d]	MCF-7[e]	KB[f]
4a	20	>100	0.52	0.28	22
4b	14	>100	0.46	0.30	21
4d	>100	>100	2.1	0.15	>100
5a	0.17	26	2.5	0.17	28
5b	0.15	22	2.8	0.18	30
5c	0.16	>100	2.4	0.14	27
5d	0.18	2.0	2.0	25	26
ADR[g]	0.13	0.18	0.16	0.15	0.19

[a] 50% growth inhibition and the values are mean of three determinations.
[b] Lung cancer.
[c] Ovarian cancer.
[d] Prostate cancer.
[e] Breast cancer.
[f] Oral cancer.
[g] Adriamycin.

(s, 2H, Ar**H**), 3.83 (s, 6H, 2×–OC**H**$_3$), 0.99 (s, 9H, –C(C**H**$_3$)$_3$), 0.12 (s, 6H, –Si(C**H**$_3$)$_3$); MS (ESI): *m*/*z* 312 (M+1)$^+$.

*5.1.2. 3-(tert-butyldimethylsilanyloxy)-4-methoxy-benzaldehyde oxime (**7b**)*

This compound was prepared according to the method described for compound **7a**, employing compound **6b** (2.66 g, 10 mmol). The crude product was chromatographed on silica gel using ethyl acetate/light petroleum (10%) as eluent and the compound **7b** was obtained as oil (1.3 g, 47%). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 7.15–7.11 (m, 2H, Ar**H**), 7.08 (s, 1H, Ar**H**), 6.84 (d, 1H, *J* = 8.3 Hz, Ar**H**), 3.84 (s, 3H, 1×–OC**H**$_3$), 1.00 (s, 9H, –C(C**H**$_3$)$_3$), 0.17 (s, 6H, –Si(C**H**$_3$)$_3$); MS (ESI): *m*/*z* 282 (M + H)$^+$.

*5.1.3. 3,4,5,-Trimethoxy-benzaldehyde oxime (**12**)*

This compound was prepared according to the method described for compound **7a**, employing compound **11** (1.96 g, 10 mmol). The crude product was chromatographed on silica gel using ethyl acetate/light petroleum (25%) as eluent and the compound **12** was obtained as pale yellow solid (1.17 g, 56%). The spectral data is in agreement with those described by Simoni et al [19].

*5.1.4. Synthesis of 1,2,3-trimethoxy-5-vinyl-benzene (**8a**)*

NaH (55%) (20 mmol), previously washed with dry hexane, was added to a stirred suspension of methyltriphenylphosphonium-bromide (10 mmol) in dry THF (15 mL) containing the appropriate aldehyde **11** (1.96 g, 10 mmol). After the suspension was stirred for 5 h at room temperature, diethyl ether (30 mL) was added, and the mixture was poured into ice-water and extracted with Et_2O. The combined organic extracts were dried and evaporated, and the residue was chromatographed on silica gel using ethyl acetate/light petroleum (10%) as eluent to obtain the product **10a** as an oil (1.59 g, 82%). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 6.59 (dd, 1H, *J* = 17.3, 11.3 Hz, olefin–**H**), 6.57 (s, 2H, Ar**H**), 5.60 (d, 1H, *J* = 17.3 Hz, olefin–**H**), 5.16 (d, 1H, *J* = 11.3 Hz, olefin–**H**), 3.86 (s, 6H, 2×–OC**H**$_3$), 3.81 (s, 3H, 1×–OC**H**$_3$); MS (ESI): *m*/*z* 195 (M + H)$^+$.

*5.1.5. tert-Butyl-(2-methoxy-5-vinyl-phenoxy)-dimethyl-silane (**13a**)*

This compound was prepared according to the method described for compound **10a**, employing compound **8a** (2.66 g, 10 mmol). The crude product was chromatographed on silica gel using ethyl acetate/light petroleum (10%) as eluent and the compound **15a** was obtained as oil (1.84 g, 70%). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 6.85 (d, 2H, *J* = 6.5 Hz, Ar**H**), 6.75 (d, 1H, *J* = 8.6 Hz, Ar**H**), 6.59 (dd, 1H, *J* = 17.6,10.5 Hz, olefin–**H**), 5.55 (d, 1H, *J* = 17.6 Hz, olefin–**H**), 5.16 (d, 1H, *J* = 10.5 Hz, olefin–**H**), 3.80 (s, 3H, 1×–OC**H**$_3$), 1.00 (s, 9H, –C(C**H**$_3$)$_3$), 0.16 (s, 6H, –Si(C**H**$_3$)$_3$); MS (ESI): *m*/*z* 265 (M + H)$^+$.

*5.1.6. Synthesis of 3-(4-(tert-butyldimethylsilyloxy)-3,5-dimethoxy phenyl)- 5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (**9a**)*

To dipolarophile **8a** (1.94 g, 10 mmol) and triethylamine (0.1 equiv) in dichloromethane were added, under argon atmosphere, a 13% aqueous solution of NaOCl (1.6 equiv) and dropwise (over a period of 1 h) at 0 °C, the appropriate oxime **7a**, (3.11 mg, 10 mmol) in dichloromethane. After being stirred at room temperature for 24 h water was added to the reaction mixture and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was chromatographed using ethyl acetate/lightpetrolium (20%) as eluent to obtain isoxazole **9a** as yellow oil (258 mg, 53% yield). Data are in agreement with those described by Simoni et al [19].

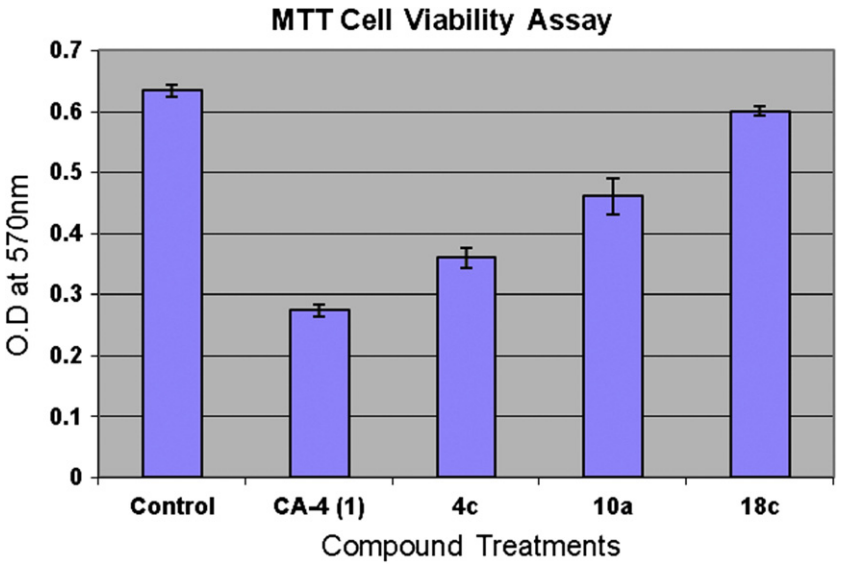


Fig. 2. The cell viability observed after the treatment with compounds at 5 μM concentration by MTT assay. MCF-7 cells have been treated with various compounds (**1**, **4c**, **10a** and **18c**) as indicated for 24 h in 96 well plates at 10,000 cells per well. Compounds **10a** and **18c** are the conjugate partners for **4c**. Control indicates the untreated cells.

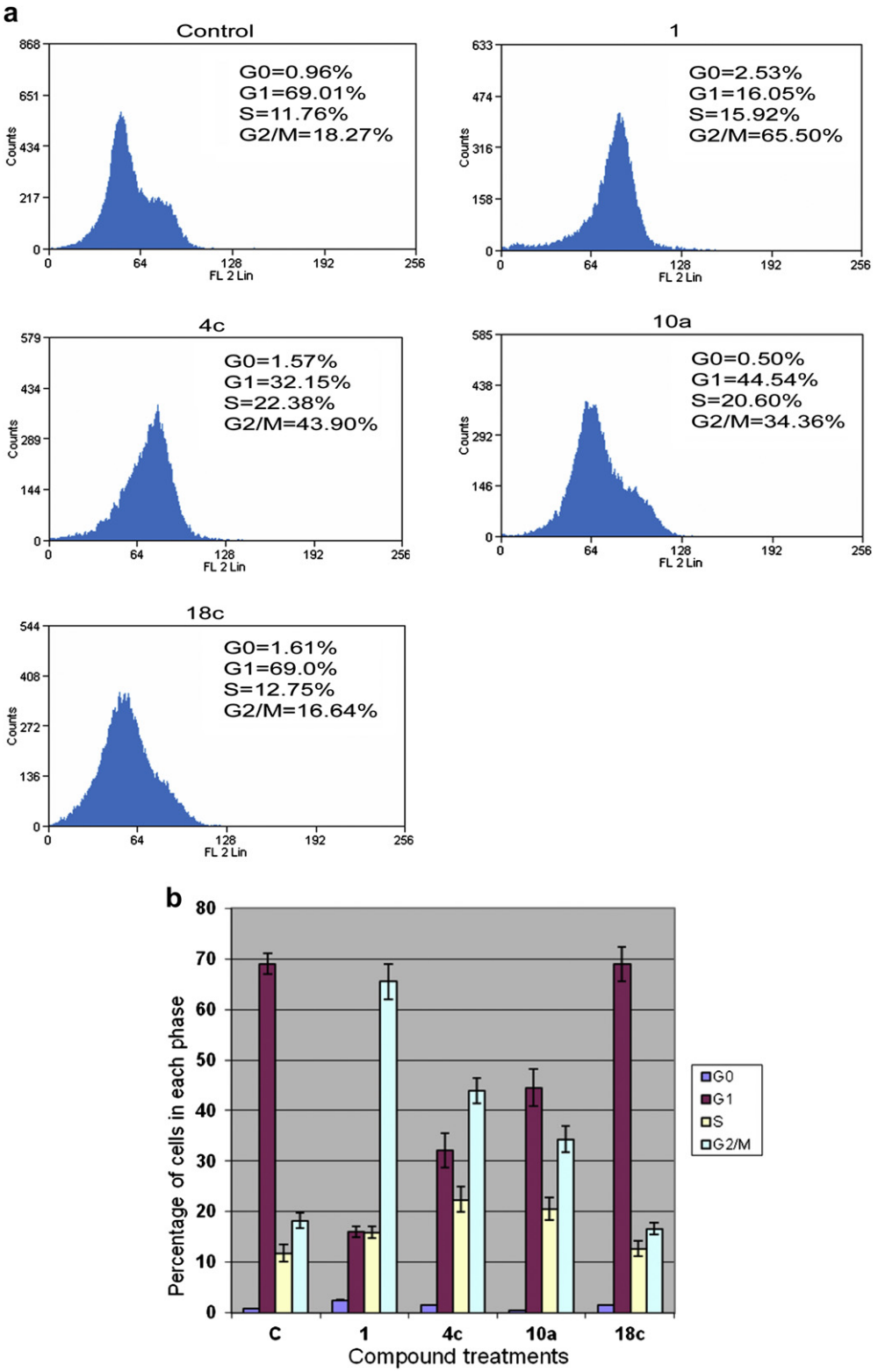


Fig. 3. (a) FACS analysis of cell cycle distribution of MCF-7 cells after treatment with compounds **1** (CA-4), **4c, 10a** and **18c** at 5 μM concentration for 24 h and control indicates the untreated cells. (b) Percentages of cells in G0, G1, S, and G2/M cell cycle phase, after the treatment of MCF-7 cells with compounds **1** (CA-4), **4c**, **10a** and **18c**.

*5.1.7. 3-[3-(tert-Butyldimethylsilanyloxy)-4-methoxyphenyl]-5-(3,4,5-trimethoxyphenyl)-isoxazole (**9b**)*

From alkyne **8b** (1.92 g, 10 mmol) and oxime **7b** (2.81 g, 10 mmol), isoxazole **9b** was obtained as a yellow oil (1.8 g, 40%) after a flash chromatography using ethyl acetate/light petroleum (10%) as eluent. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38 (d, 1H, $J = 2.1$ Hz, Ar**H**), 7.32 (dd, 1H, $J = 2.1$, 6.5 Hz, ArH), 7.04 (s, 2H, ArH), 6.88 (d, 1H, $J = 8.7$ Hz, Ar**H**), 6.61 (s, 1H, Ar**H**), 3.96 (s, 6H, 2×−OC**H**$_3$), 3.85 (s, 6H, 2×−OC**H**$_3$), 1.01 (s, 9H, −C(C**H**$_3$)$_3$), 0.18 (s, 6H, −Si(C**H**$_3$)$_3$); MS (ESI): m/z 472 $(M + H)^+$.

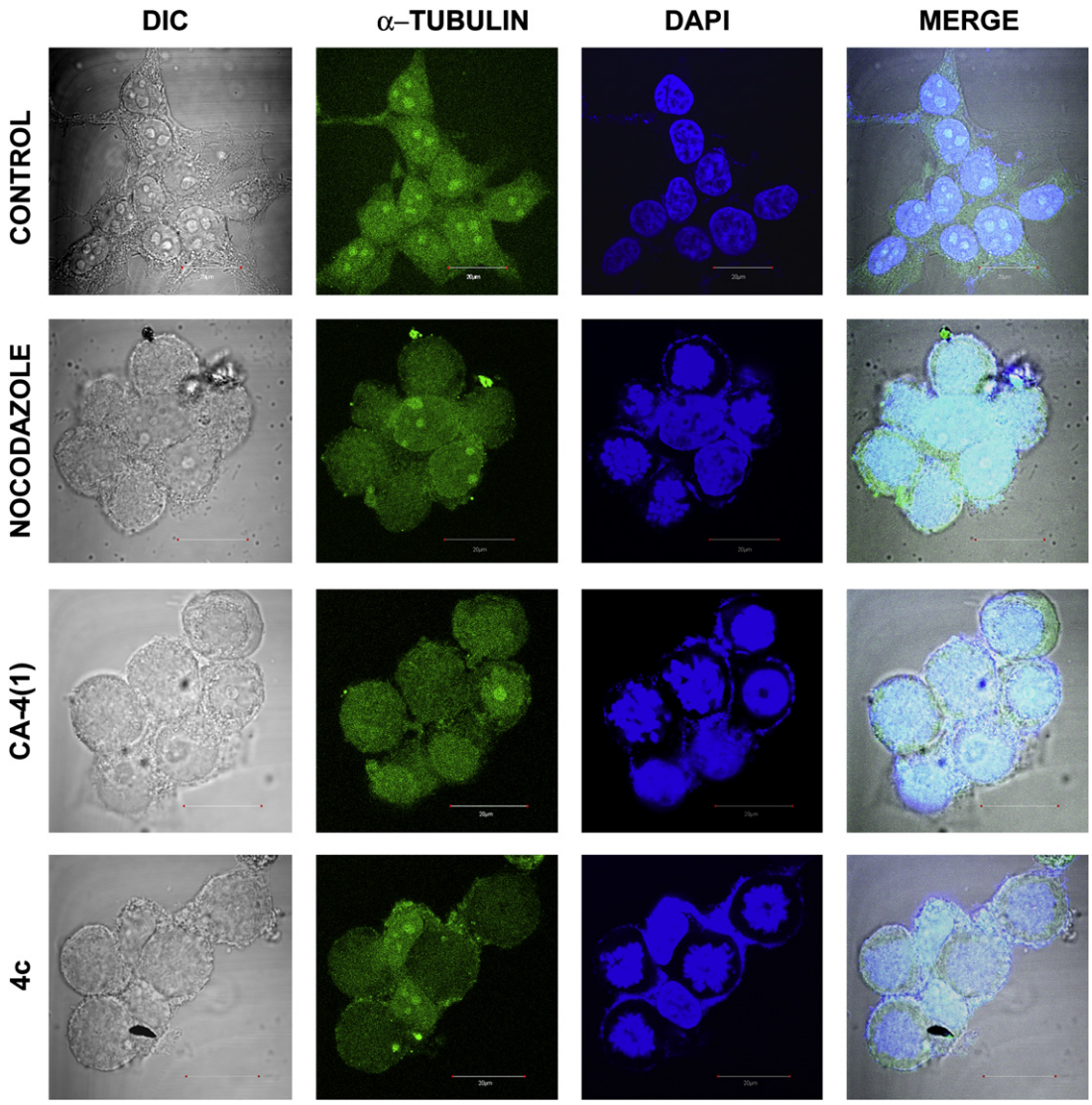


Fig. 4. Effect of compounds on the microtubule network of MCF-7 cells untreated (control), nocodazole (2.5 μM), CA-4 (1) (5 μM) and cells treated with compound **4c** (5 μM) for 48 h. Microtubules and unassembled tubulin are shown in green. DNA, stained with 4,6-diamidino-2-phenylindole (DAPI), is shown in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

*5.1.8. 5-[3-(tert-Butyldimethylsilanyloxy)-4-methoxyphenyl]-3-(3,4,5- trimethoxyphenyl)-4,5-dihydroisoxazole (**14a**)*

From alkene **13a** (2.64 g, 10 mmol) and oxime **12** (2.11 g, 10 mmol), isoxazole **14a** was obtained as a yellow oil (3.01 g, 60%) after a flash chromatography using ethyl acetate/light petroleum (40%) as eluent. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.81–6.97 (m, 5H, Ar**H**), 5.63 (dd, 1H, J = 10.7, 8.6 Hz, isoxazoline–**H**), 3.87 (s, 9H, 3×–OC**H**$_3$), 3.80 (s, 3H, 1×–OC**H**$_3$), 3.69 (dd, 1H, J = 16.4,10.7 Hz, isoxazoline–**H**), 3.28 (dd, 1H, J = 16.4, 8.7 Hz, isoxazoline–**H**), 1.00 (s, 9H, –C(C**H**$_3$)$_3$), 0.15 (s, 6H, –Si(C**H**$_3$)$_3$); MS (ESI): m/z 474 $(M + H)^+$.

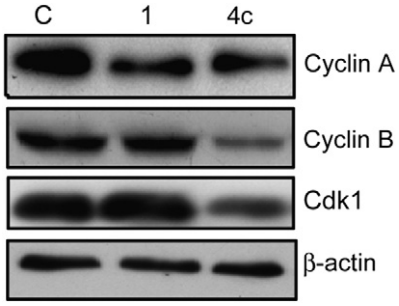


Fig. 5. Effect on Cyclin A, Cyclin B and CDK1 levels. MCF-7 cells were treated with 5 μM concentration of compounds (**1** and **4c**) for 24 h. The cell lysates have been collected and observed for Cyclin A and Cyclin B protein levels using specific antibodies. β-actin was used as loading control. C: control (untreated).

*5.1.9. 5-(4-(tert-Butyldimethylsilyloxy)-3,5-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-isoxazole (**14b**)*

From alkyne **13b** (2.94 g, 10 mmol) and oxime **12** (2.11 g, 10 mmol), isoxazole **14b** was obtained as a yellow oil (3.0 g, 60%) after a flash chromatography using ethyl acetate/light petroleum (10%) as eluent. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.01 (s, 4H, Ar**H**), 6.63 (s, 1H, Ar**H**), 3.90 (s, 6H, 2×–OC**H**$_3$), 3.88 (s, 6H, 2×–OC**H**$_3$), 3.84 (s, 3H, 1×–OC**H**$_3$), 1.02 (s, 9H, –C(C**H**$_3$)$_3$), 0.15 (s, 6H, –Si(C**H**$_3$)$_3$); MS (ESI): m/z 502 $(M + H)^+$.

*5.1.10. Synthesis of 2,6-dimethoxy-4-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-3-yl) phenol (**10a**)*

A solution of the silyl ether **9a**, (503 mg, 1 mmol) in dry THF (1 equiv) was treated with 1 M solution of tetrabutylammonium fluoride in THF (1 equiv). The mixture was stirred at room temperature

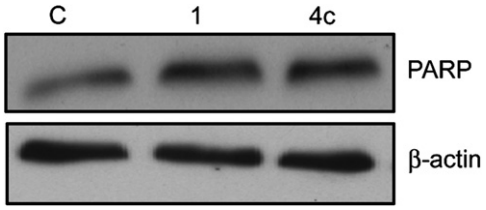


Fig. 6. Effect of compounds on the expression level of apoptotic specific cleaved PARP. MCF-7 cells were treated with 5 μM concentration of compounds (**1** and **4c**) for 24 h. The cell lysates were collected and observed for cleaved PARP using specific antibody. β-actin was used as loading control. C: control (untreated).

for 2 h, then diluted with water and extracted with EtOAc. The organic layers were washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was chromatographed using ethyl acetate/light petroleum (50%) as eluent to obtain the product **10a** as a white solid (360 mg, 90%). Data are in agreement with those described by Simoni et al [18].

*5.1.11. 2-Methoxy-5-(3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl) phenol (**10b**)*

This compound was prepared according to the method described for compound **10a**, employing compound **9b** (471.6 mg, 1 mmol). The crude product was chromatographed using ethyl acetate/light petroleum (20%) as eluent to obtain the pure product **10b** as a white solid (301 mg, 64%). Mp: 197–200 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.43 (d, 1H, J = 8.3 Hz, Ar**H**), 7.36 (d, 1H, J = 8.3 Hz, Ar**H**), 7.04 (s, 2H, Ar**H**), 6.93 (d, 1H, J = 8.3 Hz, Ar**H**), 6.70 (s, 1H, isoxazole−**H**), 5.80 (br, 1H, −O**H**), 3.95 (s, 9H, 3×−OC**H**$_3$), 3.90 (s, 3H, 1×−OC**H**$_3$); MS (ESI): m/z 358 $(M + H)^+$.

*5.1.12. 2-Methoxy-5-(3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxa zol-5-yl)phenol (**15a**)*

This compound was prepared according to the method described for compound **10a**, employing compound **14a** (473.6 mg, 1 mmol). The crude product was chromatographed using ethyl acetate/light petroleum (20%) as eluent to obtain the pure product 17a as a white solid (236 mg, 66%). Mp: 160–175 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28 (d, 1H, J = 8.1 Hz, ArH), 7.22 (d, 1H, J = 8.1 Hz, ArH), 6.90 (d, 1H, J = 8.1 Hz, ArH), 6.62 (s, 2H, ArH), 5.68 (br, 1H, −OH), 5.65 (dd, 1H, J = 10.4, 8.4 Hz, isoxazoline−H), 3.96 (s, 3H, 1×−OC**H**$_3$), 3.90 (s, 6H, 2×−OC**H**$_3$), 3.86 (s, 3H, 1×−OC**H**$_3$), 3.75 (dd, 1H, J = 16.4, 10.4 Hz, isoxazoline−H), 3.30 (dd, 1H, J = 16.4, 8.4, Hz, isoxazoline−H); MS (ESI): m/z 360 $(M + H)^+$.

*5.1.13. 2,6-Dimethoxy-4-(3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl) phenol (**15b**)*

This compound was prepared according to the method described for compound **10a**, employing compound **14b** (501.6 mg, 1 mmol). The crude product was chromatographed using ethyl acetate/light petroleum (20%) as eluent to obtain the pure product **15b** as a white solid (232 mg, 60%). Mp: 220–235 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.05 (s, 2H, Ar**H**), 7.00 (s, 2H, Ar**H**), 6.64 (s, 1H, isoxazole−**H**), 5.80 (br, 1H, −O**H**), 3.99 (s, 6H, 2×−OC**H**$_3$), 3.95 (s, 6H, 2×−OC**H**$_3$), 3.88 (s, 3H, 1×−OC**H**$_3$); MS(ESI): m/z 388 $(M + H)^+$.

*5.1.14. Synthesis of 2-(4-hydroxy-3-methoxyphenyl)-2,3-dihydroqu inazolin-4(1H)-one (**17a**)*

p-Toluenesulfonic acid monohydrate (0.1 g, 0.3 mmol) was added to a solution of benzamide (1.0 g, 7.3 mmol) and **16a** (1.0 g, 7.3 mmol) in *N*,*N*-dimethyl acetamide (DMA) (20 mL). The mixture was stirred at room temperature for 2 h and poured into ice water (200 mL). The precipitate was collected, washed with water, dried, and crystallized from ethanol to get the compound **17a** as a pale yellow solid (1.18 g, 60%). Mp: 188–190 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.67 (br, 1H, −O**H**), 8.23 (br s, 1H, N$_3$**H**), 7.67 (d, 1H, J = 7.4 Hz, Ar**H**), 7.28–7.21 (m, 2H, Ar**H**), 7.13 (br s, 1H, N$_1$**H**), 7.02 (d, 1H, J = 8.3 Hz, Ar**H**), 6.84 (d, 1H, J = 8.3 Hz, Ar**H**), 6.79–6.64 (m, 2H, Ar**H**), 5.70 (s, 1H, dihydroquinazolinone-**H**), 3.82 (s, 3H, 1×−OC**H**$_3$); MS(ESI): m/z 271 $(M + H)^+$.

*5.1.15. 2-(3-Hydroxy-4-methoxyphenyl)-2,3-dihydroquinazolin-4 (1H)-one (**17b**)*

This compound was prepared according to the method described for compound **17a**, employing compound **16b** (1.0 g, 7.3 mmol). The precipitate was collected, washed with water, dried, and crystallized from ethanol to get the compound **17b** as a pale yellow solid (1.18 g, 60%). Mp: 175–177 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.67 (br, 1H, −O**H**), 8.25 (br s, 1H, N$_3$**H**), 7.93 (d, 1H, J = 6.7 Hz, Ar**H**), 7.39–7.30 (m, 2H, Ar**H**), 7.22 (s, 1H, Ar**H**), 7.14 (br s, 1H, N$_1$**H**), 7.07 (d, 1H, J = 8.3 Hz, Ar**H**), 6.96–6.83 (m, 2H, Ar**H**), 5.83 (s, 1H, dihydroquinazolinone-**H**), 3.88 (s, 3H, 1×−OC**H**$_3$); MS (ESI): m/z 271 $(M + H)^+$.

*5.1.16. Synthesis of 2-(4-(3-bromopropoxy)-3-methoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (**18a**)*

To a solution of the compound **17a** (270 mg, 1 mmol) in DMF (5 mL) anhydrous K_2CO_3 (552 mg, 4 mmol) and the 1,3 dibromopropane (800 mg, 4 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. After completion of the reaction as indicated by TLC, ice was added to the reaction mixture followed by extraction with ethyl acetate and then washed with brine solution. The solvent was removed under vacuum. The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (20%) as eluant to afford the pure compound **18a** as a pale yellow solid (352 mg, 90%). Mp: 124–126 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.23 (br s, 1H, N$_3$**H**), 7.74 (dd, 1H, J = 7.9, 1.4 Hz, Ar**H**), 7.24–7.14 (m, 2H, Ar **H**), 7.10 (br s, 1H, N$_1$**H**), 7.01 (dd, 1H, J = 7.9, 2.1 Hz, Ar**H**), 6.85 (d, 1H, J = 7.9 Hz, Ar**H**), 6.77–6.66 (m, 1H, Ar**H**), 6.56 (dd, 1H, J = 7.5, 7.5 Hz, Ar**H**), 5.76 (s, 1H, dihydroquinazolinone-**H**), 3.99 (t, 2H, J = 6.5 Hz, 1×−OC**H**$_2$−), 3.87 (s, 3H, 1×−OC**H**$_3$), 3.48 (t, 2H, J = 6.5 Hz, 1×−C**H**$_2$Br), 2.29–2.42 (m, 2H, −C**H**$_2$−); MS (ESI): m/z 392 $(M + H)^+$.

*5.1.17. 2-(4-(4-Bromobutoxy)-3-methoxyphenyl)-2,3-dihydroquina zolin-4(1H)-one (**18b**)*

This compound was prepared according to the method described for compound **18a**, employing compound **17a** (270 mg, 1 mmol) and 1,4 dibromobutane (860 mg, 4 mmol). The crude product was chromatographed using ethyl acetate/light petroleum (20%) as eluent to obtain the pure product **18b** as a pale yellow solid (356 mg, 88%). Mp: 143–145 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.22 (br s, 1H, N$_3$**H**), 7.67 (dd, 1H, J = 7.5, 1.4 Hz, Ar**H**), 7.22–7.12 (m, 2H, Ar **H**), 7.02 (dd, 1H, J = 7.9, 2.1 Hz, Ar**H**), 6.84 (d, 1H, J = 8.3 Hz, Ar**H**), 6.79–6.64 (m, 1H, Ar**H**), 6.56 (dd, 1H, J = 7.5, 7.5 Hz, Ar**H**), 5.70 (s, 1H, dihydroquinazolinone-**H**), 4.02 (t, 2H, J = 5.8 Hz, 1×−OC**H**$_2$−), 3.82 (s, 3H, 1×−OC**H**$_3$), 3.52 (t, 2H, J = 6.0 Hz, 1×−C**H**$_2$Br), 1.87–2.14 (m, 4H, 2×−C**H**$_2$−); MS (ESI): m/z 406 $(M + H)^+$.

*5.1.18. 2-(4-(5-Bromopentyloxy)-3-methoxyphenyl)-2,3-dihydroqu inazolin-4(1H)-one (**18c**)*

This compound was prepared according to the method described for compound **18a**, employing compound **17a** (270 mg, 1 mmol) and 1,5-dibromopentane (920 mg, 4 mmol). The crude product was chromatographed using ethyl acetate/light petroleum (20%) as eluent to obtain the pure product **18c** as a pale yellow solid (378 mg, 90%). Mp: 153–155 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.20 (br s, 1H, N$_3$**H**), 7.73 (dd, 1H, J = 7.9, 1.4 Hz, Ar**H**), 7.24–7.14 (m, 2H, Ar **H**), 7.11 (br s, 1H, N$_1$**H**), 7.00 (dd, 1H, J = 7.9, 2.1 Hz, Ar**H**), 6.82 (d, 1H, J = 7.9 Hz, Ar**H**), 6.77–6.66 (m, 1H, Ar**H**), 6.56 (dd, 1H, J = 7.5, 7.5 Hz,

Ar**H**), 5.75 (s, 1H, dihydroquinazolinone-**H**), 3.99 (t, 2H, J = 6.5 Hz, 1×−OC**H**$_2$−), 3.86 (s, 3H, 1×−OC**H**$_3$), 3.45 (t, 2H, J = 6.5 Hz, 1×−C**H**$_2$Br), 1.77–2.02 (m, 4H, 2×−C**H**$_2$−), 1.59–1.72 (m, 2H, 1×−C**H**$_2$−); MS (ESI): m/z 420 $(M + H)^+$.

*5.1.19. 2-(3-(4-Bromobutoxy)-4-methoxyphenyl)-2,3-dihydroquina zolin-4(1H)-one (**18d**)*

This compound was prepared according to the method described for compound **18a**, employing compound **17b** (270 mg, 1 mmol) and 1,4 dibromobutane (860 mg, 4 mmol). The crude product was chromatographed using ethyl acetate/light petroleum (20%) as eluent to obtain the pure product **18d** as a pale yellow solid (356 mg, 88%). Mp: 143–145 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.23 (br s, 1H, N$_3$**H**), 7.70 (d, 1H, J = 7.4 Hz, Ar**H**), 7.39–7.30 (m, 2H, Ar**H**), 7.22 (s, 1H, Ar**H**), 7.10 (br s, 1H, N$_1$**H**), 7.02 (d, 1H, J = 8.3 Hz, Ar**H**), 6.96–6.83 (m, 1H, Ar**H**), 6.68 (d, 1H, J = 6.7 Hz, Ar**H**), 5.70 (s, 1H, dihydroquinazolinone-**H**), 4.03 (t, 2H, J = 5.8 Hz, 1×−OC**H**$_2$−), 3.81 (s, 3H, 1×−OC**H**$_3$), 3.52 (t, 2H, J = 6.6 Hz, 1×−C**H**$_2$Br), 1.87–2.14 (m, 4H, 2×−C**H**$_2$−); MS (ESI): m/z 406 $(M + H)^+$.

*5.1.20. Synthesis of 2-(4-(3-(2,6-dimethoxy-4-(5-(3,4,5-trimethoxy phenyl)-4,5-dihydroisoxazol-3-yl)phenoxy)propoxy)-3-methoxyphe nyl)-2,3-dihydroquinazolin-4(1H)-one (**4a**)*

To a solution compound **18a** (391 mg, 1 mmol) in DMF (5 mL) anhydrous K_2CO_3 (552 mg, 4 mmol) and the compound **10a** (389 mg, 1 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. After completion of the reaction as indicated by TLC, ice was added to the reaction mixture followed by extraction with ethyl acetate and then washed with brine solution. The solvent was removed under vacuum. The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **4a** as a pale yellow solid (559 mg, 80%). Mp: 84–86 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.22 (br s, 1H, N$_3$**H**), 7.84 (dd, 1H, J = 7.5, 1.4 Hz, Ar**H**), 7.25 (ddd, 1H, J = 7.5, 7.5, 1 Hz, Ar**H**), 7.13 (br s, 1H, N$_1$H), 7.02–6.91 (m, 3H, Ar**H**), 6.89 (s, 2H, Ar**H**), 6.86 (d, 1H, J = 7.5 Hz, Ar**H**), 6.67 (dd, 1H, J = 7.5, 7.5 Hz, Ar**H**), 6.60 (s, 2H, Ar**H**), 5.84 (s, 1H, dihydroquinazolinone-H), 5.65 (dd, 1H, J = 10.5, 8.3 Hz, isoxazoline−**H**), 4.08 (t, 2H, J = 5.6 Hz, 1×−OC**H**$_2$−), 4.03 (t, 2H, J = 5.6 Hz, 1×−OC**H**$_2$−), 3.89 (s, 3H, 1×−OC**H**$_3$), 3.87 (s, 9H, 3×−OC**H**$_3$), 3.84 (s, 6H, 2×−OC**H**$_3$), 3.74 (dd, 1H, J = 16.6, 10.5, Hz, isoxazoline−**H**), 3.31 (dd, 1H, J = 16.6, 9.0 Hz, isoxazoline−**H**), 2.33–2.18 (m, 2H, 1×−C**H**$_2$−); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.7, 156.0, 153.4, 149.7, 147.3, 136.4, 133.8, 131.1, 130.7, 128.4, 126.3, 124.5, 119.9, 119.3, 115.3, 114.4, 112.2, 110.0, 103.9, 102.5, 82.6, 72.8, 68.7, 68.5, 60.7, 56.1, 56.0, 55.8, 43.4, 29.6 ppm; MS (ESI): m/z 700 $(M + H)^+$; HRMS (ESI) m/z calcd for $C_{38}H_{41}N_3O_{10}Na$ $(M + Na)^+$, 722.2689; found, 722.2666.

*5.1.21. 2-(4-(4-(2,6-Dimethoxy-4-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)phenoxy)butoxy)-3-methoxyphenyl)-2,3-dihy droquinazolin-4(1H)-one (**4b**)*

This compound was prepared according to the method described for compound **4a**, employing compound **18b** (405 mg, 1 mmol) and **10a** (389 mg, 1 mmol). The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **4b** as a pale yellow solid (570 mg, 80%). Mp: 80–82 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.20 (br s, 1H, N$_3$**H**), 7.84 (dd, 1H, J = 7.2, 1.4 Hz, Ar**H**), 7.25 (ddd, 1H, J = 1, 7.2, 7.2 Hz, Ar**H**), 7.12 (br s, 1H, N$_1$**H**), 7.02–6.91 (m, 3 H, Ar**H**), 6.84 (s, 2H, Ar**H**), 6.79 (d, 1H, J = 8.3 Hz, Ar**H**), 6.62 (d, 1H, J = 7.2 Hz, Ar**H**), 6.54 (s, 2H, Ar**H**), 5.75 (s, 1H, dihydroquinazolinone-**H**), 5.62 (dd, 1H, J = 10.5, 8.3 Hz, isoxazoline−**H**), 4.11 (t, 2H, J = 5.6 Hz, 1×−OC**H**$_2$−), 4.01 (t, 2H, J = 5.6 Hz, 1×−OC**H**$_2$−), 3.89 (s, 3H, 1×−OC**H**$_3$), 3.87 (s, 9H, 3×−OC**H**$_3$), 3.84 (s, 6H, 2×−OC**H**$_3$), 3.74 (dd, 1H, J = 16.6, 10.5 Hz, isoxazoline−**H**), 3.31 (dd, 1H, J = 16.6, 9.0 Hz, isoxazoline−**H**), 2.15–2.02 (m, 4H, 2×−C**H**$_2$−); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.9, 156.0, 153.4, 149.6, 147.3, 136.4, 133.8, 131.1, 130.7, 128.4, 126.3, 124.5, 119.9, 119.3, 115.3, 114.4, 112.2, 110.0, 103.9, 102.5, 82.6, 72.8, 68.7, 68.5, 60.7, 56.1, 56.0, 55.8, 43.4, 26.5, 25.6 ppm; MS (ESI): m/z 714 $(M + H)^+$; HRMS (ESI) m/z calcd for $C_{39}H_{43}N_3O_{10}$ $(M + H)^+$, 714.3026; found, 714.3058.

*5.1.22. 2-(4-(5-(2,6-Dimethoxy-4-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole-3-yl)phenoxy)pentyloxy)-3-methoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (**4c**)*

This compound was prepared according to the method described for compound **4a**, employing compound **18c** (419 mg, 1 mmol) and **10a** (389 mg, 1 mmol). The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **4c** as a pale yellow solid (581 mg, 80%). Mp: 76–78 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.19 (br s, 1H, N$_3$**H**), 7.84 (dd, 1H, J = 7.4, 1.5 Hz, Ar**H**), 7.25 (ddd, 1H, J = 7.4, 7.4, 1 Hz, Ar**H**), 7.10 (br s, 1H, N$_1$**H**), 6.92 (d, 1H, J = 7.4 Hz, Ar**H**), 6.84 (s, 2H, Ar**H**), 6.79 (d, 1H, J = 8.3 Hz, Ar**H**), 6.62 (d, 1H, J = 7.4 Hz, Ar**H**), 6.54 (s, 2H, Ar**H**), 5.75 (s, 1H, dihydroquinazolinone-**H**), 5.60 (dd, 1H, J = 10.5, 8.3 Hz, isoxazoline−**H**), 4.11 (t, 2H, J = 5.6 Hz, 1×−OC**H**$_2$−), 4.01 (t, 2H, J = 5.6 Hz, 1×−OC**H**$_2$−), 3.89 (s, 3H, 1×−OC**H**$_3$), 3.84 (s, 6H, 2×−OC**H**$_3$), 3.87 (s, 9H, 3×−OC**H**$_3$), 3.74 (dd, 1H, J = 16.6, 10.5 Hz, isoxazoline−**H**), 3.31 (dd, 1H, J = 16.6, 9.0 Hz, isoxazoline−**H**), 2.11–2.01 (m, 4H, 2×−C**H**$_2$−), 1.74–1.56 (m, 2H, 1×−C**H**$_2$−); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.8, 156.0, 153.4, 149.7, 147.3, 136.4, 133.9, 131.1, 130.7, 128.5, 126.7, 124.5, 120.0, 119.4, 114.5, 112.1, 110.0, 104.0, 102.5, 82.7, 73.1, 68.8, 60.7, 56.2, 56.1, 55.9, 43.4, 29.7, 29.6, 28.7, 22.2 ppm; MS (ESI): m/z 728 $(M + H)^+$; HRMS (ESI) m/z calcd for $C_{40}H_{45}N_3O_{10}Na$ $(M + Na)^+$, 750.3002; found, 750.2999.

*5.1.23. 2-(4-Methoxy-3-(4-(2-methoxy-5-(5-(3,4,5-trimethoxyphe nyl)isoxazol-3-yl) phenoxy)butoxy)phenyl)-2,3-dihydroquinazolin-4(1H)-one (**4d**)*

This compound was prepared according to the method described for compound **4a**, employing compound **18d** (405 mg, 1 mmol) and **10b** (357 mg, 1 mmol). The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **4d** as a pale yellow solid (544 mg, 80%). Mp: 126–128 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.20 (br s, 1H, N$_3$**H**), 7.93 (dd, J = 7.5, 1.5 Hz, 1H, Ar**H**), 7.45 (dd,1H, J = 8.3, 1.9 Hz, Ar**H**), 7.33 (d, 1H, J = 1.9 Hz, Ar**H**), 7.28 (d, 1H, J = 8.3 Hz, Ar**H**), 7.23 (br s, 1H, N$_1$**H**), 7.03 (d, 1H, J = 7.9 Hz, Ar**H**), 7.04 (s, 2H, Ar**H**), 6.92 (d, 1H, J = 7.9 Hz, Ar**H**), 6.87 (d, 1H, J = 8.4 Hz, Ar**H**), 6.84 (d, 1H, J = 8.4 Hz, Ar**H**), 6.70 (s, 1H, isoxazole−**H**), 6.65 (d, 1H, J = 7.9 Hz, Ar**H**), 5.81 (s, 1H, dihydroquinazolinone-**H**), 4.11–4.24 (m, 4H, 2×−OC**H**$_2$−), 3.95 (s, 6H, 2×−OC**H**$_3$), 3.91 (s, 3H, 1×−OC**H**$_3$), 3.89 (s, 3H, 1×−OC**H**$_3$), 3.86 (s, 3H, 1×−OC**H**$_3$), 2.17–1.99 (m, 4H, 2×−C**H**$_2$−); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.0, 162.0, 153.3, 149.2, 148.1, 147.4, 145.3, 133.0, 128.3, 124.5, 120.0, 119.5, 113.1, 112.2, 111.8, 107.8, 101.4, 98.5, 74.2, 69.0, 60.8, 56.2, 55.8, 26.8, 25.2; MS (ESI): m/z 682 $(M + H)^+$; HRMS (ESI) m/z calcd for $C_{38}H_{39}N_3O_9$ $(M + H)^+$, 682.2764; found, 682.2773.

*5.1.24. 2-(3-Methoxy-4-(3-(2-methoxy-5-(3-(3,4,5-trimethoxyphe nyl)-4,5-dihydroisoxazol-5-yl)phenoxy)propoxy)phenyl)-2,3-dihyd roquinazolin-4(1H)-one (**5a**)*

This compound was prepared according to the method described for compound **4a**, employing compound **18a** (391 mg, 1 mmol) and **15a** (359 mg, 1 mmol). The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **5a** as a pale yellow solid (535 mg, 80%). Mp: 120–122 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.24 (br s, 1H, N$_3$**H**), 7.95 (dd, 1H, J = 7.5, 1.5 Hz, Ar**H**), 7.34 (ddd, 1H,

J = 7.5, 7.5, 1.0 Hz, Ar**H**), 7.18 (br s, 1H, N_1**H**), 7.01 (d, 1H, J = 7.5 Hz, Ar**H**), 6.97–6.89 (m, 7H, Ar**H**), 6.86 (d, 1H, J = 7.5 Hz, Ar**H**), 6.68 (d, 1H, J = 8.3 Hz, Ar**H**), 5.83 (s, 1H, dihydroquinazolinone-**H**), 5.67 (dd, 1H, J = 9.8, 8.3 Hz, isoxazoline–**H**), 4.12–3.99 (m, 4H, 2×–OC**H**$_2$–), 3.88 (s, 15H, 5×–OC**H**$_3$), 3.72 (dd, 1H, J = 16.6, 10.5 Hz, isoxazoline–**H**), 3.32 (dd, 1H, J = 10.6, 9.0 Hz, isoxazoline–**H**), 2.33–2.18 (m, 2H, 1×–C**H**$_2$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.8, 156.0, 153.2, 149.6, 149.3, 148.7, 133.9, 133.0, 130.7, 128.5, 124.8, 120.0, 119.4, 118.4, 115.4, 114.4, 112.2, 111.4, 110.4, 109.9, 103.9, 82.7, 68.8, 68.6, 60.8, 56.1, 55.9, 43.0, 29.6 ppm; MS (ESI): m/z 669 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{37}H_{39}N_3O_9$ $(M+H)^+$, 669.2784; found, 669.2763.

5.1.25. 2-(3-Methoxy-4-(4-(2-methoxy-5-(3-(3,4,5-trimethoxyphenyl)-4,5-dihydro isoxazol-5-yl)phenoxy)butoxy)phenyl)-2,3-dihydroquinazolin-4(1H)-one (**5b**)

This compound was prepared according to the method described for compound **4a**, employing compound **18b** (405 mg, 1 mmol) and **15a** (359 mg, 1 mmol). The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **5b** as a pale yellow solid (546 mg, 80%). Mp: 82–84 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.20 (br s, 1H, N_3**H**), 7.95 (dd, 1H, J = 7.5, 1.5 Hz, Ar**H**), 7.34 (ddd, 1H, J = 1.0, 7.5, 7.5 Hz, Ar**H**), 7.19 (br s, 1H, N_1**H**), 7.01 (d, 1H, J = 7.5 Hz, Ar**H**), 6.97–6.89 (m, 7H, Ar**H**), 6.86 (d, 1H, J = 7.5 Hz, Ar**H**), 6.68 (d, 1H, J = 8.3 Hz, Ar**H**), 5.67 (dd, 1H, J = 9.8, 8.3 Hz, isoxazoline–**H**), 5.83 (s, 1H, dihydroquinazolinone-**H**), 3.99–4.12 (m, 4H, 2×–OC**H**$_2$–), 3.88 (s, 15H, 5×–OC**H**$_3$), 3.72 (dd, 1H, J = 16.6, 10.5 Hz, isoxazoline–**H**), 3.32 (dd, 1H, J = 16.6, 9.0 Hz, isoxazoline–**H**), 2.15–2.02 (m, 4H, 2×–C**H**$_2$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.8, 156.0, 153.2, 149.6, 149.3, 148.7, 133.9, 133.0, 130.7, 128.5, 124.8, 120.0, 119.4, 118.4, 115.4, 114.4, 112.2, 111.4, 110.4, 109.9, 103.9, 82.7, 68.8, 68.6, 60.8, 56.1, 55.9, 43.0, 26.5, 25.6 ppm; MS (ESI): m/z 683 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{38}H_{41}N_3O_9$ $(M+H)^+$, 683.2764; found, 683.2773.

5.1.26. 2-(3-Methoxy-4-(5-(2-methoxy-5-(3-(3,4,5-trimethoxyphenyl)-4,5-dihydro isoxazol-5-yl)phenoxy)pentyloxy)phenyl)-2,3-dihydroquinazolin-4(1H)-one (**5c**)

This compound was prepared according to the method described for compound **4a**, employing compound **18c** (419 mg, 1 mmol) and **15a** (359 mg, 1 mmol). The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **5c** as a pale yellow solid (556 mg, 80%). Mp: 88–90 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.19 (br s, 1H, N_3**H**), 7.95 (dd, 1H, J = 7.5,1.5 Hz, Ar**H**), 7.34 (ddd, 1H, J = 7.5, 7.5, 1.0 Hz, Ar**H**), 7.20 (br s, 1H, N_1**H**), 7.01 (d, 1H, J = 7.5 Hz, Ar**H**), 6.97–6.89 (m, 7H, Ar**H**), 6.86 (d, 1H, J = 7.5 Hz, Ar**H**), 6.68 (d, 1H, J = 8.3 Hz, Ar**H**), 5.83 (s, 1H, dihydroquinazolinone-**H**), 5.67 (dd, 1H, J = 8.3, 9.8 Hz, isoxazoline–**H**), 4.12–3.99 (m, 4H, 2×–OC**H**$_2$–), 3.88 (s, 15H, 5×–OC**H**$_3$), 3.72 (dd, 1H, J = 16.6, 10.5 Hz, isoxazoline–**H**), 3.32 (dd, 1H, J = 16.6, 9.0 Hz, isoxazoline–**H**), 2.11–2.01 (m, 4H, 2×–C**H**$_2$–), 1.74–1.56 (m, 2H, 1×–C**H**$_2$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.8, 156.0, 153.2, 149.6, 149.3, 148.7, 133.9, 133.0, 130.7, 128.5, 124.8, 120.0, 119.4, 118.4, 115.4, 114.4, 112.2, 111.4, 110.4, 109.9, 103.9, 82.7, 68.8, 68.6, 60.8, 56.1, 55.9, 43.0, 28.7, 22.3 ppm; MS (ESI): m/z 697 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{39}H_{43}N_3O_9Na$ $(M+Na)^+$, 720.2897; found, 720.2886.

5.1.27. 2-(3-(4-(2,6-Dimethoxy-4-(3-(3,4,5-trimethoxyphenyl) isoxazol-5-yl)phenoxy) butoxy)-4-methoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (**5d**)

This compound was prepared according to the method described for compound **4a**, employing compound **18d** (419 mg, 1 mmol) and **15b** (387 mg, 1 mmol). The crude product thus obtained was purified by column chromatography using ethyl acetate/hexane (50%) as eluant to afford the pure compound **5d** as a pale yellow solid (568 mg, 80%). Mp: 78–81 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.25 (br s, 1H, N_3**H**), 7.93 (dd, 1H, J = 7.5, 1.5 Hz, Ar**H**), 7.33 (ddd, 1H, J = 7.5, 7.5, 1.0 Hz, Ar**H**), 7.25 (br s, 1H, N_1**H**), 7.10 (s, 2H, Ar**H**), 7.09–7.04 (m, 4H, Ar**H**), 7.03 (s, 2H, Ar**H**), 6.93–6.88 (m, 1H, Ar**H**), 6.87 (d, 1H, J = 7.5 Hz, Ar**H**), 6.73 (s, 1H, isoxazole–**H**), 6.66 (d, 1H, J = 7.5 Hz, Ar**H**), 5.79 (s, 1H, dihydroquinazolinone-**H**), 4.20–4.06 (m, 4H, 2×–OC**H**$_2$–), 3.96 (s, 6H, 2×–OC**H**$_3$), 3.91 (s, 9H, 3×–OC**H**$_3$), 3.88 (s, 3H, 1×–OC**H**$_3$), 2.18–1.90 (m, 4H, 2×–C**H**$_2$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.8, 162.8, 153.7, 147.3, 145.2, 133.9, 130.7, 128.5, 122.5, 120.0, 119.4, 111.4, 111.2, 111.1, 103.9, 103.1, 97.1, 72.8, 68.8, 68.5, 60.8, 56.2, 55.9, 26.5, 25.6 ppm; MS (ESI): m/z 712 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{39}H_{41}N_3O_{10}Na$ $(M+Na)^+$, 734.2689; found, 734.2678.

5.2. In vitro evaluation of cytotoxic activity

The synthesized compounds **4a**,**b**, **4d**, and **5a**–**d** were evaluated for their in vitro cytotoxicity in selected human cancer cell lines of breast (MCF-7), lung (A549), oral (KB), prostate (PC3) and ovarian (A2780) origin. A protocol of 48 h continuous drug exposure was used and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine, and were inoculated into 96-well microtiter plates in 90 μL at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. Aliquots of 10 μL of the drug dilutions were added to the appropriate microtiter wells already containing 90 μL of cells, resulting in the required final drug concentrations. Each compound was evaluated for four concentrations (0.1, 1, 10 and 100 μM) and each was done in triplicate wells. Plates were incubated further for 48 h, and assay was terminated by the addition of 50 μL of cold trichloro acetic acid (TCA) (final concentration, 10% TCA) and plates were again incubated for 60 min at 4 °C. The plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (50 μL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. The residual dye was removed by washing five times with 1% acetic acid. The plates were air-dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an ELISA plate reader at a wavelength of 540 nm with 690 nm reference wavelengths. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. The above determinations were repeated three times.

5.3. Cell culture

The human breast cancer cell line MCF-7 was purchased from American Type culture collection was maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen), supplemented with 10% fetal calf serum and 100 U/mL Pencillin and 100 μg/mL streptomycin sulfate (Sigma). The cell line was maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in the incubator.

5.4. MTT cell proliferation assay

MCF-7 cell lines were seeded in a 96-well plate at a cell density of 10,000 cells/well. After overnight incubation, the compounds (**1, 4c, 10a** and **18c**) were added to the culture media and incubated for 24 h. The cytotoxicity was assayed by 3-(4,5-dimethyl-2-thiozolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) dye uptake. The cell line (MCF-7) was incubated at 37 °C for 2 h with MTT at 10% (i.e. 20 μL) of the culture volume. After incubation period, cultures were removed from incubator and dissolved the resulting formazan by adding 100 μL of extraction buffer (20% SDS, 50% dimethyl

formamide). After overnight incubation at 37 °C, the absorbance (O.D) was measured at 570 nm using Multimode Varioskan Flash (Thermo Fisher Scientifics) with medium as blank.

5.5. Cell cycle analysis

5×10^5 cells each of MCF-7 cell line were seeded in 60 mm dish and were allowed to grow for 24 h. A concentration of 5 μM of **1, 4c, 10a** and **18c** was added to the culture media, and the cells were incubated for an additional 24 h. Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70% ethanol at 4 °C for 30 min, washed with PBS and incubated with 1 mg/ml RNaseA solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 μL of DNA staining solution [10 mg of Propidium Iodide (PI), 0.1 mg of trisodium citrate, and 0.03 mL of Triton X-100 were dissolved in 100 mL of sterile MilliQ water at room temperature for 30 min in the dark]. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit Software.

5.6. Tubulin polymerization assay

The assay was carried out in a 384 well plate in PEM buffer [100 mM PIPES (pH-6.9), 1 mM $MgCl_2$, 1 mM EGTA, 10% glycerol]. The reaction mixture in a total volume of 10 μL contained PEM buffer, GTP (1 mM). The reaction was initiated by the addition of GTP to all the wells. Polymerization reaction was monitored by the increase in absorbance at 340 nm using Dynex multimode plate reader at 37 °C. Absorbance was recorded at every 2 min intervals for up to 3 h. Nocodazole was used as positive controls in each assay.

5.7. Immunofluorescence and microscopy

MCF-7 cells were seeded on glass cover slips, incubated for 48 h in the presence or absence of test compound **4c** (5 μM). Following the termination of incubation, cells were fixed with 3% para-formaldehyde, 0.02% glutaraldehyde in PBS and permiabilized by dipping the cells in 100% methanol (−20 °C). Later, cover slips were blocked with 1% BSA in phosphate buffered saline for 1 h followed by incubation with a primary antitubulin (mouse monoclonal) antibody followed by FITC conjugated secondary mouse anti IgG antibody. At the end of experiments, cells were washed and fixed Photographs were taken using the confocal microscope (olympus), equipped with FITC settings and the pictures were analyzed for the integrity of microtubule network. In parallel experiment nocodazole (2.5 μM) was used as control for analyzing microtubule integrity.

5.8. Analysis of nuclear morphology

MCF-7 cells were seeded on glass cover slip, incubated for 48 h in the presence or absence of test compound **4c** (5 μM). Following the termination of incubation, cells were fixed with 3% para-formaldehyde, 0.02% glutaraldehyde in PBS, the cells were incubated with the nuclear stain DAPI, to analyze morphology of nucleus. Photographs were taken in an olyumpus fluorescence microscopy equipped with DAPI filter settings.

5.9. Protein extraction and western blot analysis

Total cell lysates from cultured MCF-7 cells were obtained by lysing the cells in ice-cold RIPA buffer (1XPBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) and containing 100 μg/mL PMSF, 5 μg/mL Aprotinin, 5 μg/mL leupeptin, 5 μg/mL pepstatin and 100 μg/mL NaF. After centrifugation at 12,000 rpm for 10 min, the protein in supernatant was quantified by Bradford method (BIO-RAD) using Multimode varioskan instrument (Thermo Fischer Scientifics). Thirty micrograms of protein per lane was applied in 12% SDS-polyacrylamide gel. After electrophoresis, the protein was transferred to polyvinylidine difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in TBS + 0.1% Tween20 (TBST) containing 5% blocking powder (Santacruz). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight (O/N). Cyclin A, (1:500), CDK1 (1:500), Cyclin B (1:500), cleaved PARP (1:500) and β-actin (1:500) antibodies were purchased from Imgenex, USA. The membrane was incubated with corresponding horseradish peroxidase-labeled secondary antibody (1:2000) (Santa Cruz) at room temperature for 1 h. Membranes was washed with TBST three times for 15 min and the blots were visualized with chemiluminescence reagent (Thermo Fischer Scientifics Ltd.). The X-ray films were developed with developer and fixed with fixer solution.

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