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Click chemistry inspired synthesis and bioevaluation of novel triazolyl derivatives of osthol as potent cytotoxic agents

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ABSTRACT

A new series of diverse triazoles linked through the hydroxyl group of lactone ring opened osthol (**1**) were synthesized using click chemistry approach. All the derivatives were subjected to 3-(4,5-Dimethylthiazol-yl)-diphenyl tetrazoliumbromide (MTT) cytotoxicity screening against a panel of seven different human cancer cell lines viz. colon (colo-205), colon (HCT-116), breast (T47D), lung (NCI-H322), lung (A549), prostate (PC-3) and Skin (A-431) to check their cytotoxic potential. Interestingly, among the tested molecules, most of the analogs displayed better cytotoxic activity than the parent osthol (**1**). Of the synthesized triazoles, compounds **8** showed the best activity with IC_{50} of 1.3, 4.9, 3.6, 41.0, 35.2, 26.4 and 7.2 μM against colon (Colo-205 and HCT-116), breast (T47D), lung (NCI-H322 and A549), prostate (PC-3) and Skin (A-431) cancer lines respectively. Compound **8** induced potent apoptotic effects in Colo-205 cells. The population of apoptotic cells increased from 11.4% in case of negative control to 24.1% at 25 μM of **8**. Compound **8** also induced a remarkable decrease in mitochondrial membrane potential ($\Delta\Psi m$) leading to apoptosis of cancer cells used. The present study resulted in identification of broad spectrum cytotoxic activity of analogs bearing electron withdrawing substituents, besides the enhanced selective activity of analogs with electron donating moieties.

1. Introduction

Natural products (NPs) have been used to treat human disease for thousands of years and play an increasingly important role in drug discovery and development. In fact, the majority of anticancer and anti-infectious agents are of natural origin [1,2]. NPs with a coumarinic moiety have been reported to have multiple biological activities including anticancer [3,4], antioxidant [5,6], anti-inflammatory [7], antimicrobial [8], antiviral [9] and enzymatic inhibitory activities [10]. Natural coumarins and their derivatives are of great interest due to their widespread pharmacological properties, and this has attracted many medicinal chemists for further derivatization and screening them as novel therapeutic agents. The coumarin ring system, present in a large number of natural products (such as the anticoagulant, Warfarin), having interesting pharmacological properties [11,12] has intrigued chemists for decades to explore the natural coumarins or their synthetic derivatives for their applicability as drugs.

Many intersecting molecules having coumarin based ring systems have been synthesized utilizing novel synthetic methodologies. Some new derivatives bearing coumarin ring including the furanocoumarins (imperatorin), pyranocoumarins (seselin) and coumarin sulfamates (coumates) have been found to be useful in photochemotherapy, antitumour and anti-HIV therapy [13,14]. Among the diverse biological activities of coumarins, the notable one being their effect against breast cancer and sulfatase and aromatase inhibitory activity [15,16].

The naturally occurring coumarin osthol **1** (Fig. 1) has been thoroughly investigated during the past years for its promising pharmacological properties, particularly in the field of cancer [17,18]. It is clinically ingested as an important component of medicinal plants and herbs in Tradition Chinese Medicine (TCM) [19–21], and it exhibits a host of biological activities [22–26]. Osthol has been found to be a promising agent for the treatment of

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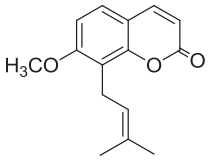


Fig. 1. Structure of osthol (**1**).

osteoporosis [27] and in reproductive system improvement properties by activation of the central cholinergic neuronal system [28]. Physiological [29], bacteriostatic and antitumour activities make these compounds attractive for further backbone derivatization and screening as novel therapeutic agent/s [30]. From the literature scan, both *in vitro* and *in vivo* studies revealed that osthol possesses anticancer effect by inhibiting human cancer cell growth and inducing apoptosis [31]. The studies on cytostatic activity in human cancer cell line MCF-7 (breast carcinoma) revealed that osthol displays some estrogenic activity by preventing the synthesis and action of estrogens (ER antagonists) indicating its potential to become a breast cancer treatment reagent [32]. This compound reportedly possesses anticancer effects by inhibiting cancer cell growth, metastasis and inducing cell apoptosis [33]. It induces G2/M arrest and apoptosis by modulating the PI3K/Akt pathway and suppresses migration and invasion through inhibition of matrix metalloproteinase-2 and matrix metallopeptidase-9 in human lung adenocarcinoma A549 cells [34]. Osthol has also been found to be effective in inhibiting the migration and invasion of breast cancer cells by wound healing and transwell assays [35]. The biological studies of osthol carried out in last few years have provided an additional dimension to the bioactivity profile of the title compound. The potential of osthol has not been fully exploited despite its biological importance; therefore, more efforts are invested towards the building of diverse libraries around its chemical structure and their biological profiles are in demand.

Click chemistry of natural products has acquired great importance in recent years. Some of the molecules studied include alkaloids [36,37], coumarins [38], saponins [39], steroids [40] and triterpenes such as betulinic acid [41–43]. Triazoles and their derivatives are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities such as antiviral, antibacterial, antifungal, anti-tuberculosis, anticonvulsant, antidepressant, anti-inflammatory and anticancer [44,45]. Triazole based compounds such as anastrozole, letrozole and vorozole are very important antineoplastic drugs (Fig. 2) [46]. Thus, the design and synthesis of novel triazole derivatives is the prospective direction for the development of novel anticancer agents with better curative effect, lower toxicity as well as higher selectivity.

Based on the above cited findings and inspiration from the potential anticancer activity of triazoles, we directed this work towards the synthesis of a diverse series of novel triazolyl derivatives of biological interest using osthol (**1**) as a key starting material. All the newly synthesized compounds were subjected to MTT [3-(4,5-Dimethylthiazol-yl)-diphenyl tetrazolium-bromide] assay against a panel of seven different human cancer cell lines viz. prostate colon (Colo-205 and HCT-116), breast (T47D), lung (NCI-H322 and A549), prostate (PC-3) and skin (A-431) to check their cytotoxic potential. This work provides the initial report on structure–activity relationship of triazolyl analogs of lactone ring opened coumarins in general and osthol (**1**) in particular.

2. Result and discussion

2.1. Chemistry

Osthol exhibits comparatively weak activity, low water solubility and limited permeability, and these properties may lower its absorption and bioavailability upon oral administration [19,20,47]. To overcome these lacunas it becomes imperative to introduce more hydrogen bond donors through its lactone ring opening and simultaneous introduction of heterocyclic ring system to improve its solubility and activity for better drug-likeness. The incorporation of heterocyclic moieties either as substituent group or as a fused component into parent coumarin nucleus alters its properties and converts it into a more useful product [48]. Keeping in view the interesting pharmacological activities of coumarins, click chemistry inspired approach involving union of terminal alkynes with organic azides has been taken up for the synthesis of regiospecific novel OH linked triazolyl derivatives of osthol in excellent yields. These click reactions exhibit remarkably broad scope and exquisite selectivity and have contrasting applications in chemistry, biology, and materials science. The target compounds **3**–**22** were synthesized as depicted in Scheme 1.

In the present study, osthol (**1**), isolated from the root parts of *Prangos pabularia* was used as the starting material. It was subjected to lactone ring opening in DMSO using NaOH as base yielding a *cis* (*Z*) product which simultaneously undergoes alkylation at OH group in presence of propargyl bromide to form (*E*)-3-(4-methoxy-3-(3-methylbut-2-en-1-yl)-2-(prop-2-yn-1-yloxy)phenyl)acrylic acid **2**. The proposed structure (**2**) was confirmed by spectral data analysis. From elemental analysis and HR-ESIMS (*m/z* 301.1459) data, this compound was assigned the molecular formula $C_{18}H_{20}O_4$. Proton singlets at δ 2.52 and 4.45 (integrating for one and two protons respectively) were assigned to terminal alkyne proton and two methylenic protons of propargylic moiety respectively. The *cis* (*Z*) behaviour of the protons of α,β-unsaturated system was depicted by the presence of two doublets at δ 5.92 and 7.26 with the coupling constant (*J*) of 12.4 Hz each. Compound **2** was allowed to undergo 1,3-dipolar cycloaddition reaction typically called Huisgen cycloaddition with various aromatic azides under sharpless click chemistry conditions ($CuSO_4.5H_2O$ and sodium ascorbate in *t*-BuOH:H_2O (1:1)) to afford regioselectively 1,4-disubstituted-1,2,3-triazoles (**3**–**22**) in good to excellent yields (Scheme 1). Under click conditions a series of such analogs was synthesized to look for structure–activity relationship studies. The structures of all the synthesised triazolyl derivatives were characterised by analytical and spectral data analysis. Formation of products could easily be confirmed by a downfield H-5 proton singlet (almost around 8.0 ppm) and other proton resonances in the aromatic region. Further characterisation of all the products was done using ^{13}C NMR-DEPT and HR-ESIMS as well as HRESI-MS.

2.2. Biology

2.2.1. Anti-proliferative activity

There are innumerable number of synthetic drugs to treat the diseased condition, but the treatment is not satisfactory, often due to their severe adverse effects, making provision for the synthesis of new and safer ones. Presently, scientists are keen to evaluate drugs from plant origin, due to their specific curative properties, healthy action, and safe and non-toxic effects. The biological studies of coumarins and related molecules containing coumarin ring; carried out in last few years have provided an additional dimension to the bioactivity. The potential of coumarins has not been fully exploited despite their biological importance; therefore, more efforts towards building the diverse libraries around its chemical structure and

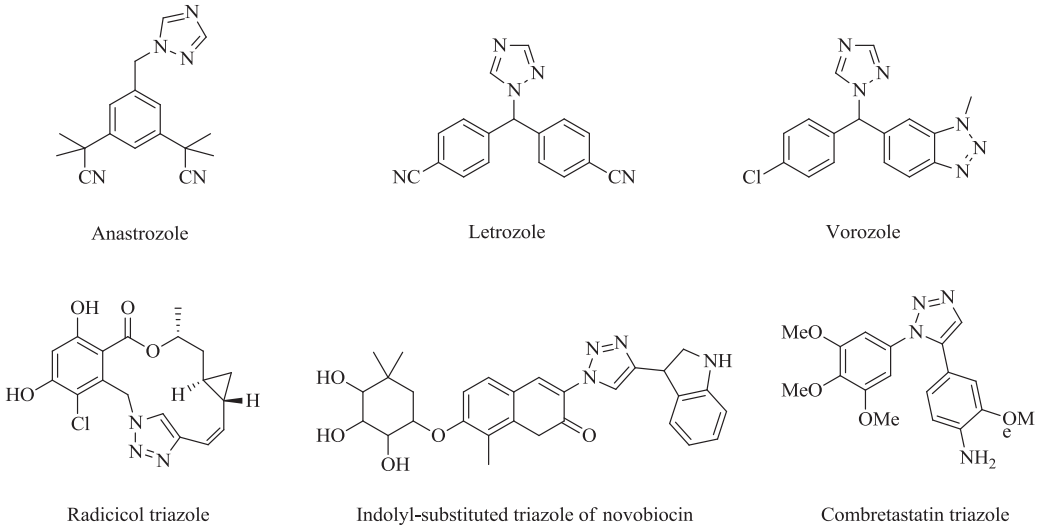


Fig. 2. Some representative anticancer triazoles.

their biological profiles are in demand. Thus, based on the reported anti-cancer activity of coumarins, it was envisaged to carry out structural modifications of osthol (**1**), for improved anti-cancer activity.

MTT cytotoxicity assay was used to screen the newly synthesized osthol analogs against a panel of seven human cancer cell lines; prostate colon (Colo-205 and HCT-116), breast (T47D), lung (NCI-H322 and A549), prostate (PC-3) and skin (A-431). The cytotoxicity profile indicates that the parent molecule, osthol (**1**) demonstrated cytotoxic effect against all the cancer cell lines. Most of the synthesized analogs displayed broad spectrum cytotoxic effect in a dose dependent manner. The analogs which exhibited significant cytotoxic effect, greater than 50% growth inhibition at the preliminary screening concentration (50 μM) were assayed using MTT assay at different concentrations (0.04–60 μM) to generate the IC_{50} values (Table 1). BEZ-235 was used as positive controls in this assay. The values are the average of triplicate analysis. Among all the tested triazolyl analogs, compound **8** exhibited the best results against colon (colo-205), colon (HCT-116), breast (T47D) and skin (A-431) cancer cell lines with IC_{50} of 1.3, 4.9, 3.6 and 7.2 μM respectively. Additionally, compound **8** exhibited superior potency to BEZ-235 against T47D and A431 cells. Compound **13**, bearing OH group in place of CN group as in case of compound **8**, is moderately active against all the tested cancer cell lines. Among compounds **5**, **6**, **9** and **12** containing a heterocyclic ring system, compound **5** displayed slightly better activity than BEZ-235 against A549 cancer cell line with IC_{50} of 5.6 μM while as it was weakly active towards the other cancer cell lines. Compounds **6**, **9** and **12** were moderately active against all the tested cancer cell lines. Compound **14** bearing an iodo group at ortho position of R moiety exhibited 3-fold increase in cytotoxic activity against A549 cancer cell line (IC_{50}: 2.2 μM) as compared to BEZ-235 (IC_{50}: 6.5 μM), while as, compound **19** having a fluoro at the same position was inactive towards lung A549 cancer cell line but strongly active towards T47D and A431 cells. These observations demonstrate the role of a particular substituent or a group in determining the biological activity against a particular target.

Compounds **4**, **16** and **17** having an electron donating OMe functionality in R group at different positions were inactive towards prostate (PC-3) cancer cell line. Compounds **16** and **17** were almost equally toxic towards the other tested cancer cell lines while as, compound **4** bearing a para OMe group in R moiety was selectively active against colon (HCT-116) and lung (A549) cancer cell line with IC_{50} of 8.7 and 10.2 μM respectively. Among **7**, **20** and **21** containing electron withdrawing group (cyano) in R moiety at different positions, **20** having cyano group at ortho position displayed better results in comparison to corresponding meta and para analogs and displayed superior potency to BEZ-235 against T47D and A431 cells with IC_{50} of 8.8 and 10.3 μM respectively. These results demonstrate the broad spectrum cytotoxic efficiency of compounds bearing electron withdrawing groups, besides the selective activity of analogs with electron donating moieties. Among all the tested cancer cell lines, colon (HCT-116) cancer cell line was sensitive towards most of these derivatives, while as, prostate (PC-3) cancer cell line was least affected cell line by these derivatives.

From these results, we come to the conclusion that it is not only the effect of a particular group but also its position plays a significant role on the bioactivity profile. The most active compounds could become a better arsenal towards the corresponding sensitive cell lines after further polishing and fine tuning.

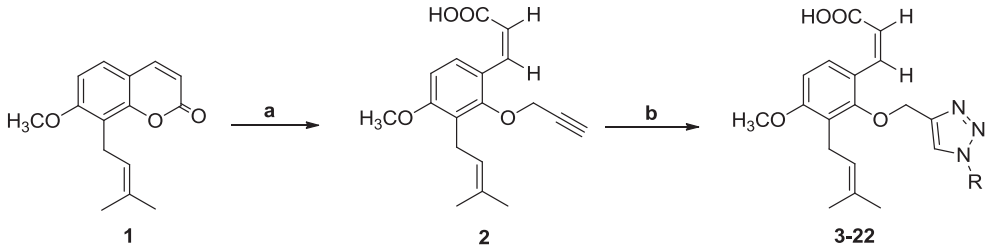
2.2.2. *Cell cycle analysis of compound **8***

The effect of the most active osthol analog, **8** on the DNA content by cell cycle phase distribution was evaluated by flow cytometry using colon (Colo-205) cancer cell line. Propidium iodide (PI) staining of Colo-205 cell line (5×10^5 cells/ml/6-well plates) exposed to different concentrations (1, 5, 10 and 25 μM) of **8** for 48 h induces apoptotic induction with apoptotic cell population of 12.5, 15.2, 15.7 and 24.1% respectively. For control cultures (untreated cells, DMSO) the apoptotic DNA content as reflected by apoptotic cell percentage of Colo-205 was 9.8%.

Also, it was found that **8** caused G1 phase arrest as percentage of cells in G1 phase increases from 45.7% in negative control to 46.8% at 5 μM. Camptothecin (1 μM) for Colo-205, used as positive controls, showed 69.4% cells in apoptotic phase (Fig. 3). Thus the molecule **8** exhibited significant apoptosis inducing effects in colon (Colo-205) in a dose dependent manner.

2.2.3. *Mitochondrial membrane potential (ΔΨm) loss*

Further studies were carried out to find the effect of **8** on the mitochondrial membrane potential (ΔΨ m) for the apoptotic induction in Colo-205 cells caused due to the loss of mitochondrial membrane potential. Colon (Colo-205) cells (5×10^5 cells/ml) were treated with concentrations of 1, 5, 10 and 25 μM and ΔΨm was



Where **R** is a substituted aromatic system

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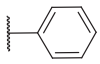
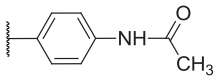
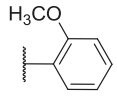
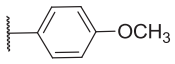
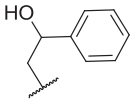
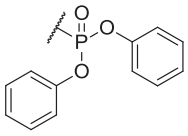
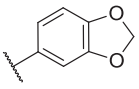
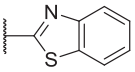
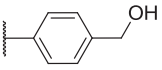
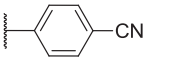
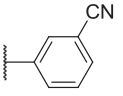
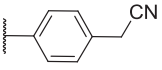
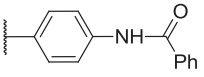
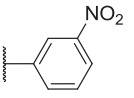
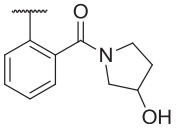
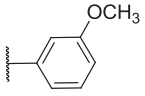
3		10		17	
4		11		18	
5		12		19	
6		13		20	
7		14		21	
8		15		22	
9		16			

Scheme 1. Reagents and conditions: (a) NaOH/DMSO, propargyl bromide, rt, 2 h (**b**) RN_3, *t*-butanol:H_2O (1:1), $CuSO_4$, sodium ascorbate, rt, 0.5–1 h.

measured by flow cytometry. In case of untreated cells having intact mitochondria, almost all the cells were bio-energetically active as evident by high rhodamine-123 (Rh-123) uptake compared to damaged mitochondria in positive control camptothecin (1 μM) for Colo-205. Compound **8** induced a remarkable increase in ΔΨm (mitochondrial membrane potential loss) which was found to be 24.4%, 26.9%, 29.7% and 29.9% at concentrations of 1, 5, 10 and 25 μM respectively (Fig. 4).

The results suggested that the apoptotic induction occurred due to the loss of mitochondrial membrane potential in Colo-205 cancer cell line. Loss of MMP indicates that mitochondrial membrane is leaky and permeable which represents a crucial stage in apoptosis in cancer cell death as it allows release of factors such as cytochrome *c* and the apoptotic inducing factor (AIF) that trigger the final and degradative stage of apoptosis. As a result of above processes, cytochrome *c*, which is normally found in the mitochondrial inter chamber, is released into the cytosol of apoptotic cells. This released cytochrome *c* through a cascade of events eventually leads to cleavage of a number of cellular proteins and the final cell death.

3. Conclusion

In conclusion, the current study demonstrates the cytotoxic activity of a novel library of triazolyl derivatives of osthol (**1**) anchored through the hydroxyl group of *cis*-ring opened lactone created through regioselective click chemistry approach and characterized by spectral data analysis. All the derivatives displayed significant broad-spectrum cytotoxic activity against the tested cancer cell lines. The most active derivative **8** exhibited potent

Table 1
IC_{50} (μM) values of analogs against colon (colo-205), colon (HCT-116), breast (T47D), lung (NCI-H322), lung (A549), prostate (PC-3) and Skin (A-431) cancer lines using MTT assay.

Entry	Colon (Colo-205)	Colon (HCT-116)	Breast (T47D)	Lung (NCI-H322)	Lung (A549)	Prostate (PC-3)	Skin (A-431)
	IC_{50} (μM)						
1	>60	30.2	42.4	30.9	46.2	24.8	23.2
3	32.3	26.0	34.1	29.2	40.5	35.0	21.2
4	nd	8.7	Nd	nd	10.2	nd	nd
5	nd	23.0	Nd	42.2	5.6	nd	32.6
6	20.2	16.4	17.2	23.0	42.1	nd	20.8
7	32.0	21	10.2	18.6	42.2	nd	23.0
8	1.3	4.9	3.6	41.0	35.2	26.4	7.2
9	25.4	32.0	13.4	nd	nd	36.2	31.5
10	22.6	nd	17.4	45.7	nd	nd	23.4
11	43.0	45.3	17.3	35.4	nd	14.2	nd
12	nd	20.4	13.2	22.7	19.4	32.0	23.1
13	19.3	31.7	40.0	27.1	nd	28.3	16.4
14	nd	23.1	Nd	42.3	2.2	nd	32.7
15	20.3	16.4	23.3	17.2	42.7	nd	21.2
16	nd	21.6	20.4	25.2	42.7	nd	23.2
17	12.2	32.2	Nd	41.5	43.0	nd	32.6
18	nd	35.5	30.6	nd	nd	36.2	43.3
19	14.5	nd	11.0	45.6	nd	24.2	12.4
20	3.2	38.3	8.8	14.4	38.6	nd	10.3
21	26.8	35.3	19.2	26.5	nd	45.5	22.9
22	6.9	27.3	15.4	43.2	nd	42.1	18.4
BEZ-235	0.085	0.044	11.2	10.3	6.5	12.3	12.5

IC_{50} values are expressed in μM concentration.
nd: not determined.
BEZ-235 was used as positive control.

anticancer activity by inducing apoptosis in Colo-205 cell line through the disruption of mitochondrial membrane potential ($\Delta\Psi m$). However, *in vivo* studies are warranted to investigate the mechanisms of action responsible for the cytotoxic activity of the most active derivatives.

4. Experimental

4.1. Chemistry

All reagents for chemical synthesis were obtained from Sigma Aldrich and the solvents used in reactions were distilled and dried prior to use. All the chemical reactions were monitored by TLC on 0.25 mm silica gel 60 F254 plates (E. Merck) using 2% ceric ammonium sulphate solution for detection of the spots. Purification of compounds was carried out by column chromatography using Silica gel 60–120 mesh stationary phase. ^{1}H NMR and ^{13}C NMR spectra (with chemical shifts expressed in δ and coupling constants in Hertz) were recorded on Bruker DPX 200, 400 and DPX 500 instruments using $CDCl_3$ or CD_3OD as the solvents with TMS as internal standard. High resolution mass spectra (HRMS) were recorded on Agilent Technologies 6540 instrument and IR recorded on an FT-IR Bruker (270-30) spectrophotometer. Melting points of compounds were recorded on Buchi melting point apparatus B-542.

4.1.1. Synthesis of (Z)-3-(4-methoxy-3-(3-methylbut-2-enyl)-2-(prop-2-ynyloxy)phenyl)acrylic acid **2** (Scheme 1)

To a solution of osthol (**1**) (2 g, 12.3 mmol) in DMSO (15 ml), crushed NaOH pellets (1.87 g, 13.1 mmol) were added and stirred for 30 min at 25 °C. Solution of propargyl bromide (1.1 ml, 14.8 mmol) in DMSO was then added slowly to the mixture and the suspension was stirred for 2–3 h. Progress of reaction was monitored using TLC at regular intervals. After the completion of reaction, the reaction mixture was extracted with ethyl acetate (3 × 30 ml) and the combined organic layer was dried over sodium sulphate and purified through column chromatography to give pure product **2** in 96% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ : 1.63 (3H, s), 1.78 (3H, s), 2.52 (s, 1H), 3.38 (d, J = 6.81 Hz, 2H), 3.86 (s, 3H), 4.45 (m, 2H), 5.17 (t, J = 6.82 Hz, 1H), 5.92 (d, J = 12.4 Hz, 1H), 6.67 (d, J = 8.7 Hz, 1H), 7.26 (d, J = 12.8 Hz, 1H), 7.58 (d, J = 8.64 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.91, 22.69, 23.40, 55.74, 62.16, 75.55, 78.89, 106.64, 117.69, 120.99, 122.31, 129.54, 131.77, 132.15, 141.65, 155.96, 159.79, 170.89. IR (KBr) ν_{max} cm^{-1}: 2941, 1682, 1608, 1593, 1486, 1425, 1384, 1366, 1314, 1266, 1162, 1118, 1088. HR-ESIMS *m/z*: calcd for $C_{18}H_{20}O_4$ $[M+H]^+$ 301.1452, found 301.1459.

4.1.2. General procedure for synthesis of azides

To a solution of particular aromatic amine in 1,4-Dioxane (at the rate of 50 mg/ml) at −15.0 °C, 5 equivalents of 2 M Sulphuric acid was added in small instalments while stirring. After 5 min 2 equivalents of 3 M sodium nitrite was added drop wise and after 30 min 3 equivalents of 3 M sodium azide was added drop wise carefully. Reaction was brought to room temperature and extracted with diethyl ether for at least three times. Organic layers were washed with saturated sodium bicarbonate solution two times, dried over anhydrous sodium sulphate and concentrated to a minimum volume under reduced pressure on Rotary evaporator without making use of heating from water bath.

4.1.3. General procedure for synthesis triazolyl derivatives (**3**–**22**)

Compound **2** (3 mmol) and different freshly prepared organic azides (3 mmol) were suspended in 10 ml of a 1:1 water:*t*-butanol mixture. Sodium ascorbate (0.3 mmol, 300 μL of freshly prepared 1 M solution in water) was added, followed by copper (II) sulphate pentahydrate (0.03 mmol, in 100 μl of water). The heterogeneous mixture was stirred vigorously till the completion of reaction. The reaction mixture was extracted with ethyl acetate (30 × 3 ml) and the combined organic layer was dried over sodium sulphate and purified through column chromatography to give pure **3**–**23** in excellent yields of 90–95%.

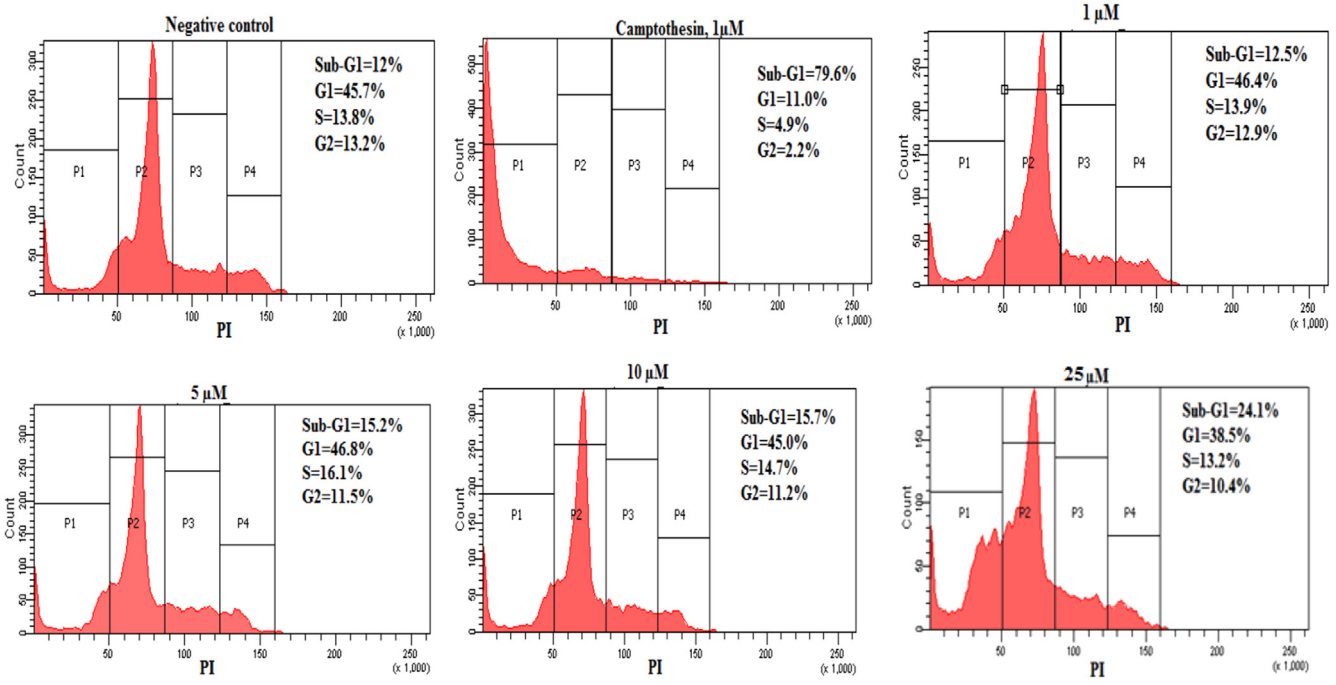


Fig. 3. Effect of **8** on cell cycle phase distribution of colon cancer cell line (Colo-205). Flow cytometric analysis of Colo-205 cells after propidium iodide staining. Cells were incubated for 48 h in presence of **8** at (0, 1, 5, 10 and 25 μM) concentration. Figures show the representative staining profile of one of two similar experiments. P1 (Sub-G1) is the population of apoptotic cells, which increases from 12.0% in case of negative control to 24.1% at 25 μM of **8**. At lower concentrations of 1 and 5 μM G1 phase cell cycle arrest of 46.4% and 46.8% respectively as compared to negative control of 45.7% was found. Camptothecin (1 μM) was used as positive control. P1, P2, P3 and P4 represents Sub-G1, G1, S and G2 phases of cell cycle in the figure respectively.

4.1.3.1. (Z)-3-(2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***3***. Colourless liquid. Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.67 (s, 3H), 1.73 (s, 3H), 3.38 (d, J = 6.5 Hz, 2H), 3.8 (s, 3H), 5.12 (s, 2H), 5.30 (t, J = 6.82 Hz, 1H), 5.93 (d, J = 12.17 Hz, 1H), 6.71 (d, J = 8.6 Hz, 1H), 7.24 (d, J = 12.29 Hz, 1H), 7.31 (m, 2H), 7.52 (m, 2H), 7.83 (m, 2H), 8.02 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.89, 23.09, 25.71, 29.60, 55.71, 67.51, 106.47, 120.53, 120.55, 120.57, 121.56, 122.76, 123.21, 123.75, 128.71, 129.26, 129.75, 129.79, 131.37, 136.98, 138.41, 155.48, 159.29, 171.07. IR (KBr) ν_{max} cm^{-1}: 2856, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{24}H_{25}N_3O_4$ [M+H] $^+$ 420.1917, found 420.1919.

4.1.3.2. (Z)-3-(2-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***4***. Colourless liquid. Yield: 93%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.67 (s, 3H), 1.73 (s, 3H), 3.35 (d, J = 6.62 Hz, 2H), 3.83 (s, 6H), 5.08 (s, 2H), 5.14 (t, J = 6.67 Hz, 1H), 5.90 (d, J = 12.17 Hz, 1H), 6.67 (d, J = 8.53 Hz, 1H), 7.01 (d, J = 8.74 Hz, 2H), 7.23 (d, J = 12.16 Hz, 1H), 7.53 (d, J = 8.38 Hz, 1H), 7.61 (d, J = 8.76 Hz, 2H), 7.89 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.94, 23.14, 25.74, 25.74, 29.69, 55.61, 55.70, 106.49, 114.77, 121.24, 121.58, 122.14, 122.66, 123.38, 123.80, 128.9, 129.46, 129.47, 130.35, 131.67, 141.37, 155.62, 159.79, 159.85, 172.01. IR (KBr) ν_{max} cm^{-1}: 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{25}H_{27}N_3O_5$ [M+H]$^+$ 450.2023, found 450.2019.

4.1.3.3. (Z)-3-(2-((1-(2-chloropyridin-3-yl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***5***. Yellow liquid. Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.67 (s, 3H), 1.73 (s, 3H), 3.34 (d, J = 6.90 Hz, 2H), 3.84 (s, 3H), 5.14 (s, 2H), 5.17 (t, J = 7.01 Hz, 1H), 5.91 (d, J = 12.01 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 14.40 Hz, 1H), 7.47 (m, 1H), 7.54 (d, J = 8.04 Hz, 1H), 8.04 (d, J = 7.95 Hz, 1H), 8.08 (s, 1H), 8.55 (d, J = 6.40 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.93, 23.17, 25.73, 33.05, 55.73, 62.15, 67.33, 106.59, 117.84, 122.59, 123.50, 125.15, 129.51, 131.71, 131.99, 136.02, 141.51, 144.07, 144.88, 150.25, 155.93, 159.80, 171.05. IR (KBr) ν_{max} cm^{-1}: 2953, 2791, 1725, 1608, 1437, 1393, 1304, 1276, 1196, 1032. HR-ESIMS *m/z*: calcd for $C_{23}H_{25}ClN_4O_4$ [M+H] $^+$ 455.1458, found 455.1452.

4.1.3.4. (Z)-3-(2-((1-(benzo[d]thiazol-2-yl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***6***. Brown liquid. Yield: 94%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.67 (s, 3H), 1.73 (s, 3H), 3.40 (d, J = 6.75 Hz, 2H), 3.83 (s, 3H), 4.46 (s, 2H), 5.17 (t, J = 6.92 Hz, 1H), 5.92 (d, J = 12.00 Hz, 1H), 6.78 (d, 1H, J = 8.16 Hz), 7.23 (d, J = 12.40 Hz, 1H), 7.41 (d, J = 8.59 Hz, 1H), 7.33–7.62 (m, 4H), 7.96 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.92, 25.75, 29.11, 31.93, 55.73, 62.15, 75.55, 78.93, 106.61, 121.04, 121.87, 122.66, 122.92, 123.30, 123.44, 125.65, 125.80, 126.18, 126.74, 129.56, 131.73, 141.31, 155.94, 159.70, 160.03, 171.06. IR (KBr) ν_{max}cm^{-1}: 3477, 2922, 1770, 1472, 1346, 1049, 778, 688, 644, 558, 548, 490. HR-ESIMS *m/z*: calcd for $C_{25}H_{24}N_4O_4S$ [M+H] $^+$ 477.1591, found 477.1580.

4.1.3.5. 5. (Z)-3-(2-((3-(4-cyanophenyl)-3H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***7***. Yellow liquid. Yield: 90%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.64 (s, 3H), 1.74 (s, 3H), 3.34 (d, 2H, J = 6.49 Hz), 3.83 (s, 3H), 5.10 (s, 2H), 5.15 (t, J = 6.67 Hz, 1H), 6.68 (d, J = 12.23 Hz, 1H), 7.10 (d, 1H, J = 8.72 Hz), 7.25 (d, J = 12.18 Hz, 1H), 7.48 (d, J = 8.67 Hz, 1H), 7.82 (d, J = 8.31, 2H), 7.90 (d, J = 8.24 Hz, 2H), 8.05 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 15.72, 20.94, 23.52, 53.52, 69.81, 79.69, 105.41, 110.22, 117.61, 118.23, 118.24, 119.26, 120.24, 122.25, 127.45, 129.26, 131.73, 138.72, 139.41, 143.32, 158.45, 159.81, 170.06. IR (KBr) ν_{max}cm^{-1}: 3732, 3314, 2918, 1764, 1681, 1585, 1461, 1299, 1170, 1126, 1050, 994, 944, 899,

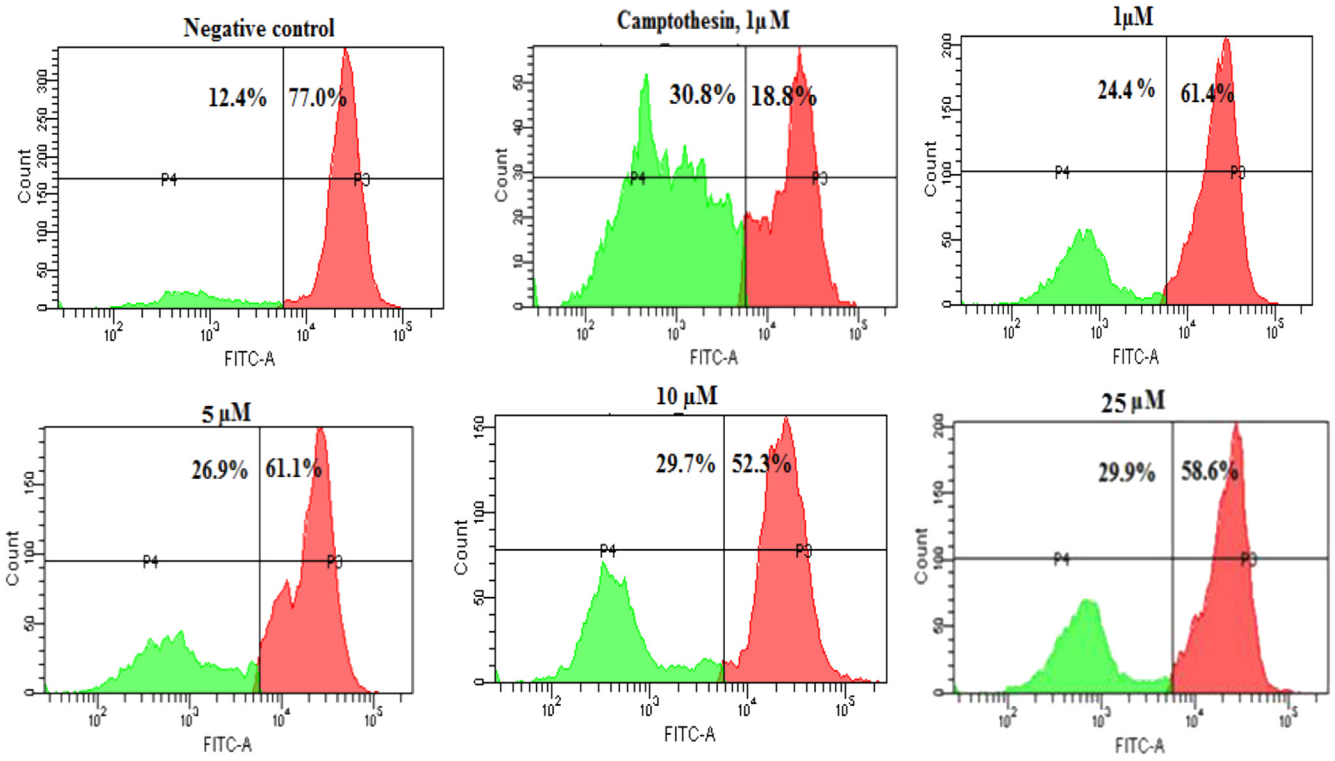


Fig. 4. Effect of **8** on mitochondrial membrane potential loss (ΔΨm). **8** induced loss of mitochondrial membrane potential (ΔΨm) in colon cancer cell line (Colo-205) incubated with the compound at different concentrations (0, 1, 5, 10 and 25 μM) in 6 well plate for 48 h treatment. Figures show the representative of one of two similar experiments. P4 is the percentage of loss of mitochondrial membrane potential (ΔΨm), which increases from 12.4% in case of negative control to 29.9% in case of 25 μM of **8**. Camptothecin (1 μM) was used as positive control. P3 and P4 represents percentage of intact and loss mitochondrial membrane potential (ΔΨm) respectively.

845, 775, 720, 654. HR-ESIMS *m/z*: calcd for $C_{25}H_{24}N_4O_4$ [M+H] 445.1870, found 445.1869.

4.1.3.6. (Z)-3-(2-((1-(benzylnitrile)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***8***. Yellow liquid. Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.78 (s, 6H), 3.35 (d, *J* = 6.53 Hz, 2H), 3.68 (s, 2H), 3.82 (s, 3H), 5.13 (s, 2H), 5.17 (t, *J* = 6.65 Hz, 1H), 5.78 (d, *J* = 12.45 Hz, 1H), 6.72 (d, *J* = 8.23 Hz, 1H), 7.25 (d, *J* = 12.20 Hz, 1H), 7.48 (d, *J* = 7.91 Hz, 1H), 7.82 (d, *J* = 8.31, 2H), 7.90 (d, *J* = 8.24 Hz, 2H), 8.10 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 17.92, 23.17, 25.71, 29.67, 55.72, 67.15, 75.52, 78.9, 106.61, 120.52, 121.71, 122.62, 123.47, 127.36, 130.28, 131.10, 131.69, 140.70, 144.17, 144.52, 155.24, 155.86, 159.80, 170.07. IR (KBr) ν_{max} cm^{-1}: 3480, 2932, 1764, 1700, 1601, 1478, 1298, 1208, 1167, 1124, 987, 949, 838, 733, 672, 571, 527. HR-ESIMS *m/z*: calcd for $C_{28}H_{26}N_4O_4$ [M+H] $^+$ 459.2026, found 459.2022.

4.1.3.7. (Z)-3-(2-((1(3-hydroxypyrrolidin-1-yl)(phenyl)methanone)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl) phenyl)acrylic acid ***9***. Brown solid. Mp: 124 °C. Yield: 94%. ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.30 (s, 3H), 1.72 (s, 3H), 1.83 (m, 2H), 2.04 (m, 1H), 3.20 (m, 2H), 3.20 (d, *J* = 6.91 Hz, 2H), 3.62 (m, 2H), 3.82 (s, 3H), 5.01 (s, 2H), 5.14 (t, *J* = 7.01 Hz, 1H), 5.89 (d, *J* = 12.07 Hz, 1H), 6.65 (d, 1H, *J* = 8.02 Hz), 7.10 (d, *J* = 13.01 Hz, 1H), 7.45 (d, *J* = 8.01 Hz, 1H), 7.51–7.59 (m, 4H), 7.99 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 17.90, 23.13, 25.72, 31.06, 33.76, 54.23, 55.71, 62.09, 67.20, 69.35, 70.28, 75.57, 106.52, 121.47, 122.68, 123.40, 124.65, 124.74, 127.4, 129.33, 129.83, 130.47, 131.65, 133.15, 155.41, 159.47, 159.52, 167.08, 170.01. IR (KBr) ν_{max} cm^{-1}: 3456, 2912, 2867, 1728, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{29}H_{32}N_4O_6$ [M+H] $^+$ 533.2394, found 533.2396.

4.1.3.8. (Z)-3-(2-((N-phenylacetamide)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***10***. White solid. Mp: 166 °C. Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.49 (s, 3H), 1.57 (s, 3H), 2.03 (s, 2H), 3.18 (d, *J* = 6.51 Hz, 2H), 3.68 (s, 3H), 4.96 (t, *J* = 6.01 Hz, 1H), 5.18 (s, 2H), 6.55 (d, *J* = 8.55 Hz, 1H), 6.97 (d, *J* = 11.94 Hz, 1H), 7.40 (d, *J* = 8.52 Hz, 1H), 7.46 (d, *J* = 13.89 Hz, 1H), 7.50 (d, *J* = 8.55 Hz, 2H), 7.65 (d, *J* = 8.59 Hz, 2H), 7.89 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 17.82, 25.62, 27.14, 29.28, 55.65, 63.4, 69.68, 106.50, 118.58, 119.27, 120.77, 121.37, 121.55, 121.81, 122.65, 123.38, 129.18, 131.55, 132.44, 139.77, 144.20, 155.29, 159.52, 169.67, 171.40. IR (KBr) ν_{max} cm^{-1}: 2967, 2851, 1767, 1634, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{26}H_{28}N_4O_5$ [M+H] $^+$ 477.2132, found 477.2132.

4.1.3.9. (Z)-3-(2-((1-Phenylethanol)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***11***. Brown liquid. Yield: 91%. ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.66 (s, 3H), 1.78 (s, 3H), 3.39 (d, *J* = 6.58 Hz, 2H), 3.81 (s, 3H), 4.48 (m, 2H), 4.86 (m, 1H), 5.01 (s, 2H), 5.16 (t, *J* = 6.39 Hz, 1H), 6.63 (d, *J* = 12.45 Hz, 1H), 7.25 (d, 1H, *J* = 7.06 Hz), 7.35 (d, *J* = 12.01 Hz, 1H), 7.38 (d, *J* = 7.63 Hz, 1H), 7.41–7.50 (m, 5H), 7.90 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 18.07, 25.74, 27.21, 31.93, 55.71, 62.14, 73.56, 73.63, 78.93, 106.63, 118.53, 119.72, 120.96, 121.35, 121.53, 121.87, 122.66, 123.39, 129.33, 131.71, 132.43, 139.71, 144.22, 155.95, 159.69, 169.5. IR (KBr) ν_{max} cm^{-1}: 3423, 2956, 2867, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z* calcd for $C_{26}H_{29}N_3O_5$ [M+H] $^+$ 464.2179, found 464.2182.

4.1.3.10. (Z)-3-(2-((1-(benzo[d][1,3]dioxol-6-yl)-1H-1,2,3-triazol-4-yl)methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***12***. Brown solid. Mp: 133 °C. Yield: 91%. ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 1.67 (s, 3H), 1.70, (s, 3H), 3.33 (d, *J* = 5.98 Hz, 2H), 3.81 (s, 3H), 5.06

(s, 2H), 5.15 (t, J = 6.09 Hz, 1H), 6.02 (s, 2H), 6.08 (d, J = 13.42 Hz, 1H), 6.67 (d, J = 8.51 Hz, 1H), 6.91 (d, J = 12.42 Hz, 1H), 7.13 (d, J = 8.45 Hz, 1H), 7.22–7.30 (m, 3H), 7.87 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.86, 23.07, 25.66, 29.62, 55.62, 67.46, 75.49, 77.31, 101.56, 102.60, 106.44, 108.73, 114.11, 121.20, 122.57, 122.62, 123.27, 129.43, 131.56, 131.65, 147.96, 148.52, 155.53, 159.62, 169.12. IR (KBr) ν_{max} cm^{-1}: 3467, 2898, 2851, 1726, 1645, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{25}H_{25}N_3O_6$ [M+H] $^+$ 464.1816, found 464.1815.

4.1.3.11. (Z)-3-(2-((1phenylmethanol)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***13***. Colourless liquid. Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.64 (s, 3H), 1.78 (s, 3H), 3.33 (d, J = 6.37 Hz, 2H), 3.78 (s, 3H), 4.45 (s, 2H), 5.08 (s, 2H), 5.13 (t, J = 6.56 Hz, 1H), 6.60 (d, J = 12.03 Hz, 1H), 7.13 (d, J = 7.63 Hz, 1H), 7.34 (m, 1H), 7.43 (m, 2H), 7.60 (d, J = 11.67 Hz, 1H), 7.63 (d, J = 7.92 Hz, 1H), 7.66 (m, 1H), 7.93 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.71, 22.94, 25.34, 29.41, 55.43, 66.95, 75.29, 77.31, 106.38, 119.09, 119.81, 121.88, 122.47, 123.01, 123.20, 126.88, 128.95, 129.51, 131.36, 131.41, 136.73, 143.39, 155.04, 159.13, 169.21. IR (KBr) ν_{max}cm^{-1}: 3432, 2923, 2851, 1745, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{25}H_{27}N_3O_5$ [M+H] $^+$ 450.2023, found 450.2025.

4.1.3.12. (Z)-3-(2-((1-(2-iodophenyl)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***14***. Brown liquid. Yield: 90%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.63 (s, 3H), 1.75 (s, 3H), 3.33 (d, J = 6.75 Hz, 2H), 3.83 (s, 3H), 5.13 (s, 2H), 5.20 (t, J = 5.96 Hz, 1H), 5.95 (d, J = 12.95 Hz, 1H), 6.99 (m, 1H), 7.26 (d, J = 12.56 Hz, 1H), 7.28 (d, J = 8.16 Hz, 1H), 7.48 (m, 1H), 7.55 (m, 2H), 7.98 (s, 1H), 7.99 (d, J = 8.00 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.14, 29.42, 22.93, 55.43, 66.91, 75.22, 77.33, 106.37, 119.01, 119.82, 121.80, 122.44, 123.01, 123.23, 126.87, 128.96, 129.54, 131.34, 131.47, 136.79, 143.35, 155.05, 159.17, 169.29. IR (KBr) ν_{max} cm^{-1}: 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{24}H_{24}IN_3O_4$ [M+H] $^+$ 456.0884, found 456.0855.

4.1.3.13. (Z)-3-(2-((N-phenylbenzamide)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***15***. Colourless solid. Mp: 142 °C Yield: 95%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.66 (s, 3H), 1.78 (s, 3H), 3.37 (d, J = 6.32 Hz, 2H), 3.83 (s, 3H), 4.45 (m, 2H), 5.12 (t, J = 6.25 Hz, 1H), 5.94 (d, J = 12.76 Hz, 1H), 6.67 (d, J = 7.65 Hz, 1H), 7.04 (d, J = 12.31 Hz, 1H), 7.07 (d, J = 7.89 Hz, 2H), 7.49–7.70 (m, 5H), 7.74 (d, J = 8.31 Hz, 1H), 7.97 (d, J = 8.01 Hz, 2H), 8.01 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.99, 24.57, 24.85, 56.79, 59.78, 108.33, 113.69, 119.51, 121.89, 123.01, 123.92, 125.24, 128.03, 128.77, 129.10, 131.68, 132.48, 133.12, 134.93, 136.00, 159.98, 161.02, 169.58. IR (KBr) ν_{max} cm^{-1}: 3446, 2956, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{31}H_{30}N_4O_5$ [M+H] $^+$539.2288, found 588.2289.

4.1.3.14. (Z)-3-(2-((3-(3-methoxyphenyl)-3H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***16***. Yellow liquid. Yield: 94%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.63 (s, 3H), 1.73 (s, 3H), 3.35 (d, J = 6.62 Hz, 2H), 3.80 (s, 3H), 3.89 (s, 3H), 5.08 (s, 2H), 5.14 (t, J = 6.67 Hz, 1H), 5.90 (d, J = 12.13 Hz, 1H), 6.67 (d, J = 8.53 Hz, 1H), 7.32 (m, 3H), 7.01 (d, J = 8.74 Hz, 1H), 7.23 (d, J = 12.16 Hz, 1H), 7.5 (m, 1H), 7.97 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.99, 24.57, 25.85, 56.04, 56.79, 59.78, 59.78, 108.07, 108.33, 113.21, 113.69, 118.30, 119.51, 121.89, 123.01, 129.10, 131.11, 131.61, 132.48, 133.10, 136.00, 137.84, 159.25, 161.02, 170.08. IR (KBr) ν_{max} cm^{-1}: 2923, 2851, 1725, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{25}H_{27}N_3O_5$ [M+H] $^+$ 450.2023, found 450.2026.

4.1.3.15. (Z)-3-(2-((3-(2-methoxyphenyl)-3H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***17***. Yellow liquid. Yield: 95%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.64 (s, 3H), 1.73 (s, 3H), 3.33 (d, J = 6.70 Hz, 2H), 3.81 (s, 3H), 3.87 (s, 3H), 5.10 (s, 2H), 5.15 (t, J = 6.67 Hz, 1H), 5.91 (d, J = 12.30 Hz, 1H), 6.67 (d, J = 8.61 Hz, 1H), 7.06–7.11 (m, 2H), 7.26 (d, J = 11.45 Hz, 1H), 7.42 (m, 1H), 7.55 (d, 1H, J = 8.52 Hz), 7.78 (d, J = 7.85 Hz, 1H), 8.10 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.91, 22.70, 29.17, 55.70, 55.93, 66.23, 67.59, 106.45, 114.08, 117.99, 121.24, 122.72, 125.57, 128.85, 129.44, 130.12, 130.21, 130.93, 139.29, 141.09, 143.05, 151.11, 155.74, 159.74, 168.23. IR (KBr) ν_{max} cm^{-1}: 3476, 2920, 1763, 1598, 1457, 1298, 1039, 947, 852, 808, 750, 699, 642. HR-ESIMS *m/z* calcd for $C_{25}H_{27}N_3O_5$ [M+H]$^+$ 450.2023, found 450.2023.

4.1.3.16. (Z)-3-(2-((diphenylphosphonate)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***18***. Yellow solid. Mp: 172 °C. Yield: 94%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.67 (s, 3H), 1.78 (s, 3H), 3.39 (d, J = 6.65 Hz, 2H), 3.81 (s, 3H), 4.45 (s, 2H), 5.15 (t, J = 6.65 Hz, 1H), 5.93 (d, J = 12.42 Hz, 1H), 6.64 (d, J = 8.64 Hz, 1H), 7.25–7.31 (m, 5H), 7.38 (d, J = 12.75 Hz, 1H), 7.57 (d, 1H, J = 8.68 Hz), 8.01 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.97, 23.41, 25.76, 55.74, 62.18, 106.65, 107.64, 112.85, 117.58, 120.23, 120.31, 122.33, 124.65, 126.15, 129.39, 129.52, 131.59, 131.94, 142.38, 155.76, 156.44, 159.57, 160.64, 170.01. IR (KBr) ν_{max} cm^{-1}: 3436, 2927, 1766, 1600, 1460, 1314, 1127, 1041, 856, 787, 564. HR-ESIMS *m/z* calcd for $C_{25}H_{27}N_3O_5$ [M+H] $^+$ 576.1923, found 576.1927.

4.1.3.17. (Z)-3-(2-((3-(2-fluorophenyl)-3H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***19***. Yellow liquid. Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.65 (s, 3H), 1.74 (s, 3H), 3.35 (d, J = 6.67 Hz, 2H), 3.84 (s, 3H), 5.12 (s, 2H), 5.19 (t, J = 6.32 Hz, 1H), 5.94 (d, J = 12.01 Hz, 1H), 6.69 (d, J = 8.01 Hz, 1H), 7.33 (d, J = 12.0 Hz, 1H), 7.36 (m, 1H), 7.45 (m, 2H), 7.54 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 8.68 Hz, 1H), 8.01 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.94, 23.41, 25.73, 55.80, 68.18, 106.57, 108.57, 112.85, 117.04, 122.06, 122.34, 122.66, 124.48, 124.88, 125.27, 129.42, 130.23, 130.39, 141.10, 142.14, 144.06, 159.54, 161.64, 169.64. IR (KBr) ν_{max} cm^{-1}: 3437, 2927, 2851, 1745, 1608, 1455, 1393, 1304, 1276, 1196, 1113, 1032, 826, 762, 615. HR-ESIMS *m/z*: calcd for $C_{25}H_{27}N_3O_5$ [M+H] $^+$ 438.1723, found 476.1927.

4.1.3.18. (Z)-3-(2-((3-(2-cyanophenyl)-3H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***20***. Colourless liquid. Yield: 90%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.65 (s, 3H), 1.74 (s, 3H), 3.37 (d, J = 6.49 Hz, 2H), 3.87 (s, 3H), 5.10 (s, 2H), 5.19 (t, J = 6.67 Hz, 1H), 5.92 (d, J = 12.03 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 12.0 Hz, 1H), 7.49 (d, J = 8.01 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 7.78–7.80 (m, 3H), 8.24 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 15.72, 20.94, 23.52, 53.52, 69.81, 79.69, 105.41, 110.22, 117.61, 118.23, 118.24, 119.26, 120.24, 122.25, 127.45, 129.26, 131.73, 132.42, 133.08,138.72, 139.41, 143.32, 158.45, 159.81, 170.06. IR (KBr) ν_{max} cm^{-1}: 3732, 3314, 2918, 1764, 1681, 1585, 1461, 1299, 1170, 1126, 1050, 994, 944, 899, 845, 775, 720, 654. HR-ESIMS *m/z*: calcd for $C_{25}H_{24}N_4O_4$ [M+H] 445.1870, found 445.1872.

4.1.3.19. (Z)-3-(2-((3-(3-cyanophenyl)-3H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***21***. Yellow liquid. Yield: 95%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.66 (s, 3H), 1.78 (s, 3H), 3.37 (d, J = 6.49 Hz, 2H), 3.87 (s, 3H), 5.10 (s, 2H), 5.19 (t, J = 6.67 Hz, 1H), 5.91 (d, J = 12.34 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 12.0 Hz, 1H), 7.49 (d, J = 8.01 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 7.68–7.74 (m, 3H), 8.04 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 15.72, 20.94, 23.52, 53.52, 69.81, 79.69, 105.41, 110.22, 117.61, 118.23, 118.24, 119.26, 120.24, 122.25, 127.45, 129.26, 131.73, 132.42, 133.08,138.72, 139.41, 143.32, 158.45, 159.81, 170.06. IR (KBr) ν_{max}

cm^{-1}: 3738, 3317, 2915, 1756, 1681, 1585, 1461, 1309, 1178, 1047, 994, 944, 899. HR-ESIMS *m/z*: calcd for $C_{25}H_{24}N_4O_4$ [M+H] 445.1870, found 445.1870.

4.1.3.20. (Z)-3-(2-((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxy-3-(3-methylbut-2-enyl)phenyl)acrylic acid ***22***. Brown liquid. Yield: 92%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.72 (s, 3H), 1.82 (s, 3H), 3.37 (d, $J = 6.67$ Hz, 2H), 3.91 (s, 3H), 5.10 (s, 2H), 5.20 (t, $J = 6.67$ Hz, 1H), 6.21 (d, $J = 12.56$ Hz, 1H), 6.94 (d, $J = 8.67$ Hz, 1H), 7.45 (d, $J = 12.18$ Hz, 1H), 7.75 (d, $J = 8.78$ Hz, 1H), 7.82 (m, 2H), 8.01 (s, 1H), 8.21 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.99, 24.57, 27.85, 56.79, 59.85, 108.33, 113.69, 119.51, 119.90, 121.49, 121.89, 123.01, 127.96, 129.10, 131.09, 131.73, 135.69, 145.64, 146.86, 157.98, 159.02, 170.60. IR (KBr) ν_{max} cm^{-1}: 3732, 2947, 1764, 1681, 1598, 1457, 1298, 1047, 994, 944, 899. HR-ESIMS *m/z*: calcd for $C_{25}H_{24}N_4O_4$ [M+H] 465.1770, found 465.1772.

4.2. Biology

RPMI-1640 medium, Penicillin, streptomycin, foetal calf serum, sodium bicarbonate, phosphate buffer saline, sulphorhodamine, trypsin, gentamycin sulphate, tryphan blue, ethanol, DMSO, paraformaldehyde were purchased from Sigma Chemicals Co. Glacial acetic acid from Fischer scientific, PBS and trichloroactetic acid (TCA) from Merck specialties private limited. All the human cancer cell lines colon (colo-205), colon (HCT-116), breast (T47D), lung (NCI–H322), lung (A549), prostate (PC-3) and Skin (A-431) were obtained from National Center for Cell Science, Ganeshkhind, Pune-4111007 (India) and National Cancer Institute, Biological Testing Branch DTP/DCTD/NCI, Frederick Cancer Research and Development Center, Fairview Center, Suite 205, 1003 West 7th Street, Frederick, MD 21701-8527 (USA).

4.2.1. MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] assay

To determine IC_{50} value, all the derivatives were further evaluated against colon (colo-205), colon (HCT-116), breast (T47D), lung (NCI-H322), lung (A549), prostate (PC-3) and Skin (A-431) (3×10^3 cells per mL per 100 μL per well) were plated into a 96 well tissue culture plate. Cells were incubated in Dulbecco's modified eagle's media containing 10% foetal calf serum, supplemented with 100 units/mL penicillin, 100 mg/L streptomycin in a humidified atmosphere in 5.0% CO_2 at 37 °C, and were subcultured at 1:5 ratio once a week. Foetal calf serum was reduced to 3% for the experiments with the test material (0.04–60 μM) for 24 h and then further treated with MTT (250 μg/mL) for 3 h. Inhibition of formation of coloured MTT formazan was taken as an index of cytotoxicity activity. The amount of coloured formazan derivative was determined by measuring optical density (OD) using TECAN microplate reader (Infinite M200 PRO) at 570 nm. Further the IC_{50} values on the cancer cells of different tissue origin used for screening were determined by non-linear regression analysis using graph pad prism software [49].

4.2.2. DNA content and cell cycle phase distribution of compound *8*

Colon (Colo-205) cancer cell line was seeded in 6-well culture plate (5×10^5 cells/ml/6-well plates). Cells were incubated for 24 h and then treated with different concentrations (1, 5, 10 and 25 μM) of compound **8**. After 48 h of treatment, cells were harvested by centrifugation for 5 min at 1000 rpm, washed twice with PBS and fixed with 70% ethanol overnight at −20 °C, and then stained with DNA staining solution (20 mg/ml PI, 0.1 mM EDTA, 10 μg/ml RNAase and 1% triton X-100 in PBS) for 30 min in dark. DNA content was measured by using a flow cytometer analysis system (BD FACS Aria). Data from 10,000 cells were collected for each data file. All the histograms were analysed using FACS Diva software.

4.2.3. Mitochondrial membrane potential ($\Delta\Psi m$) for cellular energy status

Mitochondrial membrane potential ($\Delta\Psi m$) was measured using flow cytometry with 2 μM rhodamine-123 (Rh-123), cellpermeable cationic dye that preferentially enters mitochondria based on the highly negative mitochondrial membrane potential. Depolarization of the membrane results in the loss of Rhodamine-123 from the mitochondria and a decrease in intracellular fluorescence. Colon (Colo-205) cancer cell line was seeded in 6-well culture plate (5×10^5 cells/ml/6-well plates). Cells were incubated for 24 h and then treated with different concentrations (1, 5, 10 and 25 μM) of compound **8** for 48 h treatment. Rh-123 (10 μg/ml) was added 1 h before the termination of experiment, incubated at 37 °C for 30 min and thereafter washed with PBS. The pellet collected by centrifugation, was resuspended in 300 μL of PBS. The florescence intensity of Rh-123 in cells was analysed using flow cytometer set at 485 nm.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.069.

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