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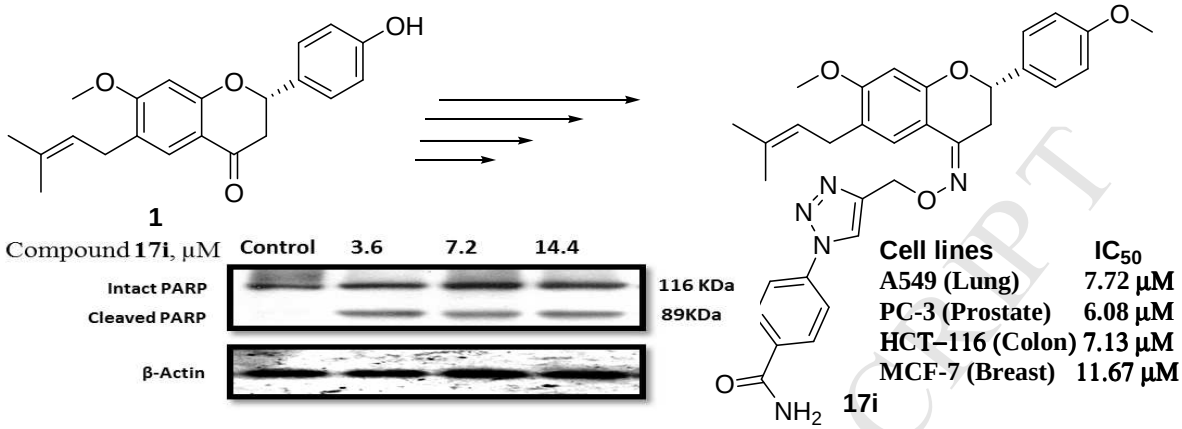

Design and synthesis of bavachinin analogues as potent cytotoxic agents

Synthesis and biological evaluation of novel bavachinin analogs as anticancer agents

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Abstract

A library of 28 analogs of bavachinin including aliphatic and aromatic ethers, epoxide, chalcone, oxime, semicarbazide, oxime ether and triazole derivatives have been synthesized and evaluated for cytotoxicity against four different human cancer cell lines. Bio-evaluation studies exhibited better cytotoxic profile for many analogs compare to bavachinin. Best results were observed for a 1,2,3-triazole analog (**17i**) with IC_{50} values 7.72, 16.08, 7.13 and 11.67 µM against lung (A549), prostate (PC-3), colon (HCT-116) and breast (MCF-7) cancer cell lines respectively. This analog showed three and four fold improvement in cytotoxicity against HCT-116 and A549 cell lines than parent molecule (**1**). Structure activity relationship (SAR) study for all synthesized analogs was carried out. Further, mechanistic study of the lead molecule (**17i**) revealed that it inhibits colony formation and *in vitro* migration of human colon cancer cells (HCT-116). Also, it induced the morphological changes and mediated the apoptotic cell death of HCT-116 cells with perturbance in mitochondrial membrane potential (MMP) and PARP cleavage.

1. Introduction

Flavanoids are one of largest classes of polyphenolic compounds that occur naturally in plants which possess broad spectrum of biological activities and are considered as suitable therapeutic agents against cancer. They generally possess a phenylbenzopyrone structure (C6-C3-C6) consisting of two aromatic rings, A and B connected by a central pyran ring C. The saturation level and opening of central pyran ring categorize various classes of flavonoids namely flavones, flavonols, isoflavones, flavanols, flavanones and flavanonols [1,2]. Unlike other flavonoids, flavanones like bavachinin **1**, hesperetin **2** and naringenin **3** (Fig.1) have been potential sources to search for new leads in the area of cancer therapy [3,4].

Insert Figure 1.

The seeds of *Psoralea corylifolia* are major source of bavachinin [5] and exhibit diverse pharmacological activities including anticancer [3], PPAR agonist [6], anti-inflammatory [7], anti-alzheimer [8] and immunomodulatory [9]. Cytotoxic effects of bavachinin have also been studied on various cancer cell lines [3, 10] and possess 20S proteasome inhibitory activity that inhibits the signaling action of NFκB leading to cell death *via* apoptosis [11]. Beside this bavachinin also inhibited *in vitro* migration of human KB cells and during *in vivo* studies in nude mice with KB xenografts, it significantly reduced tumor volume and CD31 expression [12]. Bavachinin **1** nucleus is capable of undergoing suitable chemical transformation studies at various key positions available on molecule (Fig. 1). Several bavachinin derivatives have synthesized and reported for their different biological activities. Chen et al. prepared bavachinin derivatives **4** and **5** *via* biotransformation to study their T cell differentiation effects [13]. Dai et al. reported anticancer activity of bavachinin and its enzymatically synthesized glucoside **6** [14] while Du et al. reported PPAR-γ agonist activity of several bavachinin analogs among which compounds **7** and **8** were found as most potent derivatives [15] (Fig. 2). However, further investigation is desired to develop the potent anticancer agents based on bavachinin.

Insert Figure 2.

Therefore, the natural product bavachinin was isolated in bulk quantity from the seeds of *Psoralea corylifolia* for synthesis of its analogs being our interest in isolation and structural modification of natural products for drug discovery of potent analogs in the area of anticancer [16-19]. Further the synthesized analogs were screened for cytotoxicity against four different human cancer cell lines (A549, PC3, HCT-116 and MCF-7). The most active compound was taken further to study its cytotoxic mode of action.

2. Results and discussion

2.1. Chemistry

Bavachinin **1** was isolated in gram quantities from the seeds of *P. corylifolia* and was used for structural modification studies. The configuration at C-2 an asymmetric center in the molecule was observed (*S*) based on NOESY (Fig. S1) which was also confirmed by comparing the observed specific rotation value $[\alpha]_D$ = -20 (c = 1.0, $CHCl_3$) with literature [5, 20]. Different analogs of bavachinin **1** were synthesized with modifications at ring A, B, C and prenyl chain of the molecule (Fig. 3) as shown in scheme 1 and 2. Aliphatic ether analogs of bavachinin (**9a-e**) were synthesized by reacting **1** with appropriate alkyl halides in acetone in the presence of potassium carbonate as base [21]. Analogs **9f-i**, aromatic ethers of bavachinin were synthesized through Chan-Lam coupling by reacting **1** with various aryl boronic acids in DCM in the presence of copper acetate and pyridine [22]. Epoxide analog of bavachinin (**10**) was synthesized by reacting **1** with *m*-chloroperbenzoic acid (*m*-CPBA) in DCM as per earlier report [23]. NOESY spectra (Fig S2) is provided in supporting information. Compound **11**, chalcone analog of **1** was obtained by treating it with sodium hydride in ethanol. Compound **12**, carbonyl reduction analog was synthesized by reacting **1** with sodium borohydride ($NaBH_4$) in methanol as per our earlier report [17] and its configuration was found 2*S*,4*R* at C-2 and C-4 position on the basis of 2D NMR (COSY, HSQC and HMBC) experiments including NOESY (Fig S3-8). Further the configuration was also confirmed through observed specific rotation $[\alpha]_D$ = +19 (c = 1.0, $CHCl_3$) which was found in comparison with literature [24]. Compound **12a**, double bond reduced product was prepared by hydrogenation of bavachinin in presence of 10% Pd/C [23]. Compounds **13** and **14**, oxime and semicarbazide analogs of bavachinin were synthesized by

treating **1** with hydroxylamine hydrochloride and semicarbazide hydrochloride respectively in ethanol as solvent [25].

Insert Figure 3
Insert Scheme 1.

Bavachinin triazoles were synthesized at oxime moiety to prepare anticancer analogs in the light of literature [19, 26]. For this, methyl ether of bavachinin **9a** was reacted with hydroxylamine hydrochloride to prepare its oxime analog **15**. Compound **15** was reacted with propargyl bromide in acetone in presence of potassium carbonate to furnish compound **16** which was further subjected to Cu (I) catalyzed 1,3-dipolar cycloaddition reaction (click chemistry) using various substituted aromatic azides to provide 1,2,3-triazole derivatives **17a-k** in excellent yields [27] (scheme 2). To confirm the configuration of C=N double bond in triazole analogs **17a-k**, NOESY experiment of one compound i.e. **17i** was recorded. On interpretation of data, the signal at δ 5.37 (-OCH_2) did not show any correlation with either of protons at C-3 (-CH_2) that rule out the *E* configuration while the same signal at δ 5.37 (-OCH_2) showing weak correlation with aromatic proton singlet at δ 7.63 (H-5) which indicate Z configuration of C=N (Fig. S9).

Insert Scheme 2.

2.2. Biology

2.2.1. Cell growth inhibition studies

Compound **1** and its synthesized analogs were screened against a panel of four different human cancer cell lines namely lung (A549), prostate (PC-3), colon (HCT-116) and breast (MCF-7) to evaluate their cytotoxic potential using SRB assay for 48 hours. Results are shown in table 1 and values given are the average of triplicate analysis. In preliminary screening at 50 µM, bavachinin and some of its derivatives including compounds **9e, 9i, 10**, **12**, **12a**, **13**, **17d, 17f** and **17i** exhibited significant inhibition effects (>50% inhibition) against all the experimental cancer cell

lines. Compounds **9a-f**, **9i**, **13**, **16**, **17f** and **17j** showed maximum inhibition effects against colon cancer cells (HCT-116). Compounds **10**, **11 12a,** and **14** displayed maximum inhibition effects against breast cancer cells (MCF-7) where as compounds **15** and **17c** sensitized lung cancer cells the most. Overall, compounds **17d** and **17i** exhibited most potent cytotoxic effects (100% inhibition) against all the cell lines examined while compounds **9g**, **12, 17a**, **17b**, **17e**, **17g** and **17h** did not possess significant cytotoxicity against any of the cell lines tested.

Insert Table 1.

2.2.2. IC_{50} and structure activity relationship (SAR) studies

Compounds showing significant cytotoxic effects (>75% growth inhibition) at 50 µM (Table 1) were further screened at four different concentrations 1, 5, 10 and 30 µM to calculate their IC_{50} values. The calculated IC_{50} values are shown in Table 2. Parent molecule showed IC_{50} values 30.5, 25.4, 20.7 and 19.5 µM against lung, prostate, colon and breast cancer cell lines respectively. Compound **13** displayed three fold more potent cytotoxicity than parent (**1**) against lung cancer cell line (A549). Compound **17i** was found to be three and four fold more active than the parent against lung (A549) and colon (HCT-116) cancer cell lines respectively.

Insert Table 2.

Structure activity relationship (SAR) of compound **1** was established on the basis of growth inhibition and IC_{50} data and can be summarized as follows: Alkyl and aryl ether derivatives (**9a-i**) affected colon cancer cells (HCT-116) the most than other cell lines tested but did not exhibit significant anticancer effects compared to the parent (**1**). Epoxide analog (**10**) displayed comparable cytotoxic effects where as oxime (**13**) and semicarbazide (**14**) analogs were found to be more active as compared to the parent against A549 cell line. Chalcone analog (**11**) inhibited breast cancer cells the most. Reduction analog (**12**) did not display any significant cytotoxicity against any of the examined cell line while double bond reduced prenyl analog (**12a**) showed

comparable cytotoxic effects to parent against lung cancer cell line (A549). Oxime ether analog (**16**) displayed cytotoxic effects against lung (A549) and colon cancer (HCT-116) cell lines. Among triazole derivatives (**17a-k**), analogs with electron releasing groups (**17a**, **17b** and **17h**) did not reveal any significant cytotoxicity except for the analog having *–OH* substitution at *p*-position (**17f**) showing cytotoxicity to some extent against HCT-116 cell line. Loss of activity in **17e** having *–CH_2OH* group at 3-position than **17d** having *–CH_2OH* group at 2-position showed position of substituent is essential for the activity. Analogs with electron withdrawing groups such as *-COC_6H_5* and *-CN* (**17j** and **17k**) showed cytotoxicity to some extent but analog having benzamide moiety (**17i**) was found to be most potent having IC_{50} values 7.72, 16.08, 7.13 and 11.67 µM against lung, prostate, colon and breast cancer cell lines respectively, hence chosen for further cell death mechanistic study.

*2.2.3. Compound **17i** inhibited cell proliferation during colony formation assay in colon cancer cells (HCT-116)*

In vitro clonogenic assay was performed to measure the cell proliferation capability i.e. the ability of a single cell to grow into a colony. This assay tests each and every cell in the population for its ability to undergo unlimited divisions [28]. HCT-116 cells were treated with different concentrations of **17i** at 3.6, 7.2 and 14.4 µM in which colonies were formed after 14 days treatment. It was found that compound **17i** significantly decreased colony formation in colon cancer cells (HCT-116) in a concentration dependent manner as compared to the untreated control (A) (Fig. 4).

Insert Figure 4

*2.2.4. Compound **17i** inhibited in vitro cell migration during wound healing assay in HCT-116 cells*

In vitro scratch assay or wound healing assay is a well-developed method for cell migration studies. In this method, a wound is created in a cell monolayer in order to monitor the cell migration and proliferation [29]. In this experiment, HCT-116 cells monolayer was scratched and treated with **17i** at different concentrations (3.6, 7.2 and 14.4 µM) for 24h. The wound area was measured at two different time points 0 and 24h in which the percentage reduction in cell migration was quantitatively assessed with 0h post scratch. Rate of migration was assessed as compared to the corresponding control (A). It was observed that cell migration was inhibited in a concentration dependent manner (Fig. 5).

Insert Figure 5.

*2.2.5. Compound **17i** induced morphological changes in HCT-116 cells*

The colon cancer cells (HCT-116) were treated with **17i** at 3.6, 7.2 and 14.4 µM for 24 h. Phase contrast microscopy was used to assess the morphological changes in the cells. Characteristic morphological changes were observed in the **17i** treated cells in a concentration-dependent manner (Fig. 6).

Insert Figure 6.

*2.2.6. Compound **17i** triggered mitochondrial membrane potential (MMP) loss*

MMP loss occurs due to disruption in the mitochondrial membrane permeabilisation with the release of apoptogenic stimuli leading to cell death [30]. The changes in potential of mitochondrial membrane of HCT-116 cells were analyzed by staining with a fluorescent cationic dye, rhodamine-123 (RH-123). The rate of fluorescence decay is directly proportional to MMP loss. When HCT-116 cells treated with different concentrations of **17i** (3.6, 7.2 and 14.4 µM) dramatic loss in MMP was observed in a concentration dependent manner whereas nuclei of untreated cells (control) were having intact mitochondria (Fig. 7).

Insert Figure 7

*2.2.7. Compound **17i*** mediated PARP cleavage

PARP [poly (ADP ribose) polymerase] is a nuclear protein having the main function in repairing DNA damage in response to cellular stress [31]. Cleaved PARP activity in HCT-116 cells was analyzed using Western blot analysis where it loses its nick sensor function and becomes inactive towards DNA damage. PARP cleavage started at a concentration of 3.6 µM and produced 89 KDa c-terminal fragment and continued the cleaved pattern as concentration increased with β-actin serving as a loading control. This shows that PARP played an important role in apoptotic cell pathway (Fig. 8).

Insert Figure 8.

3. Conclusion

In conclusion, a novel library of 28 analogs of bavachinin was synthesized and evaluated for cytotoxicity against a panel of four different human cancer cell lines namely lung (A549), prostate (PC-3), colon (HCT-116) and breast (MCF-7) along with the parent molecule. Cytotoxicity results revealed that many compounds exhibit significant cytotoxic effects against different cancer cell lines. More significantly, compounds **13, 14** and **17i** were found to be promising derivatives with IC_{50} <20 µM against various cancer cell lines examined. Compound **17i** was found as lead compound among the synthesized analogs with three and four fold improvement in cytotoxicity against colon and lung cancer cell lines as compared to the parent molecule. Moreover, **17i** induced apoptotic cell death through loss of MMP and PARP cleavage. It also inhibited the colony formation, cell migration and induced the morphological changes in a concentration dependent manner. All these experiments indicated the potential of compound **17i** to develop as potent anticancer agent.

4. Experimental section

4.1. Chemistry

All the reagents and solvents used for isolation, purification and synthesis were purchased from Sigma-Aldrich/E. Merck. All chemical reactions were monitored by TLC on silica gel 60 F_{254} plates (E. Merck) using 2% ceric ammonium sulphate solution as spraying reagent for detection of spots. All synthesized derivatives were purified by column chromatography using silica gel 60-120 mesh as stationary phase. NMR spectra were recorded on Bruker DPX 400 and DPX 500 instruments using CD_3OD, acetone-d6, $CDCl_3$ as the solvent taking TMS as the internal standard. The chemical shifts are expressed in δ and coupling constant in Hertz. High Resolution Mass Spectra (HRMS) were recorded on Agilent Technologies 6540 instrument. Melting points were determined (uncorrected) with a Buchi melting point apparatus B-545. The optical rotation $[\alpha]_D$ values were measured with a Perkin Elmer 241 polarimeter.

*4.1.1. Isolation of Bavachinin (**1**)*

Bavachinin was isolated in gram quantity by repeated column chromatography in 5-8% EtoAc/ Hexane of DCM: MeOH (1: 1) extract prepared from seeds of *Psoralea corylifolia*. Initially, Bakuchiol, the major constituents of the extract was isolated [17], after that bavachinin was obtained as crystalline compound which was characterized by spectroscopic techniques as reported in literature [5, 20].

*4.1.2. General procedure for the synthesis of aliphatic ether analogs (**9a-e**).* Bavachinin (100 mg, 0.30 mmol) was dissolved in acetone and to this K_2CO_3 (0.30 mmol) and respective alkyl halides (0.30 mmol) were added. The reaction mixture was stirred at room temperature for 2 h till the completion (monitored by TLC analysis). After completion, mixture was extracted with

ethyl acetate and water (3 times). The combined organic layers were dried over sodium sulphate and concentrated on rotavapour. The crude product obtained was purified by column chromatography on silica gel 60-120 mesh with 3-5% ethyl acetate/hexane as the eluent to afford the desired pure products in 90-94% yield. The spectral data of all the derivatives (**9a-e**) are given below.

*4.1.2.1. 7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-one (**9a**).* Yield: 91%, gummy mass, $[\alpha]_D$ = -12 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69 and 1.74 (3H each, s, H-4'' and H-5''), 2.75 and 3.02 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 7.3 Hz, H-1''), 3.83 and 3.84 (3H each, s, 2 × -OCH_3), 5.27 (1H, t, *J* = 8.0 Hz. H-2''), 5.37 (1H, dd, *J* = 13.0, 4.0 Hz, H-2), 6.44 (1H, s, H-8), 6.95 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.39 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.68 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.86, 164.09, 162.27, 159.97, 132.89, 131.08, 127.71 × 2, 127.1, 124.87, 121.84, 114.2 × 2, 114.04, 98.83, 79.8, 55.72, 55.34, 44.17, 27.8, 25.79 and 17.72. HRMS *m/z* calcd for $C_{22}H_{25}O_4$ $[M + H]^+$ 353.1747, found 353.1735.

*4.1.2.2. 2-(4-ethoxyphenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)chroman-4-one (**9b**).* Yield: 90%, gummy mass, $[\alpha]_D$ = -6 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (3H, t, *J* = 7.0 Hz, -CH_2CH_3), 1.69 and 1.74 (3H each, s, H-4'' and H-5''), 2.75 and 3.02 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 7.3 Hz, H-1''), 3.83 (3H each, s, -OCH_3), 4.04 (2H, q, *J* = 8.0 Hz, -CH_2CH$_3$), 5.28 (1H, t, *J* = 8.0 Hz. H-2''), 5.35 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.44 (1H, s, H-8), 6.95 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.37 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.67 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.07, 164.09, 162.32, 159.32, 133.0, 130.81, 127.75 × 2, 127.07, 124.84, 121.78, 114.71 × 2, 113.97, 98.82, 79.88, 63.56, 55.76, 44.17, 27.83, 25.89, 17.78 and 14.82. HRMS *m/z* calcd for $C_{23}H_{27}O_4$ $[M + H]^+$ 367.1904, found 367.1900.

*4.1.2.3. 7-methoxy-6-(3-methylbut-2-en-1-yl)-2-(4-propoxyphenyl)chroman-4-one (**9c**).* Yield: 91%, gummy mass, $[\alpha]_D$ = -12 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (3H, t, *J* = 7.0 Hz, -CH_3), 1.69 and 1.74 (3H each, s, H-4'' and H-5''), 1.81 (2H, q, *J* = 8.0 Hz, -C*H*$_2$CH$_3$), 2.76 and 3.02 (1H each, m, H-3a and 3b), 3.25 (2H, d, *J* = 8.0 Hz, H-1''), 3.83 (3H, s, -OC*H*$_3$), 3.94 (2H, q, *J* = 8.0 Hz, - C*H*$_2$CH$_2$CH$_3$), 5.27 (1H, t, *J* = 8.0 Hz. H-2''), 5.37 (1H, dd, *J* = 13.0, 4.0 Hz, H-2), 6.44 (1H, s, H-8), 6.94 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.38 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.67 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.07, 164.09, 162.32, 159.52, 132.99, 130.75, 127.73 × 2, 127.06, 124.82, 121.78, 114.74 × 2, 113.97, 98.83, 79.87, 69.61, 55.76, 44.16, 27.82, 25.88, 22.56, 17.78 and 10.54. HRMS *m/z* calcd for $C_{24}H_{29}O_4$ $[M + H]^+$ 381.2060, found 381.2064.

*4.1.2.4. 2-(4-butoxyphenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)chroman-4-one (**9d**).* Yield: 92%, gummy mass, $[\alpha]_D$ = -12 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98 (3H, t, *J* = 7.0 Hz, -CH_3), 1.51 (2H, q, *J* = 8.0 Hz, -C*H*$_2$CH$_3$), 1.7 and 1.74 (3H each, s, H-4'' and H-5''), 1.78 (2H, m, -C*H*$_2$CH$_2$CH$_3$), 2.77 and 3.05 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0 Hz, H-1''), 3.84 (3H, s, -OC*H*$_3$), 3.98 (2H, t, *J* = 8.0 Hz, -C*H*$_2$CH$_2$CH$_2$CH$_3$), 5.28 (1H, t, *J* = 8.0 Hz. H-2''), 5.39 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.44 (1H, s, H-8), 6.94 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.37 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.67 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.11, 164.1, 162.32, 159.54, 133.01, 130.72, 127.73 × 2, 127.06, 124.83, 121.77, 114.74 × 2, 113.97, 98.82, 79.88, 67.81, 55.77, 44.16, 27.82, 25.88, 19.25, 17.78, 13.87 and 10.54. HRMS *m/z* calcd for $C_{25}H_{31}O_4$ $[M + H]^+$ 395.2217, found 395.2234.

*4.1.2.5. 7-methoxy-6-(3-methylbut-2-en-1-yl)-2-(4-(prop-2-yn-1-yloxy)phenyl)chroman-4-one (**9e**).* Yield: 94%, gummy mass, $[\alpha]_D$ = -14 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.7 and 1.75 (3H each, s, H-4'' and H-5''), 2.54 (1H, t, *J* = 4.0 Hz, ≡C*H*), 2.77 and 3.03 (1H each,

m, H-3a and 3b), 3.25 (2H, d, *J* = 8.0 Hz, H-1''), 3.85 (3H, s, -OC*H*$_3$), 4.72 (2H, s, -OC*H*$_2$), 5.28 (1H, t, *J* = 8.0 Hz. H-2''), 5.39 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.45 (1H, s, H-8), 7.05 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.42 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.68 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.97, 164.13, 162.26, 157.82, 133.05, 131.94, 127.76 × 2, 127.08, 124.92, 121.73, 115.16 × 2, 113.95, 98.81, 79.68, 78.33, 75.8, 55.85, 55.79, 44.16, 27.83, 25.9, 17.79. HRMS *m/z* calcd for $C_{24}H_{25}O_4$ $[M + H]^+$ 377.1747, found 377.1762.

*4.1.3. General procedure for the synthesis of aromatic ether analogs (**9f-i**).* To a solution of bavachinin (0.30 mmol) in dichloromethane, $Cu(OAc)_2$ (0.45 mmol), pyridine (0.60 mmol) and respective boronic acids (0.90 mmol) were added. The reaction mixture was kept on stirring at r.t. for 2-3 h till the completion (monitored by TLC analysis). After completion of the reaction, usual workup and purification was done which afforded compounds **9f-i** in 85-90% yield.

*4.1.3.1. 7-methoxy-6-(3-methylbut-2-en-1-yl)-2-(4-phenoxyphenyl)chroman-4-one (**9f**).* Yield: 85%, gummy mass, $[\alpha]_D$ = -18 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.7 and 1.74 (3H each, s, H-4'' and H-5''), 2.77 and 3.05 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0 Hz, H-1''), 3.85 (3H, s, -OC*H*$_3$), 5.27 (1H, t, *J* = 8.0 Hz, H-2''), 5.44 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.46 (1H, s, H-8), 7.05 (4H, m, H-3', H-5', H-2''' and H-6'''), 7.13 (1H, t, *J* = 8.0 Hz, H-4'''), 7.35 (2H, m, H-3''' and H-5'''), 7.44 (2H, t, *J* = 8.0 Hz, H-2' and H-6'), 7.68 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.73, 164.15, 162.19, 157.81, 156.79, 133.56, 133.04, 129.86 × 2, 127.87 × 2, 127.12, 125.01, 123.68, 121.75, 119.24 × 2, 118.85 × 2, 113.99, 98.81, 79.69, 55.78, 44.27, 27.82, 25.87, 17.78. HRMS *m/z* calcd for $C_{27}H_{27}O_4$ $[M + H]^+$, 415.1009 found 415.1029.

*4.1.3.2. 2-4-(4-fluorophenoxy)phenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)chroman-4-one (**9g**).* Yield: 87%, gummy mass, $[\alpha]_D$ = -16 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69 and 1.74 (3H each, s, H-4'' and H-5''), 2.78 and 3.01 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0

Hz, H-1''), 3.84 (3H, s, -OC*H*$_3$), 5.27 (1H, t, *J* = 8.0 Hz, H-2''), 5.44 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.45 (1H, s, H-8), 7.0 (6H, m, H-3', 5', 2''', 3''', 5''' and H-6'''), 7.42 (2H, t, *J* = 8.0 Hz, H-2' and H-6'), 7.68 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.58, 164.14, 162.14, 160.3, 158.17, 152.47, 133.51, 132.98, 127.89 × 2, 127.13, 125.03, 121.77, 120.89, 120.81, 118.26 × 2, 116.53, 116.3, 114.02, 98.8, 79.6, 55.74, 44.24, 27.81, 25.81, 17.74. HRMS *m/z* calcd for $C_{27}H_{26}FO4$ $[M + H]^+$ 433.1810, found 433.1808.

*4.1.3.3. 7-methoxy-6-(3-methylbut-2-en-1-yl)-2-(4-(3-nitrophenoxy)phenyl)chroman-4-one (**9h**).* Yield: 88%, gummy mass, $[\alpha]_D$ = -22 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.7 and 1.74 (3H each, s, H-4'' and H-5''), 2.84 and 3.03 (1H each, m, H-3a and 3b), 3.25 (2H, d, *J* = 8.0 Hz, H-1''), 3.87 (3H, s, -OC*H*$_3$), 5.27 (1H, t, *J* = 8.0 Hz, H-2''), 5.48 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.48 (1H, s, H-8),), 7.11 (2H, d, *J* = 8.0 Hz, H-3' and 5'), 7.35 (1H, dd, *J* = 8.0 Hz, 4.0 Hz, H-6'''), 7.52 (3H, m, H-2', 6' and 5'''), 7.69 (1H, s, H-5), 7.82 (1H, t, *J* = 4.0 Hz, H-2'''), 7.96 (1H, m, H-4'''). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.54, 164.23, 162.1, 158.13, 155.9, 149.35, 135.4, 133.11, 130.46, 128.28 × 2, 127.13, 125.16, 124.47, 121.68, 119.91 × 2, 118.03, 113.95, 113.11, 98.8, 79.46, 55.82, 44.3, 27.81, 25.86, 17.77. HRMS *m/z* calcd for $C_{27}H_{26}NO_6$ $[M + H]^+$ 460.1755, found 460.1750.

*4.1.3.4. 2-(4-(4-bromophenoxy)phenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)chroman-4-one (**9i**).* Yield: 89%, gummy mass, $[\alpha]_D$ = -18 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69 and 1.74 (3H each, s, H-4'' and H-5''), 2.82 and 3.01 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0 Hz, H-1''), 3.84 (3H, s, -OC*H*$_3$), 5.27 (1H, t, *J* = 8.0 Hz, H-2''), 5.42 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.45 (1H, s, H-8), 6.9 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.03 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.44 (4H, m, H-2''', 3''', 5''' and H-6'''), 7.68 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.78, 164.24, 162.2, 157.23, 156.1, 134.12, 133.08, 132.82 × 2, 127.98 × 2, 127.15,

125.11, 121.71, 120.76 × 2, 119.03 × 2, 116.14, 113.95, 98.8, 79.58, 55.79, 44.24, 27.82, 25.86, 17.78. HRMS *m/z* calcd for $C_{27}H_{26}BrO4$ $[M + H]^+$ 493.1009, found 493.1029.

*4.1.4. Synthesis of 6-((3,3-dimethyloxiran-2-yl)methyl)-2-(4-hydroxyphenyl)-7-methoxychroman-4-one) (**10**).* Bavachinin (0.30 mmol) was dissolved in 3 mL DCM at 0^{o}C and to this *m*-CPBA (0.45 mmol) in 2 mL DCM was added slowly. The reaction mixture was stirred for 2 h at r.t. as per earlier report [23]. After completion, the mixture was extracted with ethyl acetate and water. The organic layer was dried and purified with 5% ethyl acetate/hexane as eluent to afford desired pure product **10** in 78% yield, gummy mass, $[\alpha]_D$ = -16 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 and 1.42 (3H each, s, H-4'' and H-5''), 2.81 (3H, m, 2 × H-1'' and H-3a), 3.09 (2H, m, H-2'' and H-3b), 3.83 (3H, s, -OCH_3), 5.38 (1H, t, *J* = 8.0 Hz, H-2), 6.46 (1H, s, H-8), 6.78 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.25 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.76 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.76, 164.45, 163.16, 156.96, 134.61, 133.62, 127.92 × 2, 121.1, 115.7 × 2, 113.99, 99.23, 80.05, 63.86, 60.21, 55.93, 43.83, 29.1, 24.76 and 18.88. HRMS *m/z* calcd for $C_{21}H_{23}O_5$ $[M + H]^+$ 355.1540, found 355.1571.

*4.1.5. Synthesis of (E)-1-(2-hydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**11**).* Bavachinin (0.30 mmol) was dissolved in 3.5 mL ethanol and to this NaH (0.3 mmol) was added and the mixture was kept on stirring at r.t. for half an hour. After completion of the reaction, usual workup was done and further purified with 7% ethyl acetate/hexane to furnish **11** in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69 and 1.73 (3H each, s, H-4'' and H-5''), 3.2 (2H, m, H-3), 3.83 (3H, s, -OC*H*$_3$), 5.23 (1H, t, *J* = 8.0 Hz, H-2), 6.37 (1H, s, H-8), 6.84 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.38 (1H, d, *J* = 16.0 Hz, H-2' and H-6'), 7.34 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.76 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.76, 164.7, 164.09, 159.89, 144.57, 132.85, 130.57 × 2, 129.77, 126.41, 122.19, 121.76,

117.05, 115.93 × 2, 113.22, 99.05, 55.54, 27.93, 25.56 and 17.63. HRMS *m/z* calcd for $C_{21}H_{23}O_4$ $[M + H]^+$ 339.1591, found 339.1594.

*4.1.6. Synthesis of 2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)chroman-4-ol (**12**).* Compound **1** (0.30 mmol) was dissolved in 4 mL methanol and to this $NaBH_4$ (0.45 mmol) was added slowly at 0^{o}C. The reaction mixture was stirred at r.t. for 1h and after completion of the reaction, the mixture was concentrated, diluted with water and further extracted with ethyl acetate. The organic layer was dried and purified with 8% EtOAc/hexane as eluent to provide desired product **12** in 70% yield, m. p. 141^{o}C, $[\alpha]_D$ = +19 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.71 and 1.73 (3H each, s, H-4'' and H-5''), 2.14 and 2.39 (1H each, m, H-3a and 3b), 3.25 (2H, d, *J* = 8.0 Hz, H-1''), 3.76 (3H, s, -OCH_3), 4.73 (1H, t, *J* = 4.0 Hz, H-4), 5.15 (1H, t, *J* = 8.0 Hz. H-2''), 5.29 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.43 (1H, s, H-8), 6.85 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.3 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.07 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 157.48, 156.95, 153.83, 131.97, 131.16, 127.42 × 2, 127.26, 123.11, 122.36, 117.44, 114.92 × 2, 98.45, 77.11, 64.93, 54.64, 39.78, 27.64, 24.84 and 16.89. HRMS *m/z* calcd for $C_{21}H_{24}NaO_4$ $[M + H]^+$ 363.1567, found 363.1569.

*4.1.7. Synthesis of 2-(4-hydroxyphenyl)-6-isopentyl-7-methoxy chroman-4-one (**12a**).* To a solution of bavachinin (0.30 mmol) in methanol, 10% Pd/C was added slowly while stirring at r.t. The mixture was kept on stirring for 3 h till completion [23, 26]. Then the reaction mixture was filtered, filtrate was concentrated and purified through silica gel column chromatography using 20% EtOAc/hexane as eluent which provided **12a** in 80 % yield, solid, m. p. 185^{o}C, $[\alpha]_D$ = -21 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (6H, d, *J* = 6.6 Hz, H-4'' and H-5''), 1.44 (2H, m, H-2''), 1.58 (1H, m, H-3''), 2.54 (2H, m, H-1''), 2.78 and 3.04 (1H each, m, H-3a and 3b), 3.83 (3H, s, -OC*H*$_3$), 5.39 (1H, dd, *J* = 13.4, 2.8 Hz, H-2), 6.44 (1H, s, H-8), 6.90

(2H, d, *J* = 8.6 Hz, H-3' and H-5'), 7.26 (1H, s, H-5), 7.35 (2H, d, *J* = 8.6 Hz, H-2' and H-6'). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.5, 164.47, 162.3, 156.25, 131.0, 127.98 × 2, 127.25, 126.36, 115.7 × 2, 113.85, 99.81, 79.83, 55.74, 44.14, 38.85, 27.97, 27.19, 22.58 × 2. HRMS *m/z* calcd for $C_{21}H_{25}O_4$ $[M + H]^+$ 341.1751, found 341.1754.

*4.1.8. Synthesis of 2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)chroman-4-one oxime (**13**).* To a solution of bavachinin (0.30 mmol) in ethanol was added pyridine (0.6 mmol) and $NH_2OH.HCl$ (0.45 mmol) dissolved in ethanol slowly at r.t. while stirring. The mixture was stirred for 1.5h till completion. After the usual work-up, mixture was dried and purified with 7% EtOAc/hexane as eluent to provide **13** in 85% yield, gummy mass, $[\alpha]_D$ = -9.0 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.7 and 1.72 (3H each, s, H-4'' and H-5''), 2.68 and 3.45 (1H each, m, H-3a and 3b), 3.23 (2H, d, *J* = 8.0 Hz, H-1''), 3.79 (3H, s, -OC*H*$_3$), 4.98 (1H, t, *J* = 8.0 Hz. H-2''), 5.27 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 6.44 (1H, s, H-8), 6.85 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.31 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.6 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.73, 156.97, 156.33, 149.85, 131.83, 131.06, 127.6 × 2, 123.99, 123.88, 122.53, 115.17 × 2, 110.38, 99.29, 77.4, 54.98, 30.0, 27.8, 25.14 and 17.05. HRMS *m/z* calcd for $C_{21}H_{24}NO_4$ $[M + H]^+$ 354.1700, found 354.1718.

*4.1.9. Synthesis of 2-(2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-en-1-yl)chroman-4-ylidene)hydrazinecarboxamide (**14**).* To a solution of bavachinin (0.3 mmol) in ethanol was added pyridine (0.6 mmol) and $NH_2CONHNH_2.HCl$ (0.45 mmol) dissolved in ethanol slowly at r.t. while stirring. The mixture was stirred for 1.5 h till completion. After the usual work-up, mixture was dried and purified with 10% EtOAc/hexane as eluent to provide **14** in 85% yield, white solid, m. p. 151^{o}C, $[\alpha]_D$ = +38.0 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (6H, s, H-4'' and H-5''), 2.67 and 3.03 (1H each, m, H-3a and 3b), 3.26 (2H, d, *J* = 8.0 Hz, H-

1’’), 3.8 (3H, s, -OC*H*$_3$), 5.02 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.27 (1H, t, *J* = 8.0 Hz, H-2’’), 6.45 (1H, s, H-8), 6.87 (2H, d, *J* = 8.0 Hz, H-3’ and H-5’), 7.32 (2H, d, *J* = 8.0 Hz, H-2’ and H-6’), 7.69 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.5, 160.25, 158.65, 158.42, 144.25, 133.5, 131.92, 129.08 × 3, 125.85, 124.1, 116.81 × 2, 113.24, 100.79, 78.7, 56.69, 33.26, 29.53, 26.81 and 18.87. HRMS *m/z* calcd for $C_{22}H_{26}N_3O_4$ $[M + H]^+$ 396.1918, found 396.1901.

*4.1.10. Synthesis of (Z)-7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-one oxime (**15**).* To synthesize compound **15**, firstly **9a** was prepared in gram quantities by reacting **1** (2.0 g, 5.9 mmol) with CH_3I (5.9 mmol) following the same procedure as mentioned in section 4.1.1. Then, to a solution of compound **9a** (1.9 g, 5.39 mmol) in ethanol was added pyridine (10.79 mmol) and NH_2OH. HCl (8.09 mmol) dissolved in ethanol slowly at r.t. while stirring. The mixture was stirred for 1.5 h till completion. After the usual work-up, mixture was dried and purified with 8% EtOAc/hexane as eluent to provide **15** in 90% yield, gummy mass, $[\alpha]_D = 0$ (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.68 and 1.72 (3H each, s, H-4’’ and H-5’’), 2.7 and 3.52 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0 Hz, H-1’’), 3.79 and 3.82 (3H each, s, 2 × -OC*H*$_3$), 5.01 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.28 (1H, t, *J* = 8.0 Hz. H-2’’), 6.44 (1H, s, H-8), 6.93 (2H, d, *J* = 8.0 Hz, H-3’ and H-5’), 7.4 (2H, d, *J* = 8.0 Hz, H-2’ and H-6’), 7.56 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.08, 159.73, 156.54, 150.2, 132.76, 132.01, 127.73 × 2, 124.32, 123.97, 122.25, 114.1 × 2, 109.81, 90.56, 77.26, 55.47, 55.36, 30.14, 27.91, 25.81 and 17.75. HRMS *m/z* calcd for $C_{22}H_{26}NO_4$ $[M + H]^+$ 368.1856, found 368.1868.

*4.1.11. Synthesis of (Z)-7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-oneO-prop-2-yn-1-yl oxime (**16**).* Compound **15** (5.17 mmol) was dissolved in acetone and to this K_2CO_3 (5.17 mmol) and propargyl bromide (5.17 mmol) were added. After the usual work-up, mixture was dried and purified with 8% EtOAc/hexane as eluent to provide **16** in 94% yield,

colorless solid, m.p. 154^{o}C, $[\alpha]_D$ = +5.0 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (6H each, s, H-4'' and H-5''), 2.49 (1H, t, *J* = 4.0 Hz, , ≡C*H*), 2.7 and 3.43 (1H each, m, H-3a and 3b), 3.26 (2H, d, *J* = 8.0 Hz, H-1''), 3.79 and 3.83 (3H each, s, 2 × -OCH_3), 4.77 (2H, d, *J* = 4.0 Hz, N-O-CH_2), 4.9 (1H, dd, *J* = 8.0 Hz, 4.0 Hz, H-2), 5.28 (1H, t, *J* = 8.0 Hz, H-2''), 6.43 (1H, s, H-8), 6.93 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.38 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.66 (1H, s, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.16, 159.72, 156.7, 150.46, 132.18, 131.91, 127.75 × 2, 124.56, 124.42, 122.71, 114.06 × 2, 109.61, 99.52, 79.95, 77.24, 74.33, 61.6, 55.47, 55.37, 30.94, 28.28, 25.84 and 17.85. HRMS *m/z* calcd for $C_{25}H_{28}NO_4$ $[M + H]^+$ 406.2013, found 406.2037.

*4.1.12. General procedure for the synthesis of triazolyl derivatives (**17a-k**).* Compounds (**17a-k**) were synthesized by reacting **16** (1 eq) with respective freshly prepared organic azides (1.1 eq) as per literature [27], in 6 mL water: *t*-butanol (1:1) mixture followed by the addition of sodium ascorbate and copper (II) sulphate pentahydrate (10 mol% each). The heterogeneous reaction mixture was stirred vigorously at room temperature till the completion of reaction. After completion, the reaction mixture was extracted with ethyl acetate and water. The combined organic layer was dried, concentrated and purified through column chromatography using 10-30% EtOAc:hexane mixture as eluent to furnish pure products in excellent yields. The spectral data of all triazolyl derivatives (**17a-k**) is given below.

*4.1.12.1. Synthesis of (Z)-N-(4-(4-((((7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-ylidene)amino)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)acetamide (**17a**).* Yield: 95%, gummy mass, $[\alpha]_D$ = +2 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67 (6H, s, H-4'' and H-5''), 2.13 (3H, s, -NHCOCH_3), 2.66 and 3.35 (1H each, m, H-3a and 3b), 3.2 (2H, d, *J* = 8.0 Hz, H-1''), 3.74 and 3.75 (3H each, s, 2 × -OCH_3), 4.95 (1H, dd, *J* = 12.0, 4.0 Hz, H-2),

5.23 (1H, t, *J* = 8.0 Hz. H-2’’), 5.3 (2H, s, -OC*H*$_2$C-), 6.38 (1H, s, H-8), 6.86 (2H, d, *J* = 8.0 Hz, H-3’ and H-5’), 7.31 (2H, d, *J* = 8.0 Hz, H-2’ and H-6’), 7.6 and 7.71 (2H each, d, *J* = 8.0 Hz, H-2’’’, 3’’’, 5’’’ and 6’’’), 7.62 (1H, s, H-5), 8.07 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.31, 160.19, 159.61, 156.62, 150.34, 145.23, 139.39, 132.39, 132.12, 131.85, 127.62 × 2, 124.34, 124.14, 122.53, 121.81, 121.17 × 2, 120.54 × 2, 113.92 × 2, 109.81, 99.47, 77.12, 66.85, 55.27, 55.12, 28.07, 25.54, 23.53, 22.59, 17.52. HRMS *m/z* calcd for $C_{33}H_{36}N_5O_5$ $[M + H]^+$ 582.2711, found 582.2699.

*4.1.12.2. Synthesis of (Z)-N-(4-(4-((((7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-ylidene)amino)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)benzamide (**17b**).* Yield: 91%, white solid, m. p. 205^{o}C, $[\alpha]_D$ = +5 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72 (6H, s, H-4’’ and H-5’’), 2.69 and 3.44 (1H each, m, H-3a and 3b), 3.25 (2H, d, *J* = 8.0 Hz, H-1’’), 3.78 and 3.8 (3H each, s, 2 × -OC*H*$_3$), 4.95 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.3 (1H, t, *J* = 8.0 Hz, H-2’’), 5.38 (2H, s, -OC*H*$_2$C-), 6.43 (1H, s, H-8), 6.91 (2H, d, *J* = 8.0 Hz, H-3’ and H-5’), 7.36 (2H, d, *J* = 8.0 Hz, H-2’ and H-6’), 7.5 (2H, t, *J* = 8.0 Hz, H-3’’’’ and 5’’’’), 7.57 (1H, t, *J* = 8.0 Hz, H-4’’’’), 7.66 (1H, s, H-5), 7.72 and 7.83 (2H each, d, *J* = 8.0 Hz, H-2’’’, 3’’’, 5’’’and 6’’’), 7.89 (2H, d, *J* = 8.0 Hz, H-2’’’’ and 6’’’’), 8.0 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.79, 160.14, 159.7, 156.61, 150.06, 145.62, 138.5, 134.46, 133.19, 132.29, 132.24, 131.88, 128.92 × 2, 127.73 × 2, 127.11 × 2, 124.39, 124.26, 122.65, 121.43 × 2, 121.28, 120.99 × 2, 114.04 × 2, 109.78, 99.59, 77.3, 67.37, 55.5, 55.32, 28.23, 25.87, 22.72, 17.86. HRMS *m/z* calcd for $C_{38}H_{38}N_5O_5$ $[M + H]^+$ 644.2867, found 644.2853.

*4.1.12.3. Synthesis of (Z)-(7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-oneO-((1-(2-chloropyridin-3-yl)-1H-1,2,3-triazol-4-yl)methyl)oxime (**17c**).* Yield: 90%, gummy mass, $[\alpha]_D$ = +6 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.69 (6H, s, H-4’’ and H-5’’),

2.67 and 3.42 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0 Hz, H-1''), 3.78 and 3.81 (3H each, s, 2 × -OC*H*$_3$), 4.96 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.27 (1H, t, *J* = 8.0 Hz. H-2''), 5.41 (2H, s, -OC*H*$_2$C-), 6.42 (1H, s, H-8), 6.91 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.36 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.46 (1H, dd, *J* = 8.0, 4.0 Hz, H-3'''), 7.64 (1H, s, H-5), 8.04 and 8.54 (1H each, dd, *J* = 8.0, 4.0 Hz, H-2'''and 4'''), 8.17 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.14, 159.7, 156.65, 150.2, 145.29, 144.83, 136.02, 132.24, 132.13, 131.88, 127.7 × 2, 125.07, 124.42 × 2, 124.23, 123.38, 122.64, 114.06 × 2, 109.72, 99.59, 77.24, 67.09, 55.47, 55.34, 30.94, 28.21, 25.8, 17.8. HRMS *m/z* calcd for $C_{30}H_{31}ClN_5O_4$ $[M + H]^+$ 560.2059, found 560.2034.

4.1.12.4. Synthesis of (Z)-(7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-oneO-((1-(2-hydroxymethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)oxime ***(17d)***. Yield: 92%, gummy mass, $[\alpha]_D$ = +3 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.7 (6H, s, H-4'' and H-5''), 2.69 and 3.42 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0 Hz, H-1''), 3.78 and 3.8 (3H each, s, 2 × -OC*H*$_3$), 4.47 (2H, s, -C*H*$_2$OH), 4.98 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.27 (1H, t, *J* = 8.0 Hz. H-2''), 5.4 (2H, s, -OC*H*$_2$C-), 6.43 (1H, s, H-8), 6.91 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.35 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.39 (1H, t, *J* = 8.0 Hz, H-4'''), 7.48 (2H, m, H-3''' and 6'''), 7.62 (1H, m, H-5'''), 7.66 (1H, s, H-5), 7.99 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.17, 159.71, 156.65, 150.15, 145.17, 135.95, 135.6, 132.28, 131.86, 131.55, 130.01, 129.09, 127.73 × 2, 124.61, 124.44, 124.22, 122.65, 114.05 × 2, 109.74, 99.61, 77.23, 67.26, 61.78, 55.48, 55.34, 30.92, 28.22, 25.81, 22.72, 17.82. HRMS *m/z* calcd for $C_{32}H_{35}N_4O_5$ $[M + H]^+$ 555.2602, found 555.2588.

4.1.12.5. Synthesis of (Z)-(7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-oneO-((1-(2-hydroxymethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)oxime ***(17e)***. Yield: 94%, gummy mass, $[\alpha]_D$ = +7 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.7 (6H, s, H-4'' and

H-5''), 2.7 and 3.42 (1H each, m, H-3a and 3b), 3.24 (2H, d, *J* = 8.0 Hz, H-1''), 3.77 and 3.8 (3H each, s, 2 × -OC*H*$_3$), 4.76 (2H, s, -C*H*$_2$OH), 4.97 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.28 (1H, t, *J* = 8.0 Hz. H-2''), 5.36 (2H, s, -OC*H*$_2$C-), 6.42 (1H, s, H-8), 6.9 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.34 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.38 (1H, t, *J* = 8.0 Hz, H-5'''), 7.45 (1H, t, *J* = 8.0 Hz, H-4'''), 7.57 (1H, d, *J* = 8.0 Hz, H-6'''), 7.65 (1H, s, H-5), 7.74 (1H, s, H-2'''), 8.01 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.15, 159.66, 156.61, 150.11, 143.36, 137.1, 132.24, 131.88, 129.78, 127.73 × 2, 126.99, 124.37, 124.3, 122.68, 121.46, 119.49, 118.89, 114.03 × 2, 109.76, 99.58, 77.19, 67.3, 64.2, 55.47, 55.33, 30.88, 28.25, 25.84, 22.72, 17.84. HRMS *m/z* calcd for $C_{32}H_{35}N_4O_5$ $[M + H]^+$ 555.2602, found 555.2596.

4.1.12.6. Synthesis of (Z)-(7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-oneO-((1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)oxime ***(17f)***. Yield: 93%, gummy mass, $[\alpha]_D$ = +4 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.62 (6H, s, H-4'' and H-5''), 2.64 and 3.37 (1H each, m, H-3a and 3b), 3.13 (2H, d, *J* = 8.0 Hz, H-1''), 3.69 and 3.7 (3H each, s, 2 × -OC*H*$_3$), 4.87 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.21 (1H, t, *J* = 8.0 Hz. H-2''), 5.3 (2H, s, -OC*H*$_2$C-), 6.33 (1H, s, H-8), 6.89 (2H, dd, *J* = 8.0, 16.0 Hz, H-3''' and 5'''), 6.9 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.21 (1H, t, *J* = 8.0 Hz, H-2'''), 7.26 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.57 (1H, s, H-5), 7.98 (1H, s, H-6'''), 8.04 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.2, 159.66, 158.72, 156.65, 150.43, 145.29, 137.5, 132.26, 131.88, 130.44, 130.42, 127.74 × 2, 124.4, 124.33, 122.67, 121.17, 116.85, 114.03 × 2, 109.67, 108.98, 99.57, 77.16, 66.82, 55.47, 55.33, 28.25, 25.84, 22.72, 17.84. HRMS *m/z* calcd for $C_{31}H_{33}N_4O_5$ $[M + H]^+$ 541.2384, found 541.2358.

4.1.12.7. Synthesis of (Z)-(7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-oneO-((1-(4-chloro-2-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)oxime ***(17g)***. Yield: 91%,

gummy mass, $[\alpha]_D$ = +6 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.74 (6H, s, H-4'' and H-5''), 2.22 (3H, s, -C*H*$_3$), 2.72 and 3.47 (1H each, m, H-3a and 3b), 3.26 (2H, d, *J* = 8.0 Hz, H-1''), 3.81 and 3.83 (3H each, s, 2 × -OC*H*$_3$), 5.03 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.32 (1H, t, *J* = 8.0 Hz. H-2''), 5.42 (2H, s, -OC*H*$_2$C-), 6.45 (1H, s, H-8), 6.94 (2H, dd, *J* = 8.0, 16.0 Hz, H-3''' and 5'''), 7.31 (2H, d, *J* = 8.0 Hz, H-3' and H-5'), 7.39 (3H, m, H-3''', 5''' and 6'''), 7.66 (1H, s, H-5), 7.78 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.18, 159.78, 156.61, 150.01, 144.98, 135.59, 135.03, 132.18, 131.93, 131.36, 127.68 × 2, 127.24, 126.99, 124.65, 124.38 × 2, 124.2, 122.67, 114.07 × 2, 109.82, 99.61, 77.22, 67.35, 55.46, 55.33, 30.9, 28.25, 25.84, 17.86, 17.76. HRMS *m/z* calcd for $C_{32}H_{34}ClN_4O_4$ $[M + H]^+$ 573.2263, found 573.2278.

4.1.12.8. Synthesis of (Z)-(7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-oneO-((1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)oxime ***(17h)***. Yield: 92%, gummy mass, $[\alpha]_D$ = +12 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (6H, s, H-4'' and H-5''), 2.72 and 3.41 (1H each, m, H-3a and 3b), 3.27 (2H, d, *J* = 8.0 Hz, H-1''), 3.8, 3.81, 3.9 and 3.92 (3H each, s, 5 × -OC*H*$_3$), 4.98 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.29 (1H, t, *J* = 8.0 Hz, H-2''), 5.4 (2H, s, -OC*H*$_2$C-), 6.44 (1H, s, H-8), 6.92 (2H, dd, *J* = 8.0 Hz, H-3' and 5'), 6.95 (2H, s, H-2''' and 6'''), 7.37 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.66 (1H, s, H-5), 7.68 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.09, 159.72, 156.64, 153.9 × 3, 150.02, 145.45, 132.93, 132.14, 131.9, 127.68 × 2, 124.36, 124.3, 122.71, 121.66, 114.04 × 2, 109.82, 99.61, 98.85, 77.18, 67.4, 61.01, 56.45 × 3, 55.45, 55.33, 30.9, 28.25, 25.77, 17.86. HRMS *m/z* calcd for $C_{34}H_{39}N_4O_7$ $[M + H]^+$ 615.2813, found 615.2831.

4.1.12.9. Synthesis of (Z)-4-(4-(((((7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-ylidene)amino)oxy)methyl)-1H-1,2,3-triazol-1-yl)benzamide ***(17i)***. Yield: 90%, white solid, m.p. 192^{o}C , $[\alpha]_D$ = +8 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (6H, s,

H-4'' and H-5''), 2.42 and 3.13 (1H each, m, H-3a and 3b), 2.98 (2H, d, *J* = 8.0 Hz, H-1''), 3.52 and 3.53 (3H each, s, 2 × -OC*H*$_3$), 4.74 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.0 (1H, t, *J* = 8.0 Hz. H-2''), 5.1 (2H, s, -OC*H*$_2$C-), 6.16 (1H, s, H-8), 6.64 (2H, dd, *J* = 8.0 Hz, H-3' and 5'), 7.09 (2H, s, H-2' and 6'), 7.37 (1H, s, H-5), 7.58 (2H, d, *J* = 8.0 Hz, H-3''' and H-5'''), 7.77 (2H, d, *J* = 8.0 Hz, H-2''' and H-6'''), 7.94 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.64, 161.66, 161.05, 158.05, 151.86, 147.21, 140.58, 135.09, 133.56, 133.28, 130.72 × 2, 129.03 × 2, 125.82, 125.59, 123.98, 122.96, 121.59 × 2, 115.4 × 2, 111.03, 100.96, 78.52, 68.32, 56.75, 56.61, 32.21, 30.9, 26.97, 18.98. HRMS *m/z* calcd for $C_{32}H_{34}N_5O_5$ $[M + H]^+$ 568.2554, found 568.2548.

*4.1.12.10. Synthesis of (Z)-4-(4-((((7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-ylidene)amino)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)(phenyl)methanone (**17j**).* Yield: 87%, white solid, m. p. 152^{o}C, $[\alpha]_D$ = +6 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72 (6H, s, H-4'' and H-5''), 2.71 and 3.42 (1H each, m, H-3a and 3b), 3.26 (2H, d, *J* = 8.0 Hz, H-1''), 3.79 and 3.8 (3H each, s, 2 × -OC*H*$_3$), 5.0 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.29 (1H, t, *J* = 8.0 Hz. H-2''), 5.41 (2H, s, -OC*H*$_2$C-), 6.43 (1H, s, H-8), 6.91 (2H, dd, *J* = 8.0 Hz, H-3' and 5'), 7.36 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.52 (2H, t, *J* = 8.0 Hz, H-3'''' and H-5''''), 7.62 (1H, m H-4''''), 7.66 (1H, s, H-5), 7.81 (2H, d, *J* = 8.0 Hz, H-2'''' and H-6''''), 7.89 (2H, d, *J* = 8.0 Hz, H-3''' and H-5'''), 7.97 (2H, d, *J* = 8.0 Hz, H-2''' and H-6'''), 8.11 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.26, 160.19, 159.71, 156.65, 150.2, 146.12, 139.63, 137.5, 137.05, 132.92, 132.28, 131.84, 131.77 × 2, 130.03 × 2, 128.54 × 2, 127.73 × 2, 124.41, 124.24, 122.66, 121.15, 120.02 × 2, 114.05 × 2, 109.71, 99.6, 77.22, 67.3, 55.5, 55.36, 30.92, 28.25, 25.88, 17.87. HRMS *m/z* calcd for $C_{38}H_{37}N_4O_5$ $[M + H]^+$ 629.2758, found 629.2752.

*4.1.12.11. Synthesis of (Z)-4-(4-((((7-methoxy-2-(4-methoxyphenyl)-6-(3-methylbut-2-en-1-yl)chroman-4-ylidene)amino)oxy)methyl)-1H-1,2,3-triazol-1-yl)benzonitrile (**17k**).* Yield: 86%,

gummy mass, $[\alpha]_D$ = +10 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.72 (6H, s, H-4'' and H-5''), 2.71 and 3.42 (1H each, m, H-3a and 3b), 3.25 (2H, d, *J* = 8.0 Hz, H-1''), 3.79 and 3.81 (3H each, s, 2 × -OC*H*$_3$), 4.99 (1H, dd, *J* = 12.0, 4.0 Hz, H-2), 5.28 (1H, t, *J* = 8.0 Hz, H-2''), 5.41 (2H, s, -OC*H*$_2$C), 6.43 (1H, s, H-8), 6.92 (2H, dd, *J* = 8.0 Hz, H-3' and 5'), 7.36 (2H, d, *J* = 8.0 Hz, H-2' and H-6'), 7.64 (1H, s, H-5), 7.83 and 7.91 (2H each, d, *J* = 8.0 Hz, H-2''', 3''', 5''' and H-6'''), 8.06 (1H, s, =C*H*-N-). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.09, 163.58, 160.49, 154.1, 150.4, 143.57, 137.72 × 2, 136.04, 135.68, 131.5 × 2, 128.27, 128.06, 126.51, 124.64, 124.49 × 2, 121.51, 117.89 × 2, 116.28, 113.5, 103.45, 81.0, 71.05, 59.31, 59.17, 34.72, 32.06, 29.65, 21.65. HRMS *m/z* calcd for $C_{32}H_{32}N_5O_4$ $[M + H]^+$ 550.2449, found 550.2444.

4.2. Biology

4.2.1. Cell culture and growth conditions

Human cancer cell lines A549 (Lung), PC-3 (Prostate), MCF-7 (Breast) and HCT-116 (Colon) were obtained from National Cancer Institute (NCI), USA. 5-fluorouracil was used as positive control and purchased from Sigma-Aldrich (Bangaluru, India). All four cancer cell lines were grown in tissue culture flasks in complete growth medium (RPMI-1640) supplemented with 10% fetal bovine serum, 100 µg/mL streptomycin and 100 units/mL penicillin (New Brunswick, Galaxy 170R, Eppendorf) at 37°C, 5% CO_2 and 98% RH.

4.2.2. Cytotoxicity Assay

The SRB assay [32] was executed to assess the cytotoxic potential of the potent inhibitors in which optimum cell density per well was seeded in 96 well flat bottom plates. 100 µL of cell suspension of a panel of human cancer cell lines PC-3 (7000), MCF-7 (8000), A549 (7500), and HCT-116 (7000) was plated. Following 24 h of incubation under culture conditions, the cells

were exposed to different concentration (1, 5, 10, 30 and 50 µM) of test materials containing complete growth medium along with 5-fluorouracil as positive control. The plates were kept under incubation under the same conditions for 48 h at 37°C. Further, cells were fixed with ice cold TCA for 1 h at 4°C. After 1 h, the plates were washed three times with water and allowed to air dry. Afterwards, 100 µL of 0.4% SRB dye was added for half an hour at room temperature. Plates were then washed three times with water followed by 1% v/v acetic acid to remove the unbound SRB. After drying at room temperature, the bound dye was solubilised by adding 100 µL of 10 mM Tris buffer (pH-10.4) to each well. The plates were retained on the shaker for 5 min in order to dissolve the protein bound dye. OD was taken at 540 nm in a microplate reader (Thermo Scientific) and IC_{50} was determined by using GraphPAD Prism Software Version 5.0 (1).

$$\text{The \% of cell viability} = \frac{\text{Absorbance of treated cells - Absorbance of Blank}}{\text{Absorbance of control cells - Absorbance of Blank}} \times 100$$

$$\text{\% Growth inhibition} = 100 - \text{\% of cell viability}$$

4.2.3. Colony formation assay

HCT-116 cells ($8x10^4$/mL/well) were seeded and treated with compound **17i** at different concentrations of 3.6, 7.2 and 14.4 µM and incubated for 24 h. Then, the treated cells were trypsinized, counted and re-seeded at 1000 cells/well in a six well plate. The cells were left in order to give colonies of >50 cells to assess the clonogenic ability. Thereafter, Cells were fixed with 1ml of 4% formaldehyde and stained with 0.5% crystal violet. Finally, crystal violet was aspirated carefully and rinsed with water. Clonogenic survival was expressed as the number of colony-forming units in treated cultures in comparison to untreated controls [17].

4.2.4. In vitro cell migration assay

HCT-116 cells were plated at a concentration of $8x10^5$cells/well and allowed to reach a confluenty upto 70-80%. Then, the cells were serum starved for 24h and monolayer was scraped in a straight horizontal line with a sterile 200 µL tip. Eventually, cells were treated with different concentrations (3.6, 7.2 and 14.4 µM) of **17i** for 24 h. Wounded areas were gradually photographed (20x magnification) at 0 and 24 h and the percentage of wound closure was estimated by the following equation:

% Wound closure = [1-(wound area at 0 h /wound area at 24 h) × 100%]

4.2.5. Phase contrast microscopy

HCT-116 cells were treated with 3.6, 7.2 and 14.4 µM concentration of compound **17i** for 24 h. Cells were simply photographed using phase contrast microscope after treatment.

4.2.6. Mitochondrial membrane potential (MMP)

Cells ($1x10^6$/1.5 mL/well) in 12 well plates were treated with **17i** at 3.6, 7.2 and 14.4 µM and 5-FU at 5 µM for 24 h. Rhodamine-123 (10 nM) was added 1 h before experiment termination. Cells were washed in PBS and immediately analyzed under fluorescence microscopy [18].

4.2.7. Western blot analysis

Cells (HCT-116) were treated with varying concentration of **17i** at 3.6, 7.2 and 14.4 µM for 24 h. After completion of incubation, the cells were trypsinized and washed with cold PBS. The whole cell lysates were prepared by using RIPA buffer, protease and phosphatase inhibitor cocktail. The lysates prepared were centrifuged at 12,000 g for 5 min [33]. The total protein concentration was determined with the Quantipro BCA assay kit (Sigma) in which BSA (bovine serum albumin) was taken as a standard. An equal amount of protein per lane was resolved by 10%

SDS-PAGE (sodium dodecylsulphate-polyacrylamide gel electrophoresis) and transferred to PVDF (polyvinylidene-di-fluoride membrane) (Millipore). Non-specific binding was blocked by using 5% BSA in TBST solution for 1 h at r.t. After blocking, the membrane was probed with primary PARP (1:1000) and β-actin (1:500) and incubated with the corresponding HRP conjugated secondary antibodies (CST). Immunoreactive proteins were detected with the ECL western blotting detection system and exposed to X-ray film.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at

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Legends to Figures

Fig.1. Structures of some flavanones exhibiting anticancer activity.

Fig. 2. Structures of various bavachinin derivatives.

Fig. 3. Design rationale of target anticancer compounds.

Fig. 4. Colony formation assay- P) HCT-116 cells were treated with different concentrations of **17i** at 3.6, 7.2 and 14.4µM (B, C and D). Untreated cells were taken as negative control (A). 5-fluorouracil was taken as a positive control (E). Q) Bar diagram representation of survival fraction of HCT-116 cells treated with **17i** in a dose dependent manner for each analysis versus positive control. Data represent means ± SD (n = 3). ***P < 0.001, **P < 0.01 vs. control.

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Tables

Table 1. Cytotoxic activity (% growth inhibition) of bavachinin (**1**) and its derivatives at 50µM concentration

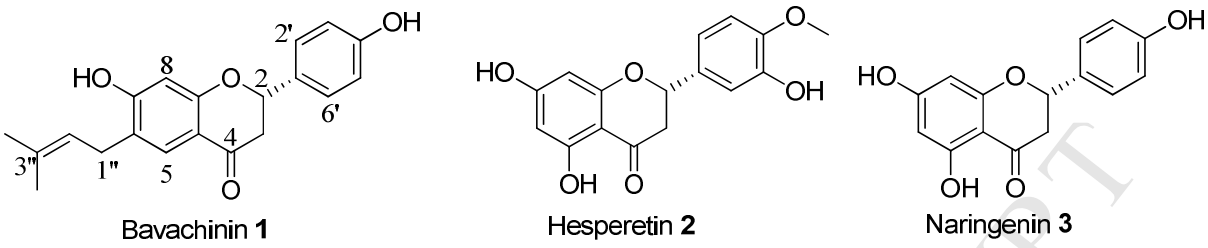
Tissue		**Lung**	**Prostate**	**Colon**	**Breast**
Cell Line		**A549**	**PC-3**	**HCT-116**	**MCF-7**
S. No.	**Compound**	**% Cytotoxicity**			
1	**1**	**85**	**91**	**100**	**99**
2	**9a**	45	**58**	**68**	**65**
3	**9b**	37	**60**	**65**	**57**
4	**9c**	43	**54**	**63**	**60**
5	**9d**	37	48	**60**	**57**
6	**9e**	**53**	**52**	**75**	**68**
7	**9f**	46	**53**	**76**	**69**
8	**9g**	27	19	29	37
9	**9h**	37	21	46	**53**
10	**9i**	**72**	**55**	**76**	**68**
11	**10**	**79**	**92**	**97**	**98**
12	**11**	**68**	44	**63**	**73**
13	**12**	48	13	37	49
14	**12a**	**75**	**78**	**77**	**81**
15	**13**	**80**	**81**	**99**	**93**
16	**14**	**77**	**67**	**87**	**93**
17	**15**	**66**	45	47	**63**
18	**16**	**64**	41	**65**	**61**
19	**17a**	19	2	6	0
20	**17b**	31	22	8	4
21	**17c**	**58**	44	47	35
22	**17d**	**100**	**100**	**100**	**100**
23	**17e**	43	34	27	42
24	**17f**	**52**	**64**	**65**	**63**
25	**17g**	35	49	20	37
26	**17h**	28	36	29	45
27	**17i**	**100**	**100**	**100**	**100**
28	**17j**	34	40	**52**	41
29	**17k**	42	50	50	40

Bold value indicates >50 % growth inhibition effects

Table 2. IC_{50} value in µM of bavachinin and its selected analogs on the panel of human cancer cell lines

Compound	Lung A549	Prostate PC-3	Colon HCT-116	Breast MCF-7
1	30.5	25.4	20.7	19.5
10	29.8	27.4	29.02	30.93
12a	30.5	36.31	34.32	34.5
13	**11.43**	31.63	29.74	23.59
14	25.29	27.7	24.34	**19.52**
17d	30.7	26.55	29.89	23.38
17i	**7.72**	**16.08**	**7.13**	**11.67**

Figures



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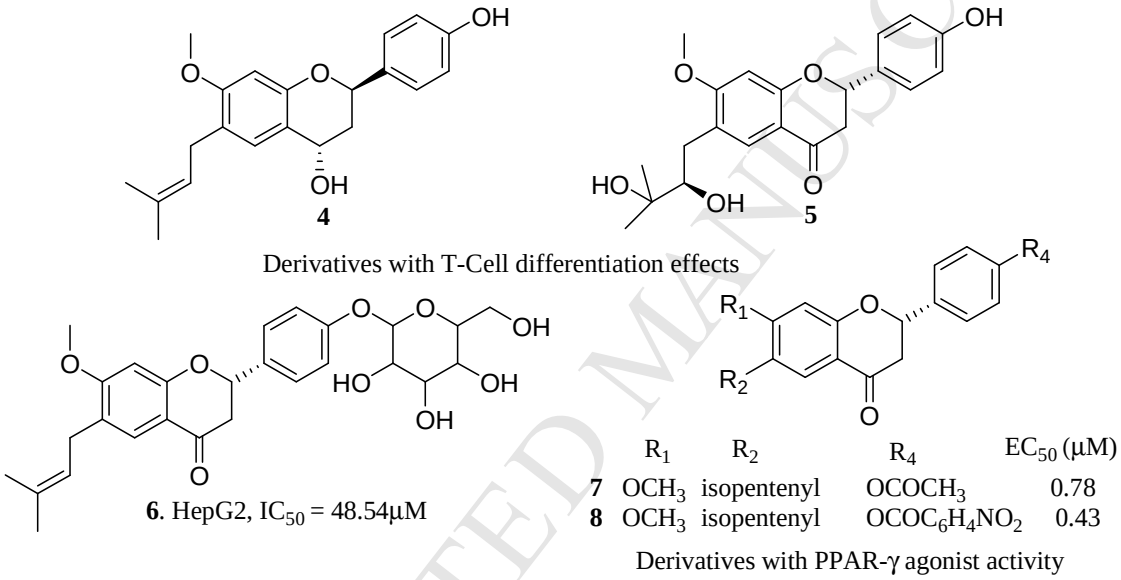
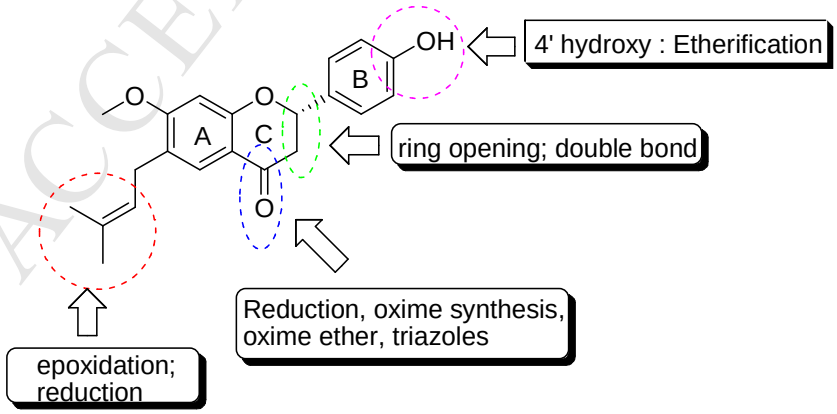


Fig. 2. Structures of various bavachinin derivatives



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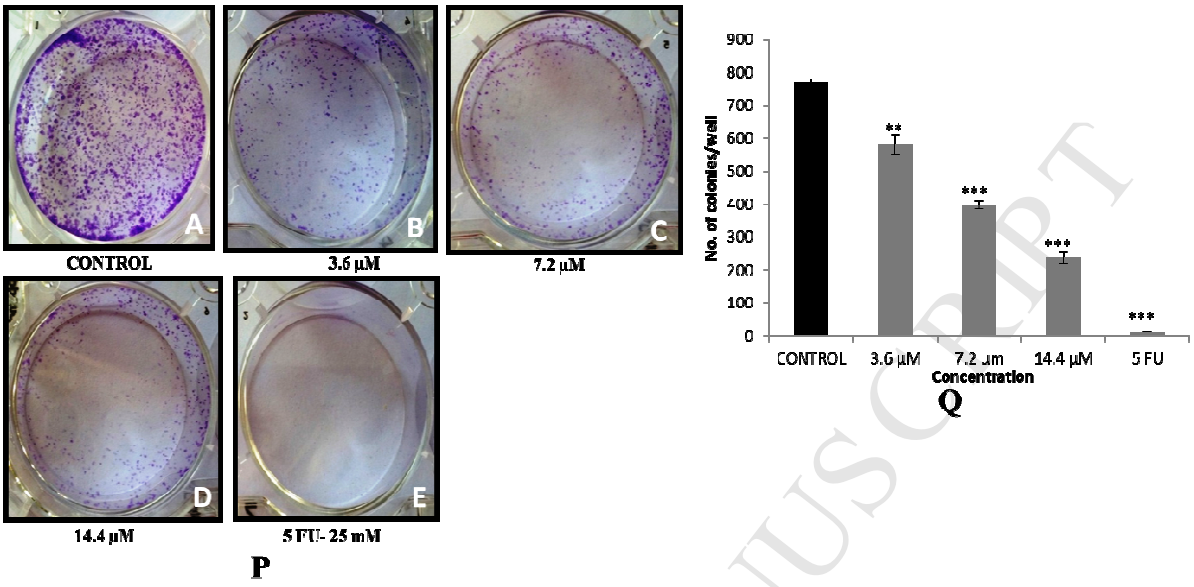

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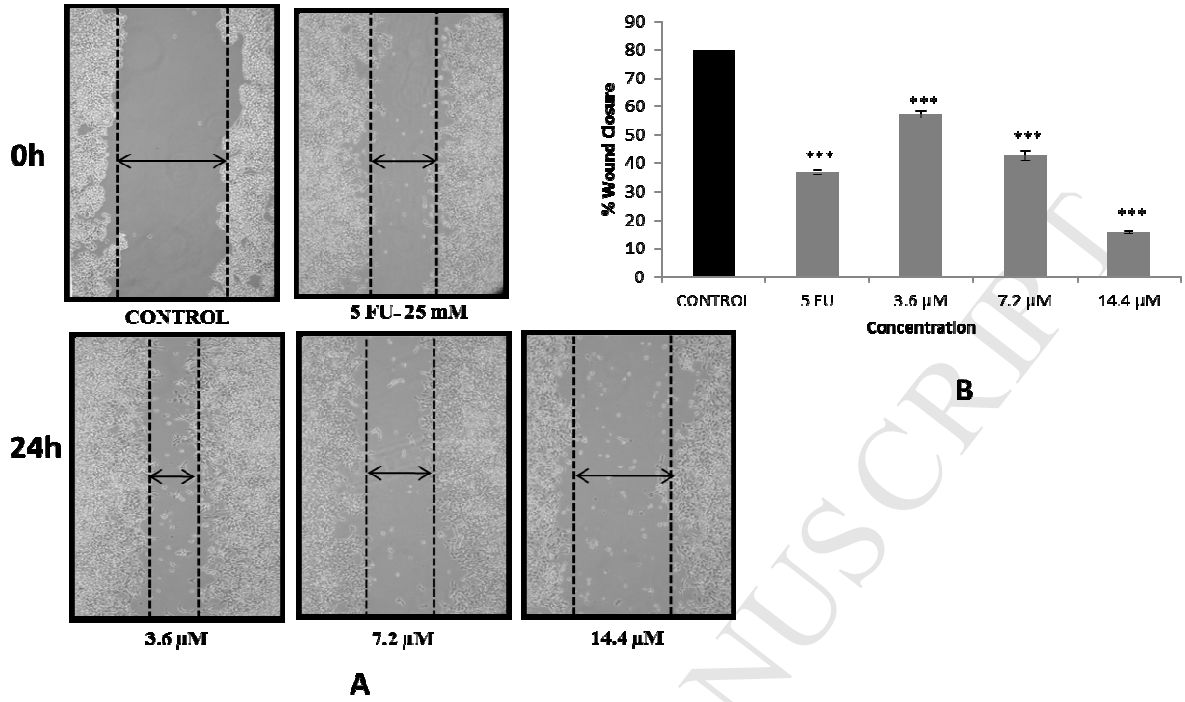

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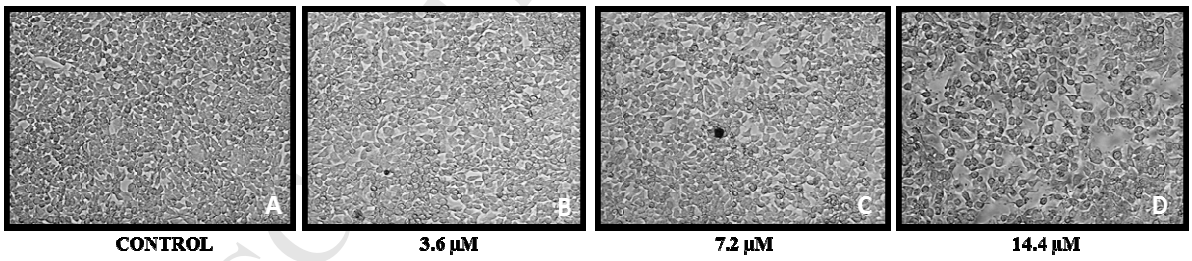

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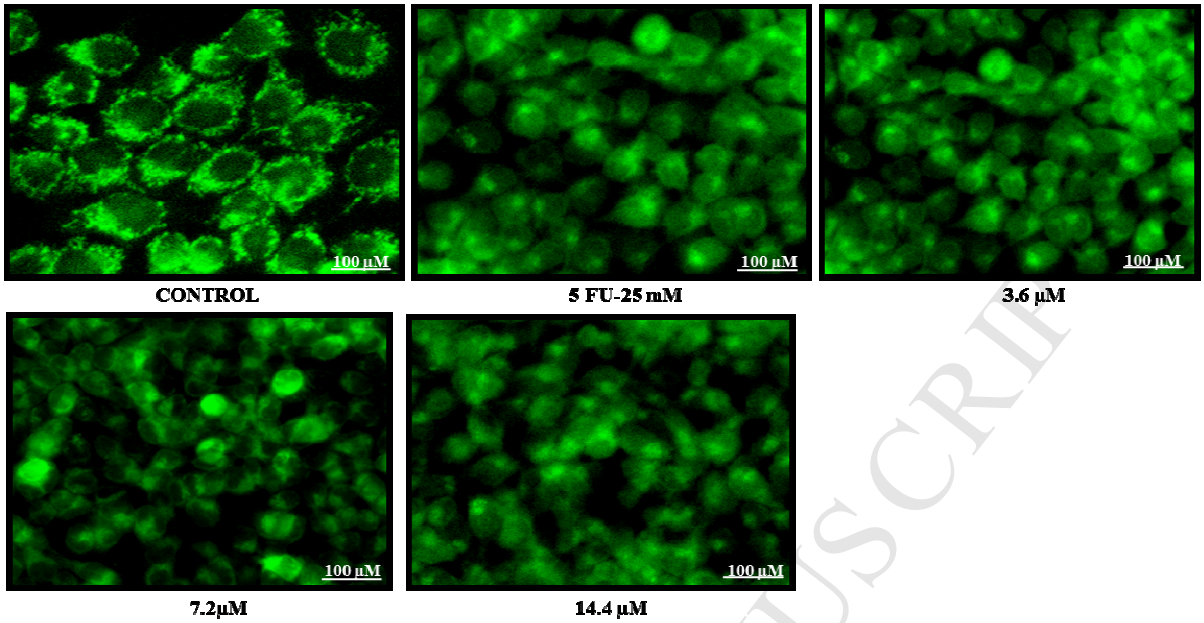

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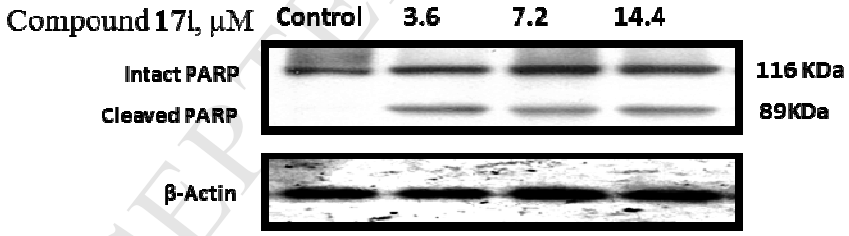

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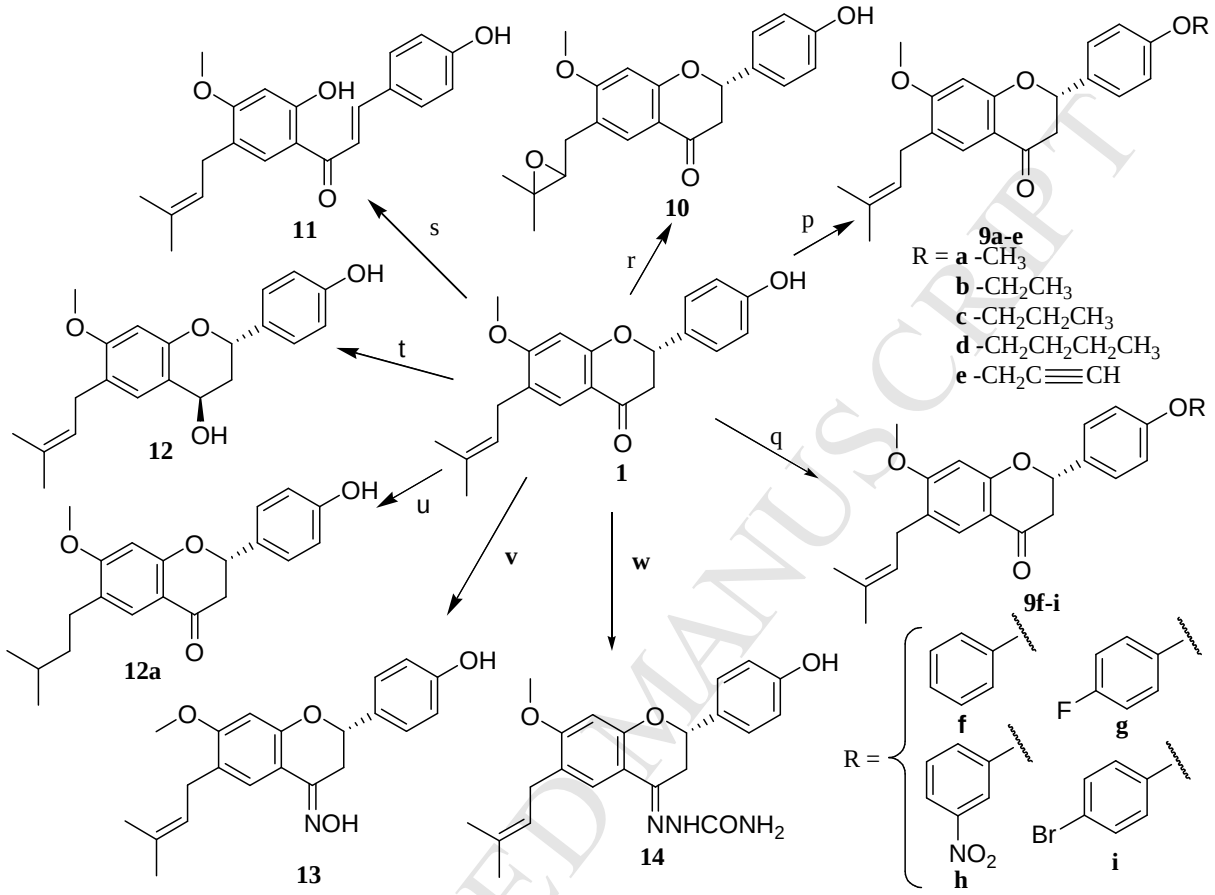
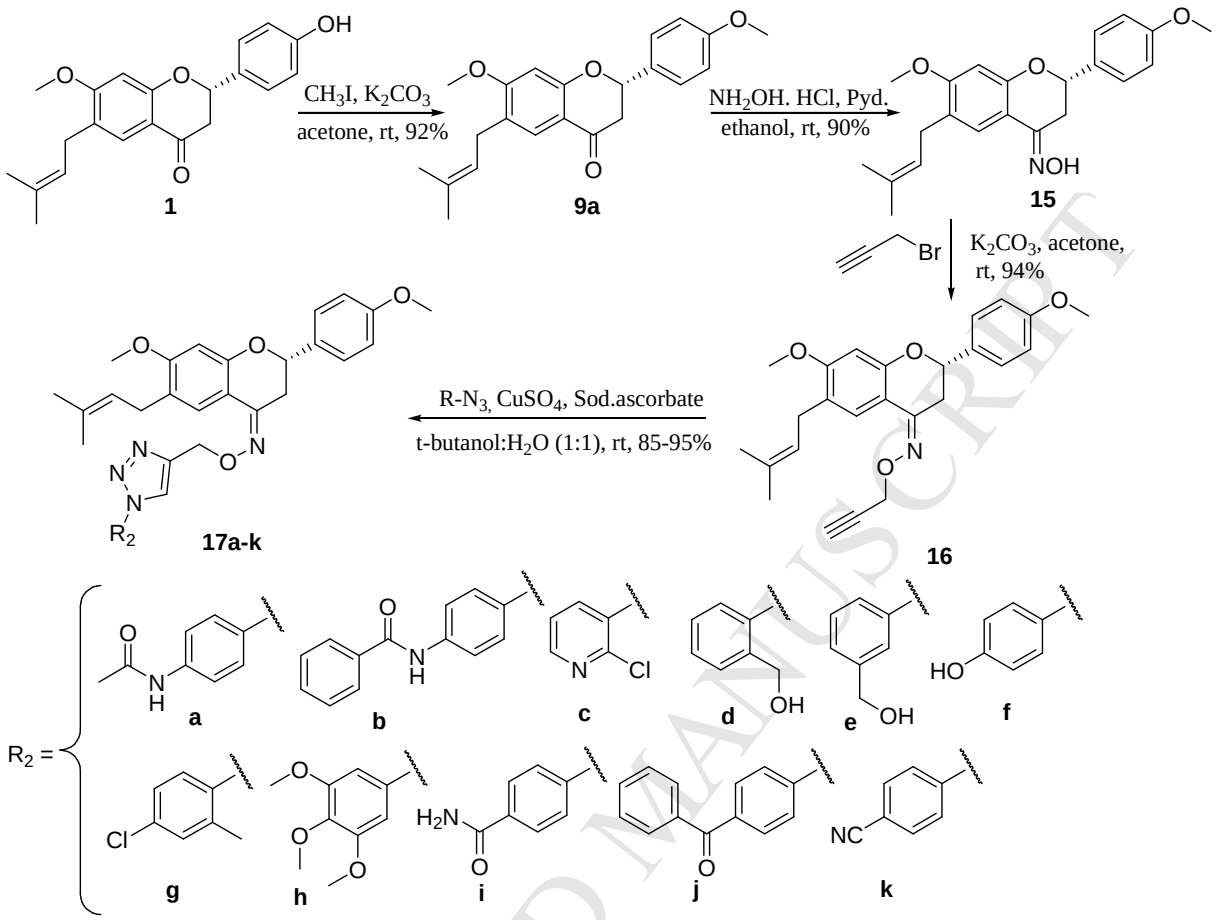
Scheme



Scheme 1. Reagents and conditions: p) R-X (-I, -Br), K_2CO_3, acetone, rt, 2h, 90-94% q) R-$B(OH)_2$, $Cu(OAc)_2$, Pyd., DCM, rt, 2-3h, 85-90% r) *m*-CPBA, DCM, 0^o-rt, 2h, 78% s) NaH, ethanol, rt, 0.5h, 90% t) $NaBH_4$, MeOH, 0^o- rt, 0.5h, 70% u) Pd/C, MeOH, rt, 2.0h, 80% v) $NH_2OH.HCl$, ethanol, rt, 1.5h, 85% w) $NH_2CONHNH_2.HCl$, ethanol, rt, 1.5h, 85%



Scheme 2: Synthesis of bavachinin 1,2,3-triazole derivatives (**17a-k**)

Research highlights

- Synthesis of 28 Bavachinin derivatives.
- Several derivatives were active against human cancer cell lines.
- The molecules inhibits the colony formation and restrict the migration of HCT-116 cells
- The molecule induced the morphological changes and mediated the apoptotic cell death of HCT-116 cells.