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# Design and synthesis of novel 1,2,3-triazole derivatives of coronopilin as anti-cancer compounds

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ABSTRACT

A series of 1,2,3-triazole coronopilin congeners have been designed and synthesized by employing click chemistry approach starting from parthenin and evaluated for their cytotoxicity against a panel of six human cancer cell lines (PC-3, THP-1, HCT-15, HeLa, A-549 and MCF-7). While many compounds exhibited significant anticancer activity, compound **3a**, was found to be the most promising analogue in this series with $IC_{50}$ values of 3.1 μM on PC-3 cell line. Flow-cytometric studies showed that 1,2,3-triazole derivative-**3a** induce dose dependent apoptosis in the sub G1 phase. This lead molecule-**3a** was further studied for NF-κB (p65) transcription factor inhibitory activity using Elisa and western blotting analysis which confirmed concentration dependent inhibitory activity against NF-κB, p65 with 80% inhibition in 24 h at 100 μM.



## 1. Introduction

Cancer is the most feared disease second only to heart disease as a leading cause of death in most of the developed countries including India. It is characterized by uncontrolled growth and spread of abnormal cells [1]. Cancer development includes a multi-step process such as induction of genetic instability, abnormal expression of genes, abnormal signal transduction, angiogenesis, metastasis, and immune evasion [2]. The high mortality rate caused by this group of diseases is an indication of the limited efficiency of the current therapies including radiation, chemotherapy and surgery [3]. Therefore development of new anticancer agents is the major focus for scientists across the world. In last few decades, research has been focussed on chemically synthesised or natural product derived compounds as anticancer entities. Although chemical synthesis is currently the dominant means to discover and produce drugs, but still the potential of bioactive plants or their extracts to provide novel products for disease treatment and prevention is enormous [4]. Compared with chemical synthesis, plant derived natural products represent an attractive source of biologically active agents since they are natural and available at affordable prices [5]. Also plants derived agents may have different mechanisms than conventional drugs, and could be of clinical importance in health care improvement [6]. Plant materials might be bioactive secondary metabolites that have the potential to treat different afflictions. In this study we focussed our attention towards parthenin[I, Fig. 1], a major constituent of the aggressive and obnoxious herb, *Parthenium hysterophorus* L. (*Compositae*) that grows wild in different regions of India. Parthenin-a sesquiterpene lactone (SL) isolated from this plant, is reported to be responsible for plethora of pharmacological effects of this plant *viz.*, anticancer, antibacterial, antiamoebic, anti-inflammatory, lipid peroxidation inhibition, and trypanocidal activity [7–15]. Of particular interest would be the anticancer activity of this bifunctional psuedoguanolide structure, which is attributed to the presence of two unsaturated double bonds on the scaffold that might act as Michael acceptor for the cysteine residues of NF-κB, thereby permanently inhibiting the transcription factors leading to apoptosis. In this direction, our group generated novel spiro derivatives of parthenin at its exocyclic unsaturation and found that some of the spiro analogs having free cyclopentenone moiety as Michael acceptor, were

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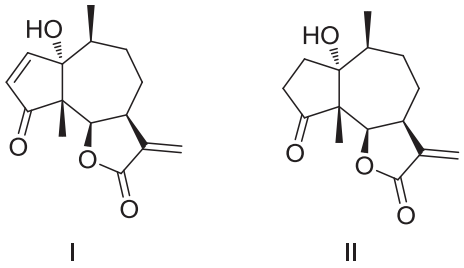
found to be the better cytotoxic entities [17]. We hypothesized that the presence of one double bond may suffice the purpose on inhibition of NF-κB, whereas, the other olefinic bond can be conveniently utilized as site of structural modification in order to derive novel semi synthetic entities with improved cytotoxicity and drug-likeness. 1,2-Dihydroparthenin also known as coronopilin [II, Fig. 1], exhibit potential anticancer activity against leukaemia to induce apoptosis [16]. In this context, herein we attempted preparation of various analogs of coronopilin by modifying endocyclic double bond of parthenin to obtain some potent derivatives, while keeping the exocyclic methylene unsaturation as possible site of attack by NF-κB cystiene residues. Such an effort should explain unequivocally, the role of individual double bonds in retaining the cytotoxicity with more clarity and also should unequivocally prove the mode of action of the pseudoguanolides as inhibitors of transcription factor. Herein, we describe the preparation and cytotoxicity of a focused library of novel derivatives at of coronopilin where 1,2,3-triazole moiety is appended at the β-position of α,β-unsaturated cyclopentenone ring of parthenin. Using Huisgen [3 + 2] cycloaddition between a terminal alkyne and an azide a series of novel regioselective 1,2,3-triazole coronopilin derivatives have been synthesized and screened for anticancer activity against a panel of human cancer cell lines. From the $IC_{50}$ values it is found that most of the compounds retained anticancer activity with improved cytotoxicity as compared to parent compound parthenin.

## 2. Results and discussion

### 2.1. Chemistry

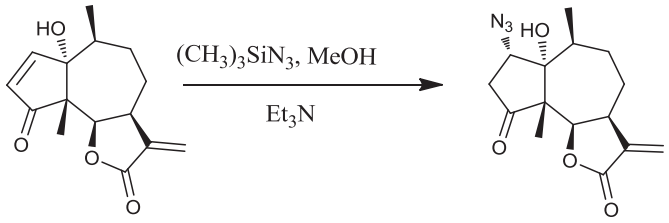
As illustrated in Scheme 1, azido group was generated on α,β-unsaturated cyclopentonone ring through Michael addition reaction of trimethylsilylazide selectively at β-position. Reaction was optimised using different bases as given in Table 1 and best results were obtained using triethylamine as base. Through this method yields for the synthesis of 2α-azido coronopilin were improved compared to those reported in the literature [18]. Addition of azido group at $\beta$-position was confirmed by $^1$H NMR, mass and IR. In $^1$H NMR disappearance of the alkene protons of cyclopentonone ring was observed. In IR absorption signal at 2100 $cm^{-1}$ corresponding to azido group was observed. Stereochemistry at C-2 position was confirmed through $^1$H NMR by observation of the position and splitting pattern of the signal for proton ($\delta$ 3.3, $t$, $J = 9.6$ Hz) which is the characteristic $J$ value of $\alpha$ orientation [19]. While for $\beta$ orientation characteristic $J$ value is 15.4 Hz [20]. Also another reason for $\alpha$ attack is the steric hindrance posed by the methyl group and the possible hydrogen bonding between the hydroxyl group and nitrogen of azide group which makes the $\alpha$ orientation stable (Table 2).

As illustrated in Scheme 2 triazole derivatives of coronopilin were synthesised through [3 + 2] cycloaddition reaction of azide generated on parthenin and terminal alkynes generated on phenols




I II

**Fig. 1.** Structures of parthenin (I) and coronopilin (II).



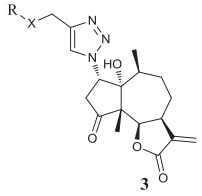

I 2

**Scheme 1.** Synthesis of 2α-azido Coronopilin.

**Table 1**
Optimization of reaction conditions for Michael addition.

| S. No | Base | Time e (h) | Yield (%) |
|---|---|---|---|
| 1 | NaOAc | 6 | 49 |
| 2 | DIEA | 6 | 50 |
| **3** | **$Et_3N$** | 3.5 | 85 |
| 4 | $C_5H_5N$ | 6 | 40 |
| 5 | Pyrolidine | 6 | 40 |

**Table 2**
Various 1,2,3-triazole derivatives of Coronopilin.

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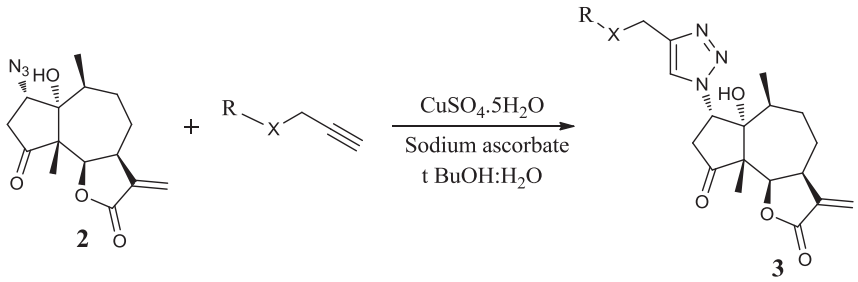
| Compound | *R* | *X* | Yield (%) |
|---|---|---|---|
| **3a** | 2Cl – $C_6H_4$- | O | 95 |
| **3b** | 2F – $C_6H_4$- | O | 92 |
| **3c** | 2Br – $C_6H_4$- | O | 95 |
| **3d** | 4Br – $C_6H_4$- | O | 90 |
| **3e** | 4$CF_3C_6H_4$- | O | 95 |
| **3f** | 4Cl – $C_6H_4$- | O | 95 |
| **3g** | 2 – $C_{10}H_9$- | O | 96 |
| **3h** | 4 Isopropyl – $2CH_3$- $C_6H_3$- | O | 95 |
| **3i** | $2CH_3$ – $C_6H_4$- | O | 97 |
| **3j** | 4 – $CH_3$ – $C_6H_4$- | O | 90 |
| **3k** | 2 – $CH_3$ – $C_6H_4$- | N | 96 |
| **3l** | 2 – F – $C_6H_4$- | N | 97 |
| **3m** | 2 – Br – $C_6H_4$- | N | 95 |
| **3n** | 5-Bromo indole | N | 94 |
| **3o** | 4 – $CF_3$ – $OCH_2$ – $C_6H_4$- | N | 96 |
| **3p** | 4F – $C_6H_4$- | N | 97 |
| **3q** | 2 – $OCH_3$ – $C_6H_4$- | N | 98 |
| **3r** | $C_6H_5$- | $CH_2$ | 93 |
| **3s** | $CH_3$- | $CH_2$ | 95 |

and anilines. The synthesis of triazole ring was confirmed through $^1$H NMR, $^{13}$C and mass spectrometry data.

### 2.2. Biological results

#### 2.2.1. Evaluation of in vitro anticancer activity

All the compounds were screened for *in vitro* cytotoxicity against a panel of six human cancer cell lines including PC-3 (prostrate), HCT-15 (colon), THP-1 (leukaemia), HeLa (cervix), A-549 (lung) and MCF-7 (breast). Mitomycin, Adriamycin and 5-FU were taken as reference compounds and the results are reported in terms of $IC_{50}$ values (Table 3). From the $IC_{50}$ values, it is clear that most of the compounds have significant cytotoxic activity against




**Scheme 2.** Synthesis of 1,2,3-triazole on ring A of Coronopilin.

prostrate, cervix, lung and breast derived cancer cell lines. However, the activity shown by these compounds was highest against HeLa, a cervix derived cancer cell line. Compound-**3a**, showed significant cytotoxicity against PC-3, HeLa and THP1 cell lines, however, its activity was found to be maximum against PC-3 and HeLa cell line with $IC_{50}$ value of 3.1, 3.6 μM respectively. Compounds **3b**, **3c**, **3e**, **3f**, **3h**, **3i** and **3l** were also found to have promising activity against the above mentioned cell lines. However, compounds **3m**, **3n**, **3o**, **3p**, **3q**, **3r** did not show significant cytotoxicity against THP and PC3 cell lines ($IC_{50}$ 100 μM). Overall, the cytotoxicity exhibited by the majority of the derivatives were below 10 μM when compared to the cytotoxicity of the parent compound parthenin ($IC_{50}$ 35.3–60 μM), there by indicating that triazolyl coronopilin derivatives prepared from parthenin could make better ligands with improved cytotoxicity against cancer cell lines.

### 2.2.2. *Cell cycle analysis of compound **3a***

To address the cell death caused by compound-**3a** the extent of apoptotic death was assessed using FACS flow cytometry through the determination of sub-G1 cell population by propidium iodide (PI) staining. Since most of the compounds showed good cytotoxicity on PC-3 cell line, therefore this cell line was chosen for further studies. The DNA cell cycle analysis of compound-**3a** (Fig. 2) revealed a concentration dependent increase in the sub G1 phase of cell cycle being **38.8**, **39.9** and **49.2** at 1, 5 and 10 μM respectively.

### 2.2.3. *NF-κB (p65), transcription factor assay*

In literature, most of the α,β- unsaturated moiety bearing potent anti-cancer compounds works by blocking the NF-κB transcription factor [21]. In our study, we visualized that derivatives of parthenin possess α,β- unsaturated functionality and it might also work through by inhibiting NF-κB transcription factor. In this direction, compound-**3a** was taken for inhibitory NF-κB (p65) transcription factor activity.

Mammals have five members of NF-κB group of transcription factors *viz*. Rel (c-Rel), Rel A (p65), RelB, NF-κB1 (p50 and its precursor p105) and NF-κB2 [22]. NF-κB plays a central role in the regulation of cancer cell proliferation and survival [23]. NF-κB has also been shown to play a vital role in the cancer cell progression by regulating the process of angiogenesis [24], Therefore, many new anti-cancer therapies are being developed against NF-κB. NF-κB binding of compound-**3a** was checked through Elisa and Western blot analysis as given in Fig. 3. Our data showed that PC-3 cells treated with compound-**3a** displayed concentration dependent inhibition of NF-κB (p65) binding with DNA response element, where, cells treated with compound-**3a** exerted highly significant (80%) effect on the binding of NF-κB (p65) with DNA at 100 μM. Thus, our results clearly indicate that compound- **3a** has a good inhibitory effect on the activity of NF-κB (p65).

## 3. Conclusion

In summary a focussed library of novel 1, 2, 3-triazole derivatives were synthesised through alkyne-azide click reaction on coronopilin. All the compounds have been then screened for their anti cancer activity. Majority of the compounds exhibited significant cytotoxicity when tested against a panel of human cancer cell lines with compound-**3a** being most active within the series of derivatives studied. This derivative also showed 80% binding with DNA at 100 μM. This reinforces the view, that compounds of this type exert their biological effects by inhibiting the NF-κB/p65. Thus, novel leads from parthenin can be further developed into potential chemotherapeutic agents in cancer therapy. Many of the new triazolyl coronopilin analogues derived from parthenin scaffold exhibited superior activity as compared to the precursor natural product-parthenin.

The present study also generated some important information about SAR of semi-synthetic sesquiterpene lactones. Various inferences drawn from the study include the requirement of single unsaturation on the scaffold should be sufficient for the inhibition of NF-κB. Cyclopentenone moiety can be utilized for structural modifications to fine tune the activity while keeping α-methylene-γ-lactone moiety un-altered to be available as Michael acceptor for the attack by cystein sulfhydryl residue of NF-κB. The triazolyl adducts appended at cyclopentenone ring prove to be the better ligands as they may facilitate better orientation of the molecule for attack by the cysteine residue of target protein and also might be improvising the drug-likeness of the molecule by improving the bioavailability of the molecule in *in-vitro* assay. Furthermore, the new analogues have shown to induce apoptosis through concentration dependent inhibition of NF-κB (p65) binding with DNA response element. Overall the present study reveals the SAR of SQLs with better clarity establishing the minimal requirement of unsaturation for NF-κB inhibition and thus, improved lead compounds with far superior cytotoxicity can be derived from parthenin by modifying the cyclopentenone double bonds while keeping the exocyclic unsaturation unmodified.

## 4. Experimental protocols

Melting points were recorded on Buchi Melting point apparatus D-545. NMR spectra were recorded on Bruker DPX500 instrument in $CDCl_3$ with TMS as an internal standard. Chemical shift values are reported in δ (ppm) and coupling constants in Hertz. Mass spectra were recorded on Maldi TOF–TOF. The progress of all reactions was monitored by TLC on 2–5 cm precoated silica gel 60 $F_{254}$ plates of

**Table 3**
$IC_{50}$ values (μM) of 1,2,3- triazolyl derivatives of coronopilin against a panel of human cancer cell lines.

| Compound | THP-1 | PC-3 | HCT-15 | Hela | A549 | MCF-7 |
|---|---|---|---|---|---|---|
| **3a** | 3.8 | 3.1 | 5.3 | 3.6 | 4.2 | 9.7 |
| **3b** | 25 | 4.3 | 23 | 3.9 | 6.3 | 4.3 |
| **3c** | 19 | 5.6 | 12 | 4.8 | 8.8 | 3.4 |
| **3d** | 10 | 4.7 | 48 | 6.5 | 14 | 16 |
| **3e** | 48 | 6.4 | 100 | 3.5 | 3.8 | 13 |
| **3f** | 12 | 5.2 | 27 | 3.6 | 4.1 | 7 |
| **3g** | 25 | 3.6 | 20 | 7.3 | 9 | 24 |
| **3h** | 33 | 7.5 | 45 | 6.8 | 21 | 32 |
| **3i** | 3.8 | 10 | 5.5 | 8.4 | 6.2 | 3.7 |
| **3j** | 26 | 7.6 | 39 | 100 | 21 | 4.2 |
| **3k** | 17 | 4.6 | 8.5 | 3.9 | 23 | 15 |
| **3l** | 100 | 3.3 | 100 | 3.7 | 34 | 5.1 |
| **3m** | 100 | 100 | 20 | 4.8 | 100 | 4.2 |
| **3n** | 100 | 100 | 26 | 7.2 | 100 | 4.5 |
| **3o** | 100 | 100 | 100 | 17 | 23 | 17 |
| **3p** | 100 | 100 | 6.5 | 15 | 100 | 15 |
| **3q** | 100 | 100 | 15 | 28 | 23 | 28 |
| **3r** | 100 | 100 | 4.3 | 16 | 5.8 | 16 |
| **3s** | 100 | 100 | 5.5 | 8.4 | 12 | 14 |
| Parthenin | 46.2 | 43.1 | 35.3 | 60 | 37.6 | 40.2 |
| Mitomycin | – | – | 0.5 | – | – | – |
| Adriamycin | – | 6 | – | – | – | 0.5 |
| 5-FU | – | 2.2 | 4.5 | 4.9 | – | – |

* Mitomycin, Adriamycin and 5-FU used as positive control for this study.

thickness 0.25 mm (Merck). The chromatograms were visualized under UV 366 nm and iodine.

### 4.1. Synthesis

#### 4.1.1. Synthesis of 2α-azido coronopilin

In a typical procedure, to a solution of parthenin (0.177 g, 1.2 mmol) in MeOH (10 mL) stirred over a period of 10 min, maintaining the temperature between 0 and 5 °C, was added trimethylsilylazide (0.262 g, 1 mmol), then triethylamine was added until the pH of solution was slightly alkaline, stirred the reaction mixture at same temperature for 15 min followed by stirring at ambient temperature for 4 h. The solvent was evaporated *in vacuo* and the crude was subjected for flash chromatography and product was obtained with 90% yield. The pure product was characterized on the basis of $^1$H NMR, $^{13}$C NMR and mass spectrometry.

*4.1.1.1. Compound* ***2***. Crystalline white solid; mp: 176–178 °C; $[\alpha]_D^{25}$ +29 (c 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 3456.39, 2958.98, 2927.09, 2857.01, 2103.89, 1749.39, 1726; $^1$H NMR (200 MHz, $CDCl_3$): 6.20 (d, 1H, $J$ = 1.29 Hz), 5.57 (d, 1H, $J$ = 2.72 Hz), 5.00 (d, 1H, $J$ = 8.8 Hz), 3.30 (t, 1H, $J$ = 9.6 Hz), 2.28 (m, 1H), 2.40 (d, 2H, $J$ = 7.5), 2.48 (m, 1H), 2.20–1.64 (m, 4H), 1.18 (d, 3H, $J$ = 7.0 Hz), 1.10 (s, 3H); $^{13}$C (100 MHz, $CDCl_3$): 10.12, 16.30, 28.21, 31.23, 34.51, 38.54, 41.21, 50.45, 65.45, 70.98, 80.21, 123.31, 140.10, 170.01, 220.11; Maldi mass: (M+Na) 328.

#### 4.1.2. Synthesis of triazoles

In a typical procedure, to a solution of coronopilin azide (0.01 g, 1 mmol) in tBuOH: $H_2O$ taken in 1:1 proportion was added 1-chloro-3-(prop-2-ynyloxy)benzene (0.025 g, 1.2 mmol), $CuSO_4.5H_2O$ (2 mmol) and sodium ascorbate (2 mmol). The reaction mixture was then stirred at room temperature for 3 h, then filtered and extracted with ethyl acetate and water. The ethyl acetate layer was then separated. The solvent was evaporated *in vacuo* and the crude was subjected for column chromatography (silica gel, 100–200 mesh, elution; *n*-hexane/EtOAc gradient) to afford pure product as colourless solid (0.3 g, 90%). The pure product was characterized on the basis of IR, $^1$H/$^{13}$C NMR, DEPT and mass spectrometry.

#### 4.1.3. Compound characterization

*4.1.3.1. Compound* ***3a***. Crystalline white solid; mp: 165–169 °C; $[\alpha]_D^{25}$ +29 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 681.49, 752.84, 997.96, 1118.39, 1162.79, 1241.73, 1277.09, 1446.84, 1484.51, 1588.92, 1753.62, 2925.18, 3400.23; $^1$H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 1H, $J$ = 11.58 Hz), 7.45 (d, 1H, $J$ = 7.99 Hz), 7.30–7.25 (m, 2H), 6.95 (d, 1H, $J$ = 1.29 Hz), 6.25 (d, 1H, $J$ = 2.72 Hz), 6.00 (t, 1H, $J$ = 9.77 Hz), 5.73 (d, 1H, $J$ = 2.35 Hz), 5.25 (t, 2H, $J$ = 6.78 Hz), 5.10 (d, 1H, $J$ = 8.28 Hz), 3.33–3.30 (m, 1H), 2.20 (d, 2H, $J$ = 4.87 Hz), 1.75–1.53 (m, 4H), 1.30 (d, 3H, $J$ = 5.6 Hz), 1.28 (s, 3H), 1.20 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 10.9, 11.5, 19.3, 27.7, 30.3, 38.0, 38.1, 42.4, 46.3, 60.3, 72.3, 73.6, 73.6, 83.4, 115.7, 120.9, 123.7, 126.6, 129.9, 138.3, 142.4, 158.8, 170.1, 220.3; ESI-MS: (M+Na) 494; Anal. Calcd for $C_{24}H_{26}ClN_3O_5$: C, 61.08; H, 5.55; N, 8.90. Found: C, 61.13; H, 5.58; N, 8.93.

*4.1.3.2. Compound* ***3b***. Colourless solid; mp: 104 °C; $[\alpha]_D^{25}$ +32 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 751.80, 816.88, 999.95, 1110.38, 1160.43, 1198.80, 1257.75, 1385.32, 1457.00, 1504.70, 1613.60, 1753.60, 2928.37, 3400.10; $^1$H NMR (200 MHz, $CDCl_3$): δ 7.75 (d, 1H, $J$ = 10.4 Hz), 7.25–6.85 (m, 4H), 6.25 (d, 1H, $J$ = 2.72 Hz), 5.50 (t, 1H, $J$ = 11.5 Hz), 5.25 (t, 2H, $J$ = 6.9 Hz), 5.00 (d, 1H, $J$ = 7.9 Hz), 4.60 (d, 1H, $J$ = 2.5 Hz), 3.75 (d, 2H, $J$ = 5.4 Hz), 3.25–3.00 (m, 1H), 2.00 (m, 1H), 1.71 (s, 3H), 1.25–1.00 (m, 7H), 0.92 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 10.96, 17.44, 22.97, 28.92, 29.68, 30.36, 37.39, 38.73, 41.32, 43.50, 80.93, 82.28, 103.30, 109.54, 116.56, 123.91, 125.93, 130.89, 144.08, 149.46, 150.62, 154.88, 173.16, 186.05; ESI-MS: (M+Na) 478; Anal. Calcd for $C_{24}H_{26}FN_3O_5$: C, 63.29; H, 5.75; N, 9.23. Found: C, 63.32; H, 5.71; N, 9.26.

*4.1.3.3. Compound* ***3c***. Colourless solid; mp: 126 °C; $[\alpha]_D^{25}$ +54 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 629.95, 751.99, 816.02, 995.37, 1051.82, 1163.06, 1239.75, 1276.08, 1478.92, 1585.52, 1753.37, 2929.66, 3150.25, 3400.57; $^1$H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 1H, $J$ = 5.326 Hz), 7.55 (d, 1H, $J$ = 7.9 Hz), 7.50–7.25 (m, 2H), 6.96 (d, 1H, $J$ = 7.8 Hz), 6.24 (d, 1H, $J$ = 2.6 Hz), 6.00 (t, 1H, $J$ = 8.5 Hz), 5.75 (d, 1H, $J$ = 2.4 Hz), 5.25 (s, 2H), 5.00 (d, 1H, $J$ = 8.4 Hz), 3.00 (d, 2H, $J$ = 9.7 Hz), 2.25 (s, 3H), 2.00–1.95 (m, 1H), 1.62–1.60 (m, 4H), 1.12 (d, 3H, $J$ = 5.9 Hz), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 10.9, 11.5, 20.2, 27.7, 30.3, 38.0, 38.1, 42.4, 44.3, 46.3, 60.3, 71.6, 73.1, 73.4, 112.2, 116.5, 123.3, 123.7, 128.8, 132.7, 138.3, 142.4, 170.8, 220.0; ESI-MS: (M+Na) 538; Anal. Calcd for $C_{24}H_{26}BrN_3O_5$: C, 55.38; H, 5.04; N, 8.10. Found: C, 55.87; H, 5.11; N, 8.12.

*4.1.3.4. Compound* ***3d***. Crystalline white solid; mp: 186 °C; $[\alpha]_D^{25}$ +28 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 658.00, 752.38, 997.27, 1030.72, 1051.18, 1162.43, 1237.60, 1384.32, 1478.16, 1628.75, 1751.16, 2927.10, 3367.93; $^1$H NMR (200 MHz, $CDCl_3$): δ 8.25 (d, 1H, $J$ = 5.36 Hz), 7.55 (d, 1H, $J$ = 7.9 Hz), 7.25 (dd, 2H, $J$ = 0.79, 6.87 Hz), 6.95 (d, 1H, $J$ = 7.8 Hz), 6.25 (d, 1H, $J$ = 2.75 Hz), 5.95 (t, 1H, $J$ = 8.1 Hz), 5.52 (d, 1H, $J$ = 2.5 Hz), 5.25 (s, 2H), 5.00 (d, 1H, $J$ = 7.8 Hz), 3.25–3.00 (m, 2H), 2.00 (m, 4H), 1.95 (m, 3H), 1.25–1.00 (m, 4H), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 7.22, 12.63, 20.3, 26.34, 31.40, 33.82, 34.30, 42.42, 50.00, 64.43, 69.31, 72.31, 82.3, 110.2, 115.43, 116.56, 120.4, 123.32, 127.00, 128.34, 132.00, 138.33, 159.46, 170.34, 220.32; ESI-MS: (M+Na) 538; Anal. Calcd for $C_{24}H_{26}BrN_3O_5$: C, 55.82; H, 5.07; N, 8.34. Found: C, 55.47; H, 5.16; N, 8.53.

*4.1.3.5. Compound* ***3e***. Off-white crystalline solid; mp: 132 °C; $[\alpha]_D^{25}$ +43 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 506.02, 771.27, 1063.08, 1116.65, 1160.56, 1273.90, 1327.00, 1615.87, 1750.39, 2924.60, 3400.14; $^1$H NMR (200 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.43 (d, 2H, $J$ = 8.2 Hz), 6.73 (d, 2H, $J$ = 8.4 Hz), 6.25 (d, 1H, $J$ = 6.02 Hz), 5.50 (t,

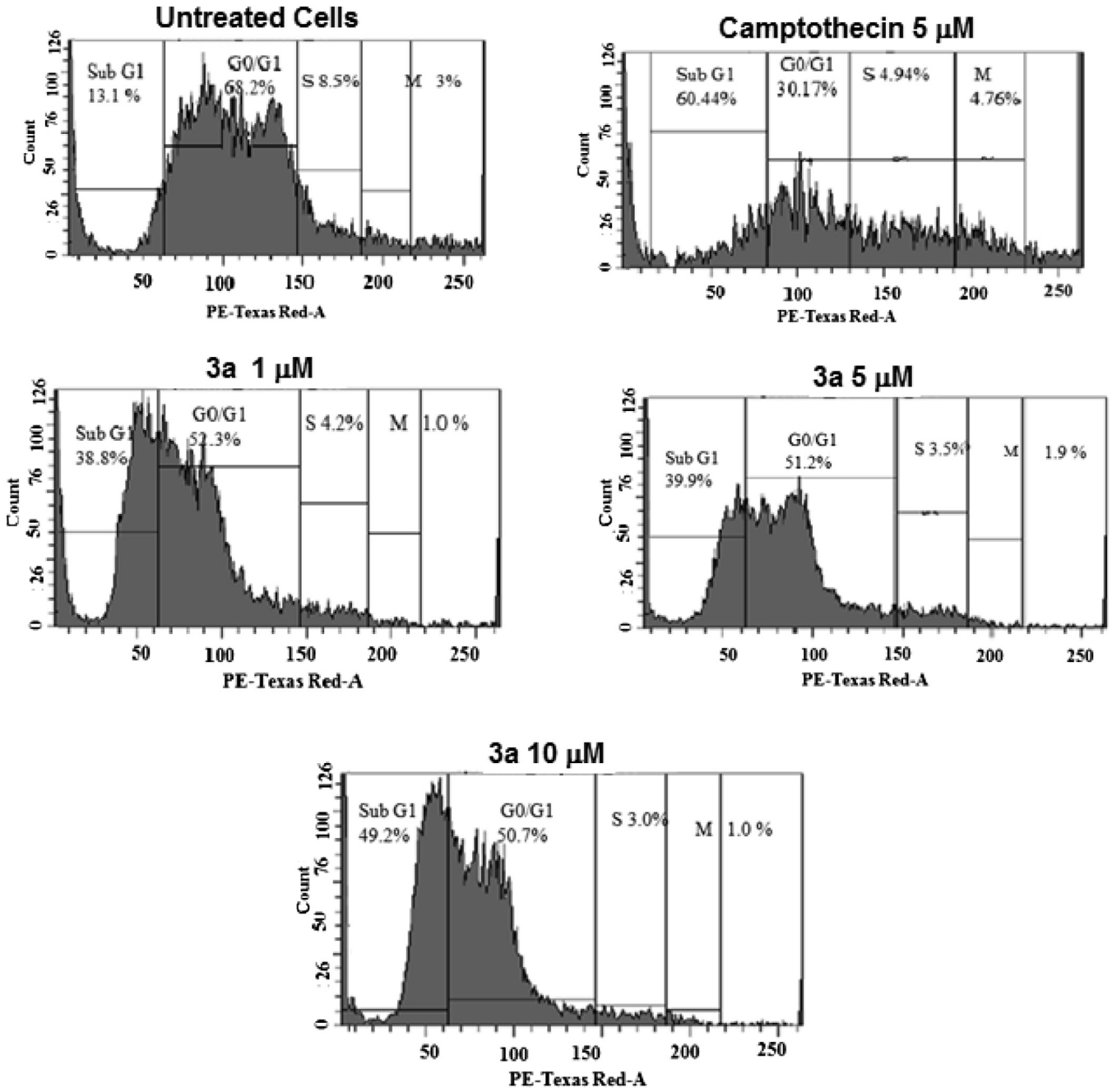



**Fig. 2.** Flow cytometric analysis of compound (**3a**) treated with PC-3 cell lines.

2H, $J = 9.6$ Hz), 5.00 (d, 1H, $J = 11.3$ Hz), 4.50–4.25 (m, 3H), 3.50 (m, 1H), 3.00 (d, 2H, $J = 10.3$ Hz), 2.00–1.96 (m, 3H), 1.55–1.25 (m, 4H), 1.12 (d, 3H, $J = 5.9$ Hz), 0.95 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): 10.12, 13.63, 13.85, 14.00, 20.23 26.68, 29.00, 29.21, 36.90, 38.13, 38.17, 43.62, 58.90, 60.47, 78.25, 80.40, 84.42, 111.71, 111.82, 121.10, 130.32, 139.71, 149.38, 169.73, 210.73; ESI-MS: (M+Na) 528; Anal. Calcd for $C_{25}H_{26}F_3N_3O_5$: C, 59.40; H, 5.18; N, 8.31. Found: C, 59.43; H, 5.24; N, 8.29.

*4.1.3.6. Compound* ***3f***. Crystalline white solid; mp: 138 °C; $[\alpha]_D^{25}$ +46 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 666.78, 770.24, 825.21, 991.52, 1097.34, 1162.13, 1237.79, 1271.49, 1393.05, 1462.42, 1490.94, 1633.33, 1750.85, 3435.23; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 7.84 (d, 1H, $J = 9.6$ Hz), 7.50 (d, 2H, $J = 4.5$ Hz), 6.98 (d, 2H, $J = 8.8$ Hz), 6.25 (d, 1H, $J = 2.75$ Hz), 5.50 (m, 2H), 5.25 (s, 2H), 5.00 (d, 1H, $J = 7.9$ Hz), 3.75 (m, 1H), 3.25 (m, 1H), 3.00 (t, 2H, $J = 10$ Hz), 1.75 (s, 3H), 1.52 (s, 3H), 1.25–1.20 (m, 4H), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 10.9, 11.5, 20.2, 25.3, 27.7, 30.3, 38.0, 38.1, 42.4, 46.3, 60.2, 72.3, 73.6, 73.6, 115.7, 120.9, 123.7, 126.6, 129.9, 138.3, 142.4, 158.8, 170.1, 220.3; ESI-MS: (M+Na) 494; Anal. Calcd for $C_{24}H_{26}ClN_3O_5$: C, 61.08; H, 5.5; N, 8.90. Found: C, 61.13; H, 5.56; N, 8.83.

*4.1.3.7. Compound* ***3g***. Colourless solid; mp: 105 °C; $[\alpha]_D^{25}$ +56 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 772.31, 1049.86, 1097.17, 1237.97, 1269.19, 1384.72, 1580.25, 1752.35, 2923.22, 3400.10; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 8.25 (d, 1H, $J = 7.8$ Hz), 7.85 (m, 2H), 7.50 (m, 4H), 7.00 (d, 1H, $J = 5.6$ Hz), 6.25 (d, 1H, $J = 2.75$ Hz), 5.53–5.25 (m, 3H), 5.00 (d, 1H, $J = 8.0$ Hz), 4.20 (d, 1H, $J = 4.3$ Hz), 3.75 (s, 1H),

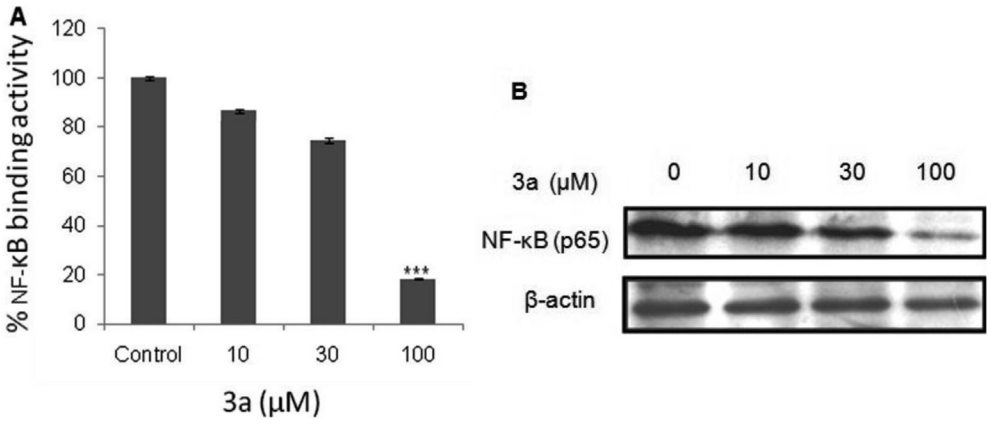



**Fig. 3.** NF-κB, p65 transcription factor assay.

3.50–3.25 (m, 1H), 3.00 (t, 2H, $J = 9.4$ Hz), 1.54 (s, 3H), 1.25 (m, 3H), 1.20–1.00 (m, 4H), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 14.10, 18.21, 19.11, 20.3, 27.37, 29.71, 30.91, 38.10, 38.83, 42.48, 44.56, 59.59, 60.83, 62.46, 79.69, 84.97, 120.96, 121.30, 123.52, 124.34, 125.37, 125.67, 125.78, 134.61, 140.32, 144.60, 153.84, 210.80; ESI-MS: (M+Na) 510; Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00; N, 8.62. Found: C, 69.02; H, 5.95; N, 8.67.

*4.1.3.8. Compound* ***3h***. Colourless solid; mp: 137 °C; $[\alpha]_D^{25}$ +46 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 754.37, 815.66, 1033.51, 1125.94, 1160.82, 1250.87, 1383.35, 1611.03, 1752.43, 2925.17, 3391.67; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 8.12 (s, 1H), 7.10 (d, 1H, $J = 3.8$ Hz), 6.92 (d, 1H, $J = 8.5$ Hz), 6.75 (d, 1H, $J = 7.6$ Hz), 6.22 (d, 1H, $J = 7.19$ Hz), 6.00 (t, 1H, $J = 9.59$ Hz), 5.65 (d, 1H, $J = 2.28$ Hz), 5.25 (s, 2H), 5.00 (d, 1H, $J = 8.2$ Hz), 3.00 (t, 2H, $J = 2.85$ Hz), 2.10 (m, 1H), 1.84 (m, 3H), 1.75 (m, 4H), 1.25–1.20 (m, 9H), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 14.18, 16.28, 27.30, 29.70, 37.44, 38.70, 44.55, 50.83, 59.55, 60.97, 62.29, 81.66, 84.97, 111.60, 111.82, 112.07, 121.38, 122.12, 124.21, 126.83, 126.91, 127.66, 130.66, 136.97, 140.34, 156.11,170.14, 210.97; ESI-MS: (M+Na) 516; Anal. Calcd for $C_{28}H_{35}N_3O_5$: C, 64.36; H, 6.48; N, 12.02. Found: C, 64.34; H, 6.40; N, 12.12.

*4.1.3.9. Compound* ***3i***. Crystalline white solid; mp: 128 °C; $[\alpha]_D^{25}$ +24 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 668.84, 754.80, 815.70, 989.38, 1121.27, 1162.34, 1189.07, 1237.92, 1273.08, 1336.93, 1391.95, 1494.14, 1601.60, 1752.83, 2941.26, 3434.86; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 7.75 (s, 1H), 7.25 (d, 2H, $J = 5.8$ Hz), 7.00 (d, 2H, $J = 8.3$ Hz), 6.25 (d, 1H, $J = 8.5$ Hz), 5.63 (t, 1H, $J = 9.8$ Hz), 5.25 (d, 2H, $J = 9.7$ Hz), 5.00 (d, 1H, $J = 8.1$ Hz), 3.75 (t, 1H, $J = 5.6$ Hz), 3.54–3.52 (m, 1H), 3.10 (t, 2H, $J = 10.1$ Hz), 2.55 (m, 1H), 2.25 (s, 3H), 1.52 (s, 3H), 1.25 (d, 3H, $J = 3.4$ Hz), 1.00 (m, 4H), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 14.18, 16.28, 22.20, 29.70, 37.44, 38.70, 44.55, 50.83, 60.90, 62.32, 84.97, 111.60, 111.82, 121.07, 121.38, 122.12, 124.23, 126.83, 126.94, 127.06, 130.06, 140.34, 156.11, 170.84, 210.97; ESI-MS: (M+Na) 474; Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65. Found: C, 68.79; H, 6.75; N, 9.69.

*4.1.3.10. Compound* ***3j***. Colourless solid; mp: 136 °C; $[\alpha]_D^{25}$ +16 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 514.99, 565.72, 754.27, 815.98, 990.45, 1052.85, 1273.53, 1392.88, 1450.56, 1510.34, 1753.97, 2927.74, 3400.66; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 7.80 (d, 1H, $J = 7.8$ Hz), 7.24 (d, 2H, $J = 8.2$ Hz), 6.75 (d, 2H, $J = 8.4$ Hz), 6.25 (d, 1H, $J = 12$ Hz), 5.50 (t, 2H, $J = 2.3$ Hz), 5.25 (s, 2H), 5.00 (d, 1H, $J = 7.9$ Hz), 3.75 (t, 1H, $J = 10.08$ Hz), 3.50–3.25 (m, 1H), 3.00 (t, 2H, $J = 9.58$ Hz), 2.25 (d, 3H, $J = 6.4$ Hz), 1.50 (s, 3H), 1.25 (d, 3H, $J = 3.4$ Hz), 1.00 (m, 4H), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 10.12, 14.11, 14.31, 20.49, 27.42, 30.31, 32.11, 38.34, 39.10, 45.05, 51.96, 59.25, 60.38, 61.54, 79.20, 83.54, 110.31,118.23, 120.12, 131.15, 140.35, 155.63, 170.51, 210.54; ESI-MS: (M+Na) 474; Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 66.50; H, 6.47; N, 9.31. Found: C, 66.54; H, 6.43; N, 9.22.

*4.1.3.11. Compound* ***3k***. Crystalline white solid; mp: 123–125 °C; $[\alpha]_D^{25}$ +13 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 566.89, 666.53, 753.88, 988.09, 1075.48, 1118.96, 1162.27, 1218.56, 1272.55, 1391.03, 1476.23, 1512.25, 1605.48, 1752.30, 2104.36, 2930.87, 3419.76; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 7.65 (s, 1H), 7.10 (dd, 2H, $J = 6.2$, 3.4 Hz), 6.75 (dd, 2H, $J = 8.3$, 2.6 Hz), 6.25 (d, 1H, $J = 2.5$ Hz), 5.65 (d, 1H, $J = 2.4$ Hz), 5.53 (t, 1H, $J = 8.5$ Hz), 5.00 (d, 1H, $J = 8.4$ Hz), 4.52 (s, 3H), 3.75 (s, 1H), 3.54–3.50 (m, 2H), 3.00 (d, 2H, $J = 9.6$), 1.63 (s, 6H), 1.25–1.20 (m, 4H); $^{13}$C (100 MHz, $CDCl_3$): 10.90, 11.52, 15.55, 20.32, 27.73, 30.31, 38.00, 38.14, 42.45, 42.70, 46.35, 60.73, 73.12, 113.42, 117.17, 123.22, 123.70, 126.23, 126.65, 129.98, 143.20, 146.52, 170.22, 220.23; ESI-MS: (M+Na) 473; Anal. Calcd for $C_{25}H_{30}N_4O_4$: C, 66.65; H, 6.71; N, 12.44. Found: C, 66.68; H, 6.74; N, 12.33.

*4.1.3.12. Compound* ***3l***. Light brown solid; mp: 153–156 °C; $[\alpha]_D^{25}$ +27 (c 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 666.56, 751.28, 991.34, 1019.59, 1118.30, 1162.16, 1237.31, 1273.62, 1384.25, 1456.95, 1504.14, 1595.87, 1752.76, 2925.51, 3400.00; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 7.75 (s, 1H), 7.50 (d, 1H, $J = 8.5$ Hz), 7.20 (t, 1H, $J = 8.4$ Hz), 6.70 (dd, 2H, $J = 8.3$, 2.6 Hz), 6.25 (d, 1H, $J = 2.6$ Hz), 5.50 (d, 1H, $J = 8.5$ Hz), 5.45 (t, 1H, $J = 2.4$ Hz), 5.00 (d, 1H, $J = 8.4$ Hz), 4.95 (m, 1H), 4.53 (d, 2H, $J = 5.4$ Hz), 3.75 (s, 1H), 3.50 (m, 1H), 3.00 (t, 2H, $J = 3.6$ Hz), 1.58 (s, 3H), 1.25 (d, 3H, $J = 3.4$ Hz), 1.00 (m, 4H), 0.95 (m, 1H); $^{13}$C (100 MHz, $CDCl_3$): 10.91, 11.53, 27.75, 30.33, 38.00, 38.15, 42.47, 42.52, 46.35, 60.00, 73.00, 73.12, 115.15, 116.83, 118.86, 123.76, 125.21, 130.55, 130.77, 138.36, 143.20, 154.92, 170.56, 220.23; ESI-MS: (M+Na) 477; Anal. Calcd for $C_{24}H_{27}FN_4O_4$: C, 63.42; H, 5.99; N, 12.33. Found: C, 63.46; H, 5.90; N, 12.28.

*4.1.3.13. Compound* ***3m***. Colourless solid; mp: 169–170 °C; $[\alpha]_D^{25}$ +31 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 581.37, 770.36, 990.46, 1053.05, 1118.05, 1219.31, 1331.19, 1394.30, 1467.22, 1751.06, 2926.80, 3400.12; $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 7.75 (s, 1H), 7.35–7.25 (m, 4H), 6.54 (d, 1H, $J = 3.2$ Hz), 6.33 (d, 1H, $J = 2.69$ Hz), 5.65 (d, 1H, $J = 2.4$ Hz), 5.52 (s, 2H), 5.00 (d, 1H, $J = 8.5$ Hz), 3.65 (s, 1H), 3.41–3.35 (m, 1H), 3.00 (t, 2H, $J = 9.8$ Hz), 2.00 (m, 1H), 1.63 (s, 3H), 1.25–1.20 (d, 3H, $J = 3.4$ Hz), 0.95 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): 14.18, 16.20, 27.41, 28.31, 37.40, 38.66, 42.78, 44.18, 59.52, 60.41, 79.74, 84.90, 101.91, 110.96, 113.18, 122.17, 123.64, 124.77, 129.08, 130.52, 134.56, 140.20, 144.31, 170.21, 211.03; ESI-MS:

(M+Na) 537; Anal. Calcd for $C_{26}H_{27}BrN_4O_4$: C, 57.89; H, 5.05; N, 10.39. Found: C, 57.85; H, 5.10; N, 10.43.

*4.1.3.14. Compound* ***3n***. Crystalline white solid; mp: 171 °C; $[\alpha]_D^{25}$ +35 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 581.37, 770.36, 990.46, 1053.05, 1118.05, 1219.31, 1331.19, 1394.30, 1467.22, 1751.06, 2926.80, 3400.12; $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 7.85 (s, 1H), 7.25–7.35 (m, 5H), 6.54 (d, 1H, $J = 3.2$ Hz), 6.33 (d, 1H, $J = 2.69$ Hz), 5.65 (d, 1H, $J = 2.4$ Hz), 5.52 (s, 2H), 5.05 (d, 1H, $J = 8.5$ Hz), 3.65 (s, 1H), 3.41–3.35 (m, 1H), 3.05 (t, 2H, $J = 9.8$ Hz), 2.00 ( d, 3H, $J = 6.2$ Hz), 1.63 (s, 3H), 1.25–1.20 (m, 4H); $^{13}C$ (100 MHz, $CDCl_3$): 14.18, 16.20, 20.31, 27.41, 28.31, 37.40, 38.66, 42.78, 44.18, 59.52, 60.41, 79.74, 84.90, 101.91, 110.96, 113.18, 122.17, 123.64, 124.77, 129.08, 130.52, 134.56, 140.20, 144.31, 170.21, 211.03; ESI- MS: (M+Na) 561; Anal. Calcd for $C_{26}H_{27}BrN_4O_4$: C, 57.89; H, 5.05; N, 10.39. Found: C, 57.80; H, 5.10; N, 10.48.

*4.1.3.15. Compound* ***3o***. Colourless solid; mp: 122–126 °C; $[\alpha]_D^{25}$ +22 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 758.05, 1048.58, 1118.82,1160.05, 1254.15, 1384.85, 1515.99, 1611.44, 1751.28, 2928.55, 3394.42; $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 7.81 (s, 1H), 6.75 (d, 2H, $J = 7.8$ Hz), 6.51 (d, 2H, $J = 8.2$ Hz), 6.25 (s, 1H), 5.62 (s, 1H), 5.53 (t, 1H, $J = 9.8$ Hz), 5.10 (d, 1H, $J = 8.4$ Hz), 4.51 (s, 2H), 3.75 (s, 2H), 3.52–3.48 (m, 2H), 3.05 (d, 2H, $J = 11$ Hz), 2.00 (d, 3H, $J = 6.2$ Hz), 1.75–1.70 (s, 3H), 1.25–1.20 (m, 4H); $^{13}C$ (100 MHz, $CDCl_3$): 10.95, 11.54, 15.50, 27.73, 30.35, 38.23, 38.12, 42.46, 42.77, 46.32, 60.54, 73.56, 73.10, 82.52, 114.11, 114.25, 116.36, 116.26, 122.47, 123.78, 138.34, 139.20, 143.26, 145.94, 170.45, 220.34; ESI-MS: 557; Anal. Calcd for $C_{26}H_{29}F_3N_3O_5$: C, 66.50; H, 6.47; N, 9.31. Found: C, 66.55; H, 6.54; N, 9.36.

*4.1.3.16. Compound* ***3p***. Crystalline white solid; mp: 125 °C; $[\alpha]_D^{25}$ +36 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 737.25, 1019.29, 1128.80, 1154.25, 1269.45, 1365.34, 1534.85, 1627.47,1759.20, 2956.52, 3378.46; $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 7.73 (s, 1H), 7.00 (t, 2H, $J = 6.5$ Hz), 6.72–6.68 (m, 2H), 6.25 (s, 1H), 5.65 (s, 1H), 5.53 (t, 1H, $J = 9.8$ Hz), 5.15 (d, 1H, $J = 8.4$ Hz), 4.52 (s, 2H), 3.75 (s, 1H), 3.53–3.48 (m, 1H), 3.00 (d, 3H, $J = 10.1$ Hz), 2.05 (t, 2H, $J = 6.2$ Hz), 1.65–1.60 (m, 3H), 1.25–1.20 (m, 4H); $^{13}C$ (100 MHz, $CDCl_3$): 10.90, 11.53, 15.55, 27.70, 30.34, 38.00, 38.13, 42.40, 42.75, 46.37, 60.33, 73.28, 73.15, 115.14, 115.16, 116.24, 116.33, 123.28, 123.71, 138.32, 143.21, 143.45, 170.13, 220.34; ESI-MS: (M+Na) 477; Anal. Calcd for $C_{24}H_{27}FN_4O_4$: C, 63.42; H, 5.99; N, 12.33. Found: C, 63.46; H, 5.90; N, 12.41.

*4.1.3.17. Compound* ***3q***. Pale yellow solid; mp: 146–147 °C; $[\alpha]_D^{25}$ +18 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 727.16, 1019.45, 1110.76,1167.32, 1256.25, 1358.53, 1551.23, 1620.27, 1767.23, 2956.51, 3370.26; $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 8.00 (d, 1H, $J = 5.5$ Hz), 7.95 (d, 2H, $J = 6.6$ Hz), 7.50 (dd, 3H, $J = 7.55, 10.01$ Hz), 6.35 (d, 1H, $J = 2.6$ Hz), 5.76 (q, 2H, $J = 5.92$ Hz), 5.15 (d, 1H, $J = 8.2$ Hz), 3.83 (s, 1H), 3.45–3.55 (m, 1H), 3.24 (t, 2H, $J = 8.8$ Hz), 1.75–1.69 (m, 3H), 1.25–1.20 (m, 8H); $^{13}C$ (100 MHz, $CDCl_3$): 11.00,14.32, 17.41, 20.31, 22.97, 37.24, 39.35, 41.36, 54.34, 85.36, 110.21, 116.08, 116.30, 124.38, 125.97, 128.97, 129.06, 129.21, 150.29, 154.77, 155.66, 162.97, 165.47, 172.18, 185.97; ESI-MS: (M+Na) 489; Anal. Calcd for $C_{25}H_{30}N_4O_4$: C, 64.32; H, 7.29; N, 11.25. Found: C, 64.36; H, 7.34; N, 11.34.

*4.1.3.18. Compound* ***3r***. Colourless solid; mp: 122–123 °C; $[\alpha]_D^{25}$ +43 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 746.15, 1034.26, 1123.73, 1154.25, 1260.32, 1378.96, 1510.90,1617.35, 1759.24, 2934.05, 3386.56; $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 7.76 (s, 1H), 6.74 (d, 2H, $J = 8.5$ Hz), 6.65 (d, 2H, $J = 8.4$ Hz), 6.23 (s, 1H), 5.63 (s, 1H), 5.55 (t, 1H, $J = 9.8$ Hz), 5.10 (d, 1H, $J = 8.4$ Hz), 4.53 (s, 2H), 4.75 (s, 3H), 3.52–3.48 (m, 2H), 3.03 (d, 3H, $J = 10.1$ Hz), 1.75–1.70 (m, 3H), 1.25–1.20 (m, 4H); $^{13}C$ (100 MHz, $CDCl_3$): 10.94, 11.50, 15.56, 27.73, 30.35, 38.05, 38.10, 42.46, 42.75, 46.36, 60.34, 73.54, 73.10, 115.23, 115.14, 116.30, 116.35, 123.23, 123.70, 138.36, 143.27, 143.30, 170.21, 220.09; ESI-MS: (M+Na) 430; Anal. Calcd for $C_{23}H_{25}N_3O_4$: C, 55.82; H, 5.07; N, 8.14. Found: C, 55.86; H, 5.13; N, 8.20.

*4.1.3.19. Compound* ***3s***. Crystalline white solid; mp: 141–143 °C; $[\alpha]_D^{25}$ +32 (*c* 0.5, $CHCl_3$); IR (KBr, $cm^{-1}$): 720.46, 1024.13, 1124.26, 1156.30, 1249.23, 1334.53, 1524.46, 1634.37, 1756.43, 2945.21, 3370.13; $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 7.95 (s, 1H), 6.23 (d, 1H, $J = 2.65$ Hz), 5.91 (t, 1H, $J = 9.71$ Hz), 5.75 (d, 1H, $J = 2.24$ Hz), 5.00 (d, 1H, $J = 8.28$ Hz), 3.31 (d, 1H, $J = 1.51$ Hz), 3.13 (d, 2H, $J = 9.58$ Hz), 2.75 (t, 2H, $J = 7.76$ Hz), 2.00 (q, 2H, $J = 8.4$ Hz), 1.67–1.75 (m, 6H), 1.351–1.41 (m, 9H); $^{13}C$ (100 MHz, $CDCl_3$): 11.01, 17.43, 24.93, 37.28, 41.37, 41.76, 54.62, 80.59, 86.04, 125.93, 127.12, 128.33, 129.18, 131.65, 135.07, 150.35, 153.97, 154.86, 171.73, 186.00; ESI-MS: (M+K) 412; Anal. Calcd for $C_{20}H_{27}N_3O_4$: C, 68.13; H, 7.15; N, 8.51. Found: C, 68.16; H, 7.20; N, 8.57.

## 5. Biological assays

### 5.1. Evaluation of in vitro anti-cancer activity

The effect of 1,2,3-triazole derivatives of coronopilin on the growth of cancer cell lines was evaluated according to the procedure adopted by the National Cancer Institute for *in vitro* anti-cancer drug screening that uses the protein-binding dye Sulphorhodamine B to estimate cell growth [25]. Briefly, cells in their log phase of growth were harvested, counted and seeded ($10^4$ cells/well in 100 mL medium) in 96-well microtitre plates. After 24 h of incubation at 37 °C and 5% $CO_2$ to allow cell attachment, cultures were treated with varying concentrations (0.1–10 μM) of test samples made with 1:10 serial dilutions. Four replicate wells were set up for each experimental condition. Test samples were left in contact with the cells for 48 h under same conditions. Thereafter cells were fixed with 50% chilled TCA and kept at 4 °C for 1 h, washed and air-dried. Cells were stained with Sulphorhodamine B dye. The adsorbed dye was dissolved in tris-buffer and the plates were gently shaken for 10 min on a mechanical shaker. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was calculated by subtracting mean OD value of the respective blank from the mean OD value of experimental set. Percentage of growth in the presence of test material was calculated considering the growth in the absence of any test material as 100% and the results are reported in terms of $IC_{50}$ values.

### 5.2. DNA cell cycle analysis

Effect of Compound-**3a** on DNA content by cell cycle phase distribution was assessed using PC-3 cells by incubating the cells $1 \times 10^6$ mL/well with compound-**3a** (1, 5 and 10 μM each) for 24 h. The cells were then washed twice with ice-cold PBS, harvested, fixed with ice-cold PBS in 70% ethanol and stored at 20 °C for 30 min [26]. After fixation, these cells were incubated with RNase A (0.1 mg/mL) at 37 °C for 30 min, stained with propidium iodide (50 mg/mL) for 30 min on ice in dark, and then measured for DNA content using BD-LSR flow cytometer (Becton Dickinson, USA) equipped with electronic doublet discrimination capability using blue (488 nm) excitation from Argon laser. Data were collected in list mode on 10,000 events for FL2-A *vs* FL2-W.

### 5.3. NF-κB (p65), transcription factor assay

Effect of the test compound on the binding of NF-κB to its consensus DNA sequence was analyzed by using NF-κB (p65), transcription factor assay Kit from Cayman Chemical. Briefly, equal quantity of nuclear protein was loaded into each well containing immobilized DNA consensus sequence for NF-κB and incubated overnight at 4 °C. Wells were washed and incubated with primary antibody against NF-κB (p65) for 1 h. HRP-conjugated secondary antibody was added for 1 h to each wells after washing. Wells were incubated for 30 min with the substrate and the reaction was stopped before taking reading at 450 nm.

### 5.4. Western blot analysis

Protein was measured by using Bio-Rad protein assay reagent and protein lysates (70 μg) were subjected to SDS-PAGE analysis. Proteins were electro transferred to PVDF membrane for 90 min at 4 °C at 100 V. Non-specific binding was blocked by incubation with 5% non-fat milk in tris-buffered saline containing 0.1% Tween-20 (TBST), for 1 h at room temperature. The blots were probed with respective primary antibodies for 3 h and washed three times with TBST. Blots were incubated with horseradish peroxidase conjugated secondary antibodies for 1 h and washed three times with TBST. Blots were incubated with ECL plus reagent and signals were detected by using hyperfilm (GE Healthcare).

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.053.

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