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Original article

Synthesis and anticancer activity of novel spiro-isoxazoline and spiro-isoxazolidine derivatives of α-santonin

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ABSTRACT

In the present study, novel spiro derivatives of α-santonin were prepared and tested for their anticancer activity against a panel of six human cancer cell lines. Spiro-isoxazoline and spiro-isoxazolidine derivatives have been generated on C-ring of α-santonin (α-methylene-γ-butyrolactone) by the 1,3-dipolar cycloaddition of α-santonin derivative **6** with nitrile oxides **7** and nitrones **9** respectively. Among all, compound **10b″** had shown IC_{50} of 0.01, 0.5 and 0.3 μM against PC-3, THP-1 and MCF-7 cell lines respectively. Further, flow cytometry studies showed that PC-3 cells treated with the spiro-isoxazolidine derivative **10b″** were arrested in the sub G1 phase of the cell cycle in a concentration dependent manner. The spiro-isoxazolidine derivative **10b″** also showed concentration dependent inhibitory activity against NF-κB, p65 with 57% inhibition in 24 h at 10 μM.

1. Introduction

Sesquiterpene lactones (SLs) are the active constituents of a variety of medicinal plants used in traditional medicine for the treatment of inflammatory diseases [1]. In recent years, sesquiterpenes has attracted a great deal of interest due to their anticancer properties and extensive work has been carried to understand the molecular mechanisms and the potential chemo-preventive and chemo-therapeutic application of sesquiterpeniods [2–5]. At present, many SLs are in different phases of clinical trials such as Artemisinin **1**, Parthenolide **2**, Thaipsigargin **3** and many of their synthetic derivatives [6–8] (Fig. 1). These data indicate that SLs and related compounds may represent a promising class of anti-leukaemic agents.

(−)-α-Santonin (**4**) is the most important and the most abundant of a number of closely related sesquiterpenoid lactones possessing the eudesmane skeleton that have been isolated from various Asian plants of the genus *Artemisia* (especially from *Artemisia maritima*). (−)-α-Santonin is also commercially available and it has a highly functionalised A ring with a cross conjugated dienone system and a lactone moiety between C(6) and C(12). Chemical and biochemical transformations of α-santonin and its pharmacological properties have been studied already for over 100 years. α-Santonin was widely used in the past as an anthelminthic [9]. Recent studies demonstrate the biological importance of α-santonin as antipyretic, anti-inflammatory, and fungicidal [10,11] agent. In addition 11,13-dehydrosantonin and other α-santonin derivatives show cytotoxicity towards KB cells (cell line derived from carcinoma of nasopharyx). The cytotoxicity exhibited by these molecules has been attributed to the presence of α-methylene-γ-lactone moiety on the scaffold. Further, since α-santonin bears several reactive centres, it offers opportunity for further chemical modification of its scaffold to generate new secondary leads [12–14]. Several such derivatives of (−)-α-santonin have been synthesized by opening the lactone ring as well and some of the compounds demonstrate potential anticancer activity [15–17].

Our continued interest towards the design and synthesis of sesquiterpene lactones based anticancer agents [18], we planned the synthesis of novel α-santonin based anticancer agents. In this direction, we present here, the synthesis and cytotoxic activity of spiro-isoxazoline and spiro-isoxazolidine derivatives of α-santonin through rational structural modifications of the scaffold. In view of

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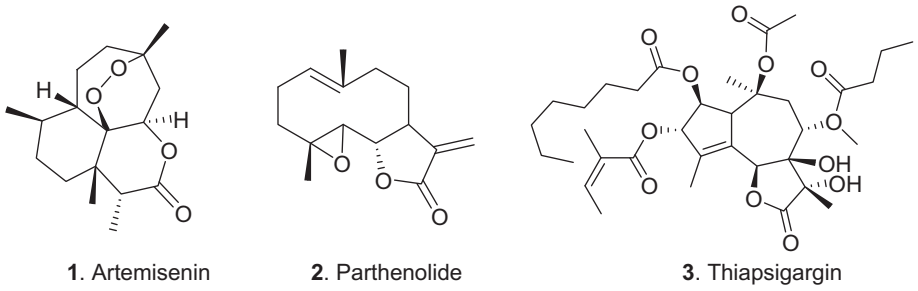
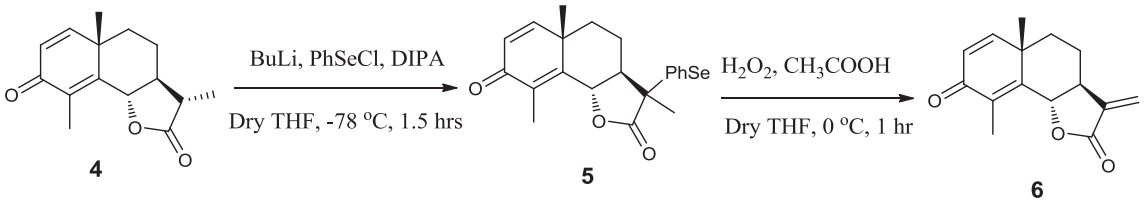


Fig. 1. Few SLs in clinical trials.



Scheme 1. Synthesis of exocyclic double bond on lactone ring of α-santonin.

the biological importance of spiro compounds together with the pharmacological significance of nitrogen heterocycles such as isoxazolines and isoxazolidines, novel spiro-heterocycles derived from α-santonin have been planned wherein the spiro-isoxazoline and spiro-isoxazolidine derivatives could be generated through 1,3-dipolar cycloaddition between corresponding nitrile oxides/nitrones and a dipolarophile (**6**) located on the α-santonin scaffold. A focussed library of 32 spiro-derivatives has been synthesized and screened for their anticancer activity against a panel of cancer cell lines. The novel spiro heterocycle appended derivatives of α-santonin thus designed have shown increased drug likeness as revealed by the parameters calculated using Schrödinger software.

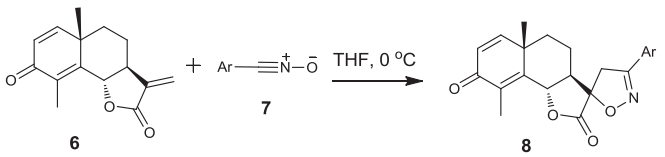
2. Results and discussion

2.1. Chemistry

In order to generate the spiro-derivatives, α-santonin (**4**) was converted into 3-oxo-7αH, 6βH,11-(phenylselenyl)-eudesma-1,4-dien-6,12-olide **5** on reaction with phenylselenylchloride and LDA followed by the subsequent oxidation with H_2O_2 in acetic acid generated the exocyclic double containing α-santonin derivative *viz*., 3-oxo-7αH, 6βH-eudesma-1,4,11-trien-6,12-olide **6** (Scheme 1) [19]. Compound **6** was then taken for 1,3-dipolar cycloaddition reaction with nitrile oxides and nitrones.

As illustrated in Scheme 2, spiro-isoxozoline derivatives of α-santonin (**8**) were prepared through 1,3-dipolar cycloaddition between α-santonin derivative (**6**) with various nitrile oxides (**7**) [20–28]. Nitrile oxides (**7**) were prepared according to the literature procedure in which various aromatic aldehydes were converted to corresponding aldoximes (*syn* and *anti*), followed by the reaction with *N*-chlorosuccinimide in DMF. Nitrile oxide cycloaddition was selectively done to the exocyclic double bond adjacent to lactone ring. The formation of spiro-isoxazoline ring is clearly confirmed by the disappearance of the alkene protons adjacent to lactone ring and the appearance of two singlets at δ 3.3–3.2 and δ 4.0–3.9 in ^{1}H NMR. These spiro-isoxozoline derivatives of α-santonin were formed regiospecifically in all cases. The regiospecific formation of cycloaddition product with exocyclic double bond present on the five membered lactone ring is attributed to steric hindrance offered by the methyl group present on the six membered ring to the approaching dipole in the transition state for the dipolar cycloaddition reaction.

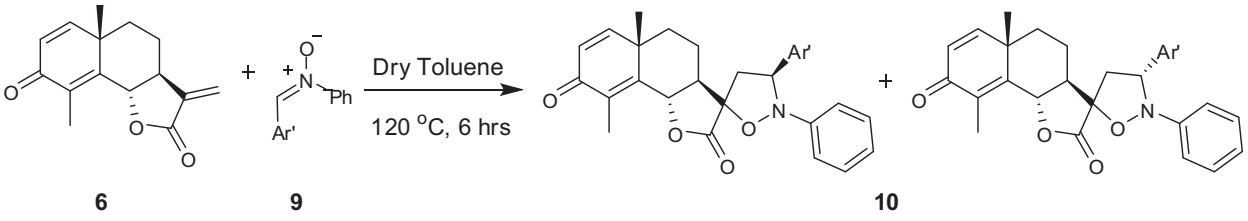
As illustrated in Scheme 3, spiro-isoxazolidine derivatives of α-santonin were prepared by the 1,3-dipolar cycloaddition between α-santonin derivative **10** and various nitrones **9** in dry benzene under reflux condition [29,30]. Nitrones **9** were prepared according to literature procedure in which nitrobenzene was reduced in the presence of Zn/NH_4Cl to get phenyl hydroxylamine followed by the condensation of various aromatic aldehydes. Nitrone cycloaddition was selectively done to the double bond adjacent to lactone ring, which is clearly confirmed by the disappearance of the alkene protons adjacent to lactone ring. Interestingly, in this case, two diastereomers were formed in 3:1 ratio in all the reactions in which one was isolated as major (non polar) while the other was minor (polar). Each diastereomer was isolated after column chromatography and characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry. A clear chemical shift deviation of benzylic proton adjacent to nitrogen atom in isoxazolidine ring between two



Ar

8a C_6H_5	**8i** 2-Napthalene
8b 4-NO_2-C_6H_4	**8j** 3,5-F-C_6H_4
8c 2,6-Cl-C_6H_4	**8k** 3-F, 4-Br-C_6H_4
8d 4-F-C_6H_4	**8l** 4-OH-C_6H_4
8e 2,4-Cl -C_6H_4	**8m** 2,5-Cl-C_6H_4
8f 3-F-C_6H_4	**8n** 2-Cl-C_6H_4
8g 4-OMe-C_6H_4	**8o** 9-Anthracene
8h 3-Br, 4-OMe-C_6H_4	**8p** 4-Br-C_6H_4

Scheme 2. Synthesis of spiro-isoxazoline derivatives of α-santonin.



Ar'

10a 4-NO_2-C_6H_4 (75%, *R* : *S* = 60 : 40)

10b 4-Br-C_6H_4 (78%, *R* : *S* = 70 : 30)

10c 2,5-Cl-C_6H_4 (82%, *R* : *S* =70 : 30)

10d 3-Br, 4F-C_6H_4 (84%, *R* : *S* = 65 : 35

10e 3,5-F-C_6H_4 (81%, *R* : *S* = 70 : 30

10f 3-Br,4-OMe-C_6H_4 (75%, *R* : *S* = 60 : 40)

10g 3-NO_2-C_6H_4 (79%, *R*: *S* = 70 : 30)

10h 1-Napthalene (86%, *R* : *S* = 60 : 40)

Scheme 3. Synthesis of spiro-isoxazolidine derivatives of α-santonin.

Table 1
IC_{50} values (μM) of various spiro-derivatives of α-santonin.

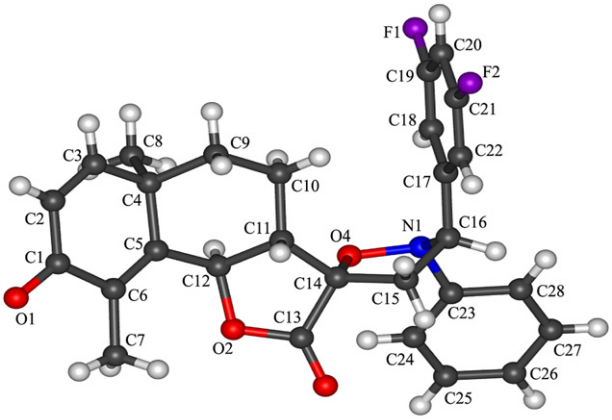
Compound	HCT-1	PC-3	THP-1	MCF-7	Hep-1	A-549
8a	100	78	100	100	100	100
8b	55	100	100	56	100	100
8c	10	100	100	27	15	100
8d	100	100	100	100	100	61
8e	29	26	35	95	29	13
8f	100	100	100	0.4	100	0.7
8g	52	100	7.9	0.02	100	0.2
8h	50	100	21	100	100	100
8i	100	0.6	0.4	30	100	2.9
8j	100	2.2	0.4	0.5	100	8.2
8k	15	2	0.6	0.9	33	3.1
8l	8.2	55	100	100	40	3.8
8m	61	100	100	39	100	100
8n	100	0.9	0.2	0.5	7.3	5.9
8o	45	0.3	0.8	0.2	50	45
8p	100	100	100	0.1	100	58
10a′	100	0.5	0.1	0.7	14	66
10a″	100	0.6	0.1	0.9	100	66
10b′	100	0.1	0.3	0.8	100	100
10b″	37	0.01	0.5	0.3	65	31
10c′	32	0.7	0.2	0.1	100	77
10c″	100	0.9	3.9	0.5	0.6	55
10d′	100	0.9	2.3	0.2	3.7	19
10d″	4.3	0.8	2.7	0.7	100	100
10e′	0.09	0.7	25	0.09	100	100
10e″	28	1.4	0.8	3.9	100	14
10f′	100	0.1	100	100	100	8.5
10f″	45	0.1	100	50	100	2.7
10g′	100	0.3	100	14	100	81
10g″	48	0.9	42	23	100	10
10h′	100	0.6	100	14	100	1.4
10h″	100	0.7	59	82	100	3.3
Mytomycine	0.5	–	–	–	–	–
Adriamycin	–	6	–	0.5	–	–
5-FU	–	2.2	1	–	4.5	4.9

′*R*-isomer, ″ *S*-isomer.

diastereomers was observed in ^{1}H NMR. In major isomer this proton appears as a triplet approximately at δ 5.2 while in the minor isomer this signal shifted towards more shielding region and appears approximately at δ 4.6. A series of nitrones were reacted with α-santonin and both diastereomers of spiro-isoxazolidine were isolated in each case. The formation of isoxazolidine ring in major isomer was confirmed by the appearance of two peaks approximately at δ 3.2 (dd) for one proton and other peak at δ 2.5 (dd) for another proton each with a coupling constant of 9.6 MHz and formation of minor isomer was confirmed by the appearance of two peaks at δ 3.2–2.8 (dd) for one proton and δ 2.5–2.6 (m) for other proton in ^{1}H NMR.

2.2. X-ray crystallography

To gain an insight into the structure and stereochemistry of the spiro-isoxazolidine derivatives of α-santonin, crystallisation of



Chiral centres: C(4) – R; C(11)– S; C(12)– R; C(14 –S; C(16)– R

Fig. 2. Molecular conformation of **10e′** in crystal.

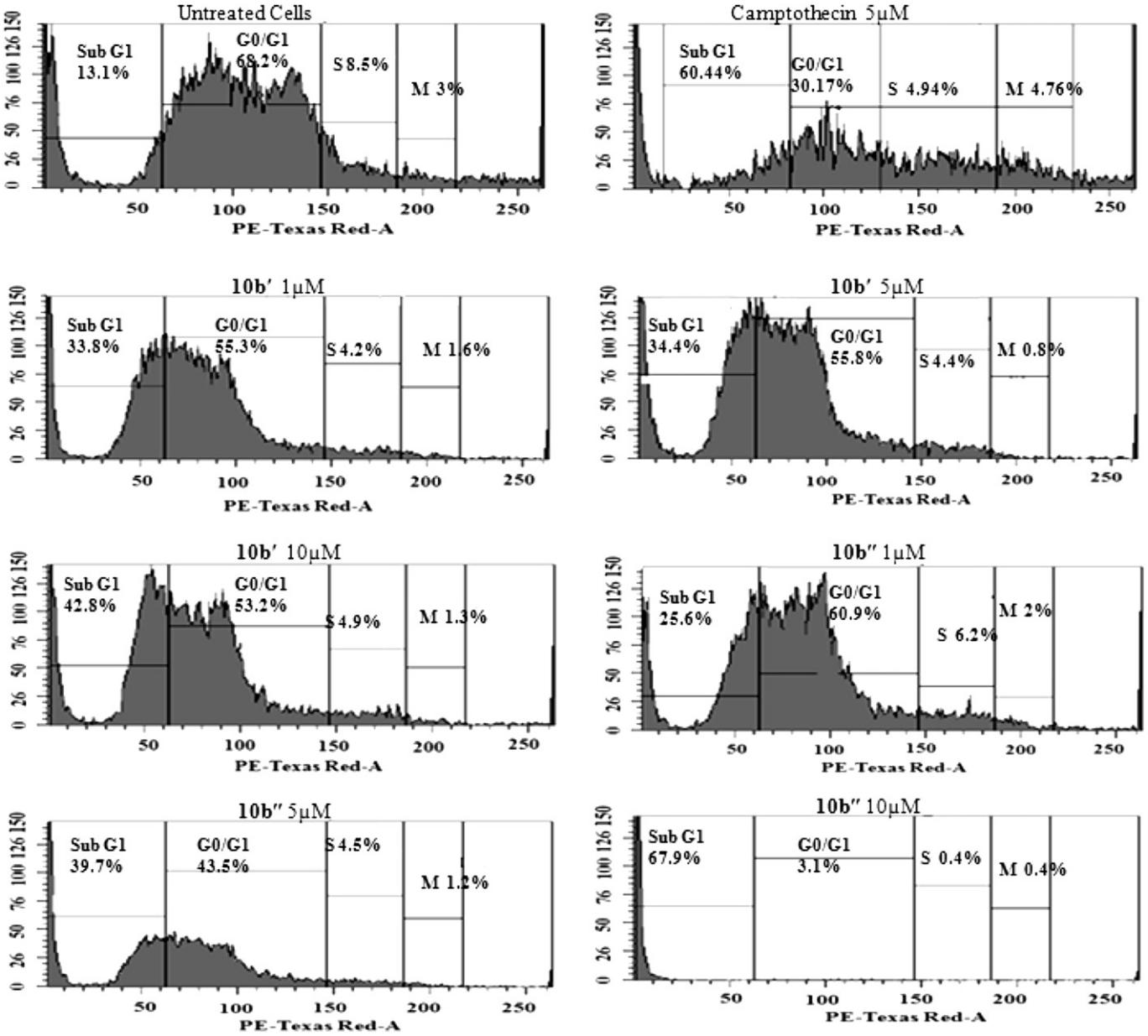


Fig. 3. Flow cytometric analysis of spiro-isoxazolidine derivatives of α-santonin **10b′** and **10b″** treated with PC-3 cell.

major diastereomer **10e′** was achieved through slow evaporation of solution of this compound in methanol containing few drops of water, and crystal structure of spiro-isoxazolidine derivative of α-santonin **10e′** was determined and its crystal data are presented in Table 1. X-ray analysis of spiro-isoxazolidine derivative of α-santonin **10e′** (structure given in Fig. 2), shows that the configuration of two newly generated chiral centre at C14 and C16 position is *S* and *R* respectively.

2.3. Biological results

2.3.1. Evaluation of in vitro anticancer activity

All the compounds were assayed for *in vitro* cytotoxicity against a panel of six human cancer cell lines including HCT-1 (colon), PC-3 (prostrate), THP-1 (leukaemia), MCF-7 (breast), Hep-2 (liver) and A549 (lung). Mitomycin, Adriamycin and 5-FU were taken as reference compounds and the results are reported in terms of IC_{50} values (results shown in Table 1). From the IC_{50} values, it is clear that majority of the compounds showed significant cytotoxicity against prostrate, leukaemia, breast and cervix derived cancer cell lines. However, it may be noted that among all the tested spiro-derivatives of α-santonin **8a**–**p** and **10a**–**h**, spiro-isoxazoline derivative **8g** and spiro-isoxazolidine derivatives **10b′**, **10b″** and **10e′** showed comparatively more potent IC_{50} value against HCT-1, PC-3 and MCF-7 cancer cell lines as compared to other derivatives.

Further, spiro-isoxazolidine derivatives of α-santonin have shown more promising activity as compared to the spiro-isoxazoline derivatives. Spiro-isoxazolidine derivatives **10b′** with 4-bromo substitution on the aromatic ring have shown IC_{50} value of 0.1, 0.3 and 0.8 μM against PC-3, THP-1 and MCF-7 cell lines respectively while its other isomer **10b″** has shown IC_{50} value of 0.01, 0.5 and 0.3 μM against PC-3, THP-1 and MCF-7 cell lines respectively. Among spiro-isoxazoline derivatives of α-santonin **8g** having 4-methoxy substitution also showed promising activity

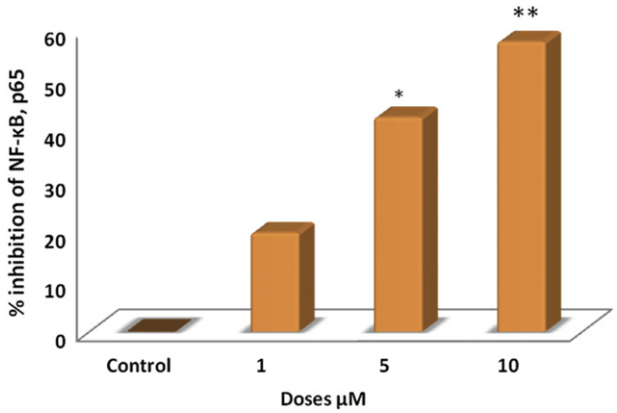


Fig. 4. NF-κB, p65 transcription factor assay. NF-κB, p65 transcription factor assay: 5×10^6 PC-3 cells were subjected to various concentrations of compound **10b″** for 24 h. Nuclear lysates were prepared and 20 μg of protein was loaded into each well containing the immobilized DNA response element for NF-κB, p65, after overnight incubation, wells were washed and incubated with the primary antibody against NF-κB, p65, followed by washing and incubation with HP conjugated secondary antibody. Well were washed again and substrate was added to the wells for 30 min. Optical density (OD) was taken at 450 nm. The activity of NF-κB, p65 in the control samples was considered to be 100% and therefore OD value of control was converted to hypothetical value of 100% and other samples were normalized accordingly. Statistical comparisons were done by using Bonferroni method and p-value <0.05 was considered to be significant, $^{*}p < 0.05$, $^{**}p < 0.001$.

with IC_{50} value of 0.02 and 0.2 μM against MCF-7 and A549 cell lines respectively. From the above results, two most potent spiro-isoxazolidine derivatives **10b′** and **10b″** were taken for cell cycle analysis.

2.3.2. *Cell cycle analysis of compounds **10b′** and **10b″***

To address the cell death caused by spiro-isoxazolidine derivatives **10b′** and **10b″**, the extent of apoptotic death in PC-3 cell lines was assessed using FACS flow cytometry through the determination of sub-G1 cell population by propidium iodide (PI) staining. The DNA cell cycle analysis of spiro-isoxazolidine derivative **10b′** revealed a concentration dependent increase in the sub G1 phase of cell cycle being 33.8, 34.7 and 42.8 at 1, 5 and 10 μM respectively. Spiro-isoxazolidine derivative **10b″** also showed a concentration dependent increase in the sub G1 phase of cell cycle being 25.6, 39.7 and 67.9 at 1, 5 and 10 μM respectively. Also complete blockage of G1 phase at 10 μM concentration after 24 h of incubation in PC-3 cells was observed with spiro-isoxazolidine derivative **10b″** (Fig. 3).

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

In literature, most of SLs having α,β-unsaturated functionality are NF-κB inhibitors [31]. In our study, we visualized that the spiro-isoxazolidine derivative **10b″** possesses α,β-unsaturated functionality and it might also work through by inhibiting NF-κB transcription factor. In this direction, the most potent spiro-isoxazolidine derivative **10b″** was taken for inhibitory NF-κB (p65) transcription factor activity.

NF-κB is structurally related and evolutionary conserved group of transcription factors with five members in mammals *viz*., Rel (c-Rel), Rel A (p65), RelB, NF-κB1 (p50 and its precursor p105) and NF-κB2 [31a]. NF-κB plays a central role in the regulation of cancer cell proliferation and survival [32]. NF-κB has also been shown to play a vital role in the cancer cell progression by regulating the process of angiogenesis [33]. Therefore, many new anticancer therapies are being developed against NF-κB. Spiro-isoxazolidine derivative **10b″** showed highest anti-proliferative activity against PC-3 among various other cancer cell lines. On account of its promising anti-cancer activity in PC-3, we choose this cell line to analyse the effect of spiro-isoxazolidine derivative **10b″** against NF-κB by using transcription factor binding assay. Our data showed that PC-3 cells treated with spiro-isoxazolidine derivative **10b″** displayed concentration dependent inhibition of NF-κB (p65) binding with DNA response element, where, cells treated with 1 μM showed around 20% of reduction in the binding of NF-κB with the consensus DNA sequence, which was further enhanced to 43% at 5 μM, and 57% at 10 μM (Fig. 4). Thus, our results clearly indicate that spiro-isoxazolidine derivative **10b″** has a good inhibitory effect on the activity of NF-κB (p65), therefore, spiro-isoxazolidine derivative of α-santonin **10b″** represented new natural product based anticancer lead.

3. Conclusion

Generation of exocyclic double bond on C ring of α-santonin and further construction of spiro heterocycles on the α-methylene-γ-butyrolactone moiety of α-santonin would possibly improved the activity through better protein modulation and enhance the bio-availability through improved drug likeness. With this aim, the exocyclic double bond was generated on the α-santonin scaffold through appropriate modifications which was further utilized for the incorporation of desired heterocyclic structural entities on the scaffold in order to develop α-santonin based anticancer agents.

In this direction, the readily available α-santonin (**4**) was used as a starting material to generate exocyclic double bond on its lactone ring. The resulting α,β-unsaturated compound **6** with exocyclic double bond on lactone ring was used as a synthon for the preparation of novel spiro-isoxozoline and spiro-isoxazolidine derivatives. During the synthesis of spiro-isoxazolidine, two diastereomers were formed whose stereochemistry was determined through X-ray crystallography. Crystal structure of one of the major isomer **10e** showed the configuration at newly generated two chiral centres at C14 and C16 position is *S* and *R* respectively. All the synthesised derivatives were evaluated against a panel of human cancer cell lines. Among all the tested spiro-derivatives of α-santonin **8a**–**p** and **10a**–**h**, spiro-isoxazolidine derivative **8g** and spiro-isoxazolidine derivatives **10b′**, **10b″** and **10e′** showed comparatively more potent IC_{50} value against HCT-1, PC-3 and MCF-7 cancer cell lines as compared to other derivatives. Further, spiro-isoxazolidine derivatives of α-santonin have shown more promising activity as compared to the spiro-isoxazoline derivatives. Spiro-isoxazolidine derivatives **10b′** with 4-bromo substitution on the aromatic ring has shown IC_{50} value of 0.1, 0.3 and 0.8 μM against PC-3, THP-1 and MCF-7 cell lines respectively while its other isomer **10b″** shown IC_{50} value of 0.01, 0.5 and 0.3 μM against PC-3, THP-1 and MCF-7 cell lines respectively. Both the compounds showed dose dependent increase in Sub G1 (apoptotic phase). Spiro-isoxazolidine derivative **10b″** showed 67.9% apoptosis at 10 μM concentration and also complete blockage of all other phases of cell cycle was observed with this compound at the same concentration. Among spiro-isoxozoline series compound **8g** was found to be most active and possesses IC_{50} value of 0.02 and 0.2 μM against MCF-7 and A549 cell lines respectively. Furthermore, spiro-isoxazolidine derivative **10b″** also displayed marked inhibitory activity against the central regulator of cancer cell growth and survival, NF-κB. Moreover, drug-like parameter calculated using Schödinger software also suggested the spiro-isoxazolidine derivative **10b″** possesses the requisite properties required for clinical candidates. These results suggested that spiro-isoxazolidine derivative of α-santonin **10b″** represents new natural product based promising candidate which could be taken for further development and detailed studies.

4. Experimental

Melting points were recorded on Buchi Melting point apparatus D-545. NMR spectra were recorded on Bruker DPX400 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 QTOF Daltonics instrument. The progress of all reactions was monitored by TLC on 2–5 cm percolated silica gel 60 F_{254} plates of thickness 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine.

4.1. Synthesis of spiro-isoxazoline derivatives of α-santonin

In a typical procedure, to a solution of benzene nitrile oxide (0.577 g, 1.2 mmol) in THF (10 mL) stirred over a period of 10 min, maintaining the temperature between 0 and 5 °C, added santonin (0.262 g, 1 mmol) and stirred the reaction mixture at same temperature for 15 min followed by stirring at ambient temperature for 2 h. The solvent was evaporated *in vacuo* and the crude was subjected for flash chromatography ethyl acetate/hexane (3:7). The pure products (**8a**–**8p**) were characterized on the basis of ^{1}H NMR, ^{13}C NMR and mass spectrometry.

4.1.1. Compound **8a**

Crystalline pale pink solid; mp: 168–170 °C; $[\alpha]_D^{25}$ +78 (*c* 1.0, $CHCl_3$); yield 81%; IR (KBr, cm^{-1}): 542.86, 760.18, 834.68, 906.40, 1039.17, 1110.19, 1243.45, 1302.20, 1360.92, 1513.90, 1637.25, 1663.28, 1791.31, 3368.25; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, 2H, J = 7.6, 1.6 Hz), 7.40–7.47 (m, 3H), 6.70 (d, 1H, J = 10 Hz), 6.30 (d, 1H, J = 10 Hz), 5.30 (dd, 1H, J = 9.6, 1.2 Hz), 4.00 & 3.96 (s, together integrating to diastereomeric 1H), 3.32 & 3.28 (s, together integrating to diastereomeric 1H), 2.20 (s, 3H), 1.95 (dd, 1H, J = 4.8, 2.4 Hz), 1.80–1.84 (m, 2H), 1.45–1.70 (m, 2H), 1.37 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.00, 17.41, 22.97, 23.77, 24.91, 28.93, 30.38, 37.24, 39.35, 41.36, 54.34, 80.63, 85.75, 116.08, 125.97, 128.97, 129.06, 150.29, 154.77, 165.47, 172.18, 185.97; Mass QTOF: 386 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{21}NO_4$: C, 72.71; H, 5.82; N, 3.85. Found: C, 72.69; H, 5.80; N, 3.8.

4.1.2. Compound **8b**

Crystalline yellow solid; mp: 206–210 °C; $[\alpha]_D^{25}$ +43 (*c* 1.0, $CHCl_3$); yield 76%; IR (KBr, cm^{-1}): 550.14, 610.38, 765.48, 934.83, 978.34, 1119.87, 1127.16, 1240.45, 1275.63, 1293.41, 1368.42, 1458.11, 1633.48, 1660.67, 1794.66, 2930.21, 3364.61; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, 2H, J = 8.8 Hz), 7.80 (d, 2H, J = 8.8 Hz), 6.70 (d, 1H, J = 9.6 Hz), 6.20 (d, 1H, J = 9.6 Hz), 5.25 (dd, 1H, J = 9.6, 1.2 Hz), 3.94 & 3.84 (s, together integrating to diastereomeric 1H), 3.29 & 3.25 (s, together integrating to diastereomeric 1H), 2.20 (s, 3H), 1.90 (t, 1H, J = 13.2 Hz), 1.70 (dd, 2H, J = 4.2, 2.4 Hz), 1.50–1.40 (m, 2H), 1.40 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.02, 17.42, 22.64, 24.94, 37.18, 38.68, 41.36, 54.19, 80.68, 86.67, 100.01, 124.18, 126.02, 127.79, 129.36, 134.07, 149.01, 150.00, 154.71, 155.20, 171.74, 185.93; Mass QTOF: 431 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{20}N_2O_6$: C, 64.70; H, 4.94; N, 6.8. Found: C, 64.65; H, 4.90; N, 6.95.

4.1.3. Compound **8c**

Crystalline white solid; mp: 163–166 °C; $[\alpha]_D^{25}$ +38 (*c* 0.5, $CHCl_3$); yield 82%; IR (KBr, cm^{-1}): 541.34, 667.00, 713.94, 754.66, 786.08, 834.39, 869.02, 946.15, 987.50, 1038.58, 1110.36, 1138.14, 1241.79, 1297.82, 1337.02, 1560.12, 1616.04, 1637.41, 1792.20, 2932.55, 3400.42; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, 1H, J = 8.4 Hz), 7.35–7.45 (m, 2H), 6.73 (d, 1H, J = 9.6 Hz), 6.35 (d, 1H, J = 9.6 Hz), 5.32 (d, 1H, J = 10.4 Hz), 4.02 & 3.97 (s, together integrating to diastereomeric 1H), 3.23 (s, together integrating to diastereomeric 1H), 2.24 (s, 3H), 2.10–2.03 (m, 1H), 1.70 (dd, 2H, J = 4.2, 2.4 Hz), 1.50–1.40 (m, 2H), 1.40 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.07, 17.43, 19.26, 24.93, 30.32, 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, 186.0; Mass QTOF: 454 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{19}Cl_2NO_4$: C, 61.12; H, 4.43; N, 3.24. Found: C, 61.09; H, 4.45; N, 3.27.

4.1.4. Compound **8d**

Crystalline white solid; mp: 157–160 °C; $[\alpha]_D^{25}$ +23 (*c* 0.5, $CHCl_3$); yield 75%; IR (KBr, cm^{-1}): 541.34, 667.00, 713.94, 754.66, 786.08, 834.39, 869.02, 946.15, 987.50, 1038.58, 1110.36, 1138.14, 1241.79, 1297.82, 1337.02, 1560.12, 1616.04, 1637.41, 1792.20, 2932.55, 3400.42; ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.70 (d, 2H, J = 6.8 Hz), 7.20 (d, 2H, J = 8.4 Hz), 6.71 (d, 1H, J = 9.6 Hz), 6.30 (d, 1H, J = 9.6 Hz), 5.42 (t, 1H, J = 10 Hz), 3.97 & 3.93 (s, together integrating to diastereomeric 1H), 3.30 & 3.25 (s, together integrating to diastereomeric 1H), 2.24 (s, 3H), 2.11 (dd, 2H, J = 4.0, 2.3 Hz), 1.93–2.01 (m, 1H), 1.39–1.41 (m, 2H), 1.27 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.01, 14.05, 17.40, 37.22, 38.72, 41.35, 80.61, 85.72, 116.10, 116.28, 124.32, 125.98, 128.80, 128.973, 129.04, 129.22, 130.89, 139.91, 150.26, 154.77, 155.63, 163.21, 165.21; Mass QTOF: 404 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{20}FNO_4$: C, 69.28; H, 5.29; N, 3.67. Found: C, 69.20; H, 5.32; N, 3.70.

4.1.5. Compound **8e**

Crystalline white solid; mp: 174–177 °C; $[\alpha]_D^{25}$ +32 (*c* 0.5, $CHCl_3$); yield 77%; IR (KBr, cm^{-1}): 758.03, 833.68, 868.62, 905.50, 987.54, 1039.09, 1109.47, 1138.80, 1204.54, 1242.12, 1303.15, 1433.63, 1637.40, 1663.54, 1790.77, 2932.81; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (d, 1H, J = 8.4 Hz), 7.54 (s, 1H), 7.32 (d, 1H, J = 2 Hz), 6.70 (d, 1H, J = 9.6 Hz), 6.35 (d, 1H, J = 9.6 Hz), 5.35 (d, 1H, J = 10.4 Hz), 4.04 & 3.99 (s, together integrating to diastereomeric 1H), 3.54 & 3.50 (s, together integrating to diastereomeric 1H), 2.21 (s, 3H), 2.13 (dd, 2H, J = 5.5, 2.3 Hz), 1.90–1.86 (m, 1H), 1.39–1.32 (m, 2H), 1.27 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.99, 17.50, 24.91, 29.68, 37.25, 41.33, 41.51, 54.42, 80.51, 86.42, 125.99, 126.08, 127.73, 129.26, 130.60, 131.53, 133.61, 137.19, 150.14, 154.71, 155.75, 171.83, 185.93; Mass QTOF: 454 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{19}Cl_2NO_4$: C, 61.12; H, 4.43; N, 3.24. Found: C, 61.15; H, 4.39; N, 3.20.

4.1.6. Compound **8f**

Crystalline white solid; mp: 162–164 °C; $[\alpha]_D^{25}$ +50 (*c* 0.5, $CHCl_3$); yield 72%; IR: (KBr, cm^{-1}): 525.76, 570.78, 667.82, 754.44, 832.03, 870.77, 904.40, 988.13, 1039.56, 1077.84, 1077.84, 1138.39, 1244.99, 1345.49, 1382.78, 1464.48, 1587.55, 1615.78, 1637.45, 1719.69, 2928.44, 3368.19; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (d, 3H, J = 1.2 Hz), 7.20–7.16 (m, 1H), 6.71 (d, 1H, J = 9.6 Hz), 6.34 (d, 1H, J = 9.6 Hz), 5.32 (t, 1H, J = 8.8 Hz), 3.97 & 3.93 (s, together integrating to diastereomeric 1H), 3.30 & 3.25 (s, together integrating to diastereomeric 1H), 2.20 (s, 3H), 2.12 (dd, 2H, J = 9.6, 1.5 Hz), 1.94 (t, 1H, J = 13.2 Hz), 1.73 (dd, 2H, J = 2.8, 1.3 Hz), 1.44 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.01, 14.05, 17.40, 37.21, 38.72, 41.35, 80.61, 85.72, 116.10, 116.28, 124.32, 127.98, 128.80, 128.97, 129.04, 129.22, 130.89, 139.91, 150.26, 154.77, 155.63, 163.21, 165.21; Mass QTOF: 404 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{20}FNO_4$: C, 69.28; H, 5.29; N, 3.67. Found: C, 69.26; H, 5.23; N, 3.71.

4.1.7. Compound **8g**

Crystalline pale yellow solid; mp: 196–198 °C; $[\alpha]_D^{25}$ +16 (*c* 0.5, $CHCl_3$); yield 81%; IR (KBr, cm^{-1}): 508.50, 674.95, 771.48, 832.71, 987.14, 1039.54, 1123.35, 1245.99, 1374.04, 1436.17, 1582.22, 1622.01, 1637.41, 1664.10, 1721.48, 1792.13, 2928.36, 3368.46; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, 1H, J = 8.8 Hz), 7.60 (dd, 1H, J = 8.4, 2.0 Hz), 6.92 (dd, 2H, J = 10, 8.8 Hz), 6.71 (d, 1H, J = 9.6 Hz), 6.32 (d, 1H, J = 9.6 Hz), 5.30 (d, 1H, J = 8.4 Hz), 3.97 (s, 3H), 3.91 (dd, 1H,

J = 17.5, 6.8 Hz), 3.31 (dd, 1H, J = 17.2, 6.4 Hz), 2.24 (s, 3H), 2.11 (dd, 2H, J = 11.2, 4.8 Hz), 2.00–1.86 (m, 2H), 1.53–1.40 (m, 2H), 1.44 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.07, 17.48, 24.98, 37.31, 37.35, 39.68, 54.46, 55.50, 85.38, 114.44, 120.56, 121.45, 123.34, 126.83, 128.62, 129.24, 150.38, 154.85, 155.35, 157.07, 161.79, 172.45, 186.12; Mass QTOF: 416 $(M + Na)^+$; Anal. Calcd for $C_{23}H_{23}NO_5$: C, 70.21; H, 5.89; N, 3.56. Found: C, 70.16; H, 5.85; N, 3.60.

4.1.8. Compound **8h**

Crystalline yellow solid; mp: 205–208 °C; $[\alpha]_D^{25}$ +56 (c 0.5, $CHCl_3$); yield 78%; IR (KBr, cm^{-1}): 755.71, 832.83, 904.62, 1039.58, 1137.88, 1276.06, 1302.64, 1419.89, 1508.68, 1606.64, 1636.59, 1663.00, 1791.25, 2929.94, 3368.51; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.65 (d, 1H, J = 6.8 Hz), 6.95 (d, 1H, J = 7.6 Hz), 6.73 (d, 1H, J = 9.6 Hz), 6.35 (d, 1H, J = 9.6 Hz), 5.32 (d, 1H, J = 10 Hz), 3.95 (s, 3H), 3.91 & 3.90 (s, together integrating to diastereomeric 1H), 3.27 & 3.23 (s, together integrating to diastereomeric 1H), 2.36 (t, 1H, J = 4 Hz), 2.12 (s, 3H), 2.03 (d, 1H, J = 8.8 Hz), 1.91 (d, 1H, J = 8.8 Hz), 1.64 (s, 3H), 1.43 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.01, 17.61, 25.08, 37.54, 41.34, 43.98, 53.46, 56.33 69.62, 80.97, 82.02, 112.13, 117.36, 121.63, 126.0, 129.14, 131.61, 132.80, 149.62, 150.70, 154.83, 173.41, 186.09; Mass QTOF: 494 $(M + Na)^+$; Anal. Calcd for $C_{23}H_{22}BrNO_5$: C, 58.49; H, 4.69; N, 2.97. Found: C, 58.44; H, 4.73; N, 3.0.

4.1.9. Compound **8i**

Crystalline white solid; mp: 182–185 °C; $[\alpha]_D^{25}$ +64 (c 0.5, $CHCl_3$); yield 76%; IR (KBr, cm^{-1}): 550.45, 612.28, 780.68, 904.83, 981.13, 1113.87, 1128.56, 1239.49, 1277.63, 1293.48, 1379.41, 1453.01, 1631.58, 1658.87, 1787.83, 2930.11, 3369.95; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (dd, 5H, J = 8.4, 2.8 Hz), 7.61–7.54 (m, 2H), 6.77 (d, 1H, J = 9.6 Hz), 6.31 (d, 1H, J = 9.6 Hz), 5.32 (t, 1H, J = 9.2 Hz), 4.12 & 4.08 (s, 1H together integrating with diastereomeric proton), 3.45 & 3.41 (s, 1H together integrating with diastereomeric proton), 2.21 (s, 3H), 2.12–1.98 (m, 1H), 1.94–1.81 (m, 2H), 1.81–1.70 (m, 2H), 1.63 (s, 2H), 1.56 (dd, 1H, J = 4.4, 1.5 Hz), 1.43 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.03, 17.39, 24.89, 37.21, 39.26, 41.33, 54.42, 80.61, 85.77, 123.32, 125.60, 125.95, 127, 127.59, 127.66, 127.90, 128.49, 128.84, 129.18, 132.86, 134.31, 150.33, 154.78, 156.78, 172.25, 185.98; Mass QTOF: 436 $(M + Na)^+$; Anal. Calcd for $C_{26}H_{23}NO_4$: C, 75.53; H, 5.61; N, 3.39. Found: C, 75.58; H, 5.65; N, 3.43.

4.1.10. Compound **8j**

Crystalline white solid; mp: 153–155 °C; $[\alpha]_D^{25}$ +30 (c 0.5, $CHCl_3$); yield 72%; IR (KBr, cm^{-1}): 553.58, 588.88, 647.36, 667.70, 797.28, 863.68, 905.73, 1038.62, 1138.90, 1153.90, 1165.94, 1260.38, 1301.85, 1376.34, 1615.73, 1637.59, 1663.42, 1791.14, 2930.71, 3367.99; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (t, 2H, J = 5.6 Hz), 6.90–6.85 (m, 1H), 6.72 (d, 1H, J = 9.6 Hz), 6.31 (d, 1H, J = 9.6 Hz), 5.34 (t, 1H, J = 9.6 Hz), 3.94 & 3.89 (s, together integrating to diastereomeric 1H), 3.27 & 3.23 (s, together integrating to diastereomeric 1H), 2.24 (s, 3H), 2.10–2.03 (m, 1H), 1.93–1.82 (m, 2H), 1.80–1.76 (m, 2H), 1.43 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.96, 14.05, 17.40, 22.49, 23.76, 28.93, 30.37, 37.18, 38.81, 41.33, 54.27, 68.18, 80.61, 86.31, 106.04, 106.29, 109.88, 109.96, 126.05, 128.81, 129.38, 130.90, 131.18, 132.48, 149.97, 155.09, 161.82, 161.94, 164.43, 171.77, 185.93; Mass QTOF: 422 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{19}F_2NO_4$: C, 66.16; H, 4.80; N, 3.51. Found: C, 66.15; H, 4.76; N, 3.54.

4.1.11. Compound **8k**

Crystalline white solid; mp: 234–236 °C; $[\alpha]_D^{25}$ +18 (c 0.5, $CHCl_3$); yield 75%; IR (KBr, cm^{-1}): 556.45, 615.28, 781.58, 914.43, 978.13, 1120.87, 1132.56, 1245.40, 1276.63, 1293.38, 1376.41, 1468.01, 1645.58, 1660.87, 1792.86, 2934.21, 3363.81; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.61 (s, 1H), 7.34 (d, 1H, J = 8 Hz), 6.72 (d, 1H, J = 9.6 Hz), 6.32 (d, 1H, J = 9.6 Hz), 5.30 (d, 1H, J = 9.6 Hz), 3.94 & 3.90 (s, together integrating to diastereomeric 1H), 3.28 & 3.24 (s, together integrating to diastereomeric 1H), 2.38 (s, 3H), 2.04–1.95 (m, 1H), 1.78–1.70 (m, 2H), 1.60–1.52 (m, 2H), 1.44 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.02, 12.99, 14.20, 37.20, 41.33, 53.43, 80.60, 86.05, 109.88, 110.10, 117, 117.23, 125.78, 126.04, 129.35, 132.23, 150.02, 154.65, 159.25, 161.77, 171.89, 185.93; Mass QTOF: 482 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{19}BrFNO_4$: C, 57.41; H, 4.16; N, 3.04. Found: C, 57.45; H, 4.20; N, 3.0.

4.1.12. Compound **8l**

Crystalline white solid; mp: 167–169 °C; $[\alpha]_D^{25}$ +24 (c 1.0, $CHCl_3$); yield 82%; IR (KBr, cm^{-1}): 542.73, 610.31, 778.48, 897.65, 985.14, 1107.57, 1125.56, 1247.43, 1275.63, 1276.44, 1345.41, 1434.12, 1634.28, 1665.35, 1778.96, 2935.21, 3456.81; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, 1H, J = 3.2 Hz), 7.92 (d, 1H, J = 14 Hz), 7.73 (dd, 2H, J = 9.6, 6.4 Hz), 6.92 (d, 1H, J = 9.6 Hz), 6.30 (d, 1H, J = 9.6 Hz), 5.32 (d, 1H, J = 11.2 Hz), 3.84 & 3.80 (s, together integrating to diastereomeric 1H), 3.55 & 3.51 (s, together integrating to diastereomeric 1H), 2.34 (t, 1H, J = 11.6 Hz), 2.20 (s, 3H), 2.16 (t, 2H, J = 4.8 Hz), 1.90–1.83 (m, 2H), 1.43 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.14, 18.23, 25.09, 38.32, 39.82, 40.11, 42.97, 48.55, 54.75, 54.83, 82.20, 87.56, 117.48, 120.47, 122.37, 124.3, 126.08, 128.03, 129.15, 130.85, 154.25, 173.94, 188.23; Mass QTOF: 402 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{21}NO_5$: C, 69.64; H, 5.58; N, 3.69. Found: C, 69.62; H, 5.59; N, 3.71.

4.1.13. Compound **8m**

Crystalline white solid; mp: 198–201 °C; $[\alpha]_D^{25}$ +53 (c 0.5, $CHCl_3$); yield 77%; IR (KBr, cm^{-1}): 553.73, 615.38, 667.01, 785.48, 904.83, 987.13, 1109.87, 1137.56, 1242.49, 1272.66, 1291.48, 1378.41, 1456.01, 1638.58, 1662.87, 1790.86, 2932.21, 3367.81; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, 1H, J = 8.4 Hz), 7.35 (d, 1H, J = 6.4 Hz), 7.25 (d, 1H, J = 1.2 Hz), 6.72 (d, 1H, J = 9.6 Hz), 6.38 (d, 1H, J = 9.6 Hz), 5.34 (d, 1H, J = 10.8 Hz), 4.02 & 3.97 (s, together integrating to diastereomeric 1H), 3.23 & 3.18 (s, together integrating to diastereomeric 1H), 2.24 (s, 3H), 2.10 (t, 1H, J = 12.4 Hz), 2.03–1.94 (m, 2H), 1.72–1.69 (m, 2H), 1.56 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.92, 12.50, 17.44, 35.27, 287.30, 37.87, 54.67, 80.58, 81.40, 125.92, 125.97, 126.14, 127.13, 128.32, 129.24, 153.96, 154.13, 154.83, 171.72, 186.03; Mass QTOF: 454 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{19}Cl_2NO_4$: C, 61.12; H, 4.43; N, 3.24. Found: C, 61.15; H, 4.39; N, 3.20.

4.1.14. Compound **8n**

Crystalline white solid; mp: 177–178 °C; $[\alpha]_D^{25}$ +61 (c 1.0, $CHCl_3$); yield 80%; IR (KBr, cm^{-1}): 553.73, 615.38, 667.01, 785.48, 904.83, 987.13, 1109.87, 1137.56, 1242.49, 1272.66, 1291.48, 1378.41, 1456.01, 1638.58, 1662.87, 1790.86, 2932.21, 3367.81; ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.60–7.54 (m, 1H), 7.51 (d, 1H, J = 1.2 Hz), 7.49 (d, 1H, J = 3.2 Hz), 7.45 (d, 1H, J = 5.2 Hz), 7.32 (d, 1H, J = 4 Hz), 6.75 (d, 1H, J = 9.6 Hz), 6.31 (d, 1H, J = 9.6 Hz), 5.36 (d, 1H, J = 10.4 Hz), 4.05 & 4.01 (s, together integrating to diastereomeric 1H), 3.57 & 3.52 (s, together integrating to diastereomeric 1H), 2.24 (s, 3H), 2.20–2.10 (m, 1H), 2.03–1.94 (m, 2H),1.75 (t, 2H, J = 6.5 Hz), 1.46 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.93, 12.51, 17.54, 23.13, 24.92, 30.92, 37.31, 41.35, 53.55, 80.41, 86.25, 126.01, 129.27, 130.69, 131.62, 132.93, 150.25, 154.75, 156.62, 171.97, 171.54, 186.05; Mass QTOF: 420 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{20}ClNO_4$: C, 66.42; H, 5.07; N, 3.52. Found: C, 66.45; H, 5.12; N, 3.54.

4.1.15. Compound **8o**

Crystalline transparent solid; mp: 220–224 °C; $[\alpha]_D^{25}$ +38 (c 1.0, $CHCl_3$); yield 75%; IR (KBr, cm^{-1}): 553.73, 615.38, 785.48, 904.83, 987.13, 1109.87, 1137.56, 1242.49, 1272.66, 1291.48, 1378.41, 1456.01, 1638.58, 1662.87, 1790.86, 2932.21, 3367.81; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, 1H, J = 8.0 Hz), 8.05 (d, 1H, J = 8.8 Hz), 7.62 (dd, 2H,

J = 5.6, 1.2 Hz), 7.51 (dd, 1H, J = 10.4, 6.8 Hz), 7.35 (s, 1H), 6.88 (d, 1H, J = 9.6 Hz), 6.31 (d, 1H, J = 9.6 Hz), 5.45 (d, 1H, J = 9.6 Hz), 4.12 & 4.08 (s, together integrating to diastereomeric 1H), 3.44 & 3.39 (s, together integrating to diastereomeric 1H), 2.42 (t, 1H, J = 7.6 Hz), 2.20–2.11 (m, 3H), 1.70–1.66 (m, 4H), 1.5 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.2, 18.6, 27.3, 37.9, 39.0, 41.3, 43.2, 75.6, 83.4, 126.0, 126.7, 127.0, 127.3, 127.6, 128.3, 128.9, 129.0, 129.2, 130.3, 130.6, 131.7, 141.0, 155.6, 156.2, 162.9, 172.3, 187.9; Mass QTOF: 486 $(M + Na)^+$; Anal. Calcd for $C_{30}H_{25}NO_4$: C, 77.74; H, 5.44; N, 3.02. Found: C, 77.77; H, 5.48; N, 3.0.

4.1.16. Compound **8p**

Crystalline white solid; mp: 214–217 °C; $[\alpha]_D^{25}$ +59 (c 0.5, $CHCl_3$); yield 73%; IR (KBr, cm^{-1}); 694.33, 756.59, 831.44, 1038.58, 1103.78, 1158.48, 1245.73, 1488.24, 1597.24, 1636.84, 1663.84, 1788.47, 2926.88, 3368.52; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (q, 4H, J = 6.8 Hz), 6.74 (d, 1H, J = 9.6 Hz), 6.35 (d, 1H, J = 9.6 Hz), 5.34 (d, 1H, J = 9.6 Hz), 3.92 & 3.89 (s, together integrating to diastereomeric 1H), 3.46 & 3.42 (s, together integrating to diastereomeric 1H), 2.22 (s, 3H), 2.12–2.05 (m, 1H), 1.71–1.64 (m, 2H), 1.53–1.41 (m, 2H), 1.40 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.2, 19.6, 27.2, 38.5, 39.1, 41.0, 42.5, 83.5, 125.4, 128.7, 131.8, 132.0, 132.5, 156.7, 157.3, 162.6, 172.4, 186.2; Mass QTOF: 464 $(M + Na)^+$; Anal. Calcd for $C_{22}H_{20}BrNO_4$: C, 59.74; H, 4.56; N, 3.17. Found: C, 59.81; H, 4.52; N, 3.15.

4.2. Synthesis of spiro-isoxazolidine derivative of santonin

In a typical procedure, to a solution of α-santonin (0.5 g, 1.9 mmol) in dry benzene (6 mL) was added appropriate nitrone (1.58 g, 2.4 mmol) in dry benzene and refluxed the reaction mixture for 8 h. The solvent was evaporated *in vacuo* to afford the crude product which was further purified by flash chromatography using ethyl acetate and hexane (2:8) as solvent system. The pure products (**10a′** and **10a″**) were isolated in good yields (75%) and characterized on the basis of ^{1}H NMR, ^{13}C NMR and mass spectrometry.

4.2.1. Compound **10a′**

Crystalline yellow solid; mp: 210–213 °C; $[\alpha]_D^{25}$ +43 (c 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 695.70, 752.29, 837.21, 856.23, 1040.85, 1105.95, 1158.20, 1267.59, 1346.69, 1453.72, 1489.70, 1521.06, 1598.02, 1636.28, 1663.06, 1790.55, 2926.44; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, 2H, J = 8.8 Hz), 7.70 (d, 2H, J = 8 Hz), 7.36–7.25 (m, 3H), 7.12 (d, 1H, J = 7.2 Hz), 6.92 (t, 1H, J = 8.8 Hz), 6.74 (d, 1H, J = 9.6 Hz), 6.34 (d, 1H, J = 9.6 Hz), 5.30–5.35 (dd, 1H, J = 10.8, 1.2 Hz), 5.10–5.15 (t, 1H, J = 8 Hz), 3.24 (dd, 1H, J = 12.5, 7.2 Hz), 2.53 (dd, 1H, J = 12.5, 8.4 Hz), 2.35 (s, 3H), 1.84 (dd, 1H, J = 12, 6.4 Hz), 1.6–1.55 (m, 4H), 1.4 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.02, 17.52, 25.09, 37.42, 41.31, 43.66, 53.06, 69.46, 80.96, 82.34, 116.78, 124.21, 124.30, 126.04, 127.97, 128.80, 128.99, 129.27, 147.24, 147.79, 149.31, 150.37, 154.71, 173.11, 186; Mass QTOF: 507 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{26}N_2O_4$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.10; H, 5.32; N, 5.81.

4.2.2. Compound **10a″**

Crystalline yellow solid; mp: 202–205 °C; $[\alpha]_D^{25}$ −28 (c 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 695.13, 753.56, 834.07, 855.33, 989.99, 1039.12, 1106.25, 1158.58, 1268.44, 1346.11, 1382.11, 1453.94, 1489.36, 1522.56, 1598.59, 1637.24, 1663.35, 1724.24, 1789.38, 2924.92, 3400.49; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (dd, 2H, J = 8 Hz), 7.72 (d, 2H, J = 8.4 Hz), 7.34 (dd, 3H, J = 9.6, 5.6 Hz), 7.12 (m, 2H), 6.75 (d, 1H, J = 9.6 Hz), 6.34 (d, 1H, J = 9.6 Hz), 5.00–5.05 (dd, 1H, J = 9.6, 6.4 Hz), 4.80–4.85 (dd, 1H, J = 12, 1.2 Hz), 2.87–2.95 (dd, 1H, J = 12.4, 6.4 Hz), 2.67–2.75 (dd, 1H, J = 12.4, 10.4 Hz), 2.59–2.66 (m, 1H), 2.1 (s, 3H), 2.05–1.93 (m, 2H), 1.62 (m, 2H), 1.45 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.91, 19.50, 24.98, 37.21, 41.12, 42.34, 51.68, 68.59, 78.32, 83.22, 117.93, 124.35, 126.12, 127.95, 128.79, 129.58, 146.23, 147.93, 149.32, 149.63, 154.41, 174.16, 185.87; Mass QTOF: 507 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{26}N_2O_4$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.10; H, 5.32; N, 5.81.

4.2.3. Compound **10b′**

Crystalline yellow solid; mp: 232–236 °C; $[\alpha]_D^{25}$ +56 (c 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 693.43, 758.43, 831.61, 1041.08, 1106.00, 1157.69, 1265.10, 1454.05, 1488.25, 1596.40, 1635.75, 1662.62, 1791.03, 2924.14, 3368.73; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, 2H, J = 8 Hz), 7.42 (d, 2H, J = 8 Hz), 7.35–7.26 (m, 2H), 7.25 (d, 1H, J = 6.8 Hz), 7.05 (d, 1H, J = 8.8 Hz), 6.75 (d, 1H, J = 12.5 Hz), 6.32 (d, 1H, J = 12 Hz), 5.35–5.30 (dd, 1H, J = 12, 1.2 Hz), 4.85 (t, 1H, J = 10.5 Hz), 3.15 (dd, 1H, J = 12.8, 7.2 Hz), 2.55 (dd, 1H, J = 12.8, 8.8 Hz), 2.25–2.18 (m, 1H), 2.10 (s, 3H), 2.00 (t, 2H, J = 11 Hz), 1.9–1.6 (m, 2H), 1.4 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.01, 17.61, 25.08, 37.54, 40.24, 41.34, 43.98, 53.46, 56.33, 69.62, 80.97, 82.02, 111.53, 112.13, 117.36, 121.63, 124.07, 126.0, 127.27, 128.78, 129.14, 131.61, 132.80, 149.62, 150.70, 154.83, 155.73, 173.41, 186.09; Mass QTOF: 543 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{26}BrNO_4$: C, 64.62; H, 5.04; N, 2.69. Found: C, 64.66; H, 5.09; N, 2.62.

4.2.4. Compound **10b″**

Crystalline yellow solid; mp: 186–188 °C; $[\alpha]_D^{25}$ −48 (c 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 758.47, 831.04, 1040.94, 1105.58, 1488.31, 1636.17, 1662.97, 1790.92, 2853.20, 2923.67, 3438.45; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, 1H, J = 8.4 Hz), 7.35 (d, 1H, J = 8.4 Hz), 7.20–7.17 (m, 5H), 7.05 (q, 2H, J = 12 Hz), 6.71 (d, 1H, J = 9.6 Hz), 6.30 (d, 1H, J = 9.6 Hz), 4.80–4.74 (m, 2H), 2.80–2.85 (dd, 1H, J = 12.4, 6.4 Hz), 2.75 (dd, 1H, J = 12.4, 10 Hz), 2.60–2.65 (m, 1H), 2.32 (d, 3H, J = 4.8 Hz), 2.00 (t, 2H, J = 12 Hz), 1.51–1.06 (m, 2H), 1.45 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.91, 19.49, 24.97, 37.25, 41.12, 42.44, 51.98, 68.99, 78.22, 82.92, 118.18, 122.21, 124.18, 126.10, 128.63, 128.76, 129.53, 132.25, 137.62, 149.53, 149.80, 154.46, 174.36, 185.95; Mass QTOF: 543 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{26}BrNO_4$: C, 64.62; H, 5.04; N, 2.69. Found: C, 64.66; H, 5.09; N, 2.62.

4.2.5. Compound **10c′**

Crystalline pink solid; mp: 195–197 °C; $[\alpha]_D^{25}$ +54 (c 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 692.93, 755.33, 863.54, 906.64, 1039.28, 1103.18, 1136.17, 1158.65, 1200.93, 1244.60, 1489.21, 1597.18, 1616.19, 1663.43, 1789.40, 2933.93; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, 1H, J = 8 Hz), 7.45 (d, 2H, J = 8 Hz), 7.30–7.24 (m, 4H), 7.14 (q, 1H, J = 6.8 Hz), 6.95 (t, 1H, J = 6.8 Hz), 6.72 (d, 1H, J = 9.6 Hz), 6.34 (d, 1H, J = 9.6 Hz), 5.32–5.37 (t, 1H, J = 8.4 Hz), 4.76–4.83 (dd, 1H, J = 12, 1.2 Hz), 3.24 (dd, 1H, J = 12.8, 7.2 Hz), 2.51 (dd, 1H, J = 12.8, 8.4 Hz), 2.20 (d, 3H, J = 6.8 Hz), 2.13–2.00 (m, 1H, J = 3.2 Hz), 2.00–1.93 (m, 2H), 1.60–1.56 (m, 2H), 1.40 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.99, 17.51, 25.03, 37.42, 41.29, 41.62, 53.08, 66.04, 80.73, 82.57, 115.52, 116.72, 118.01, 127.75, 128.72, 128.71, 128.77, 128.93, 128.97, 129.13, 129.20, 129.70, 136.81, 149.44, 150.502, 154.45, 172.95, 186.02; Mass QTOF: 532 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{25}Cl_2NO_4$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.10; H, 5.32; N, 5.81.

4.2.6. Compound **10c″**

Crystalline pink solid; mp: 187–191 °C; $[\alpha]_D^{25}$ −30 (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, 1H, J = 8.4 Hz), 7.40 (d, 1H, J = 8.4 Hz), 7.33–7.27 (m, 4H), 7.05 (dd, 3H, J = 5.6 Hz), 6.73 (d, 1H, J = 9.6 Hz), 6.34 (d, 1H, J = 9.6 Hz), 5.32 (t, 1H, J = 8.4 Hz), 4.82 (dd, 1H, J = 12, 1.2 Hz), 2.81–2.76 (dd, 1H, J = 12, 7.2 Hz), 2.53–2.48 (m, 1H), 2.20 (d, 3H, J = 7.2 Hz), 1.93 (t, 2H, J = 12.4 Hz), 1.50–1.45 (m, 2H), 1.34 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.90, 17.54, 19.54, 22.99, 24.98, 30.91, 37.44, 39.89, 41.07, 41.30, 41.65, 51.78, 53.11, 64.82, 66.07, 68.17, 80.74, 83.51, 116.75, 123.42, 126.10, 127.75, 127.99,

128.73, 128.79, 129.60, 129.78, 133.16, 134.39, 135.69, 149.35, 149.72, 154.52, 154.73, 173.83, 185.94, 206.89; Mass QTOF: 532 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{25}Cl_2NO_4$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.10; H, 5.32; N, 5.81.

4.2.7. Compound **10d′**

Crystalline white solid; mp: 153–156 °C; $[\alpha]_D^{25}$ +51 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 510.46, 550.37, 667.37, 694.02, 833.28, 859.69, 983.34, 1039.37, 1119.11, 1216.06, 1247.56, 1378.93, 1454.60, 1490.93, 1598.66, 1626.05, 1665.26, 1723.71, 1789.80, 2856.96, 2928.69; ^1H NMR (400 MHz, $CDCl_3$): δ 7.31 (m, 2H), 7.12 (dd, 3H, $J = 14.8$, 8 Hz), 7.32–7.25 (m, 4H), 6.95 (dd, 2H, $J = 8.4$, 0.8 Hz), 6.76 (d, 1H, $J = 9.6$ Hz), 6.36 (d, 1H, $J = 9.6$ Hz), 5.30–5.35 (d, 1H, $J = 10.8$ Hz), 4.95–5.00 (t, 1H, $J = 8$ Hz), 3.15–3.12 (dd, 1H, $J = 12.8$, 4.4 Hz), 2.40 (dd, 1H, $J = 12.8$, 8.4 Hz), 2.23 (s, 3H), 2.05–1.90 (m, 1H), 1.60–1.54 (m, 4H), 1.35 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.96, 14.05, 17.44, 22.97, 37.39, 38.73, 41.32, 53.05, 69.24, 80.93, 82.28, 103.30, 103.71, 109.59, 109.70, 116.56, 123.91, 125.93, 128.80, 128.90, 144.15, 149.46, 150.62, 154.88, 162.33, 164.41, 173.16, 186.05; Mass QTOF: 560 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{25}BrFNO_4$: C, 62.40; H, 4.68; N, 2.60. Found: C, 62.43; H, 4.70; N, 2.65.

4.2.8. Compound **10d″**

Crystalline white solid; mp: 178–181 °C; $[\alpha]_D^{25}$ −24 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 693.88, 757.02, 832.83, 984.06, 1040.99, 1118.82, 1248.88, 1462.85, 1491.30, 1598.24, 1624.90, 1664.02, 1789.72, 2924.31, 3394.40; ^1H NMR (400 MHz, $CDCl_3$): δ 7.71–7.68 (m, 3H), 7.12–7.00 (m, 4H), 6.85 (dd, 1H, $J = 7.2$, 3.4 Hz), 6.72 (d, 1H, $J = 9.6$ Hz), 6.34 (d, 1H, $J = 9.6$ Hz), 5.32 (t, 1H, $J = 7.6$ Hz), 4.76 (dd, 1H, $J = 9.6$, 2.4 Hz), 3.14 (dd, 1H, $J = 12.5$, 7.2 Hz), 2.54–2.48 (m, 1H), 2.33 (s, 3H), 2.00 (t, 2H, $J = 6$ Hz), 1.61–1.57 (m, 2H), 1.30 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.12, 14.67, 15.53, 21.65, 36.73, 37.90, 38.45, 40.41, 51.30, 67.65, 80.76, 81.23, 101.23, 106.56, 107.83, 109.21, 114.31, 121.67, 127.71, 128.00, 128.30, 143.25, 149.29, 151.12, 156.73, 161.83, 167.41, 175.16, 186.15; Mass QTOF: 560 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{25}BrFNO_4$: C, 62.40; H, 4.68; N, 2.60. Found: C, 62.43; H, 4.70; N, 2.65.

4.2.9. Compound **10e′**

Crystalline yellow solid; mp: 145–148 °C; $[\alpha]_D^{25}$ +47 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 694.02, 756.60, 831.26, 1038.96, 1104.96, 1159.33, 1245.20, 1491.37, 1597.43, 1636.36, 1663.37, 1788.27, 2927.74, 3368.49; ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (dd, 1H, $J = 6.8$, 2.4 Hz), 7.35 (dd, 1H, $J = 9.6$, 7.6 Hz), 7.25 (dd, 2H, $J = 16.8$, 8.4 Hz), 7.15 (d, 1H, $J = 7.6$ Hz), 7.00 (d, 1H, $J = 10$ Hz), 6.94 (d, 2H, $J = 10$ Hz), 6.72 (d, 1H, $J = 9.6$ Hz), 6.34 (d, 1H, $J = 9.6$ Hz), 5.30 (dt, 1H, $J = 11.2$, 1.2 Hz), 4.97–4.95 (t, 1H, $J = 8$ Hz), 3.15–3.13 (dd, 1H, $J = 12.8$, 7.2 Hz), 2.47–2.40 (dd, 1H, $J = 12.8$, 8.8 Hz), 2.20 (s, 3H), 2.00 (d, 1H, $J = 12.5$ Hz), 1.80 (dd, 2H, $J = 8.4$, 1.2 Hz), 1.60–1.57 (m, 2H), 1.30 (d, 3H, $J = 12.5$ Hz); ^{13}C (100 MHz, $CDCl_3$): 11.01, 17.53, 25.07, 29.69, 37.46, 41.32, 43.86, 53.18, 69.22, 80.94, 82.17, 109.58, 109.79, 116.83, 117.04, 124.13, 126.0, 127.45, 127.52, 128.89, 129.17, 131.81, 137.09, 137.13, 149.47, 150.54, 154.77, 157.49, 159.96, 173.24, 186.02; Mass QTOF: 422 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{25}F_2NO_4$: C, 70.43; H, 5.28; N, 2.93. Found: C, 70.39; H, 5.32; N, 2.96.

4.2.10. Compound **10e″**

Crystalline yellow solid; mp: 183–186 °C; $[\alpha]_D^{25}$ −83 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 691.02, 753.64, 834.12, 1035.56, 1112.76, 1154.30, 1249.24, 1497.57, 1592.40, 1632.26, 1660.27, 1785.37, 2923.54, 3365.39; ^1H NMR (400 MHz, $CDCl_3$): δ 7.75–7.72 (m, 3H), 7.20 (d, 2H, $J = 6.4$ Hz), 7.10 (t, 1H, $J = 7.2$ Hz), 6.90 (d, 1H, $J = 8$ Hz), 6.80 (t, 1H, $J = 9.6$ Hz), 6.73 (d, 1H, $J = 9.6$ Hz), 6.30 (d, 1H, $J = 9.6$ Hz), 5.31 (dd, 1H, $J = 10.8$, 6.4 Hz), 4.30 (t, 1H, $J = 8.4$ Hz), 2.92–2.97 (dd, 1H, $J = 13.2$, 7.2 Hz), 2.75–2.82 (dd, 1H, $J = 13.2$, 8.4 Hz), 2.60 (t, 1H, $J = 3.2$ Hz), 2.23 (s, 3H), 1.85 (dd, 2H, $J = 8.4$, 4.4 Hz), 1.63–1.57 (m, 2H), 1.30 (d, 3H, $J = 12.8$ Hz); ^{13}C (100 MHz, $CDCl_3$): 10.83, 17.81, 19.25, 25.19, 30.89, 37.44, 37.68, 41.29, 43.24, 50.31, 69.34, 80.28, 80.65, 103.59, 103.79, 110.62, 110.67, 118.55, 124.66, 126.04, 129.24, 143.02, 147.83, 150.64, 154.34, 164.21, 173.00, 186.10; Mass QTOF: 422 $(M + 23)^+$; Anal. Calcd for $C_{28}H_{25}F_2NO_4$: C, 70.43; H, 5.28; N, 2.93. Found: C, 70.39; H, 5.32; N, 2.96.

4.2.11. Compound **10f**

Crystalline yellow solid; mp: 152–156 °C; $[\alpha]_D^{25}$ +35 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 694.13, 756.70, 832.48, 1038.69, 1103.91, 1103.69, 1257.60, 1454.05, 1496.66, 1609.33, 1787.86, 2930.11, 3368.60; ^1H NMR (400 MHz, $CDCl_3$): 7.80 (dd, 1H, $J = 12$, 6.8 Hz), 7.42 (m, 1H), 7.25 (dd, 2H, $J = 9.6$, 2.4 Hz), 7.15 (t, 1H, $J = 8.4$ Hz), 7.00 (t, 1H, $J = 7.2$ Hz), 6.92 (d, 2H, $J = 7.2$ Hz), 6.72 (d, 1H, $J = 9.6$ Hz), 6.34 (d, 1H, $J = 9.6$ Hz), 5.35 (dd, 1H, $J = 9.6$ Hz), 4.94 (t, 1H, $J = 8$ Hz), 3.90 (s, 3H), 3.14 (dd, 1H, $J = 12.5$, 7.2 Hz), 2.55 (dd, 1H, $J = 12.5$, 8.4 Hz), 2.36–2.15 (m, 1H), 2.14 (s, 3H), 2.00–1.85 (m, 2H), 1.65–1.54 (m, 2H), 1.40 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.01, 17.61, 25.08, 37.54, 41.34, 43.98, 53.46, 56.33, 69.62, 80.97, 82.02, 111.53, 112.13, 117.36, 121.63, 124.07, 126.0, 127.27, 128.78, 129.14, 131.61, 132.80, 149.62, 150.70, 154.83, 155.73, 173.41, 186.09; Mass QTOF: 573 $(M + Na)^+$; Anal. Calcd for $C_{29}H_{28}BrNO_5$: C, 63.28; H, 5.13; N, 2.54. Found: C, 63.26; H, 5.09; N, 2.57.

4.2.12. Compound **10f′**

Crystalline brown solid; mp: 164–167 °C; $[\alpha]_D^{25}$ −37 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 514.78, 686.56, 756.02, 831.32, 906.81, 1039.37, 1138.30, 1244.51, 1303.04, 1403.39, 1500.30, 1615.59, 1636.72, 1663.22, 1791.42, 2852.88, 2923.26, 3435.92; ^1H NMR (400 MHz, $CDCl_3$): δ 7.30–7.25 (m, 2H), 7.12 (dd, 2H, $J = 9.6$, 6.8 Hz), 7.00 (d, 2H, $J = 6.24$ Hz), 6.84 (t, 1H, $J = 6.8$ Hz), 6.71 (d, 1H, $J = 9.6$ Hz), 6.32 (d, 1H, $J = 9.6$ Hz), 5.30 (d, 1H, $J = 9.6$ Hz), 5.00 (t, 1H, $J = 8.4$ Hz), 3.93 (s, 3H), 2.95–3.00 (dd, 1H, $J = 12.5$, 7.2 Hz), 2.80–2.83 (dd, 1H, $J = 12.4$, 7.8 Hz), 2.75–2.72 (dd, 1H, $J = 12.5$, 6.4 Hz), 2.57–2.65 (m, 1H), 2.20 (d, 3H, $J = 4$ Hz), 2.10 (t, 2H, $J = 4$ Hz), 2.00–1.84 (m, 2H), 1.40 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 10.90, 19.51, 25.06, 37.31, 41.16, 42.46, 43.56, 52.03, 56.34, 68.73, 78.24, 82.89, 100.01, 112.18, 112.35, 118.69, 124.24, 126.05, 127.49, 128.09, 129.52, 131.61, 132.80, 149.62, 150.70, 154.83, 155.73, 173.41, 186.09; Mass QTOF: 573 $(M + Na)^+$; Anal. Calcd for $C_{29}H_{28}BrNO_5$: C, 63.28; H, 5.13; N, 2.54. Found: C, 63.26; H, 5.09; N, 2.57.

4.2.13. Compound **10g′**

Crystalline brown solid; mp: 203–205 °C; $[\alpha]_D^{25}$ +47 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 693.80, 756.61, 778.50, 832.65, 1040.62, 1104.01, 1156.40, 1246.13, 1379.07, 1453.63, 1490.38, 1597.29, 1636.79, 1790.12, 2925.71; ^1H NMR (400 MHz, $CDCl_3$): 8.40 (s, 1H), 8.21 (dd, 1H, $J = 12$, 6.8 Hz), 7.80 (dd, 1H, $J = 12$, 6.8 Hz), 7.63 (t, 1H, $J = 6.8$ Hz), 7.25–7.20 (m, 4H), 7.10 (d, 1H, $J = 7.6$ Hz) 7.15 (t, 1H, $J = 8.4$ Hz), 6.92 (d, 2H, $J = 7.2$ Hz), 6.74 (d, 1H, $J = 9.6$ Hz), 6.30 (d, 1H, $J = 9.6$ Hz), 5.44 (dd, 1H, $J = 10.8$, 1.2 Hz), 5.13 (t, 1H, $J = 8.4$ Hz), 3.20 (dd, 1H, $J = 12.8$, 7.2 Hz), 2.55 (dd, 1H, $J = 12.8$, 7.8 Hz), 2.25 (d, 3H, $J = 4$ Hz), 2.10–2.00 (m, 1H), 2.00 (t, 2H, $J = 8.4$ Hz), 1.62–1.57 (m, 2H), 1.30 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 9.41, 11.02, 14.85, 15.74, 17.55, 22.62, 25.09, 30.32, 31.67, 37.43, 39.39, 41.32, 43.72, 69.43, 80.97, 82.35, 117.07, 121.82, 123.18, 124.32, 126.10, 128.98, 129.22, 130.05; Mass QTOF; 509 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{26}N_2O_4$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.10; H, 5.32; N, 5.81.

4.2.14. Compound **10g″**

Crystalline brown solid; mp: 213–216 °C; $[\alpha]_D^{25}$ −43 (*c* 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 693.80, 756.61, 778.50, 832.65, 1040.62, 1104.01, 1156.40, 1246.13, 1379.07, 1453.63, 1490.38, 1597.29, 1636.79, 1790.12, 2925.71; ^1H NMR (400 MHz, $CDCl_3$): δ 8.32 (t, 2H,

J = 8 Hz), 7.62 (t, 1H, J = 9.6), 7.32–7.28 (m, 5H), 7.10 (d, 2H, J = 7.8 Hz), 6.82 (d, 1H, J = 6.8 Hz), 6.71 (d, 1H, J = 9.6 Hz), 6.74 (d, 1H, J = 9.6 Hz), 6.32 (d, 1H, J = 9.6 Hz), 5.10 (dd, 1H, J = 9.6, 6Hz), 4.51 (t, 1H, J = 8.4 Hz), 2.90–2.95 (dd, 1H, J = 12.0, 5.2 Hz), 2.70 (dd, 1H, J = 12.4, 2.4 Hz), 2.67–2.62 (m, 1H), 2.20 (d, 3H, J = 4 Hz), 2.15 (t, 2H, J = 4 Hz), 1.60–1.47 (m, 2H), 1.30 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.04, 14.11, 17.81, 19.50, 29.69, 31.53, 32.52, 37.21, 37.45, 41.14, 41.31, 42.38, 43.23, 49.71, 49.97, 68.38, 68.60, 80.35, 83.19, 116.52, 116.99, 118.31, 122.02, 122.96, 123.33, 123.41, 124.52, 124.90, 126.03, 173.10, 185.94; Mass QTOF: 509 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{26}N_2O_4$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.10; H, 5.32; N, 5.81.

4.2.15. Compound **10h′**

Crystalline yellow solid; mp: 145–147 °C; $[\alpha]_D^{25}$ +28 (c 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 694.02, 756.60, 831.26, 1038.96, 1104.96, 1159.33, 1245.20, 1491.37, 1597.43, 1636.36, 1663.37, 1788.27, 2927.74, 3368.49; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (t, 2H, J = 7.2 Hz), 7.83 (d, 2H, J = 8.4 Hz), 7.30 (q, 3H, J = 7.2 Hz), 7.10 (d, 1H, J = 6.8 Hz), 7.00–6.96 (m, 5H), 6.75 (d, 1H, J = 9.6 Hz), 6.34 (d, 1H, J = 9.6 Hz), 5.47 (dd, 1H, J = 9.6, 1.2 Hz), 5.23 (t, 1H, J = 8.4 Hz), 3.28 (dd, 1H, J = 12.5, 7.2 Hz), 2.90 (dd, 1H, J = 12.5, 7.8 Hz), 2.15 (s, 3H), 2.10–2.00 (m, 1H), 1.65–1.45 (m, 4H), 1.43 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.20, 16.65, 19.30, 24.50, 30.89, 36.35, 37.60, 41.32, 42.15, 47.45, 70.96, 78.36, 80.32, 102.21, 109.12, 110.62, 111.23, 114.51, 121.21, 125.31, 128.54, 147.08, 147.56, 152.43, 160.98, 171.76, 185.21; Mass QTOF: 514 $(M + Na)^+$; Anal. Calcd for $C_{32}H_{29}NO_4$: C, 78.19; H, 5.95; N, 2.85. Found: C, 78.21; H, 5.93; N, 2.90.

4.2.16. Compound **10h″**

Crystalline yellow solid; mp: 153–155 °C; $[\alpha]_D^{25}$ −23 (c 0.5, $CHCl_3$); IR (KBr, cm^{-1}): 694.02, 756.60, 831.26, 1038.96, 1104.96, 1159.33, 1245.20, 1491.37, 1597.43, 1636.36, 1663.37, 1788.27, 2927.74, 3368.49; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (t, 2H, J = 7.2 Hz), 7.85 (d, 2H, J = 8.4 Hz), 7.30 (q, 3H, J = 7.2 Hz), 7.15 (d, 1H, J = 6.8 Hz), 7.00–6.96 (m, 5H), 6.75 (d, 1H, J = 9.6 Hz), 6.30 (d, 1H, J = 9.6 Hz), 5.40 (d, 1H, J = 9.6 Hz), 5.15 (t, 1H, J = 8.4 Hz), 3.05–3.15 (m, 1H), 2.90–2.97 (dd, 1H, J = 12.5, 4.4 Hz), 2.10 (s, 3H), 2.00–1.93 (m, 1H), 1.90 (t, 2H, J = 8.4, 5 Hz), 1.65–1.53 (m, 3H), 1.45 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): 11.07, 15.45, 19.89, 22.31, 32.34, 35.46, 37.38, 40.98, 41.23, 46.67, 67.45, 75.90, 80.76, 104.64, 103.21, 107.54, 110.98, 113.32, 121.54, 127.65, 129.43, 149.11, 152.40, 152.87, 160.78, 173.76, 186.53; Mass QTOF: 514 $(M + Na)^+$; Anal. Calcd for $C_{32}H_{29}NO_4$: C, 78.19; H, 5.95; N, 2.85. Found: C, 78.21; H, 5.93; N, 2.90.

4.3. Biological assays

4.3.1. Evaluation of in vitro anticancer activity

The effect of spiro-derivatives of α-santonin on the growth of cancer cell lines was evaluated according to the procedure adopted by the National Cancer Institute for *in vitro* anticancer drug screening that uses the protein-binding dye sulforhodamine B to estimate cell growth [34]. Briefly, cells in their log phase of growth were harvested, counted and seeded (104 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 sulforhodamine 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.

4.3.2. DNA cell cycle analysis

Effect of compounds **10b′** and **10b″** on DNA content by cell cycle phase distribution was assessed using PC-3 cells by incubating the cells 1 × 10^6 mL/well with compounds **10b′** and **10bØ** (1, 5 &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 [35]. 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.

4.3.3. Preparation of nuclear lysates

PC-3 cells (5 × 10^6) were incubated with the test compounds for 24 h at different concentrations. Cells were lysed with hypotonic buffer (10 mM HEPES/KOH pH 7.9, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM NaF, 1 mM Na_3OV_4, 10 mM KCl, 1 mM DTT, 0.5 mM PMSF, 1% (v/v) eukaryotic protease inhibitor cocktail) for 10 min on ice. Cell suspension was centrifuged at 15,000 *g* for 30 s at 4 °C and the nuclear pellet was resuspended into 100 μL of ice cold saline buffer (50 mM HEPES/KOH pH 7.9, 50 mM KCL, 300 mM NaCl, 1 mM EDTA, 1 mM NaF, 1 mM Na_3OV_4, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, 1% (v/v) eukaryotic protease inhibitor cocktail) on ice for 30 min. The suspension was centrifuged at 15,000 *g* for 5 min at 4 °C and the supernatant was collected as nuclear fraction.

4.3.4. NF-κB (p65), transcription factor assay

Effect of the test compound on the binding of NF-κB to its consensus DNA sequence was checked 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 for overnight at 4 °C. Wells were washed and incubated with primary antibody against NF-κB (p65) for 1 h. HP-conjugated secondary antibody was added for 1 h to the wells after washing. Wells were incubated with the substrate, reaction was stopped after 30 min and reading was taken at 450 nm.

4.4. X-ray crystallography of spiro-isoxazolidine derivative of α-santonin **10e′**

A single crystal of spiro-isoxazolidine derivative **10e′** was obtained by slow evaporation at room temperature, from a mixture of methanol/water. The X-ray data was collected from a dry crystal mounted on an 'Xcalibur, Sapphire3', Oxford diffractometer. The crystal structure was solved by direct method using SHELXS-97 followed by full matrix anisotropic least square refinement using SHELXL-97 [36]. All the proton atoms were located from difference Fourier map except the methyl groups. For methyl group the proton atom was fixed geometrically and refined in the final cycle as riding over the heavy atom it is bonded. All the relevant crystallographic data collection parameters and structure refinement details, bond lengths and bond angles for **10e′** are given in Supplementary information.

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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.2013.01.003.

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