2.2.1. Sesquiterpene lactones: Biological importance

Sesquiterpene lactones (SLs) are considered as major class of secondary metabolites which have been identified in several plant families such as Acanthaceae, Anacardiaceae, Apiaceae, Asteraceae, Euphorbiaceae, Hepatideae, Lauraceae, Magnoliaceae, Menispermaceae, Rutaceae and Winteraceae etc (Robles et al., 1995; Zhang et al., 2005; Chaturvedi, 2011). However, the greatest numbers are found in the Compositae (Asteraceae) family. Over 4000 different sesquiterpene lactone structures are known (Modzelewska et al., 2005; Cho et al., 2006). SLs are primarily classified on the basis of their carbocyclic skeletons into pseudoguainolides, guaianalides, germacranolides, eudesmanolides, heliangolides and hypocretenolides etc (Figure 1). However, SLs exhibit a variety of other skeletal arrangements also. They generally contain \( \alpha \)-methylene-\( \gamma \)-lactone or \( \alpha \), \( \beta \)-unsaturated carbonyl moieties as structural feature, and exhibit a variety of biological activities like anticancer, antifungal, antibacterial, antiplasmodial, anthelmintic, schistosomicidal, phytotoxic and analgesic activities (Pickman, 1984, 1986; Rodriguez et al., 1976; Lee et al., 1977; Hanson et al., 1970; Kupchan, 1970a, 1970b, 1971; Howie et al., 1976; Heilmann et al., 1976). They also cause allergic contact dermatitis in humans. These biological activities are generally the result of reaction of sesquiterpene lactones with the thiol groups of important biomolecules such as enzymes.

2.2.2. Sesquiterpenoid lactones cytotoxicity and SAR

SLs are one of the major classes of cytotoxic/antitumor compounds of plant origin. Various studies on the relationship between chemical structure and cytotoxicity of SLs revealed that the presence of unsaturated moieties like \( \alpha \)-methylene-\( \gamma \)-lactone or \( \alpha \), \( \beta \)-unsaturated carbonyl group necessary for anticancer activity (Kupchan et al., 1970a, 1970b, 1971; Schmidt, 1997; Bettina et al., 2004). The cytotoxicity of these compounds is due to the alkylation of nucleophilic groups especially sulfhydryl groups (cys-38 and cys-120) present in biological system in Michael addition fashion. From the literature it is well known that SLs are NF-\( \kappa \)B inhibitors (Lyss et al., 1997, 1998; Garcia-Pineres et al., 1997; Bettina et al., 2004).

2.2.3. *Parthenium-hysterophorus L.*

*Parthenium-hysterophorus L.* is known as congress weed, carrot grass, star weed, feverfew, white top, chatak chandani, bitter weed, ramphool, garghas etc. *Parthenium hysterophorus L.* is considered one of the ten worst weeds in the world. It was accidently introduced with imported wheat in the early 1950s to India and has since spread over most part of the country (Bose, 1996). *Parthenium* now occurs in several countries,
particularly in India, this weed is spreading in an epidemic proportion. *Parthenium hysterophorus* L. is affecting crop production and causes asthma, bronchitis, dermatitis and hay-fever in humans and livestock (Towers *et al*., 1977). *Parthenium hysterophorus* contain SLs, the major component of these SLs being parthenin (Sethi *et al*., 1987, Ramesh *et al*., 2003a, 2003b). *Parthenium hysterophorus* also contain phenolic acids such as caffeic acid, vanillic acid, anisic acid, chlorogenic acid, para-hydroxy benzoic acid and p-anisic acid which are lethal to humans and animals.

Even though *Parthenium hysterophorus* L. has several disadvantages, several bioactive compounds were isolated from it. The word *parthenium* is derived from the Latin *parthenice* suggesting medicinal uses. It possesses allelopathic activity and anti-tumor activity (Mukherjee and Chatterjee 1993; Kanchan, 1975; Patel and Hedge, 1988; Das and Das, 1995; Mew *et al*., 1982). Root decoction of *parthenium* is useful in dysentery and it is reported as promising remedy against hepatic amoebiasis (Sharma and Bhutani, 1988; Singh *et al*., 1996; Uphof, 1959). In the Caribbean and Central America, *Parthenium* is used as folk remedy in the treatment of skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments (Dominguez and Sierra, 1970).

2.2.4. Parthenin: Biological importance

Parthenin (Figure-2 & 3) is a major sesquiterpene lactone (psuedoguaianolide) isolated from *Parthenium-hysterophorus* L (Figure-3). It contains α-methylene-γ-butyrolactone and cyclopentenone moieties along with other functionalities and five chiral centres. Parthenin exhibit several biological activities like anticancer (Modzelewska *et al*., 2005, Shah *et al*., 2009), antibacterial (Ramesh *et al*., 2003), antimalarial (Hooper *et al*., 1990), antiamoebic (Sharma and Bhutani, 1988), and phytotoxic properties (Batish *et al*., 1997a, 1997b; Pandey, 1996; Kohli *et al*., 1993). Anticancer activity of parthenin is more attractive to researchers, even though parthenin is a highly a toxic molecule. Various structural modifications of parthenin (Figure-4) have recently been carried out to obtain more potent analogues with lower toxicity (Reddy *et al*., 2011; Das *et al*., 1999; Das and Das, 1995; Shah *et al*., 2009).

2.2.5. Rationale behind the synthesis of spiroisoxazolidine derivatives of parthenin

Despite numerous biological activities of parthenin, no concrete SAR model for this molecule have yet been established. As discussed above, cytotoxicity, like many other biological activities of parthenin type of molecules, is known to be mediated by the presence of potentially alkylant structural elements capable of reacting covalently with
Figure-1: Various skeleton types of sesquiterpene lactones.

Figure-2: Various sesquiterpene lactones having anticancer activity.
Figure-3: *Parthenium hysterophorus* L

Parthenin

Figure-4: Structures of some parthenin derivatives
biological nucleophiles in Michael addition type of fashion, thereby inhibiting a variety of cellular functions which directs the cells into apoptosis. In parthenin there are two alkylating structures (Figure-5), one is the exocyclic double bond and the other is the endocyclic one. To establish the role of exo/endocyclic double bonds towards the anticancer activity, a strategy to selectively react one of these double bonds has been devised. Out of few chemical transformational possibilities available to achieve the above goal, the present approach involves the selective addition of dipole, i.e., nitrone to exocyclic double bond, which should also enhance the activity due to the introduction of basic nitrogen atom into the structural framework. Thus, keeping in view the Lipinski’s rule together with the possibility to introduce nitrogen bearing (alkaloid type) structural moiety, better secondary leads could be possibly derived applying this strategy.

Both endo- as well as the exo-cyclic double bonds are Michael acceptors, but due to steric hindrance offered by the substitution at the cyclopentenone ring, nitrone cycloaddition takes place selectively on the exocyclic double bond alone. Further, exocyclic bond is more reactive since the resulting tetra substituted enolate formed as intermediate during cycloaddition, is more stable for the exocyclic olefin than the trisubstituted enolate formed by addition to the enone. The greater product stability correlates with a activation energy according to Hammond’s postulate. In this section, nitrone cycloaddition to the exocyclic double bond of parthenin to generate a focused library of novel spiro isooxazolidines is presented. By screening the anticancer activity of these novel spiro derivatives we can easily establish the pharmacological importance of cyclopentenone ring or the \( \alpha \)-methylene-\( \gamma \)-butyrolactone ring, thereby establish the SAR of the molecule unequivocally.

In this section isolation of parthenin from *Parthenium hysterophorus* L. and synthesis of a series of its isooxazolidine derivatives has been presented. All the spiro-isoaxazolidine derivatives have been screened for their anticancer activity against a panel of human cancer cell lines.

2.2.6. Extraction and isolation of parthenin

The flowers of *Parthenium hysterophorus* were collected locally. The plant material (0.5 kg) was dried under shade, powdered and extracted with n-hexane (1 litre) in a soxhlet extractor (6 hrs) to remove fatty material. Then plant material was extracted with MeOH (3 litres) (9 hrs) and the crude MeOH extract was concentrated (60 g) and the concentrated extract was further extracted with CHCl\(_3\) (1.5 litres).
Figure-5: Possible sites for the attack of biological nucleophile on parthenin

Scheme-1: Synthesis of isoxazolidines and 1,3-amino alcohols
The crude chloroform extract after concentration (21 g) was subjected to hot water extraction (5 x 100 mL) for the isolation of parthenin (1.5 g). The combined extract was concentrated and the residue was subjected to column chromatography using silica gel (100-200 mesh) and EtOAc-hexane to afford 1.6 g of parthenin as light green color crystalline solid.

2.2.7. Chemistry
As we employed 1,3-dipolar cycloaddition of nitrones to exocyclic double bond of parthenin to generate a focused library of parthenin isoxazolidine derivatives, it is important to discuss briefly the chemistry of 1,3-dipolar cycloaddition of nitrones.

2.2.7.1. 1,3-dipolar cycloaddition reactions: Nitrones
1,3-Dipolar species are 4π-electron systems, where the π-electrons are delocalised over three atomic centres. In valence bond terms the electronic structures of the 1,3-dipolar species are represented as resonance hybrids of canonical forms that involve charge separation. The 1,3-dipolar cycloaddition reaction is also known as the Huisgen cycloaddition reaction, which is a classic reaction in organic chemistry. In 1,3-dipolar cycloaddition reaction, 1,3-dipole and a dipolarophile combine to give a five-membered heterocycle (Huisgen, 1963). Huisgen cycloaddition reaction is widely used as a high yielding, efficient, regio- and stereoselective method for the synthesis of a variety of valuable five-membered heterocycles.

Nitrones, an important category of 1,3–dipolar species, undergo facile concerted \( [\pi^4 + \pi^2] \)-cycloadditions to olefins and acetylenes to yield biologically important isoxazolidines and isoxazolines, respectively. Isoxazolidines, are saturated, five membered heterocycles containing adjacent nitrogen and oxygen atoms. Isoxazolidines are considered as useful synthetic intermediates for the preparation of 1,3-amino alcohols, because of the labile nature of the N-O bond under mildly reducing conditions (Takeuchi and Furusaki, 1977). By using chiral auxiliaries or chiral starting material it has often been possible to control both the regioselectivity, endo/exo selectivity, and diastereofacial selectivity in 1,3-dipolar cycloaddition reactions (Frederickson, 1997; Gothelf and Jørgensen, 1998; Desimoni et al., 1999).

2.2.7.2. Synthesis of spiro-isoxazolidine derivatives of parthenin via 1,3-dipolar cycloaddition of nitrone to the exocyclic double bond of parthenin.
As illustrated in scheme-2, spiro-isoxazolidine derivatives of parthenin (3) were prepared in high yields through 1,3-dipolar cycloaddition of various nitrones (2) to exocyclic double bond of parthenin under reflux in benzene. Nitrones (2) were prepared according
to literature procedure in which nitrobenzene was reduced in the presence of Zn/NH₄Cl to get phenylhydroxylamine followed by the condensation with various aromatic aldehydes (Gothelf and Jørgensen, 1998; Sandler et al., 1989; Black et al., 1975; Tufariello, 1979; Huisgen, 1963). Nitrone cycloaddition was selectively carried out on the exocyclic double bond located on the lactone ring in parthenin, which was clearly confirmed by the disappearance of the corresponding lactone alkene protons in ¹H-NMR. Presumably due to steric restrictions offered by the parthenin, only two diastereomers were formed, instead of expected four. Each diastereomer was isolated after column chromatography or by preparative TLC and characterized by ¹H-NMR, ¹³C-NMR and mass spectral analysis. These diastereomers were separated by HPLC also. A significant chemical shift difference > 1.0 δ due to benzylic proton adjacent to nitrogen atom in isoxazolidine ring corresponding to two diastereomers was observed in ¹H NMR. In the major isomer obtained, this proton appeared as a triplet around δ 5.0, but in the minor isomer, this proton resonated up field at δ 4.0, indicating shielding due to anisotropic effect which is characteristic of protons anti to the proton located at the β position of the hetero atom i.e., nitrogen. We have determined the absolute configuration of the newly formed chiral centre in isoxazolidine ring by simple correlation of ¹H anisotropy followed by applying Cahn-Ingold-Prelog priority rule (Figure-14). In isoxazolidine ring the spatial arrangement of nitrogen is fixed. Due to anisotropic effect, the isomer in which the hydrogen atom located on C21 which is “anti” to the lone pair electrons present on “N” atom will be shielded and a triplet appeared up field at around δ 4.0 and the isomer generated with anti spatial arrangement will have “R” configuration when priority rules is applied. Whereas, the “syn” isomer is assigned “S” configuration at C21 proton and with no significant electronic shielding influence on it, the proton resonates at around δ 5.0. A series of diastereomers of spiroisoxazolidines isolated through dipolar cycloaddition of nitrone exhibited similar pattern of anisotropy which helped in determining the absolute configuration of each enantiomer unequivocally.

2.2.8. Biological studies

2.2.8.1. In vitro cytotoxicity assay

All the spiro-isoxazolidine derivatives of parthenin were screened for their cytotoxicity against a panel of three human cancer cell lines SW-620 (colon), DU-145 (prostate) and PC-3 (prostate). The results are summarized in Table-1. From IC₅₀ values it is clear that all the spiro isaxazolidine derivatives exhibited better cytotoxicity than parent parthenin.
Compounds 3i', 3p' exhibited highest activity against SW-620 cell line (3 μM). Compounds 3n'', 3o' exhibited highest activity against DU-145 cell line (4.1 μM). 3a'', 3j'' exhibited highest activity against PC-3 cell line (2.3 μM). Even though halogen substituted derivatives appeared to exhibit better cytotoxicity with significant cell line specificity, the overall improvement of activity may be attributed to the contribution of cytotoxicity due to newly appended isoxazolidine ring together with the fact that exocyclic double bond with its intrinsic reactivity may lead to non specific alkylation with the biological nucleophiles.

2.2.8.2. DNA fragmentation assay
DNA fragmentation is the net result of apoptosis, and is observed at the late stage of cell cycle. DNA fragmentation assay showed that compound-3i' induced DNA fragmentation in SW-620 cells at 10 and 20 μM concentrations after 24 hrs of incubation. Camptothecin taken as a positive control showed significant fragmentation after 6 hrs at 5 μM concentration, however, no fragmentation was observed in untreated cells (Figure-6).

2.2.8.3. Cell cycle analysis
To address the cell death caused by compound-3i', the extent of apoptosis 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 compound-3i' revealed a concentration dependent increase in the sub G1 phase of cell cycle being 7.02, 33.3, 42.3 and 63.54 % at 1, 5, 10 and 20 μM respectively and also complete blockage of G1 phase at 20 μM concentration after 24 hrs of incubation in SW-620 cells was observed (Figure-7).

2.2.8.4. In vivo study of compound-3i'
Solid tumor bearing mice treated with different doses of parthenin and its derivative compound-3i' exhibited dose dependent tumor growth inhibition against EAT tumor model. A highly significant (p<0.01) tumor growth inhibition up to 35.11% was observed in EAT bearing mice treated with compound-3i' at 100 mg/kg i.p. dose whereas 200 mg/kg i.p. dose induced mortality of all the test animals by fourth day of the treatment. On the contrary, a high level of toxicity without significant antitumor activity was recorded in parthenin treated groups as it caused mortality of all the animals by second and third day of treatment at 25 and 50 mg/kg i.p. doses respectively (Table-2).
Scheme-2: Synthesis of spiro-isoxazolidine derivatives of parthenin
Table 1: IC₅₀ (µM) values of various spiro-isoxazolidine derivatives of parthenin against various human cancer cell lines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
<th>SW-620</th>
<th>DU-145</th>
<th>PC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a''</td>
<td>6.7</td>
<td>5.3</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>3a'</td>
<td>8.1</td>
<td>4.2</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>3b''</td>
<td>7.5</td>
<td>7</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>3b'</td>
<td>5.1</td>
<td>6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3c''</td>
<td>7.3</td>
<td>6.4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>3c'</td>
<td>6</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3d''</td>
<td>4.8</td>
<td>5</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>3d'</td>
<td>5</td>
<td>7</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>3e''</td>
<td>7.3</td>
<td>6.4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>3e'</td>
<td>4.2</td>
<td>7.1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3f''</td>
<td>8.7</td>
<td>8.1</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>3f'</td>
<td>9</td>
<td>6.3</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>3g''</td>
<td>12</td>
<td>4.5</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>3g'</td>
<td>9</td>
<td>5.1</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>3h''</td>
<td>5.3</td>
<td>5.6</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>3h'</td>
<td>6</td>
<td>6.5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3i''</td>
<td>3</td>
<td>9</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>3i'</td>
<td>5</td>
<td>6.3</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>3j''</td>
<td>5</td>
<td>12</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>3j'</td>
<td>7.2</td>
<td>9</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>3k''</td>
<td>6.1</td>
<td>7.4</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>3k'</td>
<td>10.5</td>
<td>6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3l''</td>
<td>4.8</td>
<td>5</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>3l'</td>
<td>5.1</td>
<td>8.1</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Parthenin</td>
<td>38.7</td>
<td>31.1</td>
<td>40.3</td>
<td></td>
</tr>
</tbody>
</table>

' major diastereomer, '' minor diastereomer

Cancer cell lines: SW-620 (colon), DU-145 (prostate) and PC-3 (Prostate).

IC₅₀: Half maximal inhibitory concentration.
Figure-6. DNA fragmentation assay of compound 3i’ in SW-620 cells. Lane-1: Untreated Cells, lane-2: treated with 5µM of Camptothecin, lane-3: treated with 5 µM of compound-3i’, lane-4: treated with 10 µM of compound-3i’, lane-5: treated with 20 µM of compound-3i’.

Figure-7: Flow cytometric analysis of compound 3i’ treated with SW-620 cells.
Table-2. Effect of parthenin and its compound-3i' on Ehrlich ascitic tumor (EAT) bearing mice.

<table>
<thead>
<tr>
<th>Test group</th>
<th>Dose (mg/kg)</th>
<th>Tumor weight (mg)</th>
<th>% Tumor growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 mL</td>
<td>1708.57 ± 51.07</td>
<td>-</td>
</tr>
<tr>
<td>Parthenin</td>
<td>10</td>
<td>1542.86 ± 35.24</td>
<td>9.69</td>
</tr>
<tr>
<td>Parthenin</td>
<td>25</td>
<td>All animals died by 4th day</td>
<td></td>
</tr>
<tr>
<td>Parthenin</td>
<td>50</td>
<td>All animals died by 2nd day</td>
<td></td>
</tr>
<tr>
<td>3i'</td>
<td>10</td>
<td>1547.54 ± 35.93</td>
<td>9.54</td>
</tr>
<tr>
<td>3i'</td>
<td>25</td>
<td>1445.66 ± 41.63</td>
<td>15.90</td>
</tr>
<tr>
<td>3i'</td>
<td>50</td>
<td>1332.27 ± 32.54*</td>
<td>22.23</td>
</tr>
<tr>
<td>3i'</td>
<td>100</td>
<td>1108.54 ± 8.01**</td>
<td>35.19</td>
</tr>
<tr>
<td>3i'</td>
<td>200</td>
<td>1108.54 ± 8.01**</td>
<td></td>
</tr>
</tbody>
</table>

p>0.05 = Insignificant, * = p<0.05 = Significant, ** = p<0.01= Highly significant.

Table-3. Effect of parthenin and its derivative-3i' on Ehrlich ascitic carcinoma (EAC) bearing mice.

<table>
<thead>
<tr>
<th>Test group</th>
<th>Dose (mg/kg)</th>
<th>Ascitic fluid volume (mL)</th>
<th>Tumor cells in ascitic fluid (x 10^7)</th>
<th>% Tumor growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 mL</td>
<td>4.57 ± 0.39</td>
<td>85.00 ± 4.75</td>
<td></td>
</tr>
<tr>
<td>Parthenin</td>
<td>10</td>
<td>3.92 ± 0.38</td>
<td>74.71 ± 2.77</td>
<td>12.01</td>
</tr>
<tr>
<td>Parthenin</td>
<td>25</td>
<td>All animals died by 4th day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parthenin</td>
<td>50</td>
<td>All animals died by 2nd day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3i'</td>
<td>10</td>
<td>4.07 ± 0.35</td>
<td>78.42 ± 2.90</td>
<td>7.83</td>
</tr>
<tr>
<td>3i'</td>
<td>25</td>
<td>3.62 ± 0.32</td>
<td>68.71 ± 3.16</td>
<td>19.17</td>
</tr>
<tr>
<td>3i'</td>
<td>50</td>
<td>2.85 ± 0.37</td>
<td>61.85 ± 4.20*</td>
<td>27.36</td>
</tr>
<tr>
<td>3i'</td>
<td>100</td>
<td>2.07 ± 0.33**</td>
<td>33.57 ±</td>
<td>60.66</td>
</tr>
<tr>
<td>3i'</td>
<td>200</td>
<td>2.60**</td>
<td>All animals died by 3rd day</td>
<td></td>
</tr>
</tbody>
</table>

p>0.05 = Insignificant, * = p<0.05 = Significant, ** = p<0.01= Highly significant.
In case of Ehrlich ascetic carcinoma (EAC) bearing mice same pattern of dose dependent
tumor growth inhibition and toxicity was exhibited by both parthenin and its derivative
compound-3i'. At 100 mg/kg, i.p. dose, compound-3i' exhibited highly significant
(p<0.01) tumor growth inhibition up to 60% and its higher dose of 200 mg/kg i.p. induced
mortality of all the test animals by fourth day of the treatment. In this experiment also the
parent compound parthenin induced mortality of all the test animals by 2nd and 3rd day
of the treatment at 25 and 50 mg/kg i.p. doses respectively exhibiting high level of
toxicity to the treated animals without any significant antitumor activity (Table-3).
This clearly indicates the reduced toxicity and improved anticancer activity of the spiro-
isoxazolidine derivative of parthenin.

2.2.8.5. Western blot analysis of the inhibition of NF-kB(P65) expression by
compound-3i'
Using helenalin and parthenolide as models, it has been well established that DNA
binding of NF-kB is prevented by alkylation of cysteine in the p65/NF-kB subunit, which
is considered to be the general mechanism for SLs bearing α,β-unsaturated carbonyl
structures (Lyss et al., 1997, 1998; Garcia-Pineres et al., 1997; Bettina et al., 2004).
Based on these reports, Western blot experiments have been carried out. NF-κB(P65)
expression is completely inhibited by the compound-3i' which can be presumed due to
inhibition of expression either on transcriptional level or translational level.
NF-κB is a central mediator of the human immune response. In the majority of cell types
this protein is composed of a p50 and a p65 subunit. It is retained in an inactive
cytoplasmic complex by binding to IκB, its inhibitory subunit. A large variety of inflammatory
conditions, such as bacterial and viral infections as well as inflammatory cytokines,
rapidly induce NF-κB activity. Active NF-κB is released from the cytoplasmic complex
by phosphorylation, ubiquitination and degradation of the IκB subunit. The activated
factor then translocates to the nucleus where it stimulates the transcription of its target
genes. NF-κB regulates the transcription of various inflammatory cytokines, such as IL-1,
IL-2, IL-6, IL-8 and TNF-α, as well as genes encoding cyclo-oxygenase-II, nitric oxide
synthase, immunoreceptors, cell adhesion molecules, hematopoietic growth factors and
growth factor receptors. Pharmacological inhibition of NF-κB in vivo may thus
substantially attenuate inflammatory processes. The inhibition of NF-κB (P-65) by spiro-
isoxazolidine derivative of parthenin (Compound -3i') is shown in Figure-8.
Figure-8: Inhibition of NF-κB (P65) by spiro derivative of parthenin (Compound-3i̊)

2.2.9. Docking studies

From the literature, it is well known that parthenin type of sesquiterpenoid lactones are NF κB (p65) inhibitors and western blotting experiments (discussed above) also proved that spiro-isoxazolidine derivatives inhibit p65 subunit of NF-κB (Lyss et al., 1997, 1998; Garcia-Pineres et al., 1997; Bettina et al., 2004). As discussed earlier the cytotoxicity of these compounds is due to the alkylation of nucleophilic groups especially sulfhydryl groups (cys-38 and cys-120) present in biological system in Michael addition fashion.

To identify which “cystein” group is more approachable to cyclopentenone ring and also to have some insight of binding mode of this type of molecules to NF-κB we have done in silico studies (compounds-3a, 3b & 3e are used for this study). From the analysis of crystal structure of the target protein it was observed that p65 subunit of NF-κB is held to DNA by 3 hydrogen bonds present between residues Tyr36, Cys38 and Lys122 as shown in Figure-9.

The interaction figure of the ligands and receptor are shown in Figure 10-12. The common residues that are involved in holding the ligands in cavity are Tyr36, Cys38, Glu39, His88, Cys120, val121, Lys122, Leu154, Asn155, Asp185 and Arg187. Out of which Tyr36, cys38, cys120, val121 and leu154 are involved in holding the ligand hydrophobically. There are two strong bonds present between -OH (that is below the plane) of compound-3b with Cys38 with strength of 2.4 Å and another hydrogen bond is present with glu39 with strength of 1.9 Å. Compound-3e was also held in the same cavity with all the same residues and same hydrogen bonds but with different strengths. Compound-3e was having 2 hydrogen bond with two residues of active site i.e one is with cys38 with strength of 2.49 Å and other is with glu39 with strength of 2.0 Å. Compound-3a also contained same hydrogen bonds but different strengths of 1.18 Å and 2.43 Å respectively. The change in the overall arrangement of crucial residues was also observed before and after the placement of the ligand in the cavity as shown in Figure-
13. The very important information that was inferred from the ligand receptor interaction study was that the important residues that were involved earlier in holding DNA were eventually got engaged in binding the ligand to the receptor there by leading to no free position for binding DNA. The orientation of tyr-136 was analyzed before and after docking as shown in Figure-13 A and B and it was inferred that the orientation of tyr-136 after the binding of ligand was such that it is not capable of binding to the DNA.

In all the docking figures there is a hydrogen bonding between cys-38 and “carbonyl oxygen” present in the cyclopentenone ring. Even though hydrogen bonding is weak, it clearly shows that cys-38 is approachable to the cyclopentenone ring and likely to forms alkylation through Michael addition fashion.

2.2.10. Conclusions
The present set of data clearly reveals that spiro-isoxazolidine derivatives of parthenin presented here induce concentration dependent apoptosis in cancer cells. Spiro isoxazolidine derivatives of parthenin exhibited improved cytotoxicity as indicated by the in vitro cell based assay whereas in vivo data of select derivative clearly indicate reduced mammalian toxicity when tested in mice models. Select spiro derivatives of pathenin completely inhibited the NF-κB(P65) expression confirmed that these derivatives block p65 subunit of NF-κB. Docking studies revealed that cys-38 present in the NF-κB(P65) forms alkylation structures with cyclopentenone ring of spiro-isoxazolidine derivatives of parthenin. SAR of parthenin could be explained with clarity as it has been established through the present study that conservation of α,β-unsaturated ketone of parthenin is crucial for retaining the anticancer activity of the ligand, whereas the exocyclic double bond can be advantageously utilized to incorporate appropriate structural entities that may enhance the cytotoxicity of the parent molecule.

2.2.11. Experimental section
All chemicals and reagents were of analytical grade, purchased from Sigma-Aldrich. All the solvents were LR grade, purchased from Rankem (India). Melting points were recorded on Buchi Melting point apparatus D-545 and IR spectra (KBr) on Bruker Vector 22 instrument. 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 ESI-esquire 3000 Bruker Daltonics instrument. The progress of all reactions was monitored by TLC on 2 x 5 cm precoated silica gel 60 F254 plates of thickness 0.25 mm (Merck). The chromatograms were
visualized under UV 254-366 nm and iodine. Purity was checked with Waters analytical HPLC.

2.2.11.1. Synthesis of spiroisoxazolidine derivatives of parthenin via 1,3-dipolar cycloaddition of nitrone to the exocyclic double bond.

In a typical procedure, to a solution of parthenin (0.5g, 1.9 mmol) in dry benzene (6 mL), was added a solution of appropriate nitrone (1.58g, 2.4 mmol) in dry benzene and refluxed the reaction mixture for 8 hrs. After completion of the reaction, solvent was evaporated in vacuo and the crude was subjected for flash chromatography (silica gel, 230-400 mesh, elution; n-hexane/EtOAc gradient) to afford pure diastereomers [3a’ (92%) and 3a” (8%)] in good yields (75%). The products were characterized on the basis of \(^1\)H NMR, \(^{13}\)C NMR, IR and Mass spectrometry analysis. The ratio of the diastereomers was also confirmed by HPLC analysis [hexane:Isopropyl alcohol (85:15), Si-60 column (Merck, 5 µM, 4.0 x 25 cm, temperature (30 °C)].

N-(Phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a’)

Pale yellow colour solid; mp: 185 °C

Specific rotation: \([\alpha]_D^{25} -12\) (c 0.9, CHCl\(_3\))

\(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta 7.52-7.48\) (m, 3H), 7.39 (d, \(J = 8.4\) Hz, 2H), 7.19 (t, \(J = 8.0\) Hz, 2H), 6.93 (t, \(J = 7.3\) Hz, 1H), 6.86 (d, \(J = 8.2\) Hz, 2H), 6.26 (d, \(J = 5.9\) Hz, 1H), 5.26 (d, \(J = 5.6\) Hz, 1H), 5.14 (t, \(J = 8.0\) Hz, 1H), 3.21-3.17 (dd, \(J = 6.5, 5.6\) Hz, 1H), 2.94-2.90 (dd, \(J = 6.9, 5.6\) Hz, 1H), 2.5-2.45 (dd, \(J = 9.2, 3.3\) Hz, 1H), 2.37-2.31 (p, \(J = 7.3\) Hz, 1H), 2.24-2.17 (td, \(J = 12.6, 6.4\) Hz, 1H), 1.95-1.86 (q, \(J = 13.6\) Hz, 1H), 1.69-1.65(dd, \(J = 5.7, 8.5\) Hz, 1H), 1.47-1.43 (dd, \(J = 9.4, 5.0\) Hz, 1H), 1.34 (s, 3H), 1.09 (d, \(J = 7.8\) Hz, 3H)

\(^{13}\)C NMR (CDCl\(_3\)): 211.5, 174.4, 164.1, 151.1, 140.0, 132.1, 131.6, 128.8, 128.5, 122.5, 121.6, 114.8, 86.5, 84.2, 79.6, 70.0, 59.0, 49.4, 42.7, 39.9, 31.4, 21.7, 20.1, 18.0

IR (KBr): 3452, 2962, 2926, 1774, 1754, 1597, 1488, 1011 cm\(^{-1}\)

ESI MS (m/z): 539 (M + 1)

126
Figure-9: The DNA and p65 subunit interactions are shown here. Figure-9B is the full view of DNA and p65 subunit where as Figure-9A is enlarged view of the same. There are three hydrogen bonds observed between DNA and p65 monomer with residues viz. Tyr36, Cys38, Lys123.

Figure-10: The ligand 3a is represented in cylinder and the important residues (Cys38 & Cys120) are shown in cylinder form as well. One hydrogen bond is present with cys38 (strength 1.18 Å) and one with Glu39 (strength 2.43 Å). Also the change in orientation of tyr36 another crucial residue was observed after docking.
Figure-11: The ligand 3e is represented in cylinder and the important residues (Cys38 & Cys120) are shown in cylinder form as well. One hydrogen bond is present with cys38 (strength 2.49 Å°) and one with glu39 (strength 2.0Å°). Also the change in orientation of tyr36 another crucial residue was observed after docking.

Figure-12: The ligand 3b is represented in cylinder and the important residues (Cys38 & Cys120) are shown in cylinder form as well. One hydrogen bond is present with cys38 (strength 2.4Å°) and one with glu39 (strength 1.9 Å°). Also the change in orientation of tyr36 another crucial residue was observed after docking.
Figure-13: The change in orientation of the crucial residues was observed before (A) and after docking (B).

Table-4. Molecular properties of spiro isoxazolidine derivatives of parthenin

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</table>

LogP: partition coefficient; MW: molecular weight; nON: number of hydrogen bond acceptors; nOHNH: number of hydrogen bond donors; nV: number of violations.

Molecular properties are calculated using “Molinspiration property engine” software.
Figure-14: Determination of absolute configuration isoxazolidine derivative of parthenin.
N-(Phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a’’)

Pale yellow colour solid; mp: 179 °C

Specific rotation: $[\alpha]_D^{25} -5.5$ (c 0.75, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): 7.51-7.48 (m, 5H), 7.19-7.16 (t, $J = 7.5$ Hz, 2H), 6.96 (t, $J = 7.4$ Hz, 1H), 6.89 (d, $J = 7.7$ Hz, 2H), 6.21 (d, $J = 5.9$ Hz, 1H), 5.2 (d, $J = 5.5$ Hz, 1H), 4.315 (t, $J = 8.2$ Hz, 1H), 3.13-3.1 (dd, $J = 5.5$, 6.2 Hz, 1H), 2.92-2.88 (dd, $J = 9.2$, 4.0 Hz, 1H), 2.69-2.65 (dd, $J = 8.0$, 5.1 Hz, 1H), 2.38-2.32 (p, $J = 7.3$ Hz, 1H), 2.25-2.18 (td, $J = 12.4$, 6.3 Hz, 1H), 1.94-1.86 (q, $J = 13.5$ Hz, 1H), 1.75-1.71 (dd, $J = 5.7$, 8.4 Hz, 1H), 1.58-1.54 (dd, $J = 9.5$, 4.5 Hz, 1H), 1.37 (s, 3H), 1.1 (d, $J = 7.8$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$): 211.2, 174.5, 164.0, 152.0, 140.3, 132.1, 131.4, 128.6, 128.4, 122.4, 121.7, 114.6, 86.5, 84.8, 79.7, 70.1, 59.1, 49.5, 42.7, 40.2, 31.7, 21.9, 20.1, 18.0

IR (KBr): 3452, 2962, 2925, 1775, 1754, 1596, 1488, 1012 cm$^{-1}$

ESI MS ($m/z$): 561 (M + 23)$^+$

N-(Phenyl)-C-(4-chloro phenyl)-spiro-isoxazolidinyl parthenin (3b’)

Pale yellow colour solid; mp: 181 °C

Specific rotation: $[\alpha]_D^{25} -15.5$ (c 1, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): 7.5-7.46 (m, 3H), 7.36 (d, $J = 8.4$ Hz, 2H), 7.17 (t, $J = 8.0$ Hz, 2H), 6.93 (t, $J = 7.3$ Hz, 1H), 6.86 (d, $J = 8.3$ Hz, 1H), 6.27 (d, $J = 5.8$ Hz, 2H), 5.26 (d, $J = 5.4$ Hz, 1H), 5.14 (t, $J = 8.1$ Hz, 1H), 3.18-3.14 (dd, $J = 6.5$, 5.7 Hz, 1H), 2.95-2.91 (dd, $J = 7.0$, 5.6 Hz, 1H), 2.47-2.42 (dd, $J = 6.9$, 5.7 Hz, 1H), 2.36-2.3 (p, $J = 7.4$ Hz, 1H), 2.24-2.16 (td, $J = 12.7$, 6.34 Hz, 1H), 1.9-1.8 (q, $J = 13.6$ Hz, 1H), 1.67-1.63 (dd, $J = 8.5$, 5.7 Hz, 1H), 1.45-1.4
(dd, \( J = 9.4, 5.0 \) Hz, 1H), 1.3 (s, 3H), 1.09 (d, \( J = 7.8 \) Hz, 3H).

\(^{13}\)C NMR (CDCl\(_3\)): 210.4, 175.1, 164.0, 151.9, 140.2, 132.5, 129.5, 129.2, 128.4, 122.9, 115.3, 87.3, 84.1, 79.9, 70.3, 59.6, 49.7, 43.2, 40.5, 31.9, 21.9, 20.5, 18.4.

IR (KBr): 3450, 2963, 2910, 1751, 1594, 1220, 1071 cm\(^{-1}\)

ESI MS (m/z): 516 (M + 23)

Anal. Calcd for C\(_{28}\)H\(_{28}\)ClNO\(_5\): C, 68.08; H, 5.71; N, 2.84. Found: C, 68.02; H, 5.74; N, 2.87.

N-(Phenyl)-C-(4-chloro phenyl)-spiro-isoxazolidinyl parthenin (3b’’)

Pale yellow colour solid; mp: 188\(^\circ\)C

Specific rotation: \([\alpha]_D^{25} - 11.1 (c 1, \text{CHCl}_3)\).

\(^1\)H NMR (500 MHz, CDCl\(_3\)): 7.48-7.47 (m, 5H), 7.15 (t, \( J = 7.6 \) Hz, 2H), 6.95 (t, \( J = 7.4 \) Hz, 1H), 6.89 (d, \( J = 7.7 \) Hz, 2H), 6.21 (d, \( J = 5.9 \) Hz, 1H), 5.21 (d, \( J = 5.5 \) Hz, 1H), 4.31 (t, \( J = 8.3 \) Hz, 1H), 3.12-3.09 (dd, \( J = 6.2, 5.4 \) Hz, 1H), 2.90-2.86 (dd, \( J = 9.2, 4.0 \) Hz, 1H), 2.68-2.64 (dd, \( J = 8.0, 5.1 \) Hz, 1H), 2.37-2.31 (p, \( J = 7.4 \) Hz, 1H), 2.23-2.16 (td, \( J = 12.7, 6.3 \) Hz, 1H), 1.93-1.85 (q, \( J = 13.3 \) Hz, 1H), 1.73-1.69 (dd, \( J = 8.6, 5.7 \) Hz, 1H), 1.58-1.54 (dd, \( J = 9.4, 4.5 \) Hz, 1H), 1.36 (s, 3H), 1.1 (d, \( J = 7.6 \) Hz, 3H).

\(^{13}\)C NMR (CDCl\(_3\)): 210.7, 175.3, 164.0, 151.9, 140.5, 132.5, 129.5, 129.3, 128.6, 122.7, 115.7, 87.4, 84.1, 80.1, 70.5, 59.6, 49.7, 43.3, 40.5, 31.9, 22.4, 20.5, 18.8

IR (KBr): 3451, 2964, 2912, 1751, 1595, 1366, 1221, 1071 cm\(^{-1}\)

ESI MS (m/z): 494 (M + 1)

Anal. Calcd for C\(_{28}\)H\(_{28}\)ClNO\(_5\): C, 68.08; H, 5.71; N, 2.84. Found: C, 68.03; H, 5.75; N, 2.87.

N-(Phenyl)-C-(4-fluoro phenyl)-spiro-isoxazolidinyl parthenin (3c’)

Pale yellow colour solid; mp: 154-155 \(^\circ\)C.

Specific rotation: \([\alpha]_D^{25} - 18.3 \ (c 0.9, \text{CHCl}_3)\)

\(^1\)H NMR (500 MHz, CDCl\(_3\)): 7.52-7.45 (m, 3H), 7.41 (d, \( J = 8.3 \) Hz, 1H), 7.19
(t, J = 7.5 Hz, 2H), 6.93 (t, J = 7.2 Hz, 1H), 6.85  
(d, J = 7.6 Hz, 2H), 6.21 (d, J = 5.9 Hz, 1H),  
5.26 (d, J = 5.4 Hz, 1H), 5.19 (t, J = 8.0 Hz, 1H),  
3.22-3.20 (dd, J = 6.5, 5.4, 1H), 2.65-2.61  
(dd, J = 9.3, 3.3 Hz, 1H), 2.4-2.35 (p, J = 7.4 Hz, 1H),  
2.25-2.2 (td, J = 12.4, 6.3 Hz, 1H), 1.94-1.86  
(q, J = 13.5 Hz, 1H), 1.72-1.67(dd, J = 8.4, 5.6 Hz, 1H),  
1.47-1.44 (dd, J = 9.5, 5.0 Hz, 1H), 1.35 (s, 3H),  
1.09 (d, J = 7.8 Hz, 3H)  

$^{13}$C NMR (CDCl$_3$):  
211.2, 174.7, 164.3, 151.5, 140.3, 132.1, 130.6, 128.6, 128.4, 122.1, 121.4, 114.2, 87.1, 84.3, 79.6, 70.1, 58.8, 49.6, 42.6, 40.1, 31.7, 21.7, 20.4, 17.6.  

IR (KBr):  
3437, 2962, 2924, 1753, 1722, 1598, 1509, 1261, 1088, 1024, 801 cm$^{-1}$  

ESI MS ($m/z$):  
500 (M + 23)$^+$  

Anal. Calcd for C$_{28}$H$_{28}$FNO$_5$:  
C, 70.43; H, 5.91; N, 2.98. Found: C, 70.37; H, 5.94; N, 3.01.

N-(Phenyl)-C-(4-fluoro phenyl)-spiro-isoxazolidinyl parthenin (3c"")

Pale yellow colour solid; mp: 154-165°C  
Specific rotation:  
$[\alpha]_{D}^{25}$ -10.5° (c 0.9, CHCl$_3$)  

$^1$H NMR (500 MHz, CDCl$_3$):  
7.51-7.46 (m, 5H), 7.175(t, J = 7.6 Hz, 2H), 6.93  
(t, J = 7.5 Hz, 1H), 6.89 (d, J = 7.78 Hz, 2H), 6.21  
(d, J = 5.57 Hz, 1H), 5.25 (d, J = 5.5 Hz, 1H), 4.23  
(t, J = 8.1 Hz, 1H), 3.15-3.12 (dd, J = 6.4, 5.6 Hz, 1H),  
2.93-2.89 (dd, J = 9.2, 3.9 Hz, 1H), 2.69-2.65  
(dd, J = 7.9, 5.2 Hz, 1H), 2.38-2.33 (p, J = 7.3 Hz, 1H),  
2.24-2.17 (td, J = 12.3, 6.3 Hz, 1H), 1.95-1.87  
(q, J = 13.2 Hz, 1H), 1.74-1.7 (dd, J = 8.2, 5.6 Hz, 1H),  
1.58-1.54 (dd, J = 9.2, 4.7 Hz, 1H), 1.36 (s, 3H),  
(d, J = 7.7 Hz, 3H).  

$^{13}$C NMR (CDCl$_3$):  
211.1, 174.1, 165.3, 151.5, 140.8, 132.1, 130.2, 128.7, 128.4, 122.1, 121.7, 114.8, 87.1, 84.2, 79.6, 70.1, 59.1, 49.5, 42.3, 40.1, 31.7, 21.9, 20.5, 17.9
IR (KBr): 3439, 2961, 2925, 1755, 1723, 1597, 1510, 1262, 1085, 1021, 803 cm$^{-1}$

ESI MS ($m/z$): 478 (M + 1)$^+$.  

Anal. Calcd for C$_{28}$H$_{28}$FNO$_5$: C, 70.43; H, 5.91; N, 2.98. Found: C, 70.38; H, 5.94.; N, 3.0

N-(Phenyl)-C-(4-cyano phenyl)-spiro-isoxazolidinyl parthenin (3d)

White colour solid; mp: 114-116 °C  
Specific rotation: $[\alpha]_D^{25}$ -8.4 (c 0.6, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): 7.71-7.61 (m, 4H), 7.47 (d, $J = 5.9$ Hz, 1H), 7.19 (t, $J = 7.7$ Hz, 2H), 6.93 (t, $J = 7.2$ Hz, 1H), 6.82 (d, $J = 7.8$ Hz, 2H), 6.19 (d, $J = 5.9$ Hz, 1H), 5.26 (d, $J = 5.4$ Hz, 1H), 5.23 (t, $J = 8.0$ Hz, 1H), 3.23-3.19 (dd, $J = 6.4$, 5.6 Hz, IH), 3.0-2.94 (dd, $J = 6.8$, 5.6 Hz, 1H), 2.72-2.68 (dd, $J = 9.0$, 3.4 Hz, 1H), 2.36-2.31 (p, $J = 7.5$ Hz, 1H), 2.24-2.3 (td, $J = 12.5$, 6.4 Hz, 1H), 1.94-1.86 (q, $J = 13.5$ Hz, 1H), 1.69-1.65 (dd, $J = 8.4$, 5.5 Hz, 1H), 1.47-1.43 (dd, $J = 9.3$, 5.0 Hz, 1H), 1.34 (s, 3H), 1.1 (d, $J = 7.7$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$): 211.0, 174.1, 163.7, 150.8, 146.7, 132.8, 131.8, 128.9, 128.5, 122.4, 118.5, 114.3, 86.6, 84.5, 79.6, 69.7, 58.7, 49.1, 42.4, 40.0, 31.4, 21.4, 20.3, 17.9

IR (KBr): 3444, 2962, 2926, 2228, 1776, 1723, 1597, 1261, 1195, 1020, 802 cm$^{-1}$

ESI MS ($m/z$): 485.4 (M + 1)$^+$.  

Anal. Calcd for C$_{29}$H$_{28}$N$_2$O$_5$: C, 71.88; H, 5.82; N, 5.78. Found: C, 71.81; H, 5.85; N, 6.80.

N-(Phenyl)-C-(4-cyano phenyl)-spiro-isoxazolidinyl parthenin (3d’)

White colour solid; mp: 120-121 °C  
Specific rotation: $[\alpha]_D^{25}$ -4.1 (c 0.6, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): 7.69-7.61 (m, 4H), 7.45 (d, $J = 5.9$ Hz, 1H), 7.165 (t, $J = 7.5$ Hz, 2H), 6.95 (t, $J = 7.6$ Hz, 1H), 6.86 (d, $J = 7.8$ Hz, 2H), 6.21 (d, $J = 5.7$ Hz, 1H), 5.25 (d, $J = 5.6$ Hz, 1H), 4.41 (t, $J = 8.1$ Hz, 1H), 3.12-3.09 (dd, $J = 6.4$, 1H).
5.7 Hz, 1H), 2.9-2.84 (dd, J = 9.3, 3.9 Hz, 1H),
2.68-2.64 (dd, J = 7.9, 5.2 Hz, 1H), 2.38-2.32
(p, J = 7.4 Hz, 1H), 2.24-2.17 (td, J = 12.6, 6.4 Hz, 1H),
1.95-1.87 (q, J = 3.4 Hz, 1H), 1.75-1.71
(dd, J = 8.3, 5.6 Hz, 1H), 1.58-1.54 (dd, J = 9.4, 4.7 Hz, 1H), 1.36 (s, 3H), 1.1 (d, J = 7.8 Hz, 3H).

$^{13}$C NMR (CDCl$_3$):
211.0, 174.7, 163.7, 151.1, 146.3, 132.8, 131.7, 128.7, 128.6, 122.4, 118.5, 114.4, 86.7, 84.5, 79.4, 70.0, 58.4, 49.2, 42.1, 39.7, 31.6, 21.2, 20.0, 18.1

IR (KBr):
3444, 2959, 2925, 2230, 1776, 1722, 1597, 1260, 1197, 1021, 803 cm$^{-1}$

ESI MS (m/z):
485 (M + 1)$^+$

Anal. Calcd for C$_{29}$H$_{28}$N$_2$O$_5$:  C, 71.88; H, 5.82; N, 5.78. Found: C, 71.84; H, 5.80; N, 5.82.

N-(Phenyl)-C-(4-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3e)

White colour solid; mp: 180-182 °C
Specific rotation: $[\alpha]_D^{25}$ -12.5 (c 0.5, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 7.54-7.46 (m, 3H), 7.21 (d, J = 8.1 Hz, 2H), 7.145
t (J = 7.9 Hz, 2H), 6.91 (t, J = 7.4 Hz, 1H), 6.86 (d, J = 8.1 Hz, 2H), 6.31 (d, J = 5.8 Hz, 1H), 5.30 (d, J = 5.7 Hz, 1H), 5.165 (t, J = 8.1 Hz, 1H), 3.87 (s, 3H),
3.27-3.23 (dd, J = 6.6, 5.61 Hz, 1H), 2.99-2.95
(dd, J = 6.8, 5.5 Hz, 1H), 2.5-2.45 (dd, J = 9.1, 3.4 Hz, 1H), 2.39-2.33 (p, J = 7.4 Hz, 1H), 2.24-2.17
(td, J = 12.4, 6.5 Hz, 1H), 1.94-1.85 (q, J = 13.6 Hz, 1H),
1.71-1.67 (dd, J = 8.5, 5.7 Hz, 1H), 1.47-1.43
(dd, J = 9.4, 5.0 Hz, 1H), 1.34 (s, 3H),
1.09 (d, J = 7.7 Hz, 3H)

$^{13}$C NMR (CDCl$_3$):
210.6, 174.1, 163.6, 159.8, 151.1, 132.1, 131.4, 128.8, 128.4, 122.5, 117.8, 115.4, 86.5, 84.4, 79.5, 70.4,
59.2, 55.4, 49.6, 43.0, 40.2, 31.6, 21.7, 20.1, 18.1

IR (KBr):
3450, 2963, 2927, 1775, 1597, 1488, 1012 cm$^{-1}$

ESI MS (m/z): 490 (M + 1)$^+$
N-(Phenyl)-C-(4-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3e’’)

White colour solid; mp: 175-176 °C

Specific rotation: $[\alpha]_D^{25}$ -18.6 (c 0.5, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):

7.51-7.48 (m, 5H), 7.19-7.16 (t, $J = 7.7$ Hz, 2H),
6.9 (t, $J = 7.5$ Hz, 1H), 6.885 (d, $J = 7.8$ Hz, 2H),
6.27(d, $J = 5.8$ Hz, 1H), 5.3 (d, $J = 5.6$ Hz, 1H),
4.33 (t, $J = 8.1$ Hz, 1H), 3.14-3.11 (dd, $J = 6.3$,
5.6 Hz, 1H), 2.91-2.87 (dd, $J = 9.1$, 3.9 Hz, 1H),
2.68-2.64 (dd, $J = 7.7$, 5.0 Hz, 1H), 2.36-2.3
(p, $J = 7.3$ Hz, 1H), 2.24-2.17 (td, $J = 12.2$, 6.4 Hz, 1H),
1.94-1.86 (q, $J = 13.6$ Hz, 1H), 1.75-1.71
(dd, $J = 8.5$, 5.7 Hz, 1H), 1.58-1.54
(dd, $J = 9.3$, 4.5 Hz, 1H), 1.36 (s, 3H),
1.1 (d, $J = 7.7$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$):

210.7, 174.2, 163.6, 159.8, 151.1, 132.2, 131.3, 128.5,
128.5, 122.5, 117.6, 115.2, 86.2, 84.6, 79.5, 70.1,
59.5, 55.5, 49.7, 43.9, 40.2, 31.5, 21.7, 20.1 18.0

IR (KBr): 3452, 2925, 1775, 1595, 1490 cm$^{-1}$

ESI MS ($m/z$): 512 (M + 23)$^+$

N-(Phenyl)-C-(4-methyl phenyl)-spiro-isoxazolidinyl parthenin (3f’)

Brown colour solid; mp: 115 °C

Specific rotation: $[\alpha]_D^{25}$ -6.4 (c 0.5, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):

δ 7.47-7.38 (m, 3H), 7.30 (d, $J = 8.3$ Hz, 2H),
7.17 (t, $J = 7.8$ Hz, 2H), 6.88 (t, $J = 7.5$ Hz, 1H),
6.84 (d, $J = 8.1$ Hz, 2H), 6.2 (d, $J = 5.8$ Hz, 1H),
5.26 (d, $J = 5.6$ Hz, 1H), 5.19 (t, $J = 8.0$ Hz, 1H),
3.23-3.18 (dd, $J = 6.5$, 5.6 Hz, 1H), 2.95-2.91
(dd, $J = 6.7$, 5.8 Hz, 1H), 2.63-2.48 (dd, $J = 9.1$, 3.4
Hz, 1H), 2.37-2.31 (p, $J = 7.3$ Hz, 1H), 2.3 (s, 3H),
2.25-2.18 (td, \( J = 12.4, 6.4 \) Hz, 1H), 1.95-1.86 (q, \( J = 13.5 \) Hz, 1H), 1.72-1.68 (dd, \( J = 8.37, 5.6 \) Hz, 1H), 1.48-1.44 (dd, \( J = 9.2, 5.0 \) Hz, 1H), 1.33 (s, 3H), 1.1 (d, \( J = 7.7 \) Hz, 3H)

\(^{13}\)C NMR (CDCl\(_3\)):

210.9, 174.4, 163.7, 151.3, 137.8, 131.9, 129.3, 128.3, 126.5, 122.2, 117.5 115.0, 86.3, 84.6, 79.4, 70.4, 59.0, 49.2, 43.0, 40.0, 31.6, 29.6, 21.6, 20.0 17.9

IR (KBr):

3443, 2926, 1774, 1597, 1497, 1258, 1020 cm\(^{-1}\)

ESI MS (m/z):

496 (M + 23)

Anal. Calcd for C\(_{29}\)H\(_{31}\)NO\(_5\):

C, 73.55; H, 6.6; N, 2.96. Found: C, 73.48; H, 6.64; N, 3.0.

N-(Phenyl)-C-(4-methyl phenyl)-spiro-isoxazolidinyl parthenin (3f"

Brown colour solid; mp: 121 °C

Specific rotation: \([\alpha]_{D}^{25} -12.4 \) (c 0.6, CHCl\(_3\))

\(^1\)H NMR (500 MHz, CDCl\(_3\)):

7.47-7.43 (m, 5H), 7.18 (t, \( J = 7.6 \) Hz, 2H), 6.99 (t, \( J = 7.5 \) Hz, 1H), 6.9 (d, \( J = 7.6 \) Hz, 2H), 6.21 (d, \( J = 5.9 \) Hz, 1H), 5.25 (d, \( J = 5.7 \) Hz, 1H), 4.28 (t, \( J = 8.2 \) Hz, 1H), 3.21-3.18 (dd, \( J = 6.3, 5.5 \) Hz, 1H), 2.92-2.88 (dd, \( J = 9.2, 4.0 \) Hz, 1H), 2.67-2.63 (dd, \( J = 7.9, 5.1 \) Hz, 1H), 2.3 (s, 3H), 2.29-2.22 (p, \( J = 7.5 \) Hz, 1H), 2.25-2.18 (td, \( J = 12.6, 6.4 \) Hz, 1H), 1.94-1.86 (q, \( J = 13.6 \) Hz, 1H), 1.72-1.68 (dd, \( J = 8.5, 5.7 \) Hz, 1H), 1.54-1.50 (dd, \( J = 9.33, 4.4 \) Hz, 1H), 1.36 (s, 3H), 1.1 (d, \( J = 7.7 \) Hz, 3H)

\(^{13}\)C NMR (CDCl\(_3\)):

210.9, 174.5, 164.0, 151.8, 138.0, 132.1, 129.4, 128.6, 126.9, 121.5, 118.1, 114.6, 86.4, 84.9, 79.7, 70.5, 59.1, 49.3, 41.4, 39.8, 31.4, 29.7, 21.6, 20.2, 18.0

IR (KBr):

3444, 2927, 1775, 1597, 1258, 1022 cm\(^{-1}\)

ESI MS (m/z):

474 (M + 1)

Anal. Calcd for C\(_{29}\)H\(_{31}\)NO\(_5\):

C, 73.55; H, 6.6; N, 2.96. Found: C, 73.49; H, 6.63; N, 2.99.

N-(Phenyl)-C-(2-methyl phenyl)-spiro-isoxazolidinyl parthenin (3g"

Brown colour solid; mp: 121 °C

Specific rotation: \([\alpha]_{D}^{25} -12.4 \) (c 0.6, CHCl\(_3\))

\(^1\)H NMR (500 MHz, CDCl\(_3\)):

7.47-7.43 (m, 5H), 7.18 (t, \( J = 7.6 \) Hz, 2H), 6.99 (t, \( J = 7.5 \) Hz, 1H), 6.9 (d, \( J = 7.6 \) Hz, 2H), 6.21 (d, \( J = 5.9 \) Hz, 1H), 5.25 (d, \( J = 5.7 \) Hz, 1H), 4.28 (t, \( J = 8.2 \) Hz, 1H), 3.21-3.18 (dd, \( J = 6.3, 5.5 \) Hz, 1H), 2.92-2.88 (dd, \( J = 9.2, 4.0 \) Hz, 1H), 2.67-2.63 (dd, \( J = 7.9, 5.1 \) Hz, 1H), 2.3 (s, 3H), 2.29-2.22 (p, \( J = 7.5 \) Hz, 1H), 2.25-2.18 (td, \( J = 12.6, 6.4 \) Hz, 1H), 1.94-1.86 (q, \( J = 13.6 \) Hz, 1H), 1.72-1.68 (dd, \( J = 8.5, 5.7 \) Hz, 1H), 1.54-1.50 (dd, \( J = 9.33, 4.4 \) Hz, 1H), 1.36 (s, 3H), 1.1 (d, \( J = 7.7 \) Hz, 3H)

\(^{13}\)C NMR (CDCl\(_3\)):

210.9, 174.5, 164.0, 151.8, 138.0, 132.1, 129.4, 128.6, 126.9, 121.5, 118.1, 114.6, 86.4, 84.9, 79.7, 70.5, 59.1, 49.3, 41.4, 39.8, 31.4, 29.7, 21.6, 20.2, 18.0

IR (KBr):

3444, 2927, 1775, 1597, 1258, 1022 cm\(^{-1}\)

ESI MS (m/z):

496 (M + 23)

Anal. Calcd for C\(_{29}\)H\(_{31}\)NO\(_5\):

C, 73.55; H, 6.6; N, 2.96. Found: C, 73.48; H, 6.64; N, 3.0.
White colour solid; mp: 138-140 °C
Specific rotation: $[\alpha]_D^{25}$ -21 (c 0.6, CHCl$_3$)
$^1$H NMR (500 MHz, CDCl$_3$): δ 7.49 (d, $J = 5.9$ Hz, 1H), 7.295 (t, $J = 8.1$ Hz, 1H), 7.185 (t, $J = 7.8$ Hz, 2H), 7.11-7.09 (m, 2H), 6.93-6.87 (m, 3H), 6.85 (d, $J = 8.1$ Hz, 1H), 6.27 (d, $J = 5.8$ Hz, 1H), 5.27 (d, $J = 5.6$ Hz, 1H), 5.18-5.15 (t, $J = 7.2$ Hz, 1H), 3.87 (s, 3H), 3.18-3.15 (dd, $J = 6.9$, 5.1 Hz, 1H), 2.95-2.91 (dd, $J = 6.9$, 5.5 Hz, 1H), 2.54-2.49 (dd, $J = 9.2$, 3.2 Hz, 1H), 2.36-2.32 (p, $J = 7.1$ Hz, 1H), 2.3 (s, 3H), 2.2-2.14 (td, $J = 12.7$, 7.1 Hz, 1H), 1.92-1.86 (q, $J = 13.9$ Hz, 1H), 1.73-1.67 (dd, $J = 7.7$, 5.6 Hz, 1H), 1.47-1.43 (dd, $J = 9.1$, 5.1 Hz, 1H), 1.35 (s, 3H), 1.13 (d, $J = 7.6$ Hz, 3H).
$^{13}$C NMR (CDCl$_3$): 211.6, 174.4, 164.3, 151.4, 143.7, 139.3, 131.3, 130.6, 128.6, 122.2, 117.2, 115.4, 114.3, 86.5, 85.1, 79.7, 68.9, 59.1, 55.4, 49.3, 42.8, 40.6, 31.9, 21.4, 20.1, 18.1
IR (KBr): 3451, 2929, 1775, 1755, 1592, 1012 cm$^{-1}$
ESI MS ($m/z$): 474 (M + 1)$^+$
Anal. Calcd for C$_{29}$H$_{31}$NO$_5$: C, 73.55; H, 6.6; N, 2.96. Found: C, 73.48; H, 6.62; N, 2.99.

N-(Phenyl)-C-(2-methyl phenyl)-spiro-isoxazolidinyl parthenin (3g’’)
White colour solid; mp: 130-132 °C
Specific rotation: $[\alpha]_D^{25}$ -15 (c 0.6, CHCl$_3$)
$^1$H NMR (500 MHz, CDCl$_3$): 7.44 (d, $J = 5.9$ Hz, 1H), 7.32-7.26 (m, 2H), 7.21 (s, 1H), 7.09 (t, $J = 7.5$ Hz, 2H), 6.9-6.85 (m, 2H), 6.27 (d, $J = 5.8$ Hz, 1H), 5.16 (d, $J = 5.7$ Hz, 1H), 4.29 (t, $J = 8.4$ Hz, 1H), 3.87 (s, 3H), 3.01-2.97 (dd, $J = 6.8$, 5.5 Hz, 1H), 2.86-2.81(dd, $J = 9.0$, 4.0 Hz, 1H), 2.67-2.63 (dd, $J = 8.0$, 5.0 Hz, 1H), 2.35 (s, 3H), 2.30-2.24 (p, $J = 7.4$ Hz, 1H), 2.13-2.10 (td, $J = 12.6$, 6.4 Hz, 1H), 1.87-1.79 (q, $J = 13.4$ Hz, 1H), 1.73-1.69 (dd, $J = 8.5$, 5.6 Hz, 1H), 1.56-1.52 (dd, $J = 9.2$, 4.6 Hz, 1H), 1.18 (s, 3H),
1.06 (d, $J = 7.7$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$): 211.5, 174.2, 164.3, 151.3, 143.7, 140.0, 131.2, 130.5, 129.0, 122.1, 117.3, 115.2, 114.5, 86.5, 85.1, 79.7, 68.9, 59.1, 55.4, 48.9, 42.6, 40.0, 31.7, 21.3, 20.1, 18.1 Hz.

IR (KBr): 3449, 2960, 2926, 1779, 1757, 1725, 1591, 1495 cm$^{-1}$.

ESI MS (m/z): 496.3 (M + 23)$^+$

Anal. Calcd for C$_{29}$H$_{31}$NO$_5$: C, 73.55; H, 6.6; N, 2.96. Found: C, 73.60; H, 6.58; N, 2.95.

N-(Phenyl)-C-phenyl-spiro-isoxazolidinyl parthenin (3h$'$)

Pale yellow colour solid; mp: 165 °C

Specific rotation: $[\alpha]_D^{25}$ -2.5 (c 0.65, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): 7.47-7.29 (m, 5H), 7.20 (t, $J = 7.7$ Hz, 2H), 6.96-6.88 (m, 3H), 6.86 (d, $J = 8.1$ Hz, 1H), 6.19 (d, $J = 5.9$ Hz, 1H), 5.28 (d, $J = 5.6$ Hz, 1H), 5.22 (t, $J = 8.2$ Hz, 1H), 3.25-3.21 (dd, $J = 6.5$, 5.7 Hz, 1H), 3.01-2.97 (dd, $J = 6.9$, 5.6 Hz, 1H), 2.48-2.45 (dd, $J = 6.8$, 5.6 Hz, 1H), 2.35-2.29 (p, $J = 7.5$ Hz, 1H), 2.24-2.16 (td, $J = 12.2$, 6.4 Hz, 1H), 1.92-1.82 (q, $J = 13.6$ Hz, 1H), 1.72-1.68 (dd, $J = 8.4$, 5.6 Hz, 1H), 1.44-1.4 (dd, $J = 9.3$, 5.0 Hz, 1H), 1.32 (s, 3H), 1.1 (d, $J = 7.7$ Hz, 3H)

$^{13}$C NMR (CDCl$_3$): 211.1, 174.5, 164.0, 151.4, 140.9, 131.7, 128.9, 128.7, 128.3, 126.5, 122.1, 117.5, 114.7, 86.4, 84.5, 79.6, 70.5, 58.9, 49.2, 42.8, 39.9, 31.5, 31.6, 20.1, 17.9 Hz.

IR (KBr): 3410, 2924, 1753, 1597, 1240, 1023 cm$^{-1}$.

ESI MS (m/z): 460 (M + 1)$^+$. Anal. Calcd for C$_{28}$H$_{29}$NO$_5$: C, 73.18; H, 6.36; N, 3.05. Found: C, 73.11; H, 6.40; N, 3.08.

N-(Phenyl)-C-phenyl-spiro-isoxazolidinyl parthenin (3h$''$)

Pale yellow colour solid; mp: 172 °C

Specific rotation: $[\alpha]_D^{25}$ -6.3 (c 0.6, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): 7.47-7.3 (m, 7H), 7.05-7.01 (m, 3H), 6.86 (d, $J = 8.2$ Hz, 1H),
Hz, 1H), 6.20 (d, J = 5.8 Hz, 1H), 5.26 (d, J = 5.6 Hz, 1H), 5.225 (t, J = 8.1 Hz, 1H), 3.24-3.20 (dd, J = 6.8, 5.6 Hz, 1H), 2.5-2.46 (dd, J = 6.8, 5.4 Hz, 1H), 2.35-2.29 (p, J = 7.4 Hz, 1H), 2.25-2.17 (td, J = 12.6, 6.3 Hz, 1H), 1.92-1.82 (q, J = 13.5 Hz, 1H), 1.74-1.7 (dd, J = 8.1, 5.7 Hz, 1H), 1.45-1.41 (dd, J = 9.2, 5.1 Hz, 1H), 1.33 (s, 3H), 1.1 (d, J = 7.7 Hz, 3H).

$^{13}$C NMR (CDCl$_3$):

211.0, 174.3, 164.1, 151.1, 140.5, 131.3, 129.0, 128.9, 128.2, 125.9, 122.1, 118.1, 114.8, 86.4, 84.3, 79.5, 70.2, 58.5, 49.2, 41.4, 40.2, 31.3, 21.3, 20.2, 18.1

IR (KBr):

3415, 2924, 1754, 1600, 1488, 1024 cm$^{-1}$

ESI MS (m/z):

460 (M + 1)$^+$

Anal. Calcd for C$_{28}$H$_{29}$NO$_5$: C, 73.18; H, 6.36; N, 3.05. Found: C, 73.10; H, 6.4; N, 3.08.

N-(Phenyl)-C-(2,6-dichloro phenyl)-spiro-isoxazolidinyl parthenin (3i')

White colour solid; mp: 153-154 °C

Specific rotation: $[\alpha]_D^{25}$ -23 (c 0.5, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):

7.39 (d, J = 5.9 Hz, 1H), 7.31 (d, J = 8.0 Hz, 2H), 7.13 (t, J = 7.9 Hz, 1H), 7.085 (t, J = 7.9 Hz, 2H), 6.78 (d, J = 8.3 Hz, 2H), 6.19 (d, J = 5.8 Hz, 1H), 5.41-5.37 (dd, J = 7.3, 3.8 Hz, 1H), 5.16 (d, J = 5.8 Hz, 1H), 3.32-3.28 (t, J = 11.7 Hz, 1H), 3.08-3.04 (dd, J = 6.8, 5.7, 1H), 2.72-2.69 (dd, J = 7.3, 5.5 Hz, 1H), 2.22-2.19 (p, J = 7.6 Hz, 1H), 1.93-1.76 (td, J = 12.0, 5.2 Hz, 1H), 1.75-1.71 (q, J = 13.7 Hz, 1H), 1.6-1.55 (dd, J = 8.4, 5.9 Hz, 1H), 1.46-1.41 (dd, J = 9.2, 5.1 Hz, 1H), 1.28 (s, 3H), 1.03 (d, J = 7.8 Hz, 3H)

$^{13}$C NMR (CDCl$_3$):

211.7, 174.3, 164.1, 151.2, 135.7, 132.9, 132.3, 129.9, 129.2, 122.0, 118.0, 114.2, 113.5, 88.3, 86.6, 84.8, 66.4, 59.1, 48.5, 40.3, 37.0, 32.0, 30.1, 25.1, 20.1 18.3
IR (KBr): 3439, 2929, 1781, 1722, 1596, 1489, 1351, 1245,
1126, 754, 694 cm⁻¹
ESI MS (m/z): 528.6 (M + 1)⁺
Anal. Calcd for C_{28}H_{27}Cl_{2}NO₅: C, 63.64; H, 5.15; N, 2.65. Found: C, 63.58; H, 5.19; N, 2.68.

N-(Phenyl)-C-(2,6-dichloro phenyl)-spiro-isoxazolidinyl parthenin (3i’’)
White colour solid; mp: 159-160 °C
Specific rotation: [α]_D^{25} -13.5 (c 0.55, CHCl₃)
¹H NMR (500 MHz, CDCl₃): 7.51 (d, J = 5.9, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.205 (t, J = 8.1 Hz, 2H), 6.855 (t, J = 7.0 Hz, 2H), 6.82 (d, J = 8.2 Hz, 2H), 6.29 (d, J = 5.8 Hz, 1H), 6.055 (t, J = 8.6 Hz, 1H), 5.33 (d, J = 5.5 Hz, 1H), 3.32-3.27 (dd, J = 6.8, 5.3 Hz, 1H), 2.88-2.84 (m, 2H), 2.40-2.35 (p, J = 7.1 Hz, 1H), 2.3-2.22 (td, J = 12.8, 6.6 Hz, 1H), 1.97-1.89 (q, J = 13.5 Hz, 1H), 1.73-1.69 (dd, J = 5.9 Hz, 8.6, 1H), 1.53-1.49 (dd, J = 9.2, 4.6 Hz, 1H), 1.25 (s, 3H), 1.1 (d, J = 7.8 Hz, 3H)
¹³C NMR (CDCl₃): 211.2, 174.7, 164.9, 151.4, 135.8, 133.0, 132.3, 129.5, 129.4, 121.9, 118.1, 114.9, 114.4, 88.6, 86.8, 85.1, 66.6, 59.1, 48.5, 40.3, 36.9, 31.9, 29.8, 24.5, 20.3, 18.2
IR (KBr): 3425, 2938, 1757, 1727, 1597, 1467, 1345, 1235, 1127, 758, 698 cm⁻¹
ESI MS (m/z): 550 (M + 23)⁺
Anal. Calcd for C_{28}H_{27}Cl_{2}NO₅: C, 63.64; H, 5.15; N, 2.65. Found: C, 63.57; H, 5.19; N, 2.68.

N-(Phenyl)-C-(2,6-difluoro phenyl)-spiro-isoxazolidinyl parthenin (3j’’)
White colour solid; mp: 173-175 °C
Specific rotation: [α]_D^{25} -11.7 (c 0.9, CHCl₃)
¹H NMR (500 MHz, CDCl₃): 7.46 (d, J = 5.9 Hz, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.14 (t, J = 7.9 Hz, 1H), 7.08 (t, J = 7.9 Hz, 2H), 6.75 (d, J = 8.2 Hz, 2H), 6.21 (d, J = 5.9 Hz, 1H), 5.40-5.36 (dd, J = 7.3, 3.9 Hz, 1H), 5.21 (d, J =
5.8 Hz, 1H), 3.3 (t, J = 11.5 Hz, 1H), 3.09-3.05 (dd, J = 6.9, 5.7 Hz, 1H), 2.73-2.69 (dd, J = 7.2, 5.6 Hz, 1H), 2.23-2.19 (p, J = 7.5 Hz, 1H), 1.95-1.78 (td, J = 12.2, 5.7 Hz, 1H), 1.76-1.72 (q, J = 13.6 Hz, 1H), 1.61-1.56 (dd, J = 8.5, 5.9 Hz, 1H), 1.46-1.41 (dd, J = 9.2, 5.1 Hz, 1H), 1.3 (s, 3H), 1.09 (d, J = 7.8 Hz, 3H).

\(^{13}\text{C NMR (CDCl}_3\):} 211.4, 175.2, 164.8, 151.5, 135.7, 133.0, 132.4, 130.1, 129.1, 122.1, 118.3, 114.9, 114.0, 88.3, 87.1, 84.6, 66.1, 59.7, 49.2, 40.2, 37.0, 32.1, 30.2, 24.7, 20.1, 18.1

IR (KBr): 3442, 2927, 1781, 1723, 1596, 1490, 1350, 1246, 1128, 755, 695 cm\(^{-1}\)

ESI MS (m/z): 518 (M + 23)

Anal. Calcd for C\(_{28}\)H\(_{27}\)F\(_2\)NO\(_5\): C, 67.87; H, 5.49; N, 2.83. Found: C, 67.80; H, 5.53; N, 2.86

N-(Phenyl)-C-(2,6-difluoro phenyl)-spiro-isoxazolidinyl parthenin (3j’’)

White colour solid; mp: 181-182 °C

Specific rotation: \([\alpha]_D^{25} -33\) (c 0.65, CHCl\(_3\))

\(^1\text{H NMR (500 MHz, CDCl}_3\):} \delta 7.5 (d, J = 5.9 Hz, 1H), 7.37 (d, J = 8.1 Hz, 2H), 7.16 (t, J = 8.1 Hz, 2H), 6.82 (t, J = 7.3 Hz, 2H), 6.76 (d, J = 8.2 Hz, 2H), 6.26 (d, J = 5.9 Hz, 1H), 6.025(t, J = 8.5 Hz, 1H), 5.31 (d, J = 5.7 Hz, 1H), 3.31-3.26 (dd, J = 6.9, 5.4 Hz, 1H), 2.89-2.85 (m, 2H), 2.40-2.35 (p, J = 7.2 Hz, 1H), 2.31-2.23 (td, J = 12.2, 6.5 Hz, 1H), 1.98-1.9 (q, J = 13.4 Hz, 1H), 1.75-1.71 (dd, J = 8.5, 5.9 Hz, 1H), 1.54-1.5 (dd, J = 9.2, 4.6 Hz, 1H), 1.28 (s, 3H), 1.1 (d, J = 7.7 Hz, 3H).

\(^{13}\text{C NMR (CDCl}_3\):} 210.5, 175.1, 165.2, 150.9, 135.3, 132.7, 132.7, 129.7, 129.3, 122.5, 119.0, 115.3, 114.0, 88.6, 87.0, 84.4, 66.0, 59.4, 48.9, 40.3, 37.1, 32.2, 30.1, 24.7, 20.4, 18.0
IR (KBr): 3428, 2935, 1758, 1726, 1599, 1467, 1348, 1235, 1129, 759, 698 cm⁻¹
ESI MS (m/z): 496 (M + 1)⁺
Anal. Calcd for C₂₈H₂₇F₂NO₅: C, 67.87; H, 5.49; N, 2.83. Found: C, 67.79; H, 5.53; N, 2.86

N-(Phenyl)-C-(3-chloro phenyl)-spiro-isoxazolidinyl parthenin (3k)
White colour solid; mp: 160 °C
Specific rotation: [α]D²⁵ -5.4 (c 0.9, CHCl₃)
¹H NMR (500 MHz, CDCl₃): δ 7.55 (s, 1H), 7.49 (d, J = 5.9 Hz, 1H), 7.36-7.16 (m, 5H), 6.95 (t, J = 7.7 Hz, 1H), 6.86 (d, J = 7.7 Hz, 2H), 6.27 (d, J = 5.9 Hz, 1H), 5.27 (d, J = 5.6 Hz, 1H), 5.17 (t, J = 8.0 Hz, 1H), 3.21-3.17 (dd, J = 6.9, 5.2 Hz, 1H), 3.0-2.94 (dd, J = 6.7, 5.6 Hz, 1H), 2.51-2.47 (dd, J = 9.0, 3.4 Hz, 1H), 2.36-2.32 (p, J = 7.1 Hz, 1H), 2.21-2.14 (td, J = 12.7, 6.8 Hz, 1H), 1.95-1.87 (q, J = 13.1 Hz, 1H), 1.71-1.67 (dd, J = 8.7, 5.6 Hz, 1H), 1.49-1.45 (dd, J = 9.0, 5.1 Hz, 1H), 1.34 (s, 3H), 1.11 (d, J = 7.7 Hz, 3H).
¹³C NMR (CDCl₃): 211.0, 174.327, 164.1, 151.5, 143.9, 139.5, 130.8, 130.5, 128.5, 122.6, 118.2, 115.2, 114.3, 86.4, 84.6, 79.5, 69.6, 59.3, 49.2, 43.1, 40.3, 31.5, 21.4, 20.3, 18.4
IR (KBr): 3451, 2964, 2927, 1777, 1755, 1725, 1592, 1491, 1012, 801, 756, 716 cm⁻¹
ESI MS (m/z): 494.4 (M + 1)⁺
Anal. Calcd for C₂₉H₂₈ClNO₅: C, 68.08; H, 5.71; N, 2.84. Found: C, 67.99; H, 5.73; N, 2.86.

N-(Phenyl)-C-(3-chloro phenyl)-spiro-isoxazolidinyl parthenin (3k’)
White colour solid; mp: 165 °C
Specific rotation: [α]D²⁵ -12.6 (c 0.75, CHCl₃)
¹H NMR (500 MHz, CDCl₃): δ 7.52 (s, 1H), 7.48 (d, J = 5.7 Hz, 1H), 7.35-7.29 (m, 2H), 7.09 (t, J = 7.6 Hz, 2H), 6.93-6.88 (m, 2H), 6.85 (d, J = 7.6 Hz, 1H), 6.27 (d, J = 5.7 Hz, 1H),
N-(Phenyl)-C-(3-fluoro phenyl)-spiro-isoxazolidinyl parthenin (31')

White colour solid; mp: 148 °C

Specific rotation: \([\alpha]_D^{25} -18\) (c 0.9, CHCl₃)

\(^1\)H NMR (500 MHz, CDCl₃):

\[\delta 7.49\ (d, J = 5.9\ Hz, 1H),\ 7.36-7.30\ (m, 2H),\ 7.28\ (s, 1H),\ 7.2\ (t, J = 7.59\ Hz, 2H),\ 7.0\ (t, J = 7.6\ Hz, 1H),\ 6.93\ (t, J = 7.3\ Hz, 1H),\ 6.88\ (d, J = 7.94\ Hz, 2H),\ 6.27\ (d, J = 5.8\ Hz, 1H),\ 5.26\ (d, J = 5.5\ Hz, 1H),\ 5.19\ (t, J = 8.2\ Hz, 1H),\ 3.18-3.14\ (dd, J = 6.9, 5.4 Hz, 1H),\ 2.96-2.92\ (dd, J = 7.0, 5.4 Hz, 1H),\ 2.53-2.49\ (dd, J = 9.1, 3.3 Hz, 1H),\ 2.36-2.32\ (p, J = 7.0\ Hz, 1H),\ 2.22-2.15\ (td, J = 12.2, 6.9 Hz, 1H),\ 1.95-1.87\ (q, J = 13.1\ Hz, 1H),\ 1.72-1.68\ (dd, J = 8.9, 5.5 Hz, 1H),\ 1.48-1.44\ (dd, J = 9.1, 5.1 Hz, 1H),\ 1.35\ (s, 3H),\ 1.11\ (d, J = 7.8\ Hz, 3H)\]

\(^{13}\)C NMR (CDCl₃):

\[210.7,\ 174.2,\ 163.6,\ 151.2,\ 143.8,\ 139.3,\ 130.6,\ 130.5,\ 128.8,\ 122.2,\ 117.5,\ 114.7,\ 113.3,\ 86.5,\ 84.7,\ 79.5,\ 69.9,\ 59.0,\ 49.3,\ 42.7,\ 40.1,\ 31.5,\ 21.5,\ 20.3,\ 18.3\]
IR (KBr): 3451, 2965, 2929, 1775, 1720, 1592, 1490, 1012, 800, 755, 716 cm\(^{-1}\)
ESI MS (\(m/z\)): 500 (M + 23)\(^{+}\)
Anal. Calcd for C\(_{28}\)H\(_{28}\)FNO\(_5\): C, 70.43; H, 5.91; N, 2.98. Found: C, 70.38; H, 5.88; N, 3.02.

N-(Phenyl)-C-(3-fluoro phenyl)-spiro-isoxazolidinyl parthenin (3l"")(3l"")
White colour solid; mp: 154 °C
Specific rotation: \([\alpha]_{D}^{25}\) -13.5 (c 0.9, CHCl\(_3\))
\(^1\)H NMR (500 MHz, CDCl\(_3\)): 7.44 (d, \(J = 5.9\) Hz, 1H), 7.32-7.26 (m, 2H), 7.21 (s, 1H), 7.09 (t, \(J = 7.5\) Hz, 2H), 6.9-6.85 (m, 2H), 6.84 (d, \(J = 7.7\) Hz, 1H), 6.27 (d, \(J = 5.8\) Hz, 1H), 5.16 (d, \(J = 5.7\) Hz, 1H), 4.925 (t, \(J = 8.4\) Hz, 1H), 3.01-2.97 (dd, \(J = 6.8, 5.5\) Hz, 1H), 2.86-2.81 (dd, \(J = 9.0, 4.0\) Hz, 1H), 2.67-2.63 (dd, \(J = 8.0, 5.0\) Hz, 1H), 2.30-2.24 (p, \(J = 7.4\) Hz, 1H), 2.13-2.10 (td, \(J = 6.4\) Hz, 1H), 1.87-1.79 (q, \(J = 13.4\) Hz, 1H), 1.73-1.69 (dd, \(J = 5.6, 8.5\) Hz, 1H), 1.56-1.52 (dd, \(J = 9.2, 4.63\) Hz, 1H), 1.18 (s, 3H), 1.06 (d, \(J = 7.7\) Hz, 3H).
\(^{13}\)C NMR (CDCl\(_3\)): 211.5, 174.9, 163.7, 152.3, 143.7, 140.3, 130.5, 129.6, 128.2, 123.3, 118.6, 114.3, 113.4, 87.1, 84.6, 79.5, 70.2, 59.0, 49.5, 43.0, 40.5, 31.6, 21.4, 20.5, 18.5
IR (KBr): 3453, 2961, 2926, 1779, 1757, 1723, 1596, 1494, 1012, 803, 754, 715 cm\(^{-1}\)
ESI MS (\(m/z\)): 500 (M + 23)\(^{+}\)
Anal. Calcd for C\(_{28}\)H\(_{28}\)FNO\(_5\): C, 70.43; H, 5.91; N, 2.98. Found: C, 70.49; H, 5.89; N, 3.01.

N-(Phenyl)-C-(3-bromo, 4-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3m"')(3m"')
Yellow colour solid; mp: 168 °C
Specific rotation: \([\alpha]_{D}^{25}\) -5 (c 1, CHCl\(_3\))
\(^1\)H NMR (500 MHz, CDCl\(_3\)): 7.73 (s, 1H), 7.44 (d, \(J = 5.9\) Hz, 1H), 7.23-7.15 (m, 2H), 6.97-6.84 (m, 4H), 6.18 (d, \(J = 5.9\) Hz, 1H), 5.25 (d, \(J = 5.6\) Hz, 1H), 5.10 (t, \(J = 8.0\) Hz, 1H), 4.925 (t, \(J = 8.0\) Hz, 1H), 3.925 (t, \(J = 8.0\) Hz, 1H), 3.01-2.97 (dd, \(J = 6.8, 5.5\) Hz, 1H), 2.86-2.81 (dd, \(J = 9.0, 4.0\) Hz, 1H), 2.67-2.63 (dd, \(J = 8.0, 5.0\) Hz, 1H), 2.30-2.24 (p, \(J = 7.4\) Hz, 1H), 2.13-2.10 (td, \(J = 6.4\) Hz, 1H), 1.87-1.79 (q, \(J = 13.4\) Hz, 1H), 1.73-1.69 (dd, \(J = 5.6, 8.5\) Hz, 1H), 1.56-1.52 (dd, \(J = 9.2, 4.63\) Hz, 1H), 1.18 (s, 3H), 1.06 (d, \(J = 7.7\) Hz, 3H).
\(^{13}\)C NMR (CDCl\(_3\)): 211.5, 174.9, 163.7, 152.3, 143.7, 140.3, 130.5, 129.6, 128.2, 123.3, 118.6, 114.3, 113.4, 87.1, 84.6, 79.5, 70.2, 59.0, 49.5, 43.0, 40.5, 31.6, 21.4, 20.5, 18.5
IR (KBr): 3453, 2961, 2926, 1779, 1757, 1723, 1596, 1494, 1012, 803, 754, 715 cm\(^{-1}\)
ESI MS (\(m/z\)): 500 (M + 23)\(^{+}\)
Anal. Calcd for C\(_{28}\)H\(_{28}\)FNO\(_5\): C, 70.43; H, 5.91; N, 2.98. Found: C, 70.49; H, 5.89; N, 3.01.
3.89 (s, 3H), 3.18-3.14 (dd, J = 6.5, 5.5 Hz, 1H),
2.9-2.8 (m, 2H), 2.5-2.45 (dd, J = 9.1, 3.3 Hz, 1H),
2.36-2.3 (p, J = 7.4 Hz, 1H), 2.24-2.17 (td, J = 12.6,
6.4 Hz, 1H), 1.94-1.85 (q, J = 13.3 Hz, 1H),
1.74-1.63 (m, 2H), 1.34 (s, 3H),
1.1 (d, J =7.7 Hz, 3H).

$^{13}$C NMR (CDCl$_3$):
211.2, 174.4, 163.9, 155.5, 151.2, 134.3, 131.8,
131.3, 128.8, 127.0, 122.5, 117.7, 115.0, 112.2,
86.5, 84.6, 79.7, 69.7, 59.0, 56.4, 49.3, 42.9,
40.1, 31.6, 21.7, 20.2, 18.0

IR (KBr):
3445, 2960, 2926, 1774, 1754, 1721, 1596, 1486,
1012, 801, 756, 713 cm$^{-1}$

ESI MS (m/z):
569 (M + 1)$^+$

Anal. Calcd for C$_{29}$H$_{30}$BrNO$_6$: C, 61.27; H, 5.32; N, 2.46. Found: C, 61.23; H, 5.36;
N, 2.49.

**N-(Phenyl)-C-(3-bromo, 4-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3m)**

Yellow colour solid; mp: 168 °C
Specific rotation: $[\alpha]_D^{25}$-2.4 (c 0.9, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):
δ 7.72 (s, 1H), 7.45-7.3 (m, 3H), 7.03-6.9 (m, 4H),
6.19 (d, J = 5.9 Hz, 1H), 5.24 (d, J = 5.6 Hz, 1H),
5.25 (t, J = 8.1 Hz, 1H), 3.89 (s, 3H), 3.19-3.15
(dd, J = 6.46, 5.5 Hz, 1H), 2.88-2.81 (m, 2H),
2.48-2.44 (dd, J = 9.1, 3.4 Hz, 1H), 2.35-2.3
(p, J = 7.3 Hz, 1H), 2.25-2.18 (td, J = 12.6, 6.4 Hz,
1H), 1.94-1.85 (q, J = 13.2 Hz, 1H), 1.75-1.65
(m, 2H), 1.33 (s, 3H), 1.1 (d, J = 7.6 Hz, 3H).

$^{13}$C NMR (CDCl$_3$):
211.1, 174.1, 164.0, 155, 151.4, 133.9, 131.8, 131.2,
128.5, 127.1, 122.4, 118.3, 114.8, 112.9, 86.5, 84.59,
79.36, 68.99, 59.11, 56.14, 49.3, 41.32, 40.36, 30.46,
21.09, 20.12, 18.12

IR (KBr):
3449, 2959, 2925, 1775, 1755, 1722, 1597, 1485,
1012, 801, 755, 715 cm$^{-1}$

ESI MS (m/z):
(M + 1)$^+$ 569
Anal. Calcd for C\textsubscript{29}H\textsubscript{30}BrNO\textsubscript{6}: C, 61.27; H, 5.32; N, 2.46. Found: C, 61.20; H, 5.36; N, 2.48

**N-(Phenyl)-C-(2-chloro phenyl)-spiro-isoxazolidinyl parthenin (3n')**

Pale yellow colour solid; mp: 165 °C
Specific rotation: \([\alpha]_D^{25} -10.8 \text{ (c 0.65, CHCl}_3\text{)}

\(^1\)H NMR (500 MHz, CDCl\textsubscript{3}):
\(\delta 7.73 \text{ (d, } J = 7.6, 1\text{H}), 7.49 \text{ (d, } J = 5.9, 1\text{H}), 7.37 \text{ (d, } J = 7.8, 1\text{H}), 7.24 \text{ (t, } J = 7.5, 1\text{H}), 7.19 \text{ (t, } J = 7.5, 1\text{H}), 7.16 \text{ (t, } J = 7.9, 2\text{H}), 6.93 \text{ (t, } J = 7.7, 1\text{H}), 6.88 \text{ (d, } J = 7.8, 2\text{H}), 6.26 \text{ (d, } J = 5.9, 1\text{H}), 5.25 \text{ (d, } J = 5.5, 1\text{H}), 4.93 \text{ (t, } J = 8.2, 1\text{H}), 3.07-3.03 \text{ (dd, } J = 9.1, 4.9, 2\text{H}), 2.58-2.54 \text{ (dd, } J = 7.3, 5.6, 1\text{H}), 2.4-2.34 \text{ (p, } J = 7.3, 1\text{H}), 2.2-2.15 \text{ (td, } J = 12.6, 6.5, 1\text{H}), 1.96-1.87 \text{ (q, } J = 13.5, 1\text{H}), 1.75-1.70 \text{ (dd, } J = 8.4, 5.7, 1\text{H}), 1.64-1.6 \text{ (dd, } J = 9.2, 5.1, 1\text{H}), 1.3 \text{ (s, 3H), 1.12 \text{ (d, } J = 7.8, 3\text{H)}}\)

\(^{13}\)C NMR (CDCl\textsubscript{3}):
209.0, 176.5, 165.9, 160.0, 142.7, 133.0, 128.9, 127.6, 127.4, 126.9, 126.8, 126.7, 121.1, 115.0, 83.3, 80.4, 76.9, 68.1, 61.9, 47.3, 38.0, 33.9, 29.2, 18.7, 16.8, 15.2

IR (KBr):
3410, 2924, 2871, 1753, 1726, 1597, 1487, 1201, 974, 754, 718 cm\(^{-1}\)

ESI MS (m/z):
494.3 (M + 1)\(^+\)

Anal. Calcd for C\textsubscript{29}H\textsubscript{28}ClNO\textsubscript{5}: C, 68.08; H, 5.71; N, 2.84. Found: C, 68.0; H, 5.75; N, 2.88

**N-(Phenyl)-C-(2-chloro phenyl)-spiro-isoxazolidinyl parthenin (3n'')**

Colourless solid; mp: 171 °C
Specific rotation: \([\alpha]_D^{25} -11.4 \text{ (c 0.6, CHCl}_3\text{)}

\(^1\)H NMR (500 MHz, CDCl\textsubscript{3}):
\(\delta 7.81 \text{ (d, } J = 7.6, 1\text{H}), 7.48 \text{ (d, } J = 5.9, 1\text{H}), 7.45 \text{ (d, } J = 7.8, 1\text{H}), 7.27-7.23 \text{ (m, 3H), 6.91 \text{ (t, } J = 7.7, 1\text{H}), 6.88 \text{ (d, } J = 7.9, 2\text{H}), 6.25 \text{ (d, } J = 5.9, 1\text{H)}, 5.59 \text{ (t, } J = 7.8, 1\text{H}), 5.27 \text{ (d, } J = 5.7, 1\text{H}), 3.25-3.21 \text{ (dd, } J = 7.5, 5.3, 1\text{H)}, 3.16-3.12 \text{ (dd, } J = 6.8, 5.4, 1\text{H)}, 2.88-2.86 \text{ (dd, } J = 7.7, 6.3, 1\text{H), 1.88-1.84 \text{ (dd, } J = 11.4, 7.8, 1\text{H)}, 1.71-1.66 \text{ (dd, } J = 12.7, 6.8, 1\text{H), 1.64-1.53 \text{ (dd, } J = 12.7, 7.9, 1\text{H)}, 1.12 \text{ (d, } J = 7.7, 3\text{H}, 1.08 \text{ (dd, } J = 12.5, 5.9, 1\text{H)})}

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2.38-2.37 (dd, \( J = 7.9, 4.8, 1H \)), 2.36-2.30
(p, \( J = 7.5, 1H \)), 2.16-2.11 (td, \( J = 12.7, 6.7, 1H \)),
1.92-1.82 (q, \( J = 13.4, 1H \)), 1.72-1.69 (dd, \( J = 8.5, 5.7, 1H \)), 1.48-1.45 (dd, \( J = 9.2, 5.1, 1H \)), 1.3 (s, 3H),
1.09 (d, \( J = 7.5, 3H \))

\(^{13}\)C NMR (CDCl\(_3\)):
210.7, 174.3, 164.0, 151.1, 139.2, 132.1, 130.9, 129.8,
128.7, 128.5, 127.4, 127.2, 122.0, 116.3, 114.1, 87.1,
84.0, 80.5, 67.6, 65.9, 53.1, 49.7, 40.4, 32.1, 22.0,
20.1, 18.2

IR (KBr):
3441, 2932, 2870, 1776, 1718, 1598, 1526, 1349,
975, 756, 695 cm\(^{-1}\)

ESI MS (m/z):
494 (M + 1)

Anal. Calcd for C\(_{28}\)H\(_{28}\)ClNO\(_5\):
C, 68.08; H, 5.71; N, 2.84. Found: C, 67.81; H, 5.74;
N, 2.86.

N-(Phenyl)-C-(2-fluoro phenyl)-spiro-isoxazolidinyl parthenin (3o)
Pale yellow colour solid; mp: 160 °C
Specific rotation: \([\alpha]_D^{25} -10 \text{ (c 0.65, CHCl}_3\));

\(^1\)H NMR (500 MHz, CDCl\(_3\)):
\(\delta\) 7.75 (d, \( J = 7.9, 1H \)), 7.48 (d, \( J = 5.8, 1H \)),
7.41 (d, \( J = 7.8, 1H \)), 7.285 (t, \( J = 7.6, 1H \)),
7.23 (t, \( J = 7.4, 1H \)), 7.18 (t, \( J = 7.8, 2H \)),
6.94 (t, \( J = 7.7, 1H \)), 6.9 (d, \( J = 7.5, 2H \)),
6.25 (d, \( J = 5.9, 1H \)), 5.25 (d, \( J = 5.5, 1H \)), 4.98
(t, \( J = 8.1, 1H \)), 3.1-3.07 (dd, \( J = 9.0, 4.8, 2H \)),
2.6-2.57 (dd, \( J = 7.7, 5.5, 1H \)), 2.5-2.42
(p, \( J = 7.4, 1H \)), 2.21-2.16 (td, \( J = 12.3, 6.5, 1H \)),
1.96-1.87 (q, \( J = 13.5, 1H \)), 1.76-1.72
(dd, \( J = 8.1, 5.5, 1H \)), 1.65-1.61 (dd, \( J = 9.1, 5.3, 1H \)),
1.3 (s, 3H), 1.12 (d, \( J = 7.7, 3H \)).

\(^{13}\)C NMR (CDCl\(_3\)):
210.2, 176.2, 165.3, 160.1, 142.4, 133.0, 128.9, 127.7,
127.3, 127.0, 126.8, 126.7, 121.9, 115.3, 84.5, 81.3,
76.9, 68.8, 62.9, 47.3, 38.0, 34.0, 29.1, 18.5, 17.5, 15.5

IR (KBr):
3412, 2926, 2875, 1755, 1730, 1597, 1488, 1201,
979, 755, 721 cm\(^{-1}\)
ESI MS (m/z): 500 (M + 1)^+
Anal. Calcd for C_{28}H_{28}FNO_{5}: C, 70.43; H, 5.91; N, 2.98. Found: C, 70.37; H, 5.95; N, 3.01.

**N-(Phenyl)-C-(2-fluoro phenyl)-spiro-isoxazolidinyl parthenin (3o’’)**

Colourless solid; mp: 153 °C
Specific rotation: [α]_D^{25} -14 (c 0.66, CHCl_3)

$^1$H NMR (500 MHz, CDCl_3): δ 7.81 (d, $J = 7.7$, 1H), 7.48 (d, $J = 5.9$, 1H), 7.46 (d, $J = 7.9$, 1H), 7.28-7.24 (m, 3H), 6.94 (t, $J = 7.8$, 1H), 6.86 (d, $J = 7.7$, 2H), 6.26 (d, $J = 5.9$, 1H), 5.59 (t, $J = 7.6$, 1H), 5.27 (d, $J = 5.6$, 1H), 3.25-3.21 (dd, $J = 7.5$, 5.2, 1H), 3.18-3.15 (dd, $J = 6.9$, 5.4, 1H), 2.41-2.37 (dd, $J = 7.9$, 4.8, 1H), 2.35-2.30 (p, $J = 7.5$, 1H), 2.15-2.10 (td, $J = 12.4$, 6.7, 1H), 1.91-1.85 (q, $J = 13.2$, 1H), 1.70-1.67 (dd, $J = 8.2$, 5.78, 1H), 1.51-1.48 (dd, $J = 9.1$, 5.1, 1H), 1.3 (s, 3H), 1.1 (d, $J = 7.5$, 3H).

$^{13}$C NMR (CDCl_3): 211.2, 174.1, 163.1, 151.2, 138.9, 132.5, 131.4, 129.9, 129.3, 128.9, 127.9, 127.6, 121.5, 116.8, 114.0, 87.1, 84.5, 81.3, 68.7, 59.4, 53.6, 49.6, 39.7, 31.3, 22.1, 20.2, 17.9

IR (KBr): 3438, 2928, 2872, 1778, 1720, 1525, 1351, 976 cm$^{-1}$.

ESI MS (m/z): 478 (M + 1)^+
Anal. Calcd for C_{28}H_{28}FNO_{5}: C, 70.43; H, 5.91; N, 2.98. Found: C, 70.38; H, 5.93; N, 3.00.

**N-(Phenyl)-C-(2,3,4,5,6-penta fluoro phenyl)-spiro-isoxazolidinyl parthenin (3p’)**

White colour solid; mp: 145 °C
Specific rotation: [α]_D^{25} -9 (c 0.55, CHCl_3)

$^1$H NMR (500 MHz, CDCl_3): 7.45 (d, $J = 5.9$ Hz, 1H), 7.14 (t, $J = 7.5$ Hz, 2H), 6.9 (t, $J = 7.3$ Hz, 1H), 6.81 (d, $J = 7.7$ Hz, 2H), 6.21 (d, $J = 5.9$ Hz, 1H), 5.51 (t, $J = 8.4$ Hz, 1H), 5.23 (d, $J = 5.5$ Hz, 1H), 3.25-3.21 (dd, $J = 6.5$, 5.7 Hz, 1H), 2.8-2.76 (dd, $J = 6.7$, 5.6 Hz, 1H), 2.65-2.6
(dd, J = 9.1, 3.6 Hz, 1H), 2.26-2.23 (p, J = 7.2 Hz, 1H),
2.22-2.15 (td, J = 12.7, 6.4 Hz, 1H), 1.90-1.75
(q, J = 13.5 Hz, 1H), 1.69-1.65 (dd, J = 8.5, 5.7
Hz, 1H), 1.46-1.42 (dd, J = 9.2, 5.0 Hz, 1H),
1.34 (s, 3H), 1.1 (d, J = 7.7 Hz, 3H).

$^{13}$C NMR (CDCl$_3$):
211.6, 174.5, 164.4, 151.2, 141.1, 132.9, 131.6, 129.3,
128.7, 124.4, 122.7, 119.4, 86.1, 84.9, 79.6, 69.4, 59.0,
49.5, 42.7, 39.9, 31.4, 21.7, 20.4 17.3

IR (KBr):
3439, 2924, 1595, 1384, 1020 cm$^{-1}$

ESI MS (m/z):
550 (M + 1)$^+$

Anal. Calcd for C$_{28}$H$_{24}$F$_5$NO$_5$:
C, 61.20; H, 4.4; N, 2.55. Found: C, 61.11; H, 4.44;
N, 2.58.

N-(Phenyl)-C-(3-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3p’)

White colour solid; mp: 149 °C
Specific rotation: $[\alpha]_D^{25}$ -3 (c 0.5, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):
7.42 (d, J = 5.9 Hz, 1H), 7.14 (t, J = 7.5 Hz, 2H),
6.92 (t, J = 7.4 Hz, 1H), 6.88 (d, J = 7.7 Hz, 2H),
6.16 (d, J = 5.9 Hz, 1H), 5.11 (d, J = 5.5 Hz, 1H),
4.88 (t, J = 8.5 Hz, 1H), 3.07-3.0 (m, 2H), 2.82-2.78
(dd, J = 8.2, 4.7 Hz, 1H), 2.26-2.23 (p, J = 7.3 Hz,
1H), 2.05-1.98 (td, J = 12.9, 7.2 Hz, 1H), 1.91-1.83
(q, J = 13.5 Hz, 1H), 1.70-1.65 (dd, J = 8.4, 5.7
Hz, 1H), 1.47-1.42 (dd, J = 9.2, 4.5 Hz, 1H),
1.23 (s, 3H), 1.04 (d, J = 7.8 Hz, 3H).

$^{13}$C NMR (CDCl$_3$):
211.5, 174.4, 164.4, 151.2, 141.4, 132.8, 131.9, 129.3,
128.5, 124.3, 122.7, 119.4, 86.1, 85.2, 79.8, 69.3,
59.1, 49.6, 42.8, 39.8, 31.3, 22.1, 20.5 17.2

IR (KBr):
3440, 2925, 1591, 1381, 1018 cm$^{-1}$

ESI MS (m/z):
550 (M + 1)$^+$

Anal. Calcd for C$_{28}$H$_{24}$F$_5$NO$_5$:
C, 61.20; H, 4.4; N, 2.55. Found: C, 61.11; H, 4.44;
N, 2.57

N-(Phenyl)-C-(3-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3q’)

Pale yellow colour solid; mp: 181-183 °C

150
Specific rotation: $[{\alpha}]_D^{25} -8.8$ (c 0.45, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):

- $\delta$ 7.72 (d, $J = 7.7$ Hz, 1H), 7.5 (d, $J = 5.8$ Hz, 1H), 7.27-7.10 (m, 4H), 6.94-6.82 (m, 3H), 6.28 (d, $J = 5.9$ Hz, 1H), 5.32 (t, $J = 8.3$ Hz, 1H), 5.27 (d, $J = 5.6$ Hz, 1H), 3.75 (s, 3H), 3.24-3.20 (dd, $J = 6.5$, 5.6 Hz, 1H), 3.04-2.84 (m, 3H), 2.42-2.36 (p, $J = 7.4$ Hz, 1H), 2.34 (s, 3H), 2.25-2.18 (td, $J = 12.4, 6.4$ Hz, 1H), 1.96-1.87 (q, $J = 13.5$ Hz, 1H), 1.54-1.5 (dd, $J = 9.2, 5.1$ Hz, 1H), 1.3 (s, 3H), 1.09 (d, $J = 7.6$ Hz, 3H)

$^{13}$C NMR (CDCl$_3$):

- 211.0, 174.4, 163.8, 151.4, 138.9, 134.6, 131.8, 130.7, 128.7, 126.7, 126.0, 121.8, 117.0, 114.2, 86.4, 84.6, 79.5, 67.6, 59.0, 49.2, 41.1, 40.1, 31.6, 21.7, 20.2, 18.0

IR (KBr): 3446, 2960, 2926, 1775, 1755, 1722, 1596, 1488, 1015, 801, 755, 715 cm$^{-1}$

ESI MS (m/z): 490 (M + 1)$^+$

Anal. Calcd for C$_{29}$H$_{31}$NO$_5$: C, 71.15; H, 6.38; N, 2.86. Found: C, 71.08; H, 6.41; N, 2.90.

N-(Phenyl)-C-(3-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3q’’)

Pale yellow colour solid; mp: 185-186 °C

Specific rotation: $[{\alpha}]_D^{25} -12.8$ (c 0.55, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):

- $\delta$ 7.72 (d, $J = 7.7$ Hz, 1H), 7.5 (d, $J = 5.8$ Hz, 1H), 7.3-7.15 (m, 4H), 7.03-6.93 (m, 3H), 6.3 (d, $J = 5.8$ Hz, 1H), 5.27 (d, $J = 5.6$ Hz, 1H), 4.73 (t, $J = 8.3$ Hz, 1H), 3.75 (s, 3H), 3.25-3.21 (dd, $J = 6.7, 5.6$ Hz, 1H), 3.03-2.84 (m, 3H), 2.45-2.39 (p, $J = 7.3$ Hz, 1H), 2.34 (s, 3H), 2.24-2.17 (td, $J = 12.2, 6.6$ Hz, 1H), 1.95-1.86 (q, $J = 13.3$ Hz, 1H), 1.75-1.7 (dd, $J = 8.2, 5.7$ Hz, 1H), 1.54-1.5 (dd, $J = 9.1, 5.2$ Hz, 1H), 1.32 (s, 3H), 1.1 (d, $J = 7.8$ Hz, 3H)

$^{13}$C NMR (CDCl$_3$):

- 210.8, 174.5, 164.1, 151.6, 139.1, 134.4, 131.5, 130.9, 128.8, 126.6, 126.4, 121.7, 117.4, 114.1, 86.7, 84.6,
79.3, 65.6, 59.4, 49.7, 41.3, 39.8, 31.5, 21.5, 20.2, 18.1
IR (KBr):
3450, 2961, 2925, 1775, 1755, 1722, 1596, 1488, 1010, 803, 755, 713 cm⁻¹
ESI MS (m/z):
490 (M + 1)⁺

Anal. Calcd for C₂₉H₃₁NO₅:
C, 71.15; H, 6.38; N, 2.86. Found: C, 71.08; H, 6.42; N, 2.89.

N-(Phenyl)-C-(4-nitro phenyl)-spiro-isoxazolidinyl parthenin (3r')
Pale yellow colour solid; mp: 145-146 °C
Specific rotation:
[α]D²⁵ -5 (c 0.6, CHCl₃)
¹H NMR (500 MHz, CDCl₃):
δ 8.25 (d, J = 8.6 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 5.8 Hz, 1H), 7.24 (t, J = 8.5 Hz, 2H), 6.98 (t, J = 7.5 Hz, 1H), 6.9 (d, J = 7.5 Hz, 2H), 6.27 (d, J = 5.7 Hz, 1H), 5.28 (d, J = 5.6 Hz, 1H), 4.96 (t, J = 8.1 Hz, 1H), 3.15-3.11 (dd, J = 6.2, 5.5, 1H), 2.95-2.91 (dd, J = 9.1, 3.9, 1H), 2.69-2.65 (dd, J = 8.0, 5.1, 1H), 2.38-2.32 (p, J = 7.4, 1H), 2.24-2.18 (td, J = 12.7, 6.3, 1H), 1.95-1.85 (q, J = 13.5, 1H), 1.75-1.71 (dd, J = 8.5, 5.6, 1H), 1.54-1.5 (dd, J = 9.5, 4.5, 1H), 1.36 (s, 3H), 1.1 (d, J = 7.7, 3H).
¹³C NMR (CDCl₃):
210.9, 174.2, 164.6, 151.9, 140.2, 132.5, 132.0, 127.6, 127.5, 122.6, 121.4, 115.5, 86.1, 82.3, 80.7, 69.9, 59.1, 49.5, 42.5, 40.1, 32.1, 21.6, 20.2, 18.0
IR (KBr):
3425, 2924, 1595, 1384, 1020 cm⁻¹
ESI MS (m/z):
504.6 (M + 1)⁺

Anal. Calcd for C₂₈H₂₈N₂O₇:
C, 66.66; H, 5.59; N, 5.55. Found: C, 66.58; H, 5.62; N, 5.57

N-(Phenyl)-C-(4-nitro phenyl)-spiro-isoxazolidinyl parthenin (3r'')
Pale yellow colour solid; mp: 145-146 °C
Specific rotation:
[α]D²⁵ -5 (c 0.6, CHCl₃)
¹H NMR (500 MHz, CDCl₃):
δ 8.25 (d, J = 8.6 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 5.8 Hz, 1H), 7.24 (t, J = 8.5 Hz, 2H),
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7.0-6.96 (t, \( J = 7.5 \text{ Hz}, 1\text{H} \)), 6.9 (d, \( J = 7.5 \text{ Hz}, 2\text{H} \)), 
6.27 (d, \( J = 5.7 \text{ Hz}, 1\text{H} \)), 5.28 (d, \( J = 5.6 \text{ Hz}, 1\text{H} \)), 
4.98-4.95 (t, \( J = 8.1 \text{ Hz}, 1\text{H} \)), 3.15-3.11 (dd, \( J = 6.2 \), 
5.5 Hz, 1H), 2.95-2.91 (dd, \( J = 9.1 \), 3.9 Hz, 1H), 
2.69-2.65 (dd, \( J = 8.0 \), 5.1 Hz, 1H), 2.38-2.32 
(p, \( J = 7.4 \text{ Hz}, 1\text{H} \)), 2.24-2.18 (td, \( J = 12.3 \), 6.3 Hz, 1H), 
1.95-1.85 (q, \( J = 13.5 \text{ Hz}, 1\text{H} \)), 1.75-1.71 
(dd, \( J = 8.5 \), 5.6 Hz, 1H), 1.54-1.5 (dd, \( J = 9.5 \), 4.5 
Hz, 1H), 1.36 (s, 3H), 1.1 (d, \( J = 7.7 \text{ Hz}, 3\text{H} \)).

\(^{13}\text{C NMR (CDCl}_3\text{):}\)
210.9, 174.2, 164.5, 151.9, 140.2, 132.5, 132.0, 127.6, 
127.5, 122.5, 121.4, 115.5, 86.1, 82.3, 80.7, 69.9, 
59.1, 49.5, 42.5, 40.1, 32.1, 21.6, 20.2, 18.0

IR (KBr): 3425, 2924, 1595, 1384, 1020 cm\(^{-1}\).

ESI MS (\( m/z \)): 504.6 (M + 1)

Anal. Caled for C\(_{28}\)H\(_{28}\)N\(_2\)O\(_7\): C, 66.66; H, 5.59; N, 5.55. Found: C, 66.57; H, 
5.61; N, 5.58.

\( \text{N-(Phenyl)-C-(3-methyl phenyl)-spiro-isoxazolidinyl parthenin (3s')} \)
White colour solid; mp: 172 °C
Specific rotation: \([\alpha]_D^{25} -15.4 \)  (c 0.9, CHCl\(_3\))

\(^1\text{H NMR (500 MHz, CDCl}_3\text{):}\)
\( \delta \) 7.5 (d, \( J = 5.9 \text{ Hz}, 1\text{H} \)), 7.41 (s, 1H), 7.33-7.12 
(m, 5H), 6.91 (t, \( J = 7.9 \text{ Hz}, 1\text{H} \)), 6.84 (d, \( J = 7.8 \text{ Hz}, 
2\text{H} \)), 6.27 (d, \( J = 5.9 \text{ Hz}, 1\text{H} \)), 5.26 (d, \( J = 5.7 \text{ Hz}, 1\text{H} \)), 
5.17 (t, \( J = 8.1 \text{ Hz}, 1\text{H} \)), 3.21-3.17 (dd, \( J = 7.2 \), 5.2 Hz, 
1H), 3.0-2.94 (dd, \( J = 7.5 \), 5.5 Hz, 1H), 2.50-2.46 
(dd, \( J = 9.0 \), 4.4 Hz, 1H), 2.33-2.28 (m, 4H), 
2.20-2.13, (td, \( J = 7.3 \text{ Hz}, 1\text{H} \)), 1.95-1.87 
(q, \( J =13.5 \text{ Hz}, 1\text{H} \)), 1.72-1.67 (dd, \( J = 8.7 \), 5.6 Hz, 1H), 
1.50-1.45 (dd, \( J = 9.2 \), 5.2 Hz, 1H), 1.32 (s, 3H), 
1.1 (d, \( J = 7.7 \text{ Hz}, 3\text{H} \)).

\(^{13}\text{C NMR (CDCl}_3\text{):}\)
212.2, 175.5, 164.0, 153.1, 143.1, 136.5, 131.0, 130.1, 
128.1, 122.7, 120.0, 115.3, 115.1, 86.5, 84.3, 80.0, 
68.5, 59.5, 49.7, 44.1, 40.5, 31.5, 25.1, 21.5, 
20.4, 18.5
IR (KBr): 3445, 2929, 1775, 1729, 1592, 1492, 1012, 810 cm⁻¹
ESI MS (m/z): 496 (M + 23)⁺
Anal. Calcd for C₂₉H₃₁NO₅: C, 73.55; H, 6.6; N, 2.96. Found: C, 73.47; H, 6.58; N, 2.98.

N-(Phenyl)-C-(3-methyl phenyl)-spiro-isoxazolidinyl parthenin (3s’’)
White colour solid; mp: 155 °C
Specific rotation: [α]D²⁵ -17.6 (c 0.75, CHCl₃)
¹H NMR (500 MHz, CDCl₃): δ 7.52 (s, 1H), 7.46 (d, J = 5.7 Hz, 1H), 7.33-7.27 (m, 2H), 7.08 (t, J = 7.6 Hz, 2H), 6.91-6.86 (m, 2H), 6.83 (d, J = 7.7 Hz, 1H), 6.25 (d, J = 5.7 Hz, 1H), 5.26 (d, J = 5.8 Hz, 1H), 4.25 (t, J = 8.1 Hz, 1H), 3.16-3.12 (dd, J = 6.9, 5.5 Hz, 1H), 2.95-2.90 (dd, J = 8.8, 4.0 Hz, 1H), 2.65-2.61(dd, J = 7.8, 5.0 Hz, 1H), 2.34-2.27 (m, 4H), 2.20-2.17 (td, J = 13.0, 7.9 Hz, 1H), 1.81-1.75 (q, J = 13.3 Hz, 1H), 1.75-1.70 (dd, J = 8.8, 5.4 Hz, 1H), 1.55-1.50 (dd, J = 9.1, 4.7 Hz, 1H), 1.31 (s, 3H), 1.1 (d, J = 7.7 Hz, 3H)
¹³C NMR (CDCl₃): 211.5, 175.2, 164.6, 151.9, 144.5, 134.2, 131.6, 130.0, 128.3, 123.3, 118.5, 115.6, 114.4, 86.5, 84.8, 79.5, 69.3, 59.2, 49.2, 43.5, 41.5, 31.5, 28.5, 21.6, 20.5, 18.0
IR (KBr): 3543, 2962, 1764, 1597, 1491, 1031, 997 cm⁻¹
ESI MS (m/z): 496 (M + 23)⁺
Anal. Calcd for C₂₉H₃₁NO₅: C, 73.55; H, 6.6; N, 2.96. Found: C, 73.49; H, 6.62; N, 2.97.

N-(Phenyl)-C-(2,4-dimethoxy phenyl)-spiro-isoxazolidinyl parthenin (3t’)
White colour solid; mp: 166-167 °C
Specific rotation: [α]D²⁵ -18 (c 0.75, CHCl₃)
¹H NMR (500 MHz, CDCl₃): 7.51-6.47 (m, 2H), 7.23-7.11 (m, 2H), 6.94-6.86 (m, 3H), 6.51-6.47 (m, 2H), 6.21 (d, J = 5.9 Hz, 1H), 5.42 (t, J = 7.5 Hz, 1H), 5.26 (d, J = 5.7 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.15-3.0 (m, 2H), 2.75-2.7
(dd, $J = 7.5, 5.7$ Hz, 1H), 2.62-2.56 (dd, $J = 9.1, 4.1$ Hz, 1H), 2.3-2.2 (p, $J = 7.2$ Hz, 1H), 2.22-2.15 (td, $J = 12.6, 6.8$ Hz, 1H), 1.92-1.75 (q, $J = 13.5$ Hz, 1H), 1.70-1.65 (dd, $J = 8.6, 5.7$ Hz, 1H), 1.46-1.42 (dd, $J = 9.3, 5.0$ Hz, 1H), 1.32 (s, 3H), 1.12 (d, $J = 7.6$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$):

211.0, 174.4, 163.5, 157.3, 151.7, 149.5, 132.1, 128.7, 127.5, 121.6, 117.0, 114.2, 105.0, 104.4, 86.6, 84.7, 79.3, 65.1, 63.7, 61.7, 59.1, 55.5, 49.5, 40.3, 31.5, 21.8, 20.0

IR (KBr): 3543, 2929, 1725, 1597, 973 cm$^{-1}$

ESI MS ($m/z$): 519.3 (M + 1)$^+$

Anal. Calcd for C$_{30}$H$_{33}$NO$_7$: C, 69.35; H, 6.40; N, 2.70. Found: C, 69.30; H, 6.42; N, 2.72

N-(Phenyl)-C-(2,4-dimethoxy phenyl)-spiro-isoxazolidinyl parthenin (3t''')

White colour solid; mp: 168-170 °C

Specific rotation: $[\alpha]_D^{25}$ -25 (c 0.75, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): 7.50-6.47 (m, 2H), 7.22-7.1 (m, 2H), 6.95-6.85 (m, 3H), 6.52-6.45 (m, 2H), 6.24 (d, $J = 5.9$ Hz, 1H), 5.25 (d, $J = 5.5$ Hz, 1H), 4.6 (t, $J = 8.5$ Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.1-3.02 (m, 2H), 2.80-2.75 (dd, $J = 8.2, 4.7$ Hz, 1H), 2.25-2.22 (p, $J = 7.5$ Hz, 1H), 1.90-1.75 (q, $J = 13.6$ Hz, 1H), 1.70-1.65 (dd, $J = 8.4, 5.6$ Hz, 1H), 1.47-1.42 (dd, $J = 9.2, 4.5$ Hz, 1H), 1.22 (s, 3H), 1.1 (d, $J = 7.5$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$): 211.0, 174.5, 164.0, 157.5, 151.6, 150.0, 132.1, 128.7, 127.2, 122.5, 117.0, 114.1, 105.0, 104.2, 86.6, 84.9, 79.3, 65.0, 63.7, 61.7, 59.5, 55.5, 49.5, 40.5, 31.7, 21.9, 20.0, 18.0

IR (KBr): 3537, 2925, 1725, 1595, 1026, 981 cm$^{-1}$

ESI MS ($m/z$): 519.3 (M + 1)$^+$

Anal. Calcd for C$_{30}$H$_{33}$NO$_7$: C, 69.35; H, 6.40; N, 2.70. Found: C, 69.29; H, 6.43; N, 2.72.
N-(Phenyl)-C-(2-bromo phenyl)-spiro-isoxazolidinyl parthenin (3u')

Pale yellow colour solid; mp: 132 °C
Specific rotation: $[\alpha]_D^{25}$ -24 (c 0.8, CHCl$_3$)
$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 7.65 (d, $J = 7.7$ Hz, 1H), 7.48 (d, $J = 5.7$ Hz, 1H),
7.39 (d, $J = 7.8$ Hz, 1H), 7.30 (t, $J = 7.8$ Hz, 1H),
7.23 (t, $J = 7.7$ Hz, 1H), 7.15 (t, $J = 7.9$ Hz, 2H),
6.91 (t, $J = 7.5$ Hz, 1H), 6.86 (d, $J = 7.5$ Hz, 2H), 6.24
(d, $J = 5.9$ Hz, 1H), 5.24 (d, $J = 5.6$ Hz, 1H), 4.95
(t, $J = 8.0$ Hz, 1H), 3.2-3.08 (dd, $J = 8.2$, 4.9 Hz, 2H),
2.62-2.59 (dd, $J = 7.7$, 5.5 Hz, 1H), 2.5-2.42
(p, $J = 7.5$ Hz, 1H), 2.23-2.18 (td, $J = 12.3$, 6.6
Hz, 1H), 1.96-1.87 (q, $J = 13.3$ Hz, 1H),
1.75-1.71 (dd, $J = 8.0$, 5.7 Hz, 1H), 1.64-1.60
(dd, $J = 8.8$, 5.5 Hz, 1H), 1.3 (s, 3H),
1.12 (d, $J =7.7$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$):
211.4, 175.5, 165.6, 158.3, 143.2, 133.4, 130.3, 129.6,
127.7, 127.1, 126.9, 125.6, 122.7, 114.3, 84.5, 81.5,
77.5, 68.2, 63.3, 47.6, 38.1, 35.2, 28.5, 18.8,
17.5, 16.4
IR (KBr): 3433, 2929, 2882, 1750, 1733, 1592, 1490, 1200,
970, 755 cm$^{-1}$
ESI MS ($m/z$): 539 (M + 1)$^+$
Anal. Calcd for C$_{28}$H$_{28}$BrNO$_5$: C, 62.46; H, 5.24; N, 2.60. Found: C, 62.41; H, 5.27; N,
2.62.

N-(Phenyl)-C-(2-bromo phenyl)-spiro-isoxazolidinyl parthenin (3u'')

Colourless solid; mp: 150 °C
Specific rotation: $[\alpha]_D^{25}$ -40 (c 0.9, CHCl$_3$)
$^1$H NMR (500 MHz, CDCl$_3$):
$\delta$ 7.65 (d, $J = 7.7$ Hz, 1H), 7.46 (d, $J = 5.9$ Hz, 1H),
7.41 (d, $J = 7.9$ Hz, 1H), 7.26-7.22 (m, 3H),
6.95 (t, $J = 8.1$ Hz, 1H), 6.86 (d, $J = 7.8$ Hz, 2H),
6.25 (d, $J = 5.8$ Hz, 1H), 5.61 (t, $J = 7.8$ Hz, 1H),
5.27 (d, $J = 5.5$ Hz, 1H), 3.25-3.21 (dd, $J = 7.8$, 5.3
Hz, 1H), 3.19-3.16 (dd, $J = 7.2$, 5.5 Hz, 1H),
2.42-2.38 (dd, $J = 7.9, 4.75$ Hz, 1H), 2.33-2.38 (p, $J = 7.8$ Hz, 1H), 2.14-2.09 (td, $J = 12.7, 6.9$ Hz, 1H), 1.9-1.84 (q, $J = 13.1$ Hz, 1H), 1.71-1.68 (dd, $J = 8.0, 5.7$ Hz, 1H), 1.5-1.46 (dd, $J = 8.9, 5.4$ Hz, 1H), 1.3 (s, 3H), 1.12 (d, $J = 7.4$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$):

210.7, 175.1, 166.6, 156.5, 144.3, 133.5, 130.8, 130.7, 128.2, 127.9, 126.9, 126.4, 123.4, 115.9, 84.1, 80.5, 78.7, 68.2, 63.5, 47.8, 38.2, 35.7, 29.1, 19.4, 17.7, 16.9

IR (KBr):

3450, 2933, 2876, 1773, 1725, 1542, 1362, 988 cm$^{-1}$

ESI MS ($m/z$):

539 (M + 1)$^+$

Anal. Calcd for C$_{28}$H$_{28}$BrNO$_5$: C, 62.46; H, 5.24; N, 2.60. Found: C, 62.40; H, 5.27; N, 2.63.

N-(Phenyl)-C-(3,4,5-tri methoxy phenyl)-spiro-isoxazolidinyl parthenin (3v’)

Pale yellow colour solid; mp: 144 °C

Specific rotation: $[\alpha]_D^{25}$-25.3 (c 0.9, CHCl$_3$).

$^1$H NMR (500 MHz, CDCl$_3$):

7.49 (d, $J = 5.9$ Hz, 1H), 7.15 (t, $J = 7.9$ Hz, 2H), 6.97-6.89 (m, 3H), 6.27 (d, $J = 5.9$ Hz, 1H), 5.25 (d, $J = 5.6$ Hz, 1H), 5.13 (t, $J = 8.0$ Hz, 1H), 3.88 (s, 3H), 3.85 (s, 6H), 3.21-3.18 (dd, $J = 6.7, 5.7$ Hz, 1H), 2.95-2.85 (dd, $J = 6.8, 5.7$ Hz, 1H), 2.65-2.5 (dd, $J = 7.9, 5.6$ Hz, 1H), 2.34-2.28 (p, $J = 7.5$ Hz, 1H), 2.24-2.16 (td, $J = 12.5, 6.4$ Hz, 1H), 1.92-1.82 (q, $J = 13.5$ Hz, 1H), 1.71-1.67 (dd, $J = 8.5, 5.7$ Hz, 1H), 1.45-1.4 (dd, $J = 9.1, 5.2$ Hz, 1H), 1.32 (s, 3H), 1.15 (d, $J = 7.7$ Hz, 3H).

$^{13}$C NMR (CDCl$_3$):

212.2, 175.0, 164.5, 151.3, 145.0, 141.1, 134.6, 132.5, 131.0, 128.6, 124.6, 114.5, 85.4, 84.0, 78.4, 72.7, 65.2, 60.5, 51.2, 47.0, 42.1, 34.4, 30.7, 22.0, 21.2, 18.6, 16.2

IR (KBr):

3410, 2926, 2910, 1755, 1725, 1596, 1487, 1386, 1350, 1240, 1025, 755 cm$^{-1}$

ESI MS ($m/z$):

550.5 (M + 1)$^+$

Anal. Calcd for C$_{31}$H$_{35}$NO$_8$: C, 67.75; H, 6.42; N, 2.55. Found: C, 67.69; H, 6.44; N, 2.55.
2.57.

N-(Phenyl)- C-(3,4,5-tri methoxy phenyl)-spiro-isoxazolidinyl parthenin (3v’’)

Pale yellow colour solid; mp: 160 °C

Specific rotation: \([\alpha]_D^{25} -40\) (c 0.6, CHCl₃).

\(^1\)H NMR (500 MHz, CDCl₃):

7.59 (d, \(J = 5.8\) Hz, 1H), 7.14 (t, \(J = 7.9\) Hz, 2H),
6.98-6.88 (m, 3H), 6.25 (d, \(J = 5.7\) Hz, 1H),
5.26 (d, \(J = 5.7\) Hz, 1H), 4.35 (t, \(J = 8.2\) Hz, 1H),
3.88 (s, 3H), 3.85 (s, 6H), 3.19-3.15 (dd, \(J = 7.7, 5.6\) Hz, 1H),
2.89-2.83 (dd, \(J = 7.68, 5.7\) Hz, 1H),
2.58-2.5 (dd, \(J = 7.5, 5.7\) Hz, 1H), 2.28-2.21 (m, 2H),
1.95-1.87 (q, \(J = 13.2\) Hz, 1H), 1.75-1.69 (dd, \(J = 8.5, 5.7\) Hz, 1H),
1.55-1.48 (dd, \(J = 8.6, 5.5\) Hz, 1H),
1.30 (s, 3H), 1.12 (d, \(J = 7.7\) Hz, 3H).

\(^{13}\)C NMR (CDCl₃):

211.9, 175.2, 164.5, 151.7, 143.9, 141.5, 134.5, 131.9, 131.0, 129.1, 123.9, 115.5, 86.7, 85.5, 84.3, 75.9, 72.9, 65.5, 60.6, 50.5, 46.4, 43.1, 35.0, 30.2, 22.0, 20.9, 19.0, 16.4

IR (KBr):

3450, 2931, 2918, 1750, 1730, 1588, 1490, 1386, 1345, 1245, 1035, 766 cm⁻¹

ESI MS (m/z):

572.5 (M + 23)⁺

Anal. Calcd for C₃₁H₃₅NO₈: C, 67.75; H, 6.42; N, 2.55. Found: C, 67.70; H, 6.44; N, 2.56.

N-(Phenyl)-C-(2-methoxy, 5-bromo-phenyl)-spiro-isoxazolidinyl parthenin (3w’)

Yellow colour solid; mp: 181 °C

Specific rotation: \([\alpha]_D^{25} -25\) (c 0.9, CHCl₃)

\(^1\)H NMR (500 MHz, CDCl₃):

δ 7.80 (d, \(J = 2.4\) Hz, 1H), 7.49 (d, \(J = 5.9\) Hz, 1H),
7.42-7.37 (dd, \(J = 6.1, 2.5\) Hz, 1H), 7.23 (t, \(J = 7.6\) Hz, 2H),
6.96-6.8 (m, 4H), 6.26 (d, \(J = 4.9\) Hz, 1H), 5.48 (t, \(J = 7.4\) Hz, 1H),
5.29 (d, \(J = 5.0\) Hz, 1H), 3.89 (s, 3H), 3.19-3.04 (dd, \(J = 7.2, 5.3\) Hz, 2H),
2.36-2.05 (m, 4H), 2.0-1.88 (q, \(J = 13.23\) Hz, 1H), 1.75-1.69 (dd, \(J = 8.6, 5.7\) Hz, 1H),
1.55-1.48 (dd, \(J = 8.6, 5.5\) Hz, 1H),
1.34 (s, 3H), 1.1 (d, \(J = 7.7\) Hz, 3H).
N-(Phenyl)-C-(2-methoxy, 5-bromo-phenyl)-spiro-isoxazolidinyl parthenin (3w’’)

**Yellow colour solid; mp:** 172-174 °C

**Specific rotation:** $[\alpha]_D^{25} -36$ (c 0.9, CHCl$_3$)

**$^1$H NMR (500 MHz, CDCl$_3$):**

\[
\begin{align*}
\delta & 7.78 \ (d, J = 2.4Hz, 1H), \ 7.48-7.40 \ (m, 2H), \\
& 7.18 \ (t, J = 7.7 Hz, 2H), \ 6.95-6.8 \ (m, 4H), \\
& 6.25 \ (d, J = 5.4, 1H), \ 5.24 \ (d, J = 5.6 Hz, 1H), \\
& 4.33 \ (t, J = 8.0 Hz, 1H), \ 3.88 \ (s, 3H), \ 3.19-3.04 \\
& \text{(dd, } J = 7.3, 5.2 \ Hz, 2H), \ 2.35-2.25 \ (m, 2H), \\
& 2.22-2.15 \ (td, J = 12.5, 6.4 Hz, 1H), \ 1.96-1.86 \\
& \text{(q, } J = 13.2 \ Hz, 1H), \ 1.75-1.68 \ (dd, J = 8.5, 5.7 \\
& \text{Hz, 1H), } 1.53-1.45 \ (dd, J = 8.4, 5.5 Hz, 1H), \\
& 1.33 \ (s, 3H), \ 1.1 \ (d, J = 7.6 Hz, 3H).
\end{align*}
\]

**$^{13}$C NMR (CDCl$_3$):**

\[
\begin{align*}
211.1, \ 175.1, \ 164.4, \ 155.8, \ 151.0, \ 134.9, \ 131.4, \ 131.2, \\
128.0, \ 127.1, \ 122.4, \ 119.0, \ 114.7, \ 112.5, \ 87.0, \ 84.5, \\
79.7, \ 67.5, \ 59.1, \ 56.2, \ 49.3, \ 42.3, \ 40.4, \ 31.2, \ 21.7, \\
20.1, \ 18.1.
\end{align*}
\]

**IR (KBr):** 3417, 2925, 1774, 1720, 1597, 1489, 1048, 975 cm$^{-1}$

**ESI MS (m/z):** 569.3 (M + 1)$^+$

**Anal. Calcd for C$_{29}$H$_{30}$BrNO$_6$:** C, 61.27; H, 5.32; N, 2.46. Found: C, 61.33; H, 5.30; N, 2.48.

N-(Phenyl)-C-(2-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3x’)

**Pale yellow colour solid; mp:** 171°C

**Specific rotation:** $[\alpha]_D^{25} -33.0$ (c 0.75, CHCl$_3$)

**$^1$H NMR (500 MHz, CDCl$_3$):**

\[
\begin{align*}
\delta & 7.52 \ (d, J = 7.6, 1H), \ 7.48 \ (d, J = 5.9, 1H), \ 7.35 \\
& \text{(d, } J = 7.9, 1H), \ 7.20-7.15 \ (m, 3H), \ 6.89 \ (t, J = 7.9,
\end{align*}
\]
1H), 6.84 (d, J = 7.9, 2H), 6.25 (d, J = 5.9, 1H), 5.25 (d, J = 5.5, 1H), 5.1 (t, J = 7.9, 1H), 3.24-3.20 (dd, J = 7.6, 5.4, 1H), 3.16-3.12 (dd, J = 6.9, 5.5, 1H), 2.37-2.34 (dd, J = 7.8, 4.7, 1H), 2.35-2.29 (p, J = 7.9, 1H), 2.14-2.09 (td, J = 12.7, 6.9, 1H), 1.90-1.80 (q, J = 13.2, 1H), 1.71-1.68 (dd, J = 8.9, 5.70, 1H), 1.49-1.45 (dd, J = 9.0, 5.7, 1H), 1.3 (s, 3H), 1.12 (d, J = 7.5, 3H)

$^{13}$C NMR (CDCl$_3$):

211.0, 175.5, 166.2, 160.7, 143.6, 134.1, 129.6, 128.0, 127.3, 126.9, 126.5, 126.1, 120.6, 115.1, 84.1, 80.6, 77.9, 68.8, 63.0, 48.3, 38.1, 33.6, 29.2, 19.3, 17.2, 16.5

IR (KBr):

3420, 2926, 2872, 1753, 1726, 1599, 1484, 1200, 975, 756, 722 cm$^{-1}$

ESI MS ($m/z$):

490 (M + 1)$^+$

Anal. Caled for C$_{29}$H$_{31}$NO$_6$:  C, 71.15; H, 6.38; N, 2.86. Found: C, 71.19; H, 6.36; N, 2.88

N-(Phenyl)-C-(2-methoxy phenyl)-spiro-isoxazolidinyl parthenin (3x"")

Colourless solid; mp: 162 ºC

Specific rotation: $[\alpha]_D^{25}$ -24.4 (c 0.75, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$):

δ 7.49 (d, J = 5.9, 1H), 7.45 (d, J = 7.6, 1H), 7.33 (d, J = 7.8, 1H), 7.24 (t, J = 7.5, 1H), 7.19 (t, J = 7.6, 1H), 7.12 (t, J = 7.9, 2H), 6.93 (t, J = 7.8, 1H), 6.86 (d, J = 7.8, 2H), 6.25 (d, J = 5.9, 1H), 5.25 (d, J = 5.5, 1H), 4.38 (t, J = 8.1, 1H), 3.78 (s, 3H), 3.07-3.03 (dd, J = 9.0, 5.0, 2H), 2.65-2.56 (dd, J = 7.5, 5.5, 1H), 2.45-2.34 (p, J = 7.5, 1H), 2.21-2.16, (td, J = 12.4, 6.5, 1H), 1.96-1.87 (q, J = 13.4, 1H), 1.74-1.69 (dd, J = 8.5, 5.6, 1H), 1.65-1.61 (dd, J = 9.1, 5.1, 1H), 1.3 (s, 3H), 1.12 (d, J = 7.6, 3H).

$^{13}$C NMR (CDCl$_3$):

212.2, 174.9, 165.0, 152.1, 140.2, 133.1, 130.0, 129.1, 128.1, 127.5, 126.9, 125.1, 122.6, 116.5, 114.7, 86.5, 84.0, 80.5, 68.6, 59.5, 53.8, 49.6, 40.5, 32.1, 21.1, 19.5, 18.3
IR (KBr): 3450, 2930, 2870, 1775, 1723, 1600, 1525, 1350, 983, 750, 711 cm\(^{-1}\)

ESI MS (m/z): 490 (M + 1)

Anal. Calcd for C\(_{29}\)H\(_{31}\)NO\(_6\): C, 71.15; H, 6.38; N, 2.86. Found: C, 71.21; H, 6.35; N, 2.89.

2.2.11.2. Evaluation of in vitro cytotoxicity

The effect of spiro-isoxazolidine derivatives of parthenin 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 (Shekhan et al., 1990). Detailed explanation was given in chapter-1, section-1, experimental section.

2.2.11.3. DNA fragmentation assay

DNA fragmentation was determined by electrophoresis of extracted genomic DNA from human colon cancer cell line. Cells (2 x 10\(^6\)/6 mL medium/60mm tissue culture plate) were treated with compound 3i at 5, 10 and 20 µM for 24 hrs. Cells were harvested, washed with PBS, pellets were dissolved in lysis buffer (10mM EDTA, 50 mM tris pH 8.0, 0.5% w/v SDS and proteinase K (0.5 mg/mL) and incubated at 50 °C for 1hr. Finally the DNA obtained was heated rapidly to 70 °C, supplemented with loading dye and immediately resolved on to 1.5% agarose gel at 50 V for 2-3 hrs.

2.2.11.4. DNA cell cycle Analysis

Effect of compound-3i on DNA content by cell cycle phase distribution was assessed using SW-620 cells by incubating the cells 1x10\(^6\) mL/well with compound-3i (1, 5, 10 & 20 µM each) for 24 hrs. 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 minutes. After fixation, these cells were incubated with RNase A (0.1 mg/mL) at 37 °C for 30 minutes, stained with propidium iodide (50 µg/mL) for 30 minutes 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 (488nm) excitation from Argon laser. Data were collected in list mode on 10,000 events for FL2-A vs. FL2-W.

2.2.11.5. In vivo anticancer activity

2.2.11.5.1. Animal care and housing

Non-inbred Swiss albino mice from an in-house colony were used in the present study. The breeding and experimental animals were housed in standard size polycarbonate cages.
providing internationally recommended space for each animal. Animals were fed balanced mice feed supplied by M/s Ashirwad Industries, Chandigarh (India) and autoclaved water was available *ad libitum*. Animals were cared as per the guide for the care and use of laboratory animals (1996), ILAR, Washington DC. They were housed in controlled conditions of temperature (23 ± 2°C), humidity (50-60%) and 12:12 hrs of light: dark cycle. The study and the number of animals used were approved by the institutional animal ethics committee, IIIM-Jammu, India. The study was conducted as per the protocols of National Cancer Institute (NCI), USA (Malik *et al.*, 2007)

### 2.2.11.5.2. Anti tumor activity of parthenin and its derivative compound-3i' on Ehrlich Ascites tumor (EAT)

Parthenin and its derivative compound-3i’ were evaluated against solid Ehrlich Ascites Tumor (EAT) models at different doses. A standard procedure for experiment was as follows: Animals of the same sex weighing 20 ± 3 g were injected 1 x 10⁷ cells collected from the peritoneal cavity of non-inbread Swiss mice, bearing 8-10 days old tumor cells, into the right thigh, intramuscularly (on day 1). On next day animals were randomized and divided into test groups (7 animals in each test group) and one tumor control group (10 animals). Test groups were treated with different doses of parthenin (10 mg/kg, 25mg/kg and 50 mg/kg) and its derivative compound-3i’ (10 mg/kg, 25mg/kg and 50 mg/kg, 100 mg/kg and 200mg/kg) suspension in 1% gum acacia in their respective group intraperitoneally for nine consecutive days. Another test group was administered 5-FU @ 20 mg/kg i.p and served as positive control. The tumor control group was similarly administered normal saline (0.2 mL i.p). The percent tumor growth inhibition was measured on day 13 with respect to their tumor weight. Shortest and longest diameter of the tumor mass were measured with the help of Vernier caliper and tumor weight (mg) was calculated by using following formula:

\[
\text{Tumor weight} = \frac{\text{Length (mm)} \times \text{Width (mm)}^2}{2}
\]

The average tumor weight for each group was calculated and the percent tumor growth inhibition in treated groups was calculated as follows:

\[
\% \text{ Growth inhibition} = \frac{100 \times (A\text{varage tumor weight of control group}-A\text{verage tumor weight of test group})}{A\text{verage tumor weight of control group}}
\]
2.2.11.5.3. Anti tumor activity of parthenin and its derivative 3i’ on Ehrlich Ascites Carcinoma (EAC)

Ehrlich Ascites Carcinoma (EAC) was propagated by transplanting 1x10⁷ cells from an animal bearing 8-10 days old Ehrlich Ascites Carcinoma, into the peritoneal cavity of non-inbreed Swiss mice. For testing, mice of the same sex weighing 20 ± 3 g bearing ascites tumor were selected. 1 x10⁷ cells obtained from an animal bearing 8-10 days old ascites tumor were injected into peritoneal cavity of all animals used for testing (0 Day). On next day animals were randomized and divided into test groups (7 animals in each test group) and one tumor control group (10 animals). Test groups were treated with different doses of parthenin (10 mg/kg, 25mg/kg and 50 mg/kg) and its derivative compound-3i’ (10 mg/kg, 25mg/kg and 50 mg/kg, 100 mg/kg and 200mg/kg) suspension in 1 % gum acacia in their respective group intra-peritoneally for nine consecutive days. Another test group was administered 5-FU @ 20 mg/kg i.p and served as positive control. The tumor control group was similarly administered normal saline (0.2 mL i.p). The percent tumor growth inhibition was measured on day 12 with respect to volume of ascitic fluid and the number of tumor cells in the ascitic fluid of peritoneal cavity (Geran et al., 1972).

The percent tumor growth inhibition was calculated as follow.

\[
\% \text{ Growth inhibition} = 100 \times \frac{(\text{Average no. of cells in control group} - \text{Average no.of cells in test group})}{\text{Average no. of cells in control group}}
\]

2.2.11.6. Preparation of cytosolic and nuclear extracts for the evaluation of NF-kB by Western blotting

SW-620 cells (5 × 10⁶) were treated with compound-3i’ (5 µM) for indicated time periods. Cells were washed with ice-cold phosphate-buffered saline and centrifuged. Cytosolic and nuclear lysates were prepared as described earlier. Cell pellets were homogenized in 200 µl of buffer A (10 mM Hepes, pH 7.9, 1 mM EDTA, 1 mM EGTA, 100 mM KCl, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 5 mM NaF, 1 mM NaVO₄ and 10% protease cocktail inhibitor). The tubes were placed in ice for 10 minutes. Nonidet P-40 was added (0.5%, v/v), tubes vortexed briefly and centrifuged at 8,000x g for 15 min. The cytosolic supernatants were stored at -80 °C. The pellets obtained were resuspended in 50 µL of buffer A supplemented with 20% glycerol, 0.4 M KCl, kept on ice for 30 minutes and centrifuged at 13,000 x g for 15 minutes. The
supernatants were stored at -80 °C for analysis of nuclear NF-kB and AIF. All steps of fractionation were carried out at 4 °C. Western blot analysis for NF-kB-p65 was performed by as described by Malik et al. (2007).

2.2.11.7. Docking study: Methodology

All the parthenin derivatives were sketched in Chemsketch (www.acdlabs.com) and transferred to Schrodinger (www.schrodinger.com) for the further analysis. Each structure was assigned an appropriate bond order using ligprep script shipped by Schrödinger. The analogues were optimized by means of the OPLS2005 force field using truncated Newton algorithm of impact energy minimization module in Schrödinger.

The target for docking was retrieved from the Brookhaven Protein Data Bank with PDB ID as 1RAM which is homodimer of p65 subunit resolved at 2.4 Å. Since it’s a homodimer so we have selected one chain for docking rest all the chains including DNA were deleted. Water molecules beyond 5Å of ligand were removed and hydrogen atoms were added to the structure. The most likely positions of hydroxyl and thiol hydrogen atoms, protonation states and tautomers of His residues, and Chi ‘flip’ assignments for Asn, Gln and His residues were selected by the protein assignment script shipped by Schrödinger. Minimizations were performed by applying OPLS-AA forcefield (Jorgensen et al., 1996) until the average root mean square deviation of the non hydrogen atoms reached 0.3 Å. Active site was identified based on selected residues cys38 and cys120 inferred from literature as the important residues involved in binding the parthenin like molecules. To study the molecular basis of interaction and affinity of binding of new parthenin derivatives prepared earlier as discussed above docking was performed using Glide module of Schrödinger. Glide (Grid-Based Ligand Docking With Energetics) software from Schrodinger. Grids were prepared for each protein with the exact same center and the size of the bounding box set on 30 Å. The coordinates of the enclosing box (x = -1,1958 Å; y = 9.0149 Å; z = 19,7598 Å) were defined starting from the set of active site residues involved in hydrogen bonds with the NF-kB recognition site of DNA (Tyr36,Cys38 and Lys122) and optimised including the double strands DNA helices volume by visual inspection. The Glide algorithm is based on a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using funnel type approach. The search begins with a rough positioning and scoring phase that significantly limits the search space and reduces the number of poses to be selected for minimization on the pre computed OPLS-2001 van der Waals and electrostatic grids for the protein. The 5–10 lowest-energy poses obtained from this stage are subjected to
Monte Carlo simulations and the minimized poses accepted are then rescored using the GlideScore function, which is a more sophisticated version of ChemScore.

2.2.12. References:


Science 168: 376-378.


$^1$H NMR spectrum of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a')
Mass spectrum of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a')
IR spectrum of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a')
$^{13}$C NMR spectrum of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a')
$^1$H NMR spectra of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a"")
$^{13}$C NMR spectrum of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a'')
IR spectrum of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3a'')
Mass spectrum of N-(phenyl)-C-(4-bromo phenyl)-spiro-isoxazolidinyl parthenin (3α‘‘)