Bioorganic & Medicinal Chemistry Letters 19 (2009) 4394–4398

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Structure–activity relationship (SAR) of parthenin analogues with pro-apoptotic activity: Development of novel anti-cancer leads

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ARTICLE INFO

*Article history:*
Received 24 February 2009
Revised 28 April 2009
Accepted 16 May 2009
Available online 28 May 2009

*Keywords:*
Cytotoxicity
Apoptosis
Topoisomerase-II
Telomerase
SAR
Parthenin

ABSTRACT

Analogues of parthenin were synthesized by substitutions at different reaction centres to establish a structure–activity relationship (SAR). Some of the molecules have displayed significant cytotoxicity in human cervical carcinoma (HeLa) and human myeloid leukemia (HL-60) cells. A few of the compounds also induced apoptosis in HL-60 cells measured in terms of sub-Go/G1 DNA fraction. Also one of the lead molecules has been shown to be the inhibitor of both telomerase and topoisomerase-II.



Terpenes have attracted considerable attention of medicinal chemist in recent past due to their innumerable biological activities.[1] Sesquiterpene lactones are a group of compounds usually isolated from various genera of Asteraceae family and are known to possess diverse biological activities.[2] The cytotoxicity and structure–activity relationship (SAR) of sesquiterpene lactones has always been a subject of interest. The biological activity of these complex natural products appears to be associated with their ability to act as alkylating agents by virtue of Michael addition of biological nucleophiles to α-methylene part of lactone moiety.[3] It has been shown that α-methylene-γ-lactone react rapidly with cysteine to form stable adducts whereas endocyclic α,β-unsaturated-γ-lactones react slowly with cysteine to form unstable adducts.[4] It was also postulated that 'the reactions between α-methylene-γ-lactone and other conjugated systems with biologically important sulfhydryl groups may play a significant role in the mechanism by which these compounds exert their biological activities'.[5] It has also been observed that an increase in cytotoxicity accompanies increase in lipophilicity. Furthermore, the presence of bifunctionality, that is, either a cyclopentenone ring, α-methylene-δ-lactone or a conjugated side chain ester in addition to α-methylene-γ-lactone increases cytotoxicity.[6] The importance of bifunctionality has also been revealed in synthesized α-methylene-γ-butyrolactones.[7] Thus they may exert their biological effects by inhibiting cellular enzyme activity and not by alkylating or impairing DNA function.[8] On the other hand, it is also been reported that compounds of this class are active towards the thiol group of enzymes like DNA polymerase II and III, necessary for DNA replication.[9]

In the course of our studies we have chosen parthenin and worked towards its structural modifications to develop some potential anti-cancer therapeutic leads of higher apoptotic index compared to parent molecule. Parthenin (**1**, a sesquiterpene lactone) is mainly isolated from *Parthenium hysterophorus*[10] and reportedly possesses diverse biological activities like anti-tumor,[11] anti-inflammatory,[12] lipid peroxidation inhibition,[13] anti-inflammatory,[4] ameobicidal activity[14] and trypanocidal activity.[15] Present study is focused on target based anti-cancer therapeutic lead development from a library of semi-synthetic analogues of parthenin. The synthesized compounds were screened for in vitro cytotoxicity, besides; an attempt was made to understand their possible molecular mode of action.

The preparation of semi-synthetic analogues of parthenin using Baylis–Hillman reaction has been reported in our previous Letter.[16] In this reaction **1** was treated with the aldehydes in presence of DBU at ambient temperature, lead to the formation of atypical Baylis–Hillman adducts having a dioxolane ring. The formation of dioxolane derivatives was observed in the majority of reactions. The reaction was initially performed with formaldehyde in pres-

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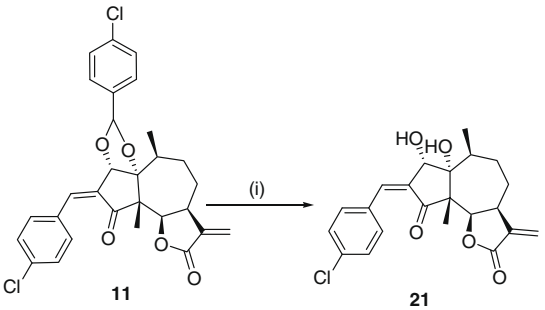
doi:10.1016/j.bmcl.2009.05.089

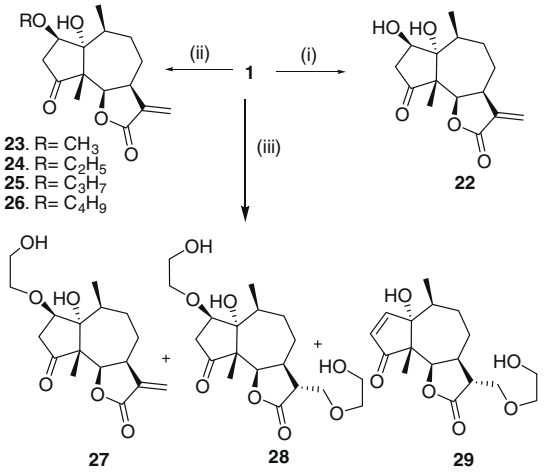
ence of DBU as a base in THF/$H_2O$ (7:3), that afforded three products, that is, compound **2** (22%), **3** (13%) and **4** (35%). The same reaction was extended to other higher aliphatic aldehydes. The reaction with lower aliphatic aldehydes, that is, paraldehyde and propionaldehyde lead to the formation of predominantly dioxolane derivative (**5** and **6**), whereas the reaction with higher homologues, that is, butyraldehyde, valeraldehyde and heptaldehyde produced normal dehydrated Baylis–Hillman product (**7**–**9**). After the accomplishment of reaction with aliphatic aldehydes, it was extended to aromatic aldehydes. The major identified products were interesting as the formation of dioxolane ring was accompanied with normal aldol type reaction leading to the formation of products **10**–**16**. These analogues with additional dioxolane ring and an exocyclic double bond may be more prone to nucleophilic attack which is interesting from bioactivity viewpoint as well SAR studies. Other than the above mentioned products, the formation of normal Baylis–Hillman products was also observed (**17**–**20**) (Scheme 1).

2-α-Hydroxylated analogue, that is, **21** was prepared by TFA (trifluoroacetic acid) catalyzed cleavage of compound **11** in almost quantitative yield (Scheme 2).

We also envisaged the preparation of *O*-alkyl products via 1,4-addition (Michael addition) of alkanols with the objective to generate more information about SAR. It was expected that the formation of adduct will be preferential at the endocyclic double bond due to stereochemical factors (lactone ring being endo, therefore less accessible to the reagent). The nucleophiles like hydroxyl and methoxy were added in a facile manner on to endocyclic double bond. The addition of hydroxyl group was achieved by reaction of water in presence of $NaHCO_3$ in a microwave oven to produce **22**. Encouraged by this reaction, methoxy, ethoxy, propoxy and butoxy groups were also introduced at C-2 by treating **1** with dry methanol, ethanol, propanol and butanol respectively, in presence of a tertiary base like triethylamine (TEA) to produce compounds **23**–**26**. With the higher homologues, there was progressive decrease in yields as compound **23** was obtained in 80%, **24** in 65%, **25** in 50% and **26** in 30% yield respectively. The conversion rate was also slower in the reactions with higher alcohols. With ethylene glycol in presence of TEA, however, three products (**27**–**29**) were isolated in 30%, 25% and 20% yields, respectively, (Scheme 3). The β-configuration at C-2 of *O*-alkyl analogues was established

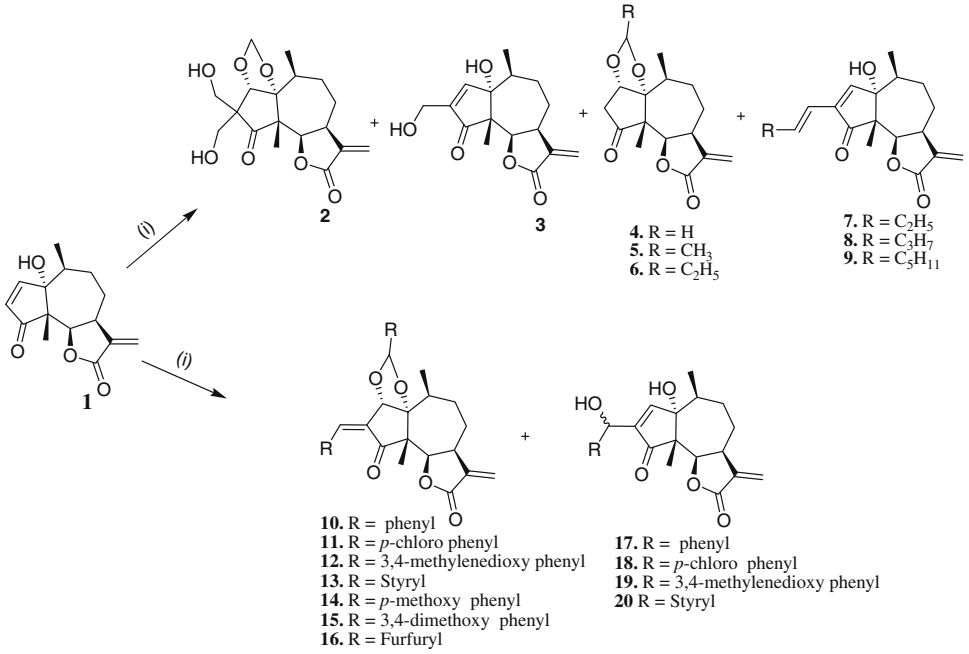



**Scheme 2.** Reagents and conditions: (i) TFA/$H_2O$ (1:1.5), 55 °C.




**Scheme 3.** Reagents and conditions: (i) $NaHCO_3$, microwave; (ii) ROH, TEA; (iii) $HOCH_2CH_2OH$, TEA.

by comparing it with a known natural product 2-β-hydroxy coronopilin,[17] which has same structure as compound **22**. The formation of these compounds may possibly be explained by the participation of C1–OH group in compound **1**, which in the presence of a




**Scheme 1.** Reagents and conditions: (i) RCHO, DBU, THF/$H_2O$ (7:3), rt.

base may give 1,2-epoxide intermediate via 1,4 addition. This is followed by the attack of a nucleophile (ROH) or a water molecule, resulting in the opening of epoxide ring and the formation of compounds **22–28**.

It was also envisaged to introduce halogen atom (bromination) in the nucleus mainly at the endocyclic double bond. Simple addition of bromine in carbon tetrachloride or acetic acid furnished a complex mixture of products; therefore, the bromination was effected with NBS. Initially, **1** was treated with 2.2 equiv of NBS in dioxane yielding dibromo product in 70% yield (**30**). On increasing the ratio of NBS to 3.4 and 4.5 equiv tribromo (**31**) (yield 60%) and tetrabromo (**32**) (yield 55%) derivatives of parthenin respectively were isolated as the major products. Compound **1** was also treated with NBS in DMF, affording bromohydrin (**33**) in 65% yield (Scheme 4).

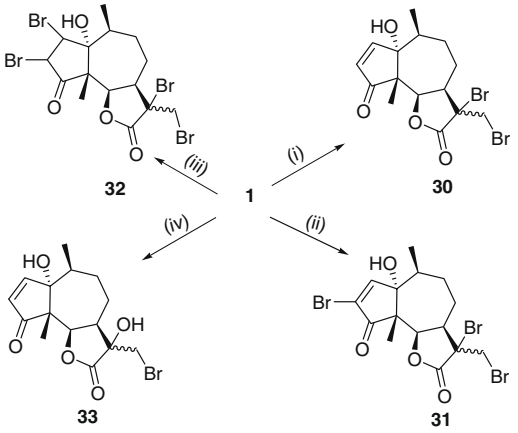
Compound **1** was also subjected to Baeyer–Villiger oxidation. The idea was to expand the cyclopentenone ring and study its role in anti-cancer activity. Therefore, **1** was treated with *m*-CPBA in DCM at room temperature with stirring to afford **34** in 70% yields (Scheme 5).

In another set of reactions the keto function of the cyclopentenone ring was subjected to reduction with $NaBH_4$ in methanol to afford **35** in 70% yield, which was subsequently converted to its acetyl derivative by treating it with acetic anhydride and DMAP in dry DCM to afford **36** in 95% yield (Scheme 6).

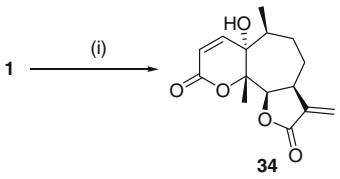
The tertiary hydroxyl group at C-1 was also removed to study its role in bioactivity. For effecting smooth dehydration, **1** was treated with $BF_3{\cdot}Et_2O$ in small instalments at room temperature yielding **37** in 80% yield (Scheme 7).

It has been amply documented that cancer cells evade apoptosis, and therefore chemotherapeutic agents that can induce cytotoxicity through apoptosis are currently the focus of anti-cancer research. The contemporary research has been focused on molecular targets which are expressed in cancer cells but are absent or under-regulated in normal cells. The present study employed two cell lines viz, HL-60 and HeLa cells. In HeLa cells, p53 gene is least functional because of its inactivation with the HPV (human papilloma virus), while in HL-60 the gene is mutated. The present studies indicated that HL-60 cells are more sensitive to cytotoxicity of semi-synthetic analogues than HeLa cells as evidenced by the extent of cytotoxicity ($IC_{50}$ value) after 48 h of treatment. The results revealed that several analogues, that is, **10**–**13**, **15**–**17**, **21** and **32** produced cytotoxicity in HL-60 cells with $IC_{50}$ value ~10 μM whereas it required several fold higher concentrations to produce a comparable effect in HeLa cells (Table 1). None of the compounds exhibited a fair degree of cytotoxicity at lower concentrations in HeLa cells with the exception of the compound **11**. Thus **11** was found to be the most effective molecule with $IC_{50}$ value of approximately 3 μM in HL-60 cells and 12 μM in HeLa cells. DMSO used as delivery vehicle (<0.2% v/v), did not affect the cell growth when treated for the same time period. Furthermore, to understand if the cytotoxicity is due to apoptosis, we analyzed various analogues for their ability to induce sub-G1 population as a measure of apoptosis induction in HL-60 cells. Hence, HL-60 cells were incubated for 6 h with 10 μM concentration of each analogue (Table 1) and the percentage of cells undergoing apoptosis was determined by flow cytometry after staining with propidium iodide (PI).[18] As expected, **11** was found to be the most effective of all analogues producing approximately 94% of apoptotic cell population at the end of 6 h treatment, suggesting that compound **11** is probably a potential candidate that can be developed into an anti-cancer therapeutic lead. As it exhibited relatively lower $IC_{50}$ values in both the cell types, it warranted more studies to understand its molecular mechanism of action. To validate the effectiveness of **11** against few among many putative molecular targets in cancer cells, we analyzed the expression of telomerase in HL-60 cells exposed to it in a time dependent manner. It was interesting to note that compound **11** lead to almost 80% inhibition of telomerase expression through 6 h treatment (Fig. 1). Immediate inhibition of telomerase however may not be a singular factor for inducing apoptosis induced by compound **11**, since it has been reported by other studies[19] that telomerase inhibition is followed by a lag time required to enable shortening of telomeres to a critical point which ensues cell death.

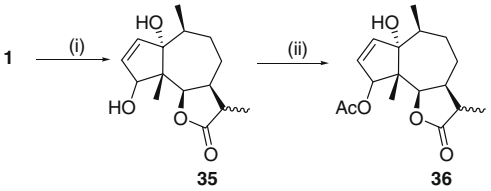
Another important target in the cancer cell is topoisomerase-I/-II and the effect of the compound **11** on topoisomerase-II was also investigated in this study. Bioactivity assay was performed to analyze the inhibitory effect of compound **11** on catalytic activity of topoisomerase-II. The results clearly showed that compound **11** even at low concentration of 10 μM was able to inhibit significantly the activity of topoisomerase-II and it was completely inhibited at



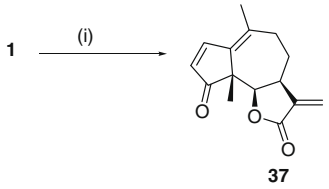

**Scheme 4.** Reagents and conditions: (i) 2.2 equiv NBS/dioxane; (ii) 3.4 equiv NBS/dioxane; (iii) 4.5 equiv NBS/dioxane; (iv) 2.2 equiv NBS/DMF.




**Scheme 5.** Reagents and conditions: (i) *m*-CPBA, DCM, rt.




**Scheme 6.** Reagents and conditions: (i) $NaBH_4$, MeOH, 0 °C to rt; (ii) $Ac_2O$, DCM, DMAP, rt.




**Scheme 7.** Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, rt.

**Table 1**
Evaluation of cytotoxicity and apoptosis induced by parthenin and its analogs

| Compound | $IC_{50}$ value (μM) | | % Sub-G0/G1 population (6 h treatment in HL-60 cells) |
|---|---|---|---|
| | HeLa cells | HL-60 cells | |
| **1** | 60 | 17 | 13.69 |
| **2** | 70 | 17 | 2.61 |
| **3** | 65 | 16 | 3.01 |
| **4** | 65 | 49 | 2.86 |
| **5** | 70 | 41 | 1.90 |
| **6** | 65 | 38 | 2.71 |
| **7** | 73 | 45 | 1.60 |
| **8** | 68 | 42 | 2.33 |
| **9** | 47 | 24 | 6.78 |
| **10** | 31 | 6 | 69.28 |
| **11** | 12 | 3 | 94.10 |
| **12** | 57 | 5 | 58.75 |
| **13** | 25 | 7 | 82.97 |
| **14** | 72 | 46 | 2.17 |
| **15** | 32 | 6 | 81.38 |
| **16** | 68 | 5 | 42.38 |
| **17** | 58 | 9 | 13.89 |
| **18** | 70 | 17 | 2.76 |
| **19** | 73 | 18 | 2.13 |
| **20** | 78 | 46 | 2.44 |
| **21** | 44 | 9 | 19.38 |
| **22** | 68 | 50 | 1.17 |
| **23** | 68 | 32 | 1.95 |
| **24** | 76 | 60 | 4.51 |
| **25** | 74 | 18 | 3.21 |
| **26** | 70 | 42 | 1.90 |
| **27** | 72 | 44 | 1.81 |
| **28** | 72 | 55 | 2.19 |
| **29** | 76 | 44 | 2.05 |
| **30** | 67 | 12 | 1.93 |
| **31** | 76 | 13 | 2.17 |
| **32** | 61 | 8 | 18.33 |
| **33** | 64 | 12 | 3.25 |
| **34** | 67 | 18 | 2.98 |
| **35** | 64 | 48 | 1.43 |
| **36** | 74 | 48 | 0.82 |
| **37** | 74 | 16 | 2.95 |

Effect of parthenin analogs on cell proliferation (IC50 values) was examined in HeLa and HL-60 cells while the apoptosis index was measured in HL-60 cells only. Briefly HL-60 cells $2.0 \times 10^4$/200 μl media and HeLa cells $10^4$/200 μl media in 96 well culture plates were treated with different concentrations (1–100 μM) of parthenin analogs for 48 h. $IC_{50}$ value was calculated in terms of 50% of cell viability as compare to untreated control. Data are representative one of three similar experiments with coefficient of variation of less than 10%. Column 4 of the table represents the percentage of cells that appeared under sub-G0/G1 peak (apoptotic population) during cell cycle analysis of HL-60 cells after 6 h treatment with parthenin and its analogs. Data are representative of one of three similar experiments.

100 μM concentration (Fig. 2). Therefore, inhibition of telomerase expression and the activity of topoisomerase-II make compound **11** an attractive lead.

The present study also generated some important information about SAR of semi-synthetic sesquiterpene lactones. Various inferences drawn from the study include (a) presence of bifunctionality,

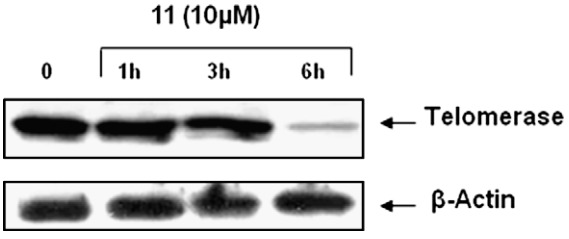



**Figure 1.** Effect of **11** on the expression of telomerase: $2 \times 10^6$ HL-60 cells were treated with or without **11**. Results are representative of one of the three similar experiments.

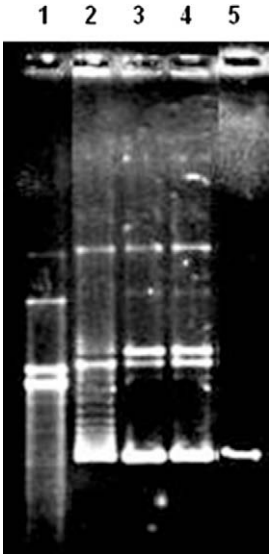



**Figure 2.** Effect of **11** on DNA relaxation activity of topoisomerase-II. Supercoiled plasmid DNA was incubated with **11** (10 and 100 μM) and topoisomerase-II. After termination of reaction samples were run on 0.8% agarose gel. Lane-1: untreated relaxed DNA, lane-2: DNA treated with topoisomerase-II in presence of 10 μM of **11**, lane-3: DNA treated with topoisomerase-II in presence of 100 μM of **11**, lane-4: DNA treated with topoisomerase-II in presence of 100 μM of etoposide, lane-5: Supercoiled DNA.

that is, a cyclopentenone ring or a conjugated side chain ester or α-methylene-δ-lactone in addition to α-methylene-γ-lactone moiety is important for cytotoxicity; (b) any modification in bifunctionality, that is, reduction of either double bond or addition of alkyl groups significantly effected the bioactivity; (c) Baylis–Hillman adducts (**10–16** and **22**) having conjugated aromatic rings displayed improved bioactivity through the generation of a new bifunctionality in the form of exocyclic ring system; (d) on the other hand Baylis–Hillman adducts with α-substitution displayed comparatively low bioactivity possibly due to the loss of bifunctionality (**2**, **4–6**) or because of comparatively lower nucleophilicity of endocyclic double bond (**3**, **7–9**, **17–20**); (e) Baeyer–Villiger oxidation product (**34**) also resulted in enhancement of activity as it resulted in the retention of bifunctionality; (f) Michael addition products e.g. *O*-alkyl analogues (**22–29**) displayed much reduced activity due to elimination of bifunctionality; (g) the brominated products (**30–33**) also displayed improved activity as bromo group can be easily replaced by biological nucleophiles like sulfhydryl group; (h) as expected reduction of keto group (analogues **35–36**) also resulted in loss of activity since it lead to the loss of bifunctionality. It apparent that both the functionalities are involved during interaction with the receptors sites and alteration in any of the functionalities with reduced nucleophilicity, generally results in decreased bioactivity.

In summary, we have synthesized a series of thirty six semi-synthetic analogues of sesquiterpene lactone parthenin with modifications in both the five membered rings, and their cytotoxicity against two cell lines evaluated. One of the molecules, that is, **11** was shown to be the potent inhibitor of both telomerase and topoisomerase-II. This reinforces the view,[9] that compounds of this type exert their biological effects by inhibiting cellular enzyme activity and not through alkylating or impairing DNA function. Thus, novel leads from parthenin can be further developed into potential chemotherapeutic agents in cancer therapy. Particularly the semisynthetic analogue **11** appears to be promising which induced higher cytotoxicity in both HeLa and HL-60 cells at relatively much lower concentrations compared to the parent natural molecule.

## Acknowledgment

We gratefully acknowledge the financial support by Department of Biotechnology, New Delhi.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.089.

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