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Aminoparthenolides as novel anti-leukemic agents: Discovery of the NF-κB inhibitor, DMAPT (LC-1)

Sundar Neelakantan [a], Shama Nasim [a], Monica L. Guzman [b], Craig T. Jordan [b], Peter A. Crooks [a,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA
[b] James P. Wilmot Cancer Center, University of Rochester, Rochester, NY 14642, USA

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ABSTRACT

A series of aminoparthenolide analogs (**6**–**37**) were synthesized and evaluated for their anti-leukemic activity. Eight compounds exhibited good anti-leukemic activity with LD_{50}'s in the low μM range (1.5–3.0 μM). Compounds **16**, **24** and **30** were the most potent compounds in the series, causing greater than 90% cell death at 10 μM concentration against primary AML cells in culture, with LD_{50} values of 1.7, 1.8 and 1.6 μM.

Parthenolide (PTL, **1**, Fig. 1) isolated from *Tanacetum parthenium* (commonly referred as feverfew), is a naturally occurring sesquiterpene lactone used in the treatment of fever, migraine headaches, rheumatoid arthritis, and also as an anti-inflammatory agent.[1–3] PTL is a neutral, lipophilic lactone with low polarity and contains an α-methylene-γ-lactone ring and an epoxide moiety that interact with nucleophilic sites on biological macromolecules.[4] The PTL molecule and several structurally related analogs have become topics of recent interest because of their potent anti-tumor and cytotoxic properties.[5–13]

PTL and its analogs have been shown to promote apoptosis by inhibiting the activity of the NF-κB transcription factor complex, and thereby down-regulating anti-apoptotic genes under NF-κB control.[4,6,14–17] Recently, it has been shown that parthenolide induces robust apoptosis of primary acute myeloid leukemic (AML) cells.[18,19] Notably, PTL causes cell death of AML stem and progenitor cells in vitro, with no observed toxicity towards normal hematopoietic cells. The apoptosis induced by PTL is not solely due to NF-κB inhibition, but rather arises from a broad set of biological responses, which include activation of p53 and an increase in reactive oxygen species. However, despite promising in vitro activity, this potent natural product has a major limitation which precludes its further development as a therapeutic agent, that is, its poor water-solubility (0.169 μM/mL maximum solubility in serum),[20] thus limiting its potential as a promising clinical agent.

Initial structure–activity studies carried out on the PTL molecule involved reduction of the exocyclic double bond by catalytic hydrogenation over palladium-on-charcoal to afford compound **2**,[21] which was inactive when evaluated for anti-leukemic activity against primary AML cells in culture (Table 1). Thus, the α-methylene-γ-lactone moiety in PTL appears to be a critical functionality for its cytotoxic effect. Interestingly, introduction of an additional epoxide moiety at the C1–C10 double bond with *m*-chloroperoxy benzoic acid, afforded compound **3**, which was also inactive in the 10 μM probe cytotoxicity assay (Table 1). Oxidation of the C14 methyl group of PTL via selenium dioxide/*t*-butyl hydroperoxide afforded a mixture of aldehyde **4** and the corresponding alcohol, **5**,[22] both of which were inactive as anti-leukemic agents (Table 1).

Our laboratory has recently been successful in overcoming the poor water-solubility of PTL without loss of its anti-leukemic activity, by derivatizing PTL into several aralkylamino analogs, which can then be converted into water-soluble organic salts.[23] We have now carried out the synthesis, structure–activity relationships (SAR) and anti-leukemic activity of a novel series of second generation water-soluble aminoparthenolide analogs derived from a variety of aliphatic primary and secondary amines.

To obtain a sub-library of these water-soluble PTL analogs, we exploited the highly electrophilic α-methylene-γ-lactone ring nat-

* Corresponding author. Tel.: +1 859 257 1718; fax: +1 859 257 7585.
E-mail address: pcrooks@email.uky.edu (P.A. Crooks).

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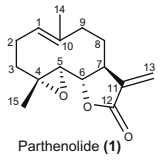
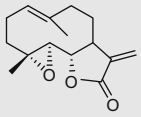
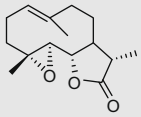
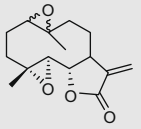
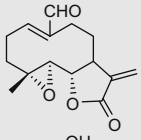
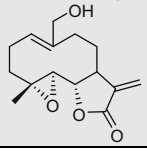


Figure 1. The structure of parthenolide (PTL, **1**).

Table 1
Anti-leukemic activity of parthenolide (**1**) and analogs **2-5**

Compound #	Structure	Anti-leukemic activity (% primary AML cell death at 10 μM)
1		84
2		0
3		0
4		0
5		0

urally present in the molecule. Compounds containing an α-methylene-γ-lactone ring are excellent Michael acceptors, and undergo facile Michael addition. Michael addition reactions were performed using a variety of primary and secondary aliphatic amines, and the amino parthenolide analogs thus obtained (**6**–**37**) were converted into water-soluble organic acid salts, thereby increasing their water solubility and bioavailability (see Fig. 2).

The Michael addition of aliphatic amines with PTL was found to be highly stereospecific and yielded exclusively a single stereoisomer with the *R*-configuration at the newly formed C-11 chiral carbon. None of the alternative diastereomer with the '*S*' configuration at C-11 was observed in the reaction mixture. We have confirmed the C-11 *R*-configuration in many of our synthesized aminoparthenolide analogs by single crystal X-ray analysis. The ORTEP structure of a representative compound, 13-(*N*,*N*-dimethyl)amino-4α,5β-epoxy-4,10-dimethyl-6α-hydroxy-12-oic acid-γ-lactone-germacra-1(10)-ene mono-fumarate (**16**), is illustrated in Figure 3.[24] The exclusive formation of the C-11 *R* diastereomer is due to

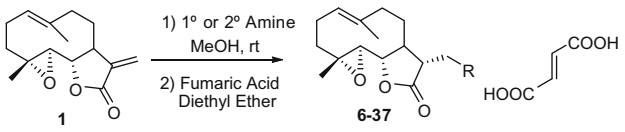


Figure 2. Synthesis of aminoparthenolide analogs.

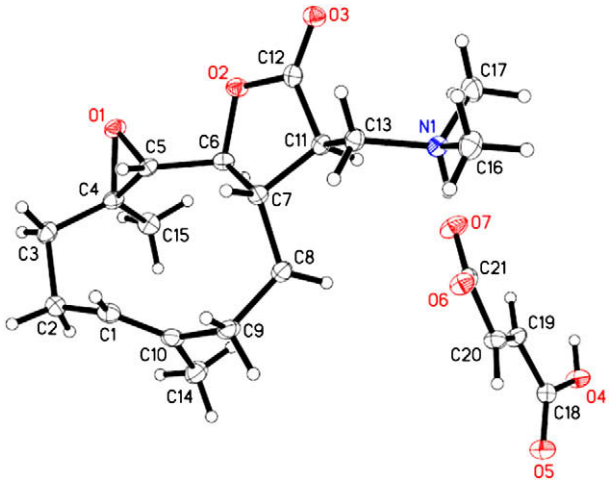


Figure 3. Single crystal structure of 13-(*N*,*N*-dimethyl)amino-4α,5β-epoxy-4,10-dimethyl)-6α-hydroxy-12-oic acid-γ-lactone-germacra-1(10)-ene (**16**, DMAPT, LC-1).

the protonation of the enolate formed during Michael addition, which occurs exclusively from the *exo* face of the molecule, resulting in an *R* configuration at C-11 in the product. The above observation is consistent with the structure of Michael addition products from other structurally related sesquiterpene lactones reported in the literature.[25,26]

The aminoparthenolide analogs (**6**–**37**) were synthesized by reacting PTL with a variety of aliphatic primary and secondary amines. PTL (25 mg, 0.1 mmol) was dissolved in 8 mL of methanol, the appropriate amine (0.15 mmol) added, and the mixture was stirred under ambient conditions for 6–8 h. The crude product was subjected to flash silica gel column chromatography to afford the pure aminoparthenolide. The compounds synthesized and their anti-leukemic activities (10 μM concentration) in the probe assay are shown in Table 2. Selected analogs were also evaluated in full dose-response assays to generate LD_{50} values, which are also given in Table 2.

The 10 μM probe assays were carried out as follows: one million primary acute myelogenous leukemia (AML) cells, obtained from volunteer donors with informed consent, were cultured in serum free medium (SFM).[27] Cells were treated for 24 h in the presence or absence of the appropriate PTL analog at a probe concentration of 10 μM. Selected analogs were then evaluated in a dose–response assay over the concentration range 2.5–20 μM, to obtain LD_{50} values. Cell viability was determined as previously described;[28] briefly, cells were washed with cold PBS and resuspended in 200 μL of Annexin binding buffer (10 mM HEPES/NaOH pH 7.4; 140 mM NaCl; 2.5 mM $CaCl_2$). Annexin-V and 7-amino-actinomycin (7-AAD) were added and the tubes were incubated at ambient temperature in the dark for 15 min. Cells were then diluted with 200 μL of Annexin binding buffer and analyzed immediately by flow cytometry. Viable cells were scored as Annexin V negative/7-AAD negative. Data provided are normalized to untreated control specimens. LD_{50} values were determined using Calcusyn software (Biosoft).

From the structure–activity data shown in Table 2, it is evident that several of the aminoparthenolide analogs obtained from the reaction of secondary aliphatic amines with PTL (compounds **16**–**36**) exhibit significant anti-leukemic activity in the AML assay compared to those analogs obtained from primary amines (compounds **6**–**15**). In the acyclic aliphatic secondary amine series, the methylpropylamino analog **21** was the most potent analog in the 10 μM probe assay, and exhibited an LD_{50}

Table 2
Anti-leukemic activity of amino parthenolide analogs against AML cells in culture

Compound	R	Anti-leukemic activity (% cell death at 10 μM)	LD_{50}[a] (μM)
1	–	84	1.4
6	H_3C–NH	4.0	18
7	H_3C NH	2.0	31
8	NH	6.0	–
9	NH	7.0	–
10	HO NH	0	–
11	HO NH	0	–
12	H_2N NH	0	–
13	H_2N NH	0	–
14	N–NH	0	–
15	NH	0	–
16	N	93	1.7
17	N CH_3	80	2.5
18	N	60	–
19	H_3C N	78	–
20	N	20	–
21	H_3C N H CH_3	95	3.2
22	H_3C N H CH_3	68	2.8
23	H_3C N CH_3	46	4.6
24	H_3C N OH	86	1.8
25	H_3C N CH_3	0	–
26	H_2C N CH_2	0	–
27	N	22	–
28	N	82	2.7
29	N	76	2.4
30	H_3C N	83	1.6
31	O N	20	14
32	N	15	–
33	N	13	–
34	N	12	–
35	N CH_3	0	15
36	HO OH HO O HN OH	0	–
37	HO OH HO O H_3C–N OH	0	–

[a] Concentration of analog causing death of 50% of the cell population in primary cultures of AML cells.

of 3.2 μM. The dimethylamino analog **16** was the second most potent analog in the series (93% cell death at 10 μM in the probe assay; LD_{50} = 1.7 μM). In the symmetrical secondary aliphatic amine series, as one moves from dimethylamino (**16**) to higher dialkylamino analogs, that is, diethylamino (**18**), diisopropylamino (**20**), dipropylamino (**25**), and diallylamino **(26)**, the anti-leukemic activity gradually decreases. It was also observed that aminoparthenolide analogs with at least one *N*-methyl group exhibited better antileukemic activity than secondary aminoparthenolide analogs lacking an *N*-methyl group. In secondary aminoparthenolide analogs that contained an *N*-methyl group, increasing the chain length of the other alkyl moiety resulted in a gradual decrease in antileukemic activity. Thus, the *N*-methyl, *N*-propyl analog (**21**), the *N*-methyl, *N*-butyl analog (**22**), and the *N*-methyl, *N*-pentyl analog (**23**) exhibited 95%, 68% and 46% cell death in the AML probe assay, respectively. The *N*-methyl, *N*-2-hydroxyethyl analog (**24**) exhibited 86% cell death in the probe assay and afforded an LD_{50} of 1.8 μM, while no observable cell death was observed at 10 μM for the structurally related N-demethylated analog **10**.

Among the aliphatic cyclic amino analogs examined, ring size appeared to play an important role in antileukemic activity. The optimum ring size was found to be between five and six; analogs with smaller or greater ring size had significantly reduced antileukemic activity in the 10 μM probe assay. Thus, the pyrrolidino analog (**28**) and the piperidino analog (**29**) afforded 82% and 76% cell death in the 10 μM probe assay, with LD_{50}'s of 2.7 and 2.4 μM, respectively, whereas the 3-, 7- and 8-membered cyclic amino analogs **27**, **32** and **33** exhibited values in the range 12–22% cell death in the probe assay.

Introduction of a heteroatom, such as oxygen, into the six-membered cyclic amine moiety (e.g., analog **29**, 76% cell death at 10 μM; LD_{50} = 2.4 μM) to afford a morpholino analog (analog **31**, 20% cell death at 10 μM) resulted in a significant decrease in cytotoxicity, whereas introduction of a methyl group at C-4 of the piperidine ring in analog **29** to afford **30** (83% cell death at 10 μM; LD_{50} = 1.6 μM), increased anti-leukemic activity (Table 2). Thus, compounds **16**–**17**, **21**–**22**, **24**, **28**–**30** exhibited the most promising anti-leukemic activities, with LD_{50} values in the low μM range (1.6–2.7 μM); full characterization data on four of the more potent analogs are provided.[29]

In summary, 31 aminoparthenolide analogs have been synthesized and evaluated for anti-leukemic activity against primary AML cells in culture. All of the synthesized compounds were more water-soluble than PTL; some analogs exhibited 100-fold greater water-solubility than PTL. A range of antileukemic activities was observed, with several analogs exhibiting LD_{50} values between 1 and 2 μM.

Compounds **16**, **24**, and **30** were the most potent compounds in the series causing 80–90% cell death of AML cells in the 10 μM probe assay, and affording LD_{50} values of 1.7, 1.8 and 1.6 μM, respectively. 13-(*N*,*N*-Dimethyl)amino-4α,5β-epoxy-4,10-dimethyl-6α-hydroxy-12-oic-acid-γ-lactone-germacra-1(10)-ene (**16**; DMAPT, LC-1) was selected as a lead compound, based on favourable pharmacokinetic and pharmacodynamic properties (unpublished results).

Aminoparthenolides have been reported to undergo retro-Michael degradation to parthenolide and the parent amine in the presence of nucleophiles.[30,21] Thus, we were concerned that the aminoparthenolides might be degrading in the cell culture buffer during the AML cell assay. Stability studies on DMAPT in the HEPES buffer utilized in the antileukemic cell assay by HPLC over the time-course of the experiment indicated that <3% of DMAPT had degraded to parthenolide at 24 h. This indicates that DMAPT is not degrading and releasing significant amounts of parthenolide into the culture media over time.

This stability data for DMAPT is consistent with the variable SAR obtained for the 31 aminoparthenolide analogs examined. Furthermore, pharmacokinetic studies in the rat indicate that DMAPT has an oral bioavailability of around 70%, and is biotransformed into a major metabolite: 13-(*N*-methyl)amino-4α,5β-epoxy-4,10-dimethyl-6α-hydroxy-12-oic-acid-γ-lactone-germacra-1(10)-ene via oxidative N-demethylation (unpublished data). Only very small amounts of parthenolide were detected in plasma over 8 h post oral dosing.

Recently, more detailed investigations into DMAPT's anti-leukemic activity have been carried out against cultured AML, CML and CLL cells, as well as in in vivo studies in dogs with canine leukemia.[31,32] In addition, we have also shown that DMAPT causes a dose-dependent decrease in the binding of the Rel-A subunit of NF-κB to DNA, thereby triggering apoptosis.[33] Also, transcription of three NF-κB-regulated genes, *CFLAR*, *BCL2*, and *BIRC5*, which have been implicated in CLL cell survival and resistance to chemotherapy, were all significantly suppressed by DMAPT.[32] DMAPT is currently being evaluated in AML patients in a first-in-man study in the United Kingdom.

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29. Representative characterization data for four of the most active compounds **16**, **17**, **24,** and **30** are provided below: *13-(N,N-dimethyl)-amino-4α,5β-epoxy-4,10-di methyl-6α-hydroxy-12-oic acid-γ-lactone-germacra-1(10)-ene monofumarate* (**16**): mp: 183–184 °C; yield, 95%. ^{1}H NMR (300 MHz, D_2O): δ 6.67 (2H, s, (HOOC–C*H*)$_2$–), 5.24 (1H, dd, *J* = 2.1, 12.0 Hz, 1-C*H*), 4.30 (1H, t, *J* = 9.0 Hz, 6-C*H*), 2.98 (6H, s, *N*–(CH_3)$_2$), 1.88–3.60 (13H, m, 2-CH_2, 3-CH_2, 8-CH_2, 9-CH_2, 13-CH_2, 5-C*H*, 7-C*H*, 11-C*H*), 1.70 (3H, s, 14-CH_3), 1.34 (3H, s, 15-CH_3) ppm. ^{13}C NMR (75 MHz, D_2O): δ 179.8 ((HOOC–CH)$_2$–), 173.8 (12-C=O), 138.2 ((HOOC–CH)$_2$–),137.2 (10-C), 127.3 (1-C), 85.8 (6-C), 69.2 (*N*–(CH_3)$_2$), 67.1 (5-C), 58.3 (4-C), 49.6 (7-C), 44.9 (11-C), 42.5 (9-C), 38.1 (3-C), 30.9 (8-C), 26.1(2-C), 18.7 (15-C), 18.6 (14-C) ppm. Elemental Anal. Calcd for $C_{21}H_{31}NO_7$: C, 61.60; H, 7.63; N, 3.42. Found: C, 61.81; H, 7.64; N, 3.47. *13-(N-ethyl-N-methyl)-amino-4α,5β-epoxy-4,10-dimethyl}-6α-hydroxy-12-oicacidγ-lactone-germacra-1(10)-ene monofumarate* (**17**): mp: 156–157 °C; yield, 80%. ^{1}H NMR (400 MHz, D_2O) δ 6.70 (2H, s, (HOOC–C*H*)$_2$–), 5.17 (1H, app d, *J* = 12.0 Hz, 1-C*H*), 4.33 (1H, t, *J* = 12.0 Hz, 6-C*H*), 2.95 (3H, s, *N*-CH_3), 1.71 (3H, s, 14-CH_3), 1.36 (3H, s, 15-CH_3), 1.27–3.75 (15H, m, 2-CH_2, 3-CH_2, 8-CH_2, 9-CH_2, 13-CH_2, *N*-CH_2-CH$_3$ 5-C*H*, 7-C*H*, 11-C*H*) 1.24 (3H, t, *J* = 12.0 Hz, *N*–CH$_2$–CH_3) ppm; ^{13}C NMR (75 MHz, D_2O) δ 179.1 ((HOOC-CH)$_2$-), 173.0 (12-C=O), 137.3 ((HOOC–CH)$_2$–), 136.3 (10-C), 126.5 (1-C), 85.2 (6-C), 68.4 (*N*-CH$_3$), 66.6 (5-C), 55.5 (4-C), 55.2 (13-C), 52.2 (*N*–CH$_2$–CH$_3$), 49.1 (7-C), 44.2 (11-C), 42.0 (9-C), 37.6 (3-C), 30.4 (8-C), 25.6 (2-C), 18.1 (15-C), 18.0 (14-C), 10.7 (*N*-CH$_2$-CH$_3$) ppm. MALDI-TOFMS: 308 *m/z* [M$^+$]: Calcd. $C_{18}H_{29}NO_3$ (307.2142) observed (307.2147), EI MS *m/z* 307. Elemental Anal. Calcd for $C_{22}H_{33}NO_7$: C, 62.39; H, 7.85; N, 3.31. Found: C, 61.98; H, 7.85; N, 3.30. *13-(N-(2-hydroxyethyl)-N-methyl)-amino-4α,5β-epoxy-4,10-dimethyl-6α-hydroxy-12-oicacid-γ-lactone germacra-1(10)-ene monofumarate* (**24**): mp: 122–124 °C; yield, 78%. ^{1}H NMR (400 MHz, D_2O) δ 6.68 (2H, s, (HOOC–C*H*)$_2$–), 5.10 (1H, app. d, *J* = 10.0 Hz, 1-C*H*), 4.34 (1H, t, *J* = 9.2 Hz, 6-C*H*), 3.95 (2H, t, *J* = 5.2 Hz, *N*–CH$_2$–CH_2–OH), 3.01 (3H, s, *N*-CH_3), 1.34 (3H, s, 14-CH_3), 1.69 (3H, s, 15-CH_3), 1.89–3.70 (15H, m, 2-CH_2, 3-CH_2, 8-CH_2, 9-CH_2, 13-CH_2, *N*–CH_2, 5-C*H*, 7-C*H*, 11-C*H*) ppm; ^{13}C NMR (75 MHz, D_2O): δ 179.5 ((HOOC–CH)$_2$–), 173.0 (12-C=O), 137.7 ((HOOC–CH)$_2$–), 136.5 (10-C), 126.8 (1-C), 85.3 (6-C), 68.7 (*N*-CH$_3$), 67.0 (5-C), 58.4 (4-C), 57.0 (*N*–CH$_2$–CH$_2$–OH) 56.5 (13-C), 52.5 (*N*–CH$_2$–CH$_2$–OH), 49.3 (7-C), 44.3 (11-C), 42.0 (9-C), 37.6 (3-C), 30.3 (8-C), 25.6 (2-C), 18.2 (15-C), 18.0 (14-C). MALDI-TOFMS: 324 *m/z* [M$^+$]: Calcd. $C_{18}H_{29}NO_4$ (323.2091) observed (323.2090). EI MS (M$^+$) *m/z* 323. Elemental Anal. Calcd for $C_{22}H_{33}NO_8{\cdot}H_2O$: C, 57.75; H, 7.71; N, 3.06. Found: C, 57.73; H, 7.32; N, 3.42. *13-(4-Methylpiperidin-1-yl)-amino-4α,5β-epo xy-4,10-dimethyl-6α-hydroxy-12-oic acid-γ-lactone-germacr-a-1(10)-ene monofumarate* (**30**): mp: 132–134 °C; yield, 70%. ^{1}H NMR (400 MHz, CD_3OD) δ 6.60 (2H, s, (HOOC–C*H*)$_2$–), 5.19 (1H, d, *J* = 10.0 Hz, 1-C*H*), 4.21 (1H, t, *J* = 9.2 Hz, 6-C*H*), 1.29 (3H, s, 14-CH_3), 1.65 (3H, s, 15-CH_3), 1.12–3.60 (22H, m, 2-CH_2, 3-CH_2, 8-CH_2, 9-CH_2, 13-CH_2, 2 × *N*–CH_2, 2 × *N*–CH$_2$–CH_2–, piperidino 4′-C*H*, 5-C*H*, 7-C*H*, 11-C*H*), 0.97 (3H, d, *J* = 6.3 Hz, piperidino 4-CH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD) δ 177.3 ((HOOC-CH)$_2$-), 170.9 (12-C=O), 136.1 ((HOOC–CH)$_2$–), 135.7 (10-C), 126.2 (1-C), 84.2 (6-C), 67.3 (5-C), 63.3 (4-C), 55.1 (2 × piperidino *N*-CH$_2$), 56.2 (13-C), 49.5 (7-C), 44.5 (11-C), 41.9 (9-C), 37.6 (3-C), 32.5 (2 × piperidino *N*–CH$_2$–CH$_2$), 30.0 (piperidino H$_3$C–C*H*), 29.9 (8-C), 25.1 (2-C), 21.4 (piperidino-4-CH_3), 17.5 (15-C), 17.1 (14-C) ppm. MALDI-TOFMS: *m/z* 348 [M$^+$]: Elemental Anal. Calcd for $C_{25}H_{37}NO_7$: C, 64.77; H, 8.05; N, 3.02. Found: C, 64.73; H, 8.29; N, 3.27. EI MS (M$^+$) *m/z* 347.
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