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Antileukemic activity of aminoparthenolide analogs

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ABSTRACT

A series of aminoparthenolide analogs have been synthesized through a diastereoselective conjugate addition of several primary and secondary amines to the α-methylene-γ-butyrolactone function of the very lipophilic sesquiterpene lactone, parthenolide. Seventeen of the above amines derivatives were evaluated in a full panel of 60 cancer cell lines for anticancer activity. Compound **12**, derived from tyramine, was found to be cytostatic as well as cytotoxic toward acute lymphoblastic leukemia cells (ALL, CCRF-CEM) at nanomolar concentrations, while the (*R*)-(1,2,3,4-tetrahydro-1-naphthyl)amino derivative **9** was found to be cytostatic toward human anaplastic large T-cell lymphoma (SR) cells at concentrations below 10 nM.

The cyclization of Farnesyl pyrophosphate (FPP) in plants gives rise to the sesquiterpene germacrene A (**1**) (Fig. 1), which is oxidized and cyclized through a number of biosynthetic transformations to a subfamily of structurally related sesquiterpenes. Parthenolide (**2**) is one such metabolite that results from the epoxidation of costunolide (**3**) (Fig. 1) at the 4,5-position.[1] Parthenolide has been isolated from several different species in the *Asteraceae* (Composita) family, feverfew (*Tanacetum parthenium*) being one of them. In the past few years, an increasing interest has been observed in this sesquiterpene lactone, due to its remarkable cytotoxic, antitumor, antiviral, and antileishmanic properties.[2–8] Recently, it has been demonstrated that parthenolide may induce tumor apoptosis through the inhibition of NF-κB activity.[9] Unfortunately, parthenolide has very poor water-solubility, making it unsuitable for clinical development as a drug entity.

In the past few years, our laboratories have been involved in the design and synthesis of water-soluble analogs of parthenolide.[2] The conjugated exocyclic olefinic moiety at C-13 readily undergoes Michael addition when treated with amines in a protic solvent. Earlier, an extensive series of analogs resulting from the addition of aliphatic amines to parthenolide was evaluated as potential antileukemic agents. Generally, the antileukemic activity of parthenolide was retained in these water-soluble analogs, which were active in the 5–10 μM concentration range. Additionally, the high survival rates for normal cells treated with the aminoparthenolide analogs were found to be similar to those observed with parthenolide.[2] Preliminary pharmacokinetic analysis (unpublished results) indicates that the water-soluble aminoparthenolide analogs possess markedly improved drugability and bioavailability compared to the parent compound.

In this report, we describe the synthesis and antileukemic properties of a series of parthenolide analogs derived from arylalkyl, heteroarylalkyl and alkyl amines. The synthesis of parthenolide analogs **4**–**20** was carried out utilizing the one-step procedure outlined in Scheme 1.

Treatment of a 40 mM methanolic solution of **2** with one equivalent of an appropriate amine led to significant consumption of **2** within 12 h at room temperature. The reaction product was purified by chromatography and/or recrystallization. In all cases, a moderate yield (40–70%) of a single diastereomeric product was obtained, together with unreacted **2**, which persisted even after prolonged reaction times, addition of excess amine, or increases in temperature. The ^{1}H NMR spectra of these amine analogs showed the absence of the two olefinic proton resonances at 5.62 and 6.34 ppm in the parthenolide molecule, and the appearance of new methine and methylene resonances in the DEPT spectrum of ^{13}C NMR was apparent, indicating that amination at C13 of the conjugated exocyclic double bond had occurred. In every reaction product, the ^{13}C NMR spectrum was also consistent with a single diastereomeric form of the expected product.[10] Single crystal X-ray analysis of representative compounds **4** (a benzylamine ad-

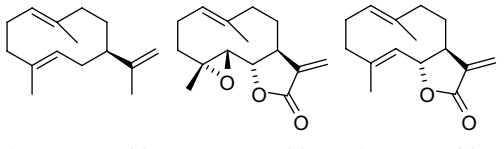

Figure 1. Structures of parthenolide (**2**) and related germacrenes.

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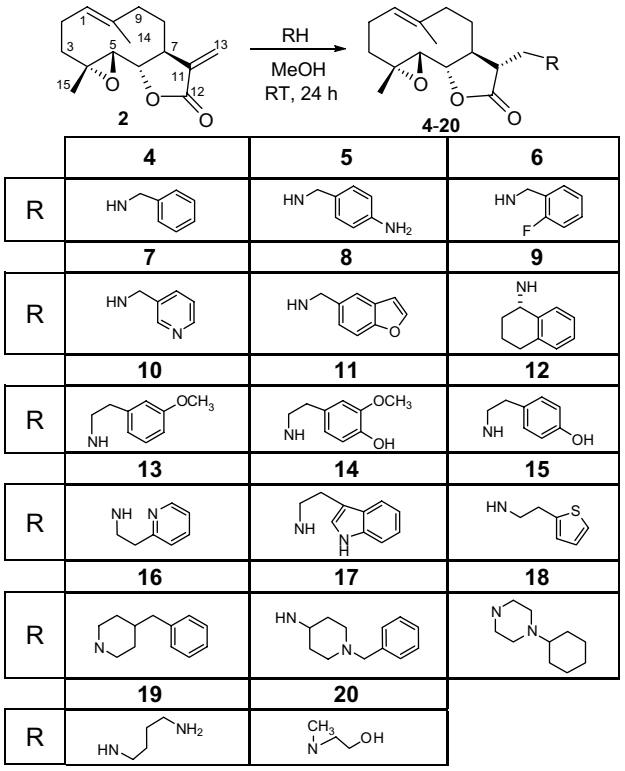

Scheme 1. Synthesis of 17 aminoparthenolide analogs.

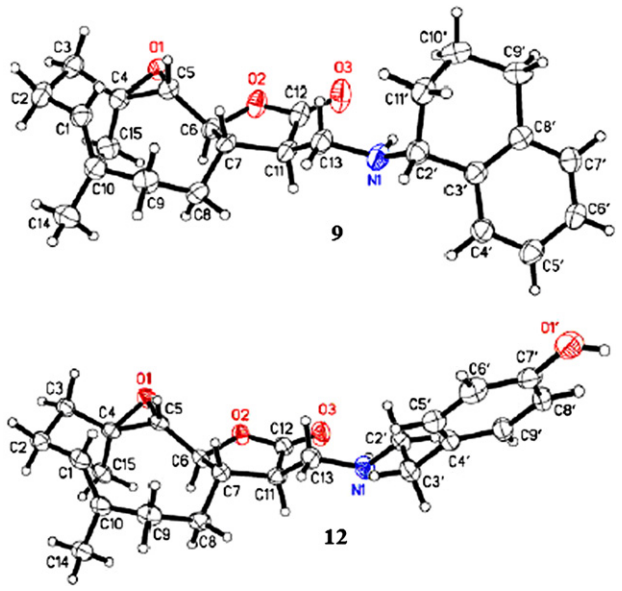

Figure 2. X-ray crystal structures of **9** and **12**.

duct), **9** [an (*R*)-(1,2,3,4-tetrahydro-1-naphthylamino) adduct)], and **12** (a tyramine adduct) established the *R* configuration at the newly formed stereocenter at C-11 in all three analogs (Fig. 2).[11–15] The seventeen amine adducts (Scheme 1) were then treated with 1 equiv. of fumaric acid in diethyl ether to obtain the fumarate salts.

The conjugate addition of amines to α,β-unsaturated esters is known to be a reversible phenomenon in many cases, especially when carried out in a protic solvent (vide infra). It is likely that the addition of amines to parthenolide under the reaction conditions employed would be thermodynamically controlled. Quenching of the intermediate enolate **21** of the conjugate addition from the *endo* face of the exocyclic double bond would give rise to diastereomer **22** (Fig. 3), while quenching from the *exo* face would give rise to diastereomer **23**. While **23** is sterically hindered by 1,3-diaxial interactions of the C-6 methine hydrogen with the axial hydrogens of the C-8 methylene group and the new C-13 methyleneamino group, no such strain is visible in **22**. This difference serves to explain the high diastereoselectivity observed in this conjugate addition reaction.

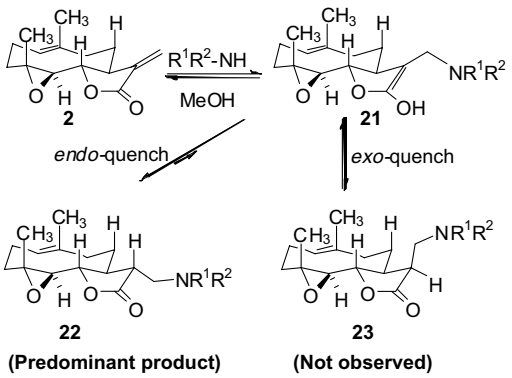

Figure 3. Proposed mechanism for diastereoselective conjugate formation.

All of the aminoparthenolide analogs (**4**–**20**) were submitted to the National Cancer Institute (Bethesda, MD) and were selected for evaluation in the full panel of 60 human cancer cell lines derived from nine human cancer cell types. These cell lines have been grouped into disease sub-panels that represent leukemia, lung, colon, central nervous system (CNS), melanoma, renal, ovary, breast, and prostate cancer cells. In this screen, growth inhibitory and cytotoxic effects were measured as a function of the variation of optical density as a percentage of control.[16,17] All the analogs have been evaluated over the concentration range 10^{-4} to 10^{-8} M.[16,17] In Tables 1 and 2, anticancer effects are expressed in terms of three parameters: the GI_{50} value (denoting the concentration that causes 50% inhibition in cell growth), the TGI value (denoting the concentration that causes complete inhibition of cell growth), and LD_{50} (the concentration that is lethal to 50% of all cells). If any of the above effects are not observed or are exceeded over the concentration range 10^{-4} to 10^{-8} M, the value for that parameter is expressed as greater or lesser than the highest or lowest concentration tested.[16] All the aforementioned aminoparthenolide analogs were found to possess weak anticancer activity in all the 54 non-leukemic cell lines in which their GI_{50}, TGI, and LD_{50} values were consistently greater than 10 μM. However, two compounds, **9** and **12**, were found to possess potent antileukemic activity, details of which are shown in Tables 1 and 2. It is clear from these data that compound **12**, which incorporates a tyramine moiety, is both cytostatic and cytotoxic toward human caucasian acute lymphoblastic leukemia (CCRF-CEM) cells at concentrations lower than 10 nM.

Compound **12** potently inhibits the growth of CCRF-CEM cells, exhibiting GI_{50} and TGI values of less than 10 nM. Compound **12** is also weakly active against HL-60 (human caucasian acute lymphoblastic leukemia), K-562 (human caucasian chronic myelogenous leukemia), and SR (human anaplastic large T-cell lymphoma) cell lines, exhibiting growth inhibition in the range of 200–400 nM. Compound **9**, which incorporates the (*R*)-(1,2,3,4-tetrahydro-1-naphthyl)amino substituent, appears to be selectively cytostatic toward the SR (human anaplastic large T-cell lymphoma) cell line, showing 50% growth inhibition at concentrations lower than 10 nM and fully inhibiting growth at a concentration of 41 nM. Table 2 shows the cytotoxic activity of parthenolide

Table 1
Growth inhibitory effects of parthenolide analogs **4**–**20** on six-cultured cancer cell lines

Compound	CCRF-CEM* (μM)		HL-60 (TB)* (μM)		K-562* (μM)		MOLT-4* (μM)		RPMI-A226* (μM)		SR* (μM)	
	GI_{50}[a]	TGI[b]	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
4	4.96	31.50	19.60	46.70	36.00	100	23.40	62.80	13.10	37.10	Nd[c]	Nd[b]
5	16.50	75.10	20.70	50.50	31.00	>100	31.70	95.00	19.00	58.50	Nd[b]	Nd[b]
6	17.50	44.00	11.50	28.20	6.28	19.30	21.30	47.40	20.90	46.50	11.00	29.80
7	23.40	86.70	30.40	70.60	42.00	>100	38.50	>100	24.90	89.80	6.93	36.10
8	6.13	25.10	10.30	25.70	5.02	18.40	18.00	40.70	17.00	36.80	15.00	35.10
9	Nd[b]	Nd[b]	5.06	26.60	2.29	56.20	1.55	1.05	15.20	39.80	<0.01	0.041
10	Nd[b]	Nd[b]	4.09	14.20	3.49	11.00	20.70	44.90	13.00	30.00	10.10	27.40
11	5.29	68.30	16.40	41.80	29.80	>100	23.80	66.20	12.00	50.20	1.79	6.78
12	<0.01	<0.01	2.38	10.80	3.07	12.20	36.20	22.00	12.60	28.70	0.351	13.60
13	6.11	36.50	18.10	40.80	35.00	95.80	19.50	48.00	13.30	40.20	Nd[b]	Nd[b]
14	6.87	63.30	22.70	49.80	32.50	>100	34.20	>100	14.00	42.70	Nd[b]	Nd[b]
15	8.87	58.40	16.70	40.70	28.30	>100	25.70	74.60	13.20	44.20	Nd[b]	Nd[b]
16	6.41	32.20	18.50	43.00	24.70	90.80	24.80	64.60	12.70	35.00	Nd[b]	Nd[b]
17	22.10	56.60	13.50	29.60	12.90	27.70	20.30	48.20	22.20	52.10	18.10	42.60
18	>100	>100	19.10	47.70	31.30	>100	29.60	>100	45.20	>100	43.90	>100
19	17.40	51.70	15.40	37.10	18.60	46.40	19.50	46.60	19.80	51.20	12.10	37.60
20	2.28	6.79	4.12	18.80	5.81	41.90	4.84	26.70	3.66	19.00	Nd[b]	Nd[b]

*CCRF-CEM, human caucasian acute lymphoblastic leukemia; HL-60 (TB), human promyleocytic leukemia cells with Tryptan Blue staining; K-562, human caucasian chronic myelogenous leukemia; MOLT-4, human acute T lymphoblastic leukemia; RPMI-A226, human B-lymphocyte cell line; SR, human anaplastic large T-cell lymphoma.
[a] GI_{50}, concentration of compound that halves cellular growth.
[b] TGI, concentration of compound that halts cellular growth.
[c] Nd, not determined.

Table 2
Cytotoxic effects of parthenolide analogs **4**–**20** on six leukemia cell lines expressed as LC_{50} values

Compound	CCRF-CEM (μM)	HL-60 (TB) (μM)	K-562 (μM)	MOLT-4 (μM)	RPMI-A226 (μM)	SR (μM)
4	>100[a]	>100	>100	>100	>100	Nd[b]
5	>100	>100	>100	>100	>100	Nd[b]
6	>100	69.10	46.40	>100	>100	80.80
7	>100	>100	>100	>100	>100	>100
8	94.90	63.80	50.60	92.00	79.40	82.10
9	>100	>100	>100	>100	>100	16.00
10	Nd[b]	56.10	56.60	97.10	69.20	74.60
11	>100	>100	>100	>100	>100	>100
12	<0.01	42.30	61.90	69.20	65.40	50.00
13	>100	91.80	>100	>100	>100	Nd[b]
14	>100	>100	>100	>100	>100	Nd[b]
15	>100	98.80	>100	>100	>100	>100
16	>100	>100	>100	>100	95.90	Nd[b]
17	>100	65.00	59.40	>100	>100	>100
18	>100	>100	>100	>100	>100	>100
19	>100	89.30	>100	>100	>100	>100
20	83.60	67.20	>100	>100	>100	Nd[b]

[a] LC_{50}, concentration of compound that halves cell population through cell death.
[b] Nd, not determined.

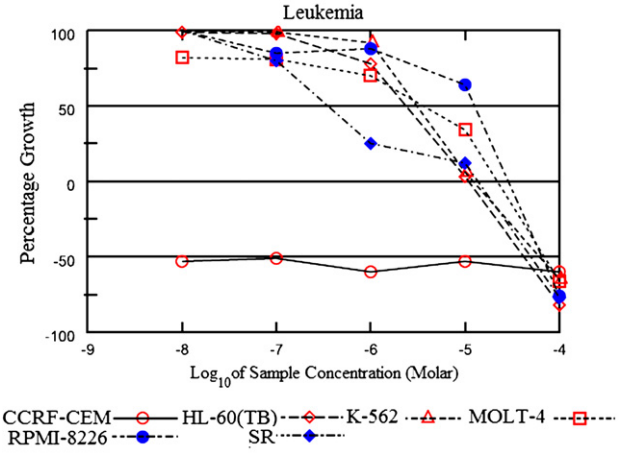

Figure 4. Growth inhibition curve showing the effect of **12** on human leukemia cell lines.

analogs **4**–**20**. Compound **12** was lethal to CCRF-CEM cells at 10 nM, while its toxicity toward the other leukemia cell lines in this screen was relatively weak. It is also clear that although analog **9** exhibits growth inhibition in SR-60 cells at concentrations lower than 10 nM, it is lethal toward this cell line only at much higher concentrations (16 μM). The antiproliferative activity of parthenolide and the underlying mechanism of its cytotoxicity against cancer cells have recently been the subject of several recent studies. Earlier studies have shown that parthenolide induces robust apoptosis in primary human acute myelogenous leukemia (AML) cells and blast crisis chronic myelogenous leukemia (CML) cells, while sparing normal hematopoietic cells. In addition, parthenolide has been shown to preferentially target AML progenitor and stem cell populations. There exists a direct correlation between NF-κB inhibition and the propensity for AML cells to undergo apoptosis.[9] Parthenolide-mediated apoptosis is strongly associated not only with inhibition of NF-κB, but also with pro-apoptopic activation of p53, and an increase in reactive oxygen species (ROS). Additionally, we have recently shown that the dimethylamino adduct of parthenolide (LC-1) causes a decrease in the binding of the Rel-A subunit of NF-κB to DNA, triggering an apoptotic state.[4] Previous studies[2,9] have demonstrated that parthenolide is cytotoxic toward acute and chronic myelogenous leukemia cells (AML and CML) at low micromolar concentrations. Studies by Ralstin et al.[18] showed that parthenolide concentrations between 2.5 and 10 μM caused growth inhibition in Hep3B, HepG2, and PLC cells, in addition to causing apoptosis in the Hep32 and HepG2 cell lines. Compound **12**, however, shows antileukemic properties at far lower concentrations, and is lethal at concentrations below 10 nM rather than at the low micromolar range usually observed with other parthenolide analogs (Fig. 4).[2–4,9,16] Furthermore, compound **12** is particularly lethal to the CCRF-CEM cell line at concentrations lower than 10 nM. This lethal effect of **12** (Table 2) appears to be selective for the CCRF-CEM cell line, since this compound was far less effective against any of the other 59 cell lines in the screen (LD_{50} = 40 to above 100 μM, data not shown). The activity of **12** appears

to arise from a very specific structural motif (i.e., the 4-hydroxyphenethylamine moiety), since other structurally related aromatic analogs, such as the 3-methoxyphenethyl analog (**10**) and the 3-methoxy-4-hydroxy analog (**11**), possessed anticancer activity only in high micromolar concentration range of 17 μM to above 100 μM against the 60 cell lines in this screen. These results suggest that **12** is a novel, potent parthenolide analog with selective cytotoxicity for CCRF-CEM cells.

Compound **9** possesses significant cytostatic activity against the SR cell line, as shown in Table 1, but its lethal effect is apparent only at μM concentrations. Compound **9** exhibited negligible activity against the other 59 cell lines in this screen, with GI_{50} values ranging from 10 μM to above 100 μM, and lethal effects were seen only above 100 μM in all cell lines.

In conclusion, through functionalization of the exocyclic C-11 double bond of parthenolide, we have synthesized a diverse series of aminoparthenolide analogs derived from various arylalkyl, heteroarylalkyl, and alkyl amines, and have identified the first potent parthenolide analog, **12**, with selective carcinocidal activity in the low nanomolar range against CCRF-CEM leukemia cells in culture. We also have identified analog **9**, which is a selective carcinostatic agent for SR leukemia cells in culture. Further pharmacological evaluation of **12** is underway. Future SAR studies will focus on the structural optimization of these lead analogs, their mechanism of action, and the molecular basis for their observed selectivity.

Acknowledgments

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10. Representative characterization data for four compounds. (**4**): white crystalline Needles; mp 120–122 °C (mono fumarate salt); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.85 (br s, 2H), 7.34 (m, 5H), 6.57 (s, 2H), 5.17 (app. d, J = 10.0 Hz, 1H), 4.00 (t, J = 9.2 Hz, 1H), 3.86 (d of d, J = 13.8 Hz, 2H), 2.92–2.63 (m, 4H), 2.36–1.05 (m, 9H), 1.61 (s, 3H), 1.18 (s, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.1, 166.5, 137.5, 134.2, 128.4, 128.1, 127.2, 124.3, 81.7, 65.3, 61.1, 52.1, 46.3, 45.6, 44.8, 38.6, 36.1, 28.8, 23.6, 16.8, 16.6 ppm; EI-MS 355.0; Anal. Calcd for $C_{22}H_{29}NO_3$. $C_4H_4O_4$: C, 66.22; H, 7.05; N, 2.97. Found C, 66.25; H, 6.89; N, 3.18. (**9**): white powder; mp 144–146 °C (mono fumarate salt); ^{1}H NMR (300 MHz, CD_3OD) δ 7.37 (m, 1H), 7.10 (m, 3H), 6.68 (s, 2H), 5.18 (app. d, J = 12.0 Hz, 1H), 4.10 (m, 2H), 3.18 (m, 1H), 3.01 (m, 1H), 2.83–1.08 (m, 17H), 1.67 (s, 3H), 1.31 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.0, 166.6, 139.1, 137.4, 134.7, 134.5, 129.0, 128.9, 126.6, 125.8, 124.7, 82.0, 65.9, 61.4, 55.2, 48.1, 45.7, 43.3, 36.4, 29.3, 27.9, 24.0, 19.2, 17.2, 16.9 ppm; EI-HR MS 395.2454; $C_{25}H_{33}NO_3$ (M^+) Calcd 395.2446. Anal. Calcd for $C_{25}H_{33}NO_3$. $C_4H_4O_4$: C, 68.08, H, 7.29, N, 2.74. Found C, 68.21, H, 7.06, N, 2.95. (**12**): colorless microcrystalline powder; mp 135–137 °C (mono fumarate salt); ^{1}H NMR (400 MHz, CD_3OD): δ 7.15 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.68 (s, 2H), 5.17 (app. d, J = 10.4 Hz, 1H), 4.20 (t, J = 9.2 Hz, 1H), 3.37–2.89 (m, 9H), δ 2.45–1.09 (m, 8H), 1.63 (s, 3H), 1.27 (s, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 178.4, 172.6, 155.6, 136.6, 135.7, 131.1, 128.9, 125.8, 116.8, 84.2, 67.7, 65.8, 58.4, 50.3, 47.9, 46.3, 44.7, 41.0, 36.6, 31.7, 31.3, 29.5, 24.6, 17.9, 17.2 ppm; EI-HRMS: found 385.2245, $C_{23}H_{31}NO_4$ (M^+) Calcd 385.2247; Anal. Calcd for $C_{23}H_{31}NO_4$. $C_4H_4O_4{\cdot}H_2O$: C, 62.41; H, 7.18; N, 2.70. Found C, 62.32; H, 7.17; N, 2.66. (**19**): white powder; mp 122–124 °C (mono fumarate salt); ^{1}H NMR (400 MHz, D_2O): δ 6.67 (s, 2H), 5.26 (app. d, J = 12.0 Hz, 1H), 4.31 (t,J = 9.2 Hz, 1H), 3.48–3.02 (m, 7H), δ 2.49–1.15 (m, 14H), 1.70 (s, 3H), 1.34 (s, 3H) ppm. ^{13}C NMR (75 MHz, D_2O) δ 178.5, 172.6, 136.7, 135.6, 125.8, 84.1, 67.5, 65.7, 48.5, 48.4, 47.7, 46.0, 44.8, 40.8, 36.4, 29.3, 24.7, 24.4, 23.3, 16.9, 16.8 ppm; EI-HR MS: found 336.2402, $C_{19}H_{32}N_2O_3$ (M^+) Calcd 336.2407; Anal. Calcd for $C_{19}H_{32}N_2O_3{\cdot}C_4H_4O_4$: C, 61.04; H, 8.02; N, 6.19. Found C, 61.23; H, 8.12; N, 6.26.
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