Bioorganic & Medicinal Chemistry Letters 19 (2009) 234–236

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

TRAIL-enhancing activity of Erythrinan alkaloids from *Erythrina velutina*

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ARTICLE INFO

Article history:
Received 6 August 2008
Revised 6 October 2008
Accepted 25 October 2008
Available online 31 October 2008

Keywords:
TRAIL
Enhanced activity
Erythrinan alkaloid
Medicinal plant
Erythrina velutina

ABSTRACT

A new Erythrinan alkaloid (**1**), erythodine N-oxide, was isolated from the seeds of *Erythrina velutina* together with seven known Erythrinan alkaloids (**2**–**7**, **9**) and an indole alkaloid (**8**). The structure of new compound (**1**) was elucidated by spectroscopic methods. Six of the nine compounds showed enhanced activity when combined with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Erysotrine (**4**) did not show cytotoxic activity by itself, but exhibited significant cytotoxicity when combined with TRAIL.

In search for biological active compounds derived from tropical medicinal plants,[1] we investigated the constituents of the seeds of *Erythrina velutina* Willd (Leguminosae).[2] This plant is commonly called 'Mulungu' in Brazil, and its bark is used for sedation, hypnogenesis, the control of convulsions, and nervous coughs in the northern region of Brazil.[3] In a previous paper, we described the isolation and characterization of an indole derivative, hypaphorine (**8**), and its sleep-inducing effect in normal mice.[4] In this paper, we describe the isolation and structural characterization of a new Erythrinan alkaloid, erysodine N-oxide (**1**), together with eight known alkaloids (**2**–**9**) from the seeds of *E. velutina*, and these isolated compounds were evaluated regarding their TRAIL enhanced activity (Fig. 1).[5]

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is an important member of the TNF superfamily.[6] TRAIL selectively induces the apoptosis of a wide variety of cancer and transformed cells without damaging most normal cells. However, the clinical application of TRAIL in cancer therapy is limited as many cancer cells have been identified as resistant to the cytotoxicity of TRAIL.

Therefore, natural products, which have synergistic activity with TRAIL but minimal toxicity would be promising tools for cancer therapy. Recent studies on natural products showing enhanced activity identified flavonoidal compounds.[7] However, the present report focuses on alkaloidal compounds from natural resources, possibility leading to the expansion of promising candidates.

The seeds of *E. velutina* (954 g) were crushed and then extracted with MeOH. The MeOH-soluble materials (19.0 g) were succes-

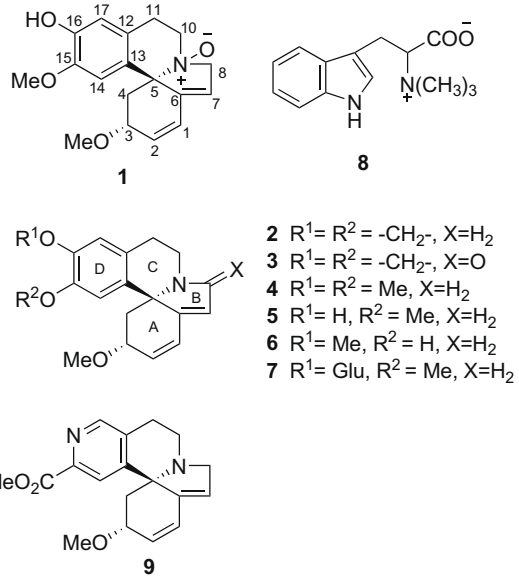

Figure 1. Chemical structures of compounds **1**–**9**.

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doi:10.1016/j.bmcl.2008.10.111

sively partitioned between petroleum ether, EtOAc, and 3% aqueous tartaric acid. Water-soluble materials were adjusted to pH 10 with Na_2CO_3 and then partitioned between $CHCl_3$ (6.07 g) and *n*-BuOH (3.48 g), to obtain the alkaloidal portions.

The $CHCl_3$-soluble materials (3.11 g) were separated in a silica gel column ($CHCl_3$–MeOH, 100:0 → 0:100, Wako-gel C-300, Wako Ltd) to obtain 20 fractions (F1∼F20). The second fraction (F2, 131 mg) was further separated using a silica gel column (EtOAc–MeOH, 99:1) to isolate erythraline (**2**, 9.0 mg),[8] 8-oxo-erythraline (**3**, 87.0 mg),[9] and erysotrine (**4**, 5.0 mg),[8] F4 (11 mg) was separated using silica gel sheets (EtOAc–MeOH, 85:15, Si60-F_{254S}, aluminum sheets, Merck) to give erysodine (**5**, 4.5 mg)[10] and erysovine (**6**, 3.3 mg),[8] and the last fraction (F-20, 349 mg) was separated using a silica gel column (MeOH/acetone, 99:1) to afford glycoerysodine (**7**, 5.0 mg)[10] and hypaphorine (**8**, 44.0 mg).[11]

On the other hand, the second extracts of constituents were extracted by 80% aqueous MeOH to obtain soluble materials (24.0 g), and these were partitioned using the same method to obtain alkaloidal fractions of $CHCl_3$-soluble (C-2, 440 mg,) and *n*-BuOH-soluble (B-2, 4.86 g) materials. The C-2 fraction was separated using an amino silica gel column (Hexane–EtOAc, 90:10 → 0:100, Chromatorex-NH, Fuji Silysia Ltd) to give 11 fractions (F_2-1∼11). The third fraction (F_2-3, 26.4 mg) was purified by employing a silica gel column to obtain erymelanthine (**9**, 1.4 mg).[12] Furthermore, the B-2 extracts were separated using an amino silica gel column ($CHCl_3$–MeOH, 100:0 → 90:10), followed by a silica gel column ($CHCl_3$–MeOH, 100:0 → 90:10) to give a new Erythrinan compound (**1**, 2.6 mg), erysodine N-oxide.

Compound **1** {$[\alpha]_D^{25}$ +172.0 (*c* 0.061, $CHCl_3$)} was obtained as a colorless, amorphous solid. Its molecular formula was determined as $C_{18}H_{21}NO_4$ by HREIMS [*m*/*z* 315.1456 $(M)^+$, calcd for 315.1471] and the IR spectrum implied the presence of a hydroxyl group (3396 cm^{-1}), an aromatic moiety and a conjugate olefin (1647, 1609, 1516, and 1457 cm^{-1}), respectively. The UV spectrum showed λ_{max} (MeOH) at 204 (logε 5.69), 221 (logε 5.18), and 234 (logε 5.24). The ^1H and ^{13}C NMR[13] and HMQC data for **1** indicated the presence of four methylenes, one sp^3 oxy-methine, one sp^3 quaternary carbon, three sp^2 methines, one sp^2 quaternary carbon, two aromatic quaternary carbons, two aromatic methine carbons, two aromatic oxy-quaternary carbons, and two methoxy groups. With two double bonds and an aromatic ring being accounted for six of nine unsaturations, it was concluded that **1** contains four rings including one aromatic ring. The ^1H–^1H COSY spectrum revealed connectivities of C-1 to C-4, C-7 to C-8, and C-10 to C-11 (Fig. 2). HMBC correlations (Fig. 2) were observed: H-17/C-11, 12 and 16, H-14/C-5 (δ_C 82.94; a spiro-carbon),13,15, H-11/C-10, 12, 13, 17, H-10/C-5, 11, H-8/C-6, 7, H-7/C-5, 6, H-1/C-2, 6, H-2/C-1, 3, H-3/C-4, 5 and H-4/C-3, 5, 6. These correlations showed that **1** was a typical Erythrinan alkaloid having a characteristic spiro-carbon positioned in center of rings of A, B, and C with two olefins of $\Delta^{1,2}$ (δ_H 6.60 dd, δ_C 125.2/δ_H 6.18 br d, δ_C 134.2) and $\Delta^{6,7}$ (δ_C 139.1/δ_H 5.68 br s, δ_C 117.2) and one aromatic hydroxyl group attached to the C-16 position. Further, two methoxy groups were attached to C-3 and C-15, as deduced from the HMBC correlations of H_3-3-OMe and H_3-15-OMe, to each methoxy-bearing carbons, respectively. Finally, the molecular formula of **1** indicated the presence of one more oxygen atom. On ^{13}C NMR, the spiro-carbon (C-5) and methylene carbons C-8 and C-10 were markedly deshielded by 16.1, 18.7, and 15.7 ppm in comparison with those of erysodine (**3**), respectively. These facts suggested that the nitrogen atom was oxygenated. The direction of the oxygen atom of N-oxide might be α-facing due to the downfield shifts of H-4α (δ_H 3.24). The NOESY correlations (Fig. 3) of H_3-15-OMe/H-14, H-14/H-4β, H-14/H-3, and H_3-15-OMe/H-4β suggested that the methoxy group was placed at C-15. Another methoxy group located at C-3 had an α-configuration from the NOESY correlations of H_3-3-OMe/H-2, H_3-3-OMe/H-3, and H_3-3-OMe/H-4α. Other NOESY correlations were satisfied with the relative stereochemistry of the new compound **1** depicted in Figure 3. Therefore, compound **1** was assigned as erysodine N-oxide (**1**) (Fig. 2). The CD spectrum of **1** showed a Cotton effect at λ_{max} (MeOH) 238 nm ($\Delta\varepsilon$ +25.1), which suggested that the absolute

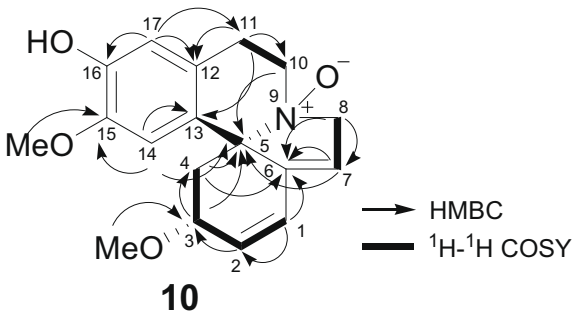

Figure 2. ^1H–^1H COSY and selected HMBC correlations of compound **1**.

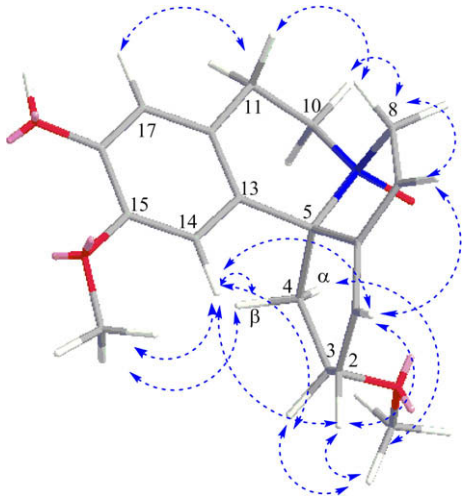

Figure 3. Selected NOESY correlations of compound **1**.

Table 1
IC_{50} (μg/mL) values of compounds (**1**–**9**) isolated from *E. velutina* with TRAIL for Jurcat cells.[a]

Compound	No TRAIL[b]	With TRAIL[c]
1	>50 (2.4)	>50 (18.9)
2	23.0	5.0
3	>50 (37)	22.0
4	>50 (45)	7.2
5	10.8	4.9
6	24.0	14.2
7	>50 (4.7)	44.0
8	>50 (5.8)	>50 (20.0)
9	>50 (1.1)	>50 (24.2)
Apigenin[d]	30.0	6.7
Vincristine[e]	3.1 (ng/mL)	ND

[a] 0.125 μg/mL of TRAIL caused a 21.0% growth inhibition of Jurcat cells.
[b] Numbers in parenthesis indicate the growth inhibitory % at a dose of 50 μg/mL.
[c] The results are expressed relative to the cell prolification rate at 0.125 μg/mL.
[d] Positive control as TRAIL enhancement.
[e] Positive control for Jurcat cells.

configuration of the 3-methoxy group of **1** was *R*.[14] Therefore, the absolute configuration of **1** was assigned as depicted.

We examined the enhancing effects of the various compounds isolated from the seeds of *E. velutina* on the cytotoxicity of TRAIL against Jurcat cells. The results of proliferation tests are shown in Table 1. Most of the compounds tested showed an enhanced cytotoxicity against Jurcat cells when combined with TRAIL. Erythraline (**2**), erysodine (**5**), and erysovine (**6**) showed IC_{50} values of 23.0, 10.8, and 24.0 μg/mL, respectively, and their cytotoxicities were further enhanced by the addition of TRAIL, up to an IC_{50} values of 5.0, 4.9, and 14.2 μg/mL, respectively. However, these compounds showed some cytotoxicity even if their activities were moderate in the absence of TRAIL. The other compounds tested, 8-oxo-erythraline (**3**), erysotrine (**4**), and grycoerysodine (**7**), showed promising data, namely, these compounds exhibit no cytotoxicity by themselves, but they act synergistically when combined with TRAIL. Erysotrine (**4**) showed the superior data when combined with TRAIL (0.125 μg/mL) among the samples tested, and exhibited no cytotoxicity (>50 μg/mL) by itself.

The results of Erythrinan alkaloids regarding the synergistically enhancing effect of TRAIL indicated the expansion of candidates beyond flavonoidal or related compounds.

Acknowledgment

We thank Dr. G. Hashimoto (Centro de Pesquisas de História Natural, São Paulo, Brazil) for the identification of *Erythrina velutina*.

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13. NMR data for **1**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.6 0 dd, J = 10.1, 2.1, H-1), 6.18 (br d, J = 10.1, H-2), 4.21 (br dd, J = 6.0, 11.6, H-3), 2.09 (dd, J = 6.0, 11.6, H-4), 3.24 (t, J = 11.6, H-4), 5.68 (br s, H-7), 4.13 (dd, J = 14.4, 3.3, H-8), 4.43 (br d, J = 14.4, H-8), 3.81–3.85 (m, H_2-10), 3.03–3.16 (m, H_2-11), 6.79 (s, H-14), 6.56 (s, H-17), 3.35 (s, H_3-3-OMe), 3.86 (s, H_3-15-OMe); ^{13}C NMR ($CDCl_3$, 125 Hz), δ 125.2 (C-1), 134.2 (C-2), 75.7 (C-3), 31.2 (C-4), 82.9 (C-5), 139.1 (C-6), 117.2 (C-7), 70.9 (C-8), 59.7 (C-10), 27.2 (C-11), 120.0 (C-12), 129.0 (C-13), 112.6 (C-14), 147.6 (C-15), 146.3 (C-16), 110.3 (C-17), 56.0 (C-3-OMe), 55.9 (C-15-OMe).
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