Pergamon

Tetrahedron Letters 42 (2001) 1255–1257

TETRAHEDRON LETTERS

Diterpenoid pyrones, novel blockers of the voltage-gated potassium channel Kv1.3 from fungal fermentations

Michael A. Goetz,* Deborah L. Zink, Gabe Dezeny, Anne Dombrowski, Jon D. Polishook, John P. Felix, Robert S. Slaughter and Sheo B. Singh*

Merck Research Laboratories, RY80Y-355, PO Box 2000, Rahway, NJ 07065-0900, USA

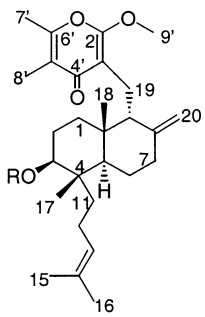
Received 2 October 2000; revised 6 December 2000; accepted 7 December 2000

Abstract—The isolation, structure elucidation and chemical modification of nalanthalide, a novel diterpenoid pyrone blocker of the voltage-gated potassium channel Kv1.3 are reported. The structure–activity relationship of the derivatives with respect to various associated biological activities is also discussed. © 2001 Elsevier Science Ltd. All rights reserved.

The human lymphocyte specific voltage-gated potassium channel, Kv1.3, has a central role in the control of membrane potential in human lymphocytes where it sets the resting potential. The depolarization that results from blockade of the channel causes a reduction in calcium entry and consequently a decrease in lymphokine release and synthesis from calcium-dependent pathways. Thus, blockers of the channel suppress activation and proliferation of human T cells. Kv1.3 is therefore thought to represent a novel target for immunosuppression. Peptide inhibitors [charybdotoxin (ChTX), margatoxin (MgTX) and noxiustoxin] have been shown to block Kv1.3 and thereby inhibit lymphokine production and the rise in intracellular calcium.[1] Our laboratories have recently reported on the discovery of correolide,[2] which potently inhibits Kv1.3, and as expected, depolarizes T cells and inhibits T cell activation events.[3,4]

Continued screening of natural products led to the discovery of nalanthalide (**1**), a diterpenoid pyrone, as a novel inhibitor of Kv1.3. We now report on the discovery including the isolation, structure elucidation, chemical modifications and structure–activity relationship of the diterpenoid pyrone class of inhibitors.

Isolation: Nalanthalide (**1**) was produced in submerged fermentations by the fungal culture MF 5638, a *Nalanthamala* sp.[5] Yields of compound **1** ranged from 80 to 140 mg/l broth after a purification scheme which included extraction with methyl ethyl ketone, column chromatography on silica gel and finally HPLC on a Rainin Dynamax C18 column with 75% acetonitrile in water. On a larger scale, **1** could be more conveniently prepared by repeating the silica gel step and triturating the resulting enriched material with methanol to give an amorphous powder of pure compound.[6] Another source of **1** was *Chaunopycnis alba*, MF 6799.[7]



1: R = $COCH_3$
2: R =H
3: R = CONH-*p*-Br-C_6H_4

Structure elucidation: High resolution EIMS analysis of nalanthalide (**1**)[6] showed a molecular ion at m/z 484.3197 (calcd 484.3189 for $C_{30}H_{44}O_5$) and suggested the presence of nine degrees of unsaturation. The formula was corroborated by analysis of the ^{13}C NMR spectrum, which displayed 30 carbons. Of these, eight were methyls, eight were methylenes, and four were methines as determined by the APT spectrum. Of the remaining quaternary carbons, two were of sp^3 type and the rest were *sp* type, including two carbonyl carbons assigned to a conjugated ketone and an ester. These carbon types were confirmed by the analysis of the ^1H NMR spectrum together with ^1H–^1H COSY and

* Corresponding authors.

0040-4039/01/$ - see front matter
PII: S0040-4039(00)02258-9

^1H–^{13}C COSY (HETCOR). The carbons bearing protons were assigned by analysis of the latter spectrum. The COSY spectrum of **1** revealed the presence of four isolated spin systems consisting of C1–C3, C5–C7, C11–C13, and C9–C19. The H_2–20 and H–13 showed long range allylic couplings to H-7 (δ 2.35, m) and the allylic methyl groups, respectively. These fragments were connected to each other and to the remainder of the molecule with the help of a long range HETCOR spectrum. The H_3-18 methyl group showed two- and three-bond proton–carbon correlations to C-1, C-9 and C-10; H_3-17 methyl group produced correlations to C-3, C-4, C-5 and C-11; H_3-15 and H_3-16 methyl groups to C-13, C-14 and to each other; H_2-20 (the exocyclic methylene protons) showed correlations to C-7, C-8 and C-9; and H_2-19 exhibited correlations to C-3′ of the pyrone ring. The methyl and the methoxy groups of the pyrone ring were accordingly assigned by the long-range HETCOR correlations.

The mass spectrum of **1** produced two major fragment ions at m/z 343.2262 ($C_{22}H_{31}O_3$) and m/z 167.0707 ($C_9H_{11}O_3$). The former ion is produced as a result of the concomitant loss of the acetate group at C-3 and the side-chain at C-4, and the latter fragment is derived from the pyrone ring as shown in Fig. 1.

Stereochemistry: The relative stereochemistry of nalanthalide (**1**) was elucidated by measurements of the scalar couplings and by NOE difference spectroscopy. The methine proton at C-3 appeared as a doublet of doublets at δ 4.85 ppm and showed a 9 Hz coupling with the axial proton at C-2, confirming its axial orientation in a chair conformation. The H_a-19 (δ 2.42) and H_b-19 (δ 2.62) showed a large ($J = \sim 12$ Hz) and small couplings ($J = \sim 3.5$ Hz) with H-9 (δ 1.95), respectively, indicating a *anti* and *syn* geometric relationship, which was confirmed by the observation of NOE effect from H_b-19 to H-9 (Fig. 2). The H_b-19 also gave NOE enhancements to the terminal methyl groups (C-15 and C-16) of the side-chain suggesting the spatial proximity of these groups to H_b-19, at least in solution. Irradiation of H-3 produced NOE effects to H-5 suggesting their 1,3-diaxial disposition. Similarly the C-17 and

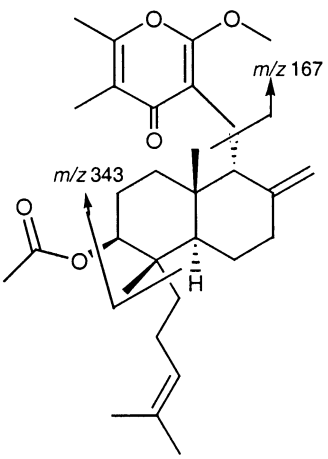


Figure 1.

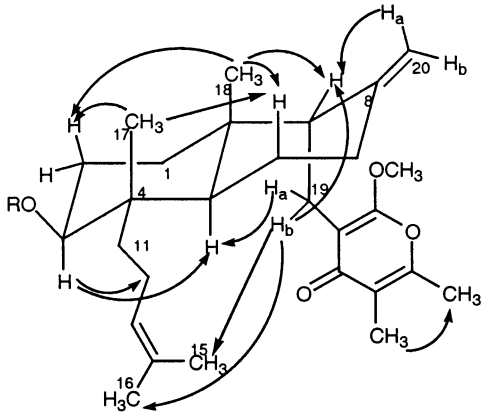


Figure 2. Selected NOE of **1**.

C-18 angular methyl groups gave NOE enhancements to both H-2 and H-6 axial protons thus establishing the respective 1,3-diaxial positions. Direct NOE effects of H_3-17 and H_3-18 could not be accomplished due to partial saturation of each other during irradiation experiments. These measurements together with others established the relative stereochemistry and conformation of nalanthalide as shown in Fig. 2, which was supported by a model generated from the ChemDraw 3D minimized structure shown in Fig. 3. The stereochemistry and the solution conformation of **1** appears to be similar to the stereochemistry and X-ray derived solid state conformation of viridoxins.[8] Structurally related pyrones, subglutinols[9] and sesquicillin[10] have been reported to show immuosuppressive and glucocorticoid mediated signal transduction inhibitory activities, respectively.

Chemical modification: To explore the chemistry and evaluate structure–activity relationship, a few derivatives of nalanthalide were prepared by chemical modifications. Mild basic hydrolysis with potassium carbonate in a mixture of THF and methanol gave 90% yield of the hydroxy compound **2**. The reaction of compound **2** with *p*-bromophenyl isocyanate in methylene chloride quantitatively produced the desired urethane **3**. Epoxidation of **1** with *m*-CPBA exclusively produced mono epoxide **4**, leaving the exocyclic methylene group intact. Zone's oxidation of **2** cleanly afforded the 3-keto derivative **5**. Selective hydrogenation with

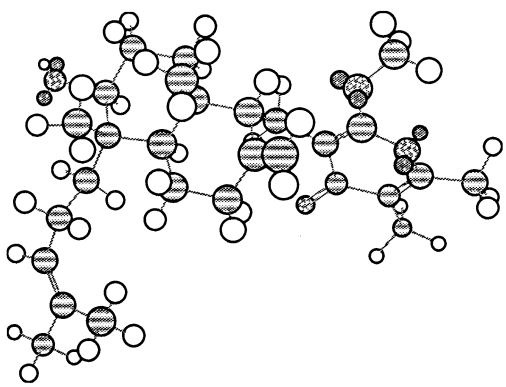
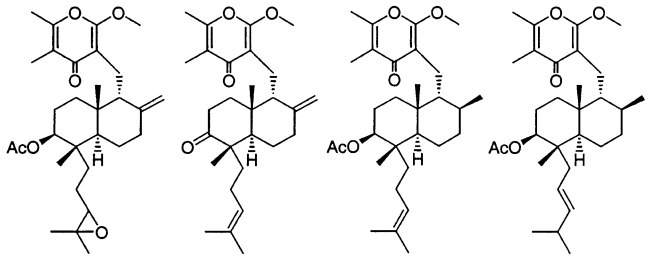


Figure 3. ChemDraw 3D minimized model of **1**.

Table 1. Biological activities of nalanthalide (**1**) and its derivatives



4 **5** **6** **7**

Compound	^{125}IChTX binding to Jurkat membranes, IC_{50} (μM)	$^{86}Rb^+$ reflux in CHO-Kv1.3 cells IC_{50} (μM)
1	3	3.9
2	11	3
3	>30	25
4	6	5.1
5	>10	3
6	5	31
7	6	16

Wilkinson's catalyst in benzene at 40 psi furnished a 9:1 mixture of dihydro compounds **6** and **7**. As expected, the hydrogenation was stereospecific and hydrogen was introduced from the less hindered pseudo equatorial bottom face and thus produced an axial methyl at C-8. It was interesting to note that a small amount of the compound went through olefin isomerization to give compound **7**.

Biological activity and SAR: Nalanthalide (**1**) inhibits the binding of ChTX to Jurkat membranes with an IC_{50} of 3 μM. It blocked Rb^+ efflux in CHO-Kv1.3 cells with an IC_{50} value of 3.9 μM. In electrophysiological measurements it depolarizes human T cells to the same extent as MgTX with an EC_{50} of 500 nM. The biological properties of **1** and its derivatives are summarized in Table 1. It appears that the acetate group at C-3 plays a role in the ChTX binding activity and has less of an effect on Rb^+ efflux activity. For example, the hydrolysis of acetate (**2**) and subsequent oxidation to C-3 ketone (**5**) did not affect the Rb^+ efflux activity, but reduced the binding activity by 3-fold. Substitution of acetate with bulky *p*-bromophenylurethane group (compound **3**) led to significant reduction in both activities. Epoxidation (compound **4**) of the side-chain olefin caused some reduction in both activities. The reduction of exocyclic methylene group (compounds **6** and **7**) had little (2-fold reduction) effect on the binding activity, but had a greater impact (4–8-fold reductions) on Rb^+ efflux activity. These compounds also blocked L-type Ca^{2+} channels in GH3 cells with the general same potency.

Nalanthalide and derivatives represent a novel class of voltage-gated potassium (Kv1.3) channel blockers and are potential immunosuppressants.

References

1. Lin, C. S.; Boltz, R. C.; Blake, J. T.; Nguyen, M.; Talento, A.; Fischer, P. A.; Springer, M. S.; Sigal, N. H.; Slaughter, R. S.; Garcia, M. L.; Kaczorowski, G. J.; Koo, G. C. *J. Exp. Med.* **1993**, *177*, 637.
2. Goetz, M. A.; Hensens, O. D.; Zink, D. L.; Borris, R. P.; Morales, F.; Tamayo-Castillo, G.; Slaughter, R. S.; Felix, J. P.; Ball, R. G. *Tetrahedron Lett.* **1998**, *39*, 2895.
3. Felix, J. P.; Bugianesi, R. M.; Schmalhofer, W. A.; Borris, R. P.; Goetz, M. A.; Hensens, O. D.; Bao, J.-M.; Kayser, F.; Parsons, W. H.; Rupprecht, K.; Garcia, M. L.; Kaczorowski, G. J.; Slaughter, R. S. *Biochemistry* **1999**, *38*, 4922.
4. Koo, G. C.; Blake, J. T.; Shah, K.; Staruch, M. J.; Dumont, F.; Wunderler, D.; Sanchez, M.; McManus, O. B.; Sirotina-Meisher, A.; Fischer, P. A.; Boltz, R. C.; Goetz, M. A.; Baker, R.; Bao, J.-M.; Kayser, F.; Rupprecht, K. M.; Parsons, W. H.; Tong, X.-C.; Ita, I. E.; Pivnichny, J.; Vincent, S.; Cunningham, P.; Hora, D.; Feeney, W.; Kaczorowski, G. J.; Springer, M. S. *Cell Immun.* **1999**, *197*, 99.
5. Frozen vegetative mycelium was inoculated into a seed medium containing yeast extract, malt extract, glucose and junlon; after 3 days at 25°C, 220 rpm, a vermiculite base mixed with a solution containing glucose, fructose, sucrose, casamino acids, asparagine, yeast extract, dibasic sodium phosphate, magnesium sulfate, calcium chloride and trace elements was inoculated with vegetative seed. Incubation at 25°C in bottles rolling on a Wheaton machine at 4 rpm was carried out for 20 days.
6. Physical data of nalanthalide (**1**): mp 96.5–98°C; $[\alpha]_D^{25}$ −58.2 (*c* 0.275, $CHCl_3$); UV (CH_3OH) λ_{max}: 216 (3.7), 260.5 (3.8); IR (ZnSe) ν_{max}: 2900–3000, 1732, 1671, 1601, 1252, 1241 cm^{-1}, ^1H and ^{13}C NMR (300 and 400 MHz, $CDCl_3$), position (δC; δH, m, *J* in Hz): C-1 (33.8; 1.98, m; 1.35, m), C-2 (24.1; 1.95, m; 1.35, m); C-3 (76.2; 4.82, dd, $J=9$, 7 Hz); C-4 (40.0), C-5 (39.1; 1.72, dd, $J=12.3$, 2.9 Hz); C-6 (22.7; 1.45, m; 1.30, m); C-7 (30.7; 2.10, m; 2.35, m); C-8 (148.7); C-9 (55.6; 1.95, dd, $J=12$, 3.5 Hz); C-10 (37.4); C-11 (37.8; 1.15, m; 1.35, m); C-12 (21.7; 1.90, m); C-13 (124.6; 5.06, t, $J=7.2$ Hz); C-14 (131.3); C-15 (17.5; 1.59, brs); C-16 (25.7; 1.67, brs); C-17 (18.1; 0.85, s); C-18 (22.9; 0.95, s); C-19 (19.9; 2.67, dd, $J=12.7$, 3.7 Hz [H_b]; 2.42, t, $J=12.0$ Hz [H_a]); C-20 (109.5; 4.51, t, $J=2.3$ Hz [H_b]; 4.19, t, $J=2.3$ Hz [H_a]); C-2′ (162.8); C-3′ (103.2); C-4′ (180.3); C-5′ (118.6); C-6′ (154.9); C-7′ (17.0; 2.23, s); C-8′ (10.0; 1.89, s); C-9′ (55.3; 3.84, s); C-1″ (176.7); C-2″ (21.3; 2.03, s).
7. Same as Ref. 5, except production medium contained glucose, urea, NZ amine type A, dibasic sodium phosphate, magnesium sulfate, potassium chloride, zinc sulfate and calcium carbonate.
8. Gupta, S.; Krasnoff, S. B.; Renwick, J. A. A.; Roberts, D. W.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 1062. Compound **1** was reported as semisynthetic derivative derived from viridoxin A while our studies were in progress, but no spectroscopic data was reported.
9. Lee, J. C.; Lobkovsky, E.; Pliam, N. B.; Strobel, G.; Clardy, J. *J. Org. Chem.* **1995**, *60*, 7076.
10. Engel, B.; Erkel, G.; Anke, T.; Sterner, O. *J. Antibiot.* **1998**, *51*, 518.