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Leporin B: A Novel Hexokinase II Gene Inducing Agent from an Unidentified Fungus

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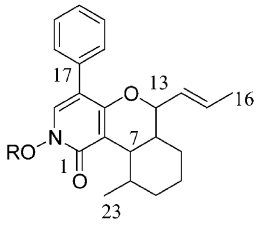
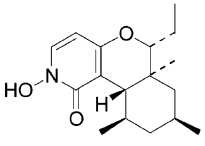
Abstract—Leporin B (**1**), a novel demethylated analogue of leporin A (**2**), was isolated from a taxonomically unidentified fungal strain as part of an effort to discover compounds with the ability to increase expression levels of the enzyme hexokinase II. The structure was determined by spectral methods, including 1D and 2D NMR, and HRMS. The relative stereochemistry was assigned by NOESY experiments and coupling constants.

Hexokinase II (HKII) is the major enzyme in the muscle responsible for insulin-stimulated glucose phosphorylation. It catalyzes the committed step in glucose utilization by phosphorylating glucose and trapping it in the cell. In type 2 diabetic patients, HKII mRNA expression and its activity are reduced.[1,2] Therefore, enhancement of HKII transcription may be an effective means in controlling hyperglycemia in type 2 diabetes. In an effort to discover small-molecule HKII transcription activators for the treatment of type 2 diabetes, we constructed a cell line derived from L6, a rat myoblast cell line, that expressed the reporter gene luciferase (luci) under the control of the HKII promoter. HKII transcription activators are expected to increase the cellular luci activity (HKII-luci signal). We used this reporter cell line to screen for compounds and fungal extracts that enhanced the luci signal. Hits that enhanced the luci signal were then verified for transcription activation by measuring the level of HKII mRNA in L6 myotubes treated with compounds. In this report we describe an *N*-hydroxy-pyridone, leporin B, that was discovered during this screening effort.

N-hydroxy-pyridones with a variety of biological activities have previously been described from numerous fungi. Several of these compounds have been shown to have cytotoxic and antifungal properties, including fusaricide (**3**),[4] tenellin (**4**),[5] and pyridoxatin (**5**).[6] Interestingly, the *N*-hydroxy-pyridone 8-methylpyridoxatin (**6**) has been shown to induce transcription of the erythropoietin gene, possibly by a similar mechanism to the (HKII) induction activity observed for leporin B (**1**).[7]



1 R=H
2 R=CH_3



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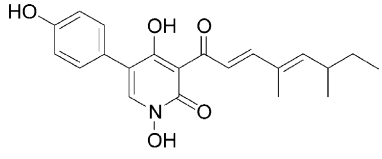

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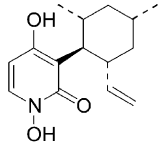

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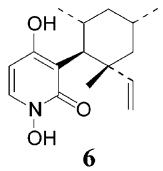

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Results and Discussion

Fungal strain OSI 54042 was collected from colonies growing on wood and twigs in a dry oak forest outside of Victoria, Mexico. The crude MeOH extract of the freeze-dried fermentation was partitioned between EtOAc and water and the material in the EtOAc layer was subsequently dissolved in acetonitrile and extracted with hexane. Further purification of the hexane partition by reversed phase HPLC provided a novel compound, which we named leporin B (**1**) due to its similarity to leporin A (**2**). The structure of compound **1** was established by analysis of spectral data including NMR, MS, and IR, as well as comparison with literature compounds.[1–5]

A HRFABMS experiment suggested a molecular formula for compound **1** of $C_{22}H_{25}NO_3$, requiring 11 degrees of unsaturation (*m*/*z* 352.1891, calcd 352.1911, Δ +5 ppm). The ^{13}C NMR spectrum displayed 20 carbon signals (assignments and values provided in Table 1), including 13 sp^2 carbons (accounting for 2 symmetrical carbons in the phenyl ring), 2 of which are *trans* olefinic (C14 and C15) as determined by coupling constants between their attached protons ($J = 16$ Hz), and one oxygenated methine. The proton and DEPT NMR indicated the presence of 24 protons requiring one exchangeable proton to complete the molecular formula.

Analysis of ^1H NMR and ^{13}C NMR spectra indicated the presence of a phenyl group. The presence of a pyridone ring was supported by both ^1H and ^{13}C data assigned using HMBC correlations as well as a carbonyl absorption in the IR spectrum at 1634 cm^{-1}. Proton H3 showed several 2 and 3 bond HMBC correlations to the carbons of the pyridone ring as well as to C17 of the phenyl ring to establish substructure A (Table 1). The resulting chemical shift assignments are in good agreement with model pyridone systems. Further analysis of the HMBC, HMQC, COSY, and TOCSY data established substructure B. Unit B was elucidated primarily by use of COSY spectra along with several key HMBC correlations. Substructures A and B were connected using key HMBC correlations, including H7 to C1, C5 and C6 that indicated the bond between C6 and C7. The down field chemical shift value for C5 (156.7) supports oxygenation at this position.

Table 1. NMR Chemical shift assignments for leporin B

Compd	^{13}C[a]	^1H	mult	*J*	Int.	HMBC	COSY
1	158.5						
2							
3	132.4	7.62	s		1H	1, 4, 5, 6, 17	
4	111.2						
5	156.7						
6	114.1						
7	38.2	2.81	dd	11, 3.5	1H	1, 5, 6, 12,13	8, 12
8	36.3	1.63	m		1H		7, 23
9	35.7	1.65	m		1H		10
10	20.7	1.42	m		1H		9
11	26.8	1.68	m		1H		12
12	35.7	1.73	m		1H		7, 11, 13
13	77.9	4.85	dd	11, 8	1H	15	12, 14
14	130.3	5.40	ddt		1H	16	13, 15
15	131.7	5.78	dt	15, 6.5	1H	13, 16	14, 16
16	18.0	1.67	dd	6.5, 1.5	1H	14, 15	15
17	134.2						
18/22	129.6	7.44	m		2H	4, 20	
19/21	129.3	7.33	m		2H	17	
20	128.4	7.27	m		1H	18/22	
23	21.1	0.87	d	8	3H	7,9	

[a]Values for all chemical shifts were measured in $CDCl_3$ at 25 °C.

The relative stereochemistry of **1** was determined by analysis of ^1H–^1H coupling constants and a NOESY experiment (Table 1). NOE correlations of H13 with H8 and axial H10 indicated that they are on the same face of the ring. The coupling constant between H7 and H8 ($J = 11$ Hz) supported the result that H8 is located at the axial position. The small coupling constant between H7 and H12 indicated that they reside on opposite faces of the ring. Both the NOE correlation between H12 and H14 and the large H12-H13 coupling constant ($J = 11$ Hz) support the assignment of H12 and H13 having a *trans* geometry.

The *N*-hydroxy group of **1** was methylated (CH_2N_2) in order to prepare a sample for biological evaluation and for comparison of the optical rotation value of the resulting leporin A to the previously published value. The optical rotation of the semi-synthetically prepared leporin A was identical to the value originally reported for the natural product,[1] suggesting that both leporin A and leporin B have the same absolute stereochemistry, though the assignment remains undetermined. Leporin A showed no activity in the hexokinase II induction assay.

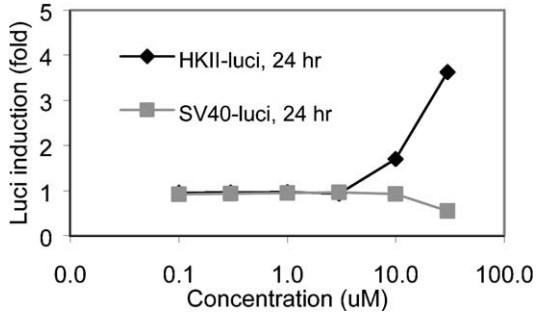


Figure 1. Luci induction data for leporin B in the HKII-luciferase transcription induction assay compared to the SV40-luci counter screen.

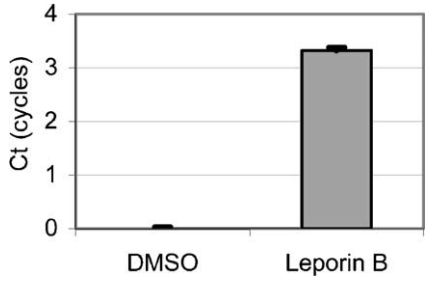
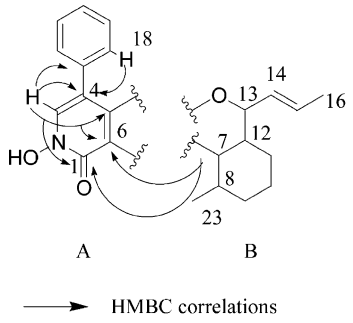


Figure 2. Induction of HKII mRNA by leporin B.



Leporin B displayed an induction of the HKII-luci signal in the reporter cell line, with an IC_{50} of approximately 10 μM (Fig. 1). L6 myotubes were then treated with 10 μM of leporin B, and HKII mRNA and total RNA isolated from the compound-treated cells was subjected to quantitation by reverse transcription-polymerase chain reaction (RT-PCR). HKII mRNA was induced approximately 8-fold (corresponding to three cycles of PCR amplification) in the leporin-treated sample compared to the DMSO-treated sample (Fig. 2). Therefore, leporin B is an HKII transcription activator.

L6 myotubes were treated with 4 μg/mL of leporin B in 0.2% DMSO or DMSO alone for 8 h, and total RNA was isolated. HKII mRNA in the total RNA was quantitated by TaqMan PCR following reverse transcription using a set of HKII-specific primers and probe. The ΔCt value represents the difference in cycle in which the PCR amplification signal rose above a background threshold signal between a sample and the DMSO control. By definition, the ΔCt value for the DMSO control is 0. Standard deviations are depicted by vertical error bars. A ΔCt value of 1 means 2-fold induction. A ΔCt value of 2 means 4-fold induction.

References and Notes

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