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Nothospondin, a new AP-1 inhibitory quassinoid from the Cameroonian plant *Nothospondias staudtii*

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ABSTRACT

A high throughput screen for inhibitors of the oncogenic transcription factor activator protein-1 (AP-1) was applied to the NCI repository of natural product extracts. The lipophilic extract of the plant *Nothospondias staudtii* (Simaroubaceae) displayed significant AP-1 inhibition. Bioassay-guided fractionation of the extract lead to a new quassinoid named nothospondin (**1**), and the known compound glaucarubinone (**2**). The structure of **1** was elucidated by spectroscopic methods. Compounds **1** and **2** showed potent, dose-dependent AP-1 inhibition at noncytotoxic concentrations.

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Oncogenic transcription factors help regulate the transcription of numerous genes associated with cell growth and cell cycle progression, and an increase in their transcriptional activity is required to support the transformation and unrestrained growth of cancer cells. Activator protein-1 (AP-1) is a transcription factor that resides within the nucleus and regulates various cellular processes including differentiation, proliferation, and apoptosis.¹ AP-1 is activated by signal transduction through the mitogen activated protein kinase (MAPK) cascade that can be induced by extracellular stimuli such as growth factors or cytokines.² Carcinogenesis is a multi-step process comprised of tumor initiation, promotion, and progression. Activation of AP-1 is required for tumor promotion, and elevated AP-1 activity is believed to play a pivotal role in tumor progression.³ Since the rate limiting stages of carcinogenesis occur during tumor promotion and progression, small molecule inhibitors of AP-1 function could serve as potential therapeutic agents. AP-1 exists as a dimer of Jun proteins (c-Jun, JunB, JunD) or as a heterodimer of Jun and Fos proteins, and dimerization is required for activation and DNA binding.⁴ Inhibition of AP-1 by the dominant negative c-Jun mutant TAM67 prevented cellular transformation but it did not significantly diminish *in vitro* cell survival, and it effectively blocked tumorigenesis *in vivo*.⁵ AP-1 inhibitors that can mimic the effect of TAM67 could have utility for the prevention

and treatment of cancer. As part of an ongoing molecularly targeted discovery effort, extracts from the NCI Natural Products Repository were screened in a high throughput assay for AP-1 inhibitors.⁶ The lipophilic extract of the tropical plant *Nothospondias staudtii* (Simaroubaceae),^{7,8} collected in Cameroon, showed potent inhibition of AP-1 activity. Bioactivity-directed fractionation of this extract afforded a new quassinoid,⁹ nothospondin A (**1**), and glaucarubinone (**2**).¹⁰

Nothospondin (**1**, Fig. 1, Table 1)¹¹ provided a [M+H]⁺ ion at *m/z* 393.1927 in the HREIMS consistent with a molecular formula of C₂₁H₂₈O₇. Analysis of the ¹³C and gHSQC NMR spectra revealed that the 21 carbon resonances consisted of 4 methyls, 2 methylenes, 7 methines, 7 quaternary carbons, and one methoxyl group. An oxymethine doublet assigned to H-2 (δ_{H} 4.71) showed HMBC correla-

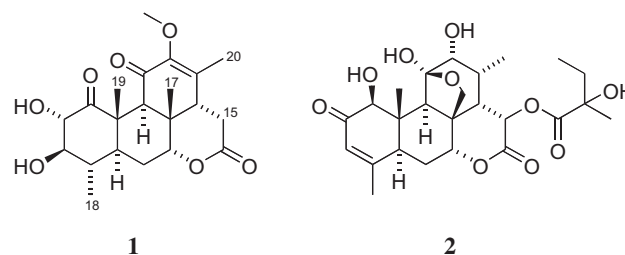


Figure 1. Structure of nothospondin (**1**) and glaucarubinone (**2**).

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Table 1
NMR data (CDCl₃) for nothospondin (**1**)^a

No.	δ_C	δ_H (J in Hz)	gHMBC
1	209.1		
2	76.9	4.71 d (8.7)	1, 3, 19
3	83.7	2.93 dd (10.1, 8.7)	2, 4, 18
4	34.7	1.96 m	3, 5, 18
5	41.6	1.30 m	19
6a	25.7	1.82 ddd (14.8, 12.4, 2.1)	5, 10
6b		2.10 dt (14.8, 3.6)	4, 5, 8, 10
7	82.1	4.29 brt (2.9)	5, 9
8	36.9		
9	47.5	3.15 s	1, 7, 8, 10, 11, 17, 18
10	48.1		
11	190.8		
12	148.4		
13	140.5		
14	47.3	2.42 dd (12.1, 7.0)	8, 9, 12, 13, 17, 20
15a	31.7	2.55 dd (18.6, 12.1)	12, 13, 14
15b		2.97 dd (18.6, 7.0)	8, 9, 16
16	169.0		
17	23.2	1.20 s	7, 8, 9, 14
18	14.9	1.04 d (6.5)	3, 4, 5
19	15.6	1.46 s	1, 5, 9, 10
20	16.0	1.89 s	12, 13, 14
OCH ₃	60.0	3.67 s	12

^a Data were acquired with a Bruker Avance 111 600 MHz spectrometer; chemical shifts are in ppm and referenced to the residual solvent signal; J in Hz; HMBC correlations are from the proton(s) stated to the indicated carbon.

tions with a ketone at C-1 (δ_C 209.1) and an oxymethine at C-3 (δ_C 83.7), and it had a 8.7 Hz coupling to H-3 (δ_H 2.93) that indicated a diaxial orientation for these two protons. Assignment of the H₃-18 doublet (δ_H 1.04) was based on COSY coupling to H-4 and HMBC correlations with C-3, C-4 (δ_C 34.7), and C-5 (δ_C 41.6). Substitution of H₃-19 (δ_H 1.46) on C-10 was established by HMBC correlations with C-1, C-5, and C-10 (δ_C 48.1). These data and COSY correlations between H-3/H-4 (δ_H 1.96) and H-4/H-5 (δ_H 1.30) were consistent with the structure proposed for the A ring. Assignment of ring B was facilitated by the HMBC correlations of H-9 (δ_H 3.15) with C-8 (δ_C 36.9), C-10, C-17 (δ_C 23.2), and C-19 (δ_C 15.6). In addition, HMBC correlations of H-7 (δ_H 4.29) with C-5 and C-9 (δ_C 47.5) and COSY correlations between H-5/H-6b (δ_H 2.10) and H-6b/H-7 unambiguously established the connectivity from C-5 through C-7. Ring C was revealed by a carbonyl at δ_C 190.8 (C-11) consistent with a conjugated ketone and HMBC correlations from H-9 to C-11, from H-14 (δ_H 2.42) to C-12 (δ_C 148.4), C-13 (δ_C 140.5), and C-20 (δ_C 16.0), and from the methoxyl group (δ_H 3.67) to C-12. Finally, the presence of a six-membered lactone ring that encompassed an ester carbonyl at C-16 (δ_C 169.0) linked to the oxygen at C-7 was deduced from the low field shift of H-7 (δ_H 4.29) and HMBC correlations from H-15a (δ_H 2.55) to C-13, C-14 (δ_C 47.3), and C-16, and from H-15b (δ_H 2.97) to C-8, C-14, and C-16. The relative stereochemistry of **1** was established from a series of selective 1D ROESY experiments. Irradiation of H₃-19 produced ROESY

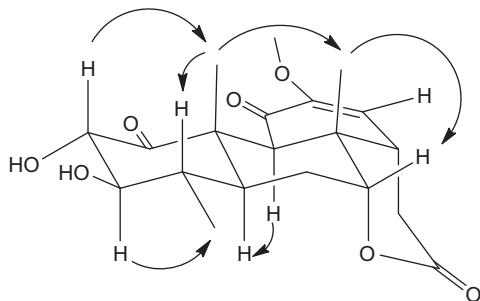


Figure 2. Key ROESY correlations for nothospondin (**1**).

enhancements in H-2, H-4, H-6a (δ_H 1.82) and H₃-17 (δ_H 1.20) that indicated these groups are located at the top (β) face of the molecule (Fig. 2). In addition, H₃-17 had ROESY interactions with H-7 and H-14 that established these protons as β as well. Substituents on the bottom (α) face of **1** were defined by ROESY interactions between H₃-18/H-3, H₃-18/H-6b and H-5/H-9. These data allowed the structural and relative configurational assignment of nothospondin (**1**) as a new tetracyclic quassinoid. The identity of compound **2** was established by comparison of its ¹H and ¹³C data with published values for glaucarubinone.¹⁰

The AP-1 inhibitory activity of compounds **1** and **2** was determined by a β -lactamase driven reporter assay using fluorescence resonance energy transfer (FRET) technology, followed by an XTT assay to test for cytotoxicity.¹² Glaucarubinone (**2**) showed the most potent AP-1 inhibition with an EC₅₀ of 0.13 μ M and it was noncytotoxic at a high-test concentration of 80 μ M. Nothospondin (**1**) was less potent against AP-1 (EC₅₀ 1.49 μ M) and it showed some cytotoxicity (IC₅₀ approximately 10 μ M). The potent AP-1 inhibitory activity of **2**, which has an ether bridge between C-17 and C-11, was consistent with a prior structure–activity study in which all of the AP-1 active quassinoids had C-17/C-11 or C-17/C-13 ether bridges.¹³ Nothospondin (**1**) is the first quassinoid without an ether link that can inhibit AP-1, albeit at significantly reduced potency.

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11. *Nothospondin* (**1**): Yellow amorphous solid; $[\alpha]_D^{25} + 4.15$ (c 0.021, MeOH); UV (MeOH) λ_{max} (ϵ) 207 (8815), 244 (5064) nm; IR (thin film) 3444 (br), 2849, 1731, 1682, 1639, 1462, 1379, 1240, 1093, 1032, 896, 730 cm^{-1} ; ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1; HRESIMS m/z ($\text{M}+\text{H}$)⁺ 393.1927 ($\text{C}_{21}\text{H}_{29}\text{O}_7$ requires 393.1908).
12. *AP-1 assay*: Inhibition of AP-1 activity by compounds **1** and **2** was assessed using a FRET based β -lactamase reporter assay that was described previously.⁶ In brief, AP-1-*bla* HEK293T cells that stably express a plasmid containing β -lactamase under the control of an AP-1 promoter were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat deactivated dialyzed fetal bovine serum (FBS), penicillin–streptomycin, nonessential amino acids, HEPES, sodium pyruvate and blasticidin. The cells at 70% confluency were trypsinized, resuspended in media that did not have blasticidin, seeded into 384-well assay plates (40 μL) and incubated (37 °C) for 18 h. DMSO solutions of the test materials were diluted with media and then added to the cell culture wells. The cells were then incubated at 37 °C for 1 h, treated with TPA to induce AP-1 activity, and incubated for an additional 18 h. β -Lactamase activity was quantified with the LiveBLAzer FRET substrate and the fluorescence was read on a Tecan Safire fluorescent plate reader (409 nm excitation and 460 nm emission, followed by 409 nm excitation and 530 nm emission). The β -lactamase activity was calculated from the ratio of emission at 460 nm to the emission at 530 nm.
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