

Supporting Information for

Austalide K, from the Fungus *Penicillium rudallense* Prevents LPS-Induced Bone Loss in Mice by Inhibiting Osteoclast Differentiation and Promoting Osteoblast Differentiation

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Structural elucidation of austalide K

The molecular formula of austalide K was determined to be $C_{25}H_{32}O_5$ based on analysis of its molecular ion peak in the HRFABMS spectrum and ^{13}C NMR data. The IR spectrum of austalide K showed characteristic absorption at 1748 cm^{-1} and 1700 cm^{-1} , indicating presence of an ester carbonyl and a ketone group. The UV maxima (λ_{max} 216 and 267 nm) indicated the existence of a phthalide moiety in the molecule.

The 1H and ^{13}C NMR spectra exhibited signals of pentacyclic backbone in austalide K. Six singlet methyls including one aromatic methyl group (H25 : δ 2.04), one methoxy group (H11 : δ 4.11), four aliphatic groups (H19 : δ 1.19, H20 : δ 1.12, H21 : δ 1.03, H24 : δ 0.72) were observed. Additionally, six methylenes (C15 : δ 29.9, C16 : δ 38.5, C18 : δ 34.2, C22 : δ 19.2, C23 : δ 18.4) with one oxygenated (C10 : δ 68.4) were determined. Two methines (C12 : δ 54.3, C14 : δ 47.2) and nine non protonated carbons were observed (Supplementary Table 1). The ^{13}C NMR spectrum showed signals at δ 216.8 (C-1) and δ 169.6 (C-2), which were assigned to ketone carbonyl and ester carboxyl carbons, respectively. The HMBC correlations established the structure of the rings and the linkage of the pentacyclic moiety (Fig 1)

Table S1 ^1H (700 MHz) and ^{13}C NMR (175 MHz) data of austalide
K in CDCl_3 (J in Hz)

No.	δ_{C}	δ_{H} , mult ^b (J in Hz)
1	216.8	
2	169.6	
3	158.8	
4	155.5	
5	145.7	
6	115.4	
7	114.6	
8	107.5	
9	76.3	
10	68.4	5.12 (s)
11	62.1	4.11 (s)
12	54.3	1.48 (d, m)
13	47.4	
14	47.2	1.49 (m)
15	39.9	2.29 (dt, 14.1, 2.80) 1.66 (td, 14.1, 4.3)
16	38.5	2.10 (ddd, 11.7, 7.0, 3.8) 1.51 (m)
17	37.8	
18	34.2	2.53 (ddd, 16.1, 11.7, 7.0) 2.41 (ddd, 16.1, 6.4, 3.8)
19	27.2	1.19 (s)
20	26.8	1.12 (s)
21	21.8	1.03 (s)
22	19.2	1.81 (qd, 13.0, 3.5) 1.52 (m)
23	18.4	2.93 (d, 18.5) 2.81 (dd, 18.5, 8.1)
24	14.3	0.72 (s)
25	10.8	2.04 (s)

Figure S1 ^1H NMR spectrum (700 MHz, CDCl_3) of austalide K

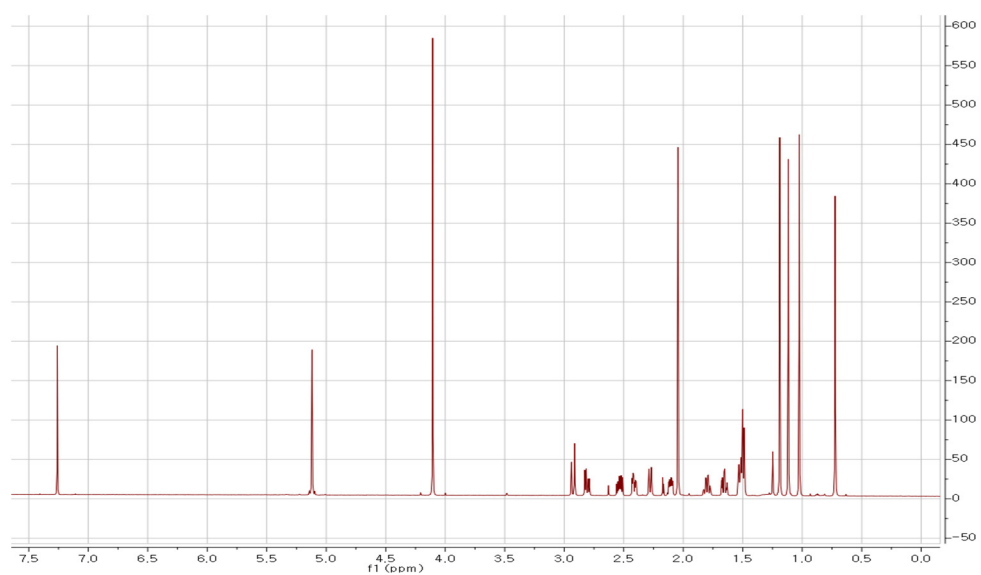


Figure S2 ^{13}C NMR spectrum (175 MHz, CDCl_3) of austalide K

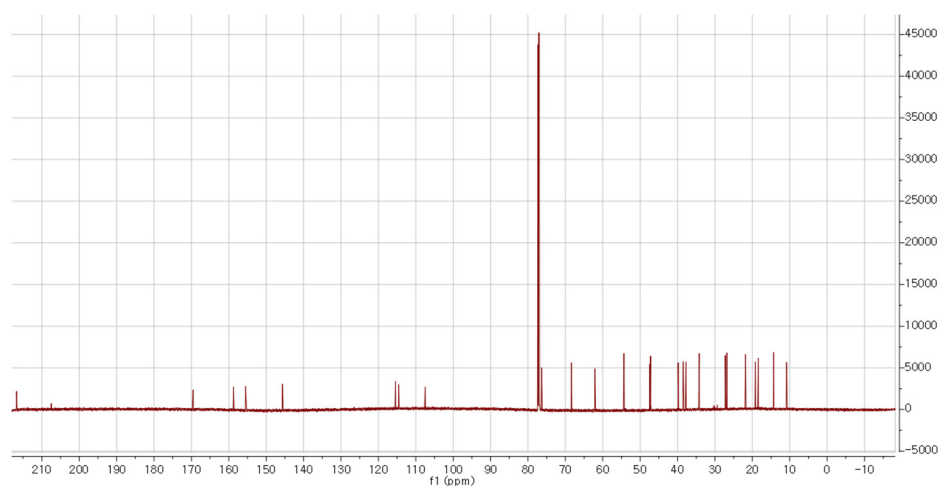


Figure S3 HSQC spectrum (700 MHz, CDCl₃) of austrialide K

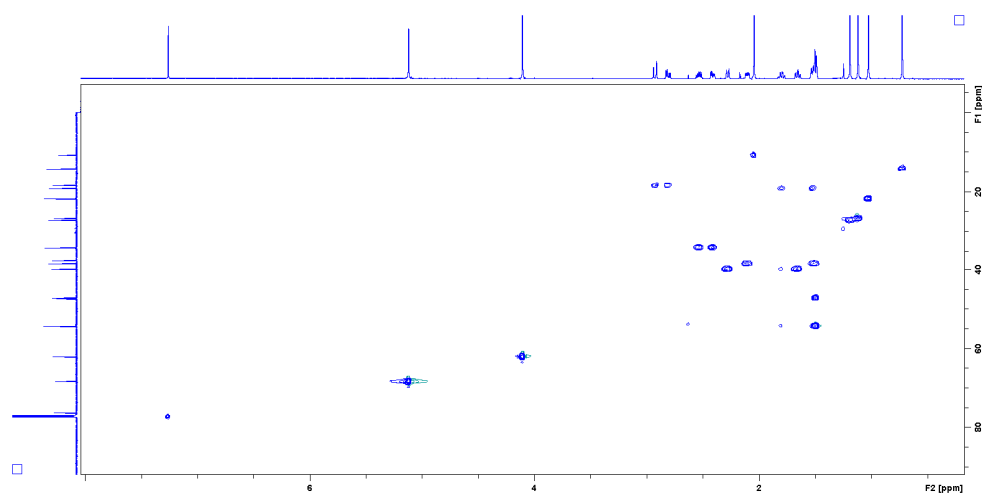


Figure S4 HMBC spectrum (700 MHz, CDCl₃) of austrialide K

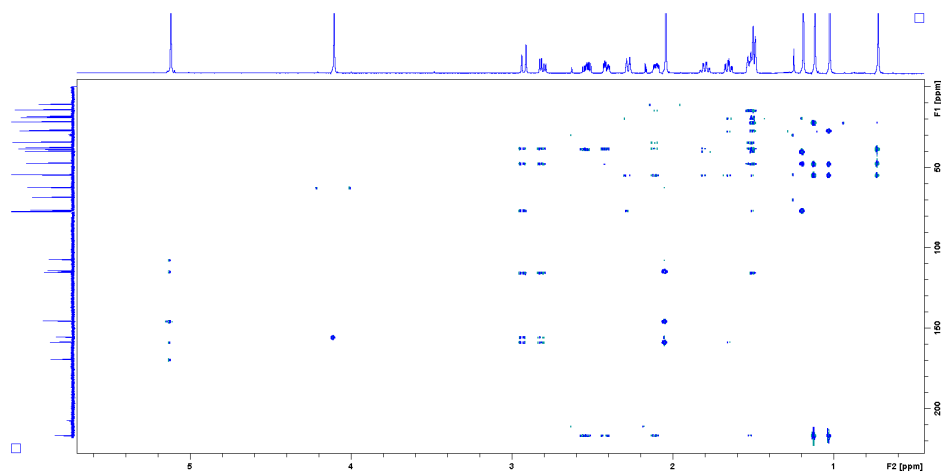


Figure S5 COSY spectrum (175 MHz, CDCl₃) of austalide K

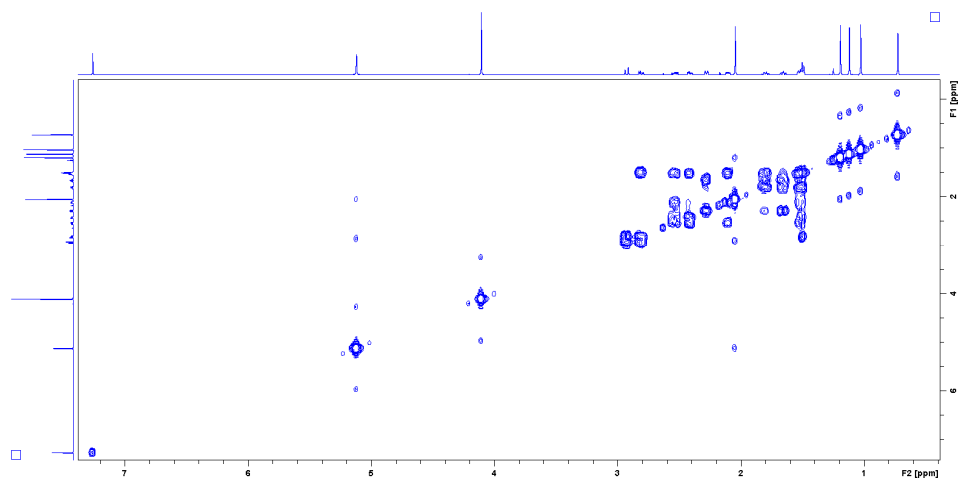


Figure S6 ROESY spectrum (175 MHz, CDCl₃) of austalide K

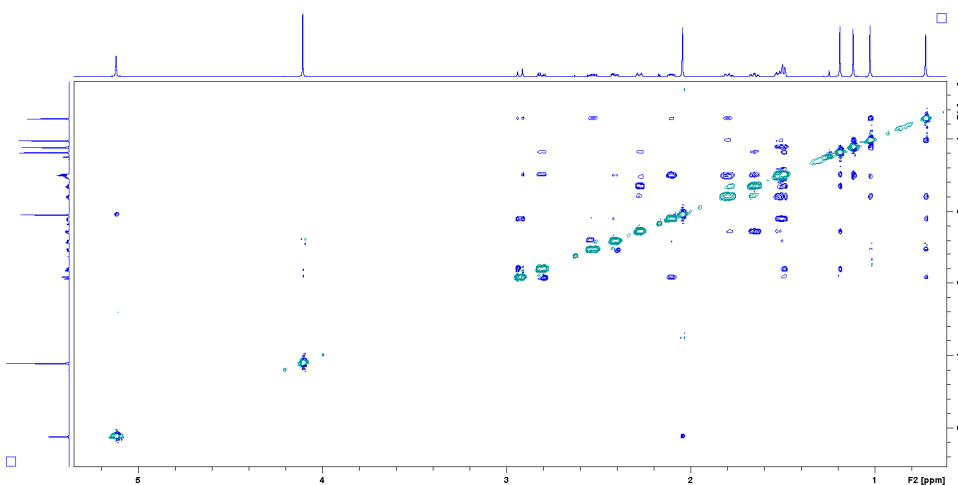


Figure S7 The expression level of NFATc1 was measured by using western blot analysis.

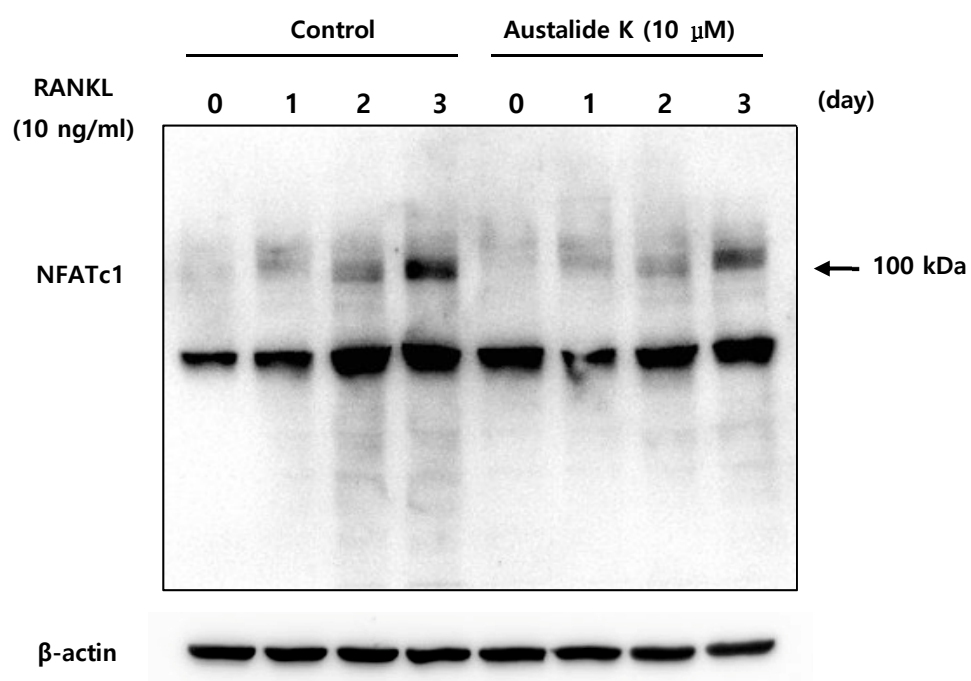


Figure S8 Survival rate (%) of RANKL-induced BMM with treatment of austalide K.

