SUPPLEMENTARY MATERIAL

New pimarane diterpenes and other antimycobacterial metabolites from *Anisochilus verticillatus*

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ABSTRACT

Phytochemical investigation of the acetone extract of the aerial parts of *Anisochilus verticillatus* afforded a new 8,9-secopimarane diterpene (**1**), two new isopimarane diterpenes (**2**, **3**), and the known ursolic acid (**4**), α -amyrin (**5**), β -amyrin (**6**), stigmast-5-en-3-one (**7**), and hydroxychavicol (**8**). Structures of the new compounds were elucidated with the help of 1D and 2D nuclear magnetic resonance spectroscopic data, and single crystal X-ray crystallography of compound **3**. Compounds **2** and **8** inhibited *Mycobacterium tuberculosis* H37Ra with an IC₅₀ 11.3 μ g/mL (IC₉₀ 20.0 μ g/mL) and 12.5 μ g/mL, respectively. Correspondingly, molecular docking studies with Extra Precision Glide revealed a correlation between score and biological activity for these compounds to describe the molecular basis for the most significant SAR results.

Keywords: *Anisochilus verticillatus*, isopimarane diterpenes, molecular docking, antimycobacterium, phytochemicals

Table of Contents

Table S1. NMR Spectroscopic Data (400 and 100 MHz, CDCl ₃) for Compounds 1- 3	S3
Figure S1. HR-ESI-MS spectrum and value of Compound 1	S4
Figure S2. ¹ H NMR spectrum of Compound 1 (Chloroform- <i>d</i>)	S5
Figure S3. ¹³ C NMR spectrum of compound 1 (Chloroform- <i>d</i>)	S6
Figure S4. DEPT 135 spectrum of compound 1 (Chloroform- <i>d</i>)	S7
Figure S5. COSY, NOESY and HMBC correlations of compound 1	S8
Figure S6. Comparison of β- (A) and α- (B) oriented epoxy bridge in MM2 stabilized model of Compound 1	S9
Figure S7. HRESIMS spectrum of Compound 2	S10
Figure S8. ¹ H NMR spectrum of Compound 2 (Chloroform- <i>d</i>)	S11
Figure S9. ¹³ C NMR spectrum of compound 2 (Chloroform- <i>d</i>)	S12
Figure S10. DEPT 135 spectrum of compound 2 (Chloroform- <i>d</i>)	S13
Figure S11. COSY, NOESY and HMBC correlations of compound 2	S14
Figure S12. HREIMS spectrum of Compound 3	S15
Figure S13. ¹ H NMR spectrum of Compound 3 (Chloroform- <i>d</i>)	S16
Figure S14. ¹³ C NMR spectrum of compound 3 (Chloroform- <i>d</i>)	S17
Figure S15. DEPT 135 spectrum of compound 3 (Chloroform- <i>d</i>)	S18
Figure S16. ORTEP drawing of the X-Ray Structure Analysis of compound 2	S19
Table S2. Crystal data and structure refinement for compound 2	S20
Figure S17. COSY, NOESY and HMBC correlations of compound 3	S21
Table S3. Docking Scores for D-cycloserine, compounds 2 and 8	S22
Figure S18. Binding mode of cycloserine	S23
Figure S19. Binding mode of compound 2	S24
Figure S20. Binding mode of compound 8	S25
Table S4. The per-residue interactions observed for compound 8 with key amino acid residues in the receptor. Figures in bracket signify the quantitative values of interaction (in kcal/mol).	S26

Table S1: NMR Spectroscopic Data (400 and 100 MHz, CDCl₃) for Compounds **1- 3**

Position	1		2		3	
	δ_C , type	δ_H (J in Hz)	δ_C , type	δ_H (J in Hz)	δ_C , type	δ_H (J in Hz)
1ax 1eq	31.3, CH ₂	1.18 m 1.38 m	34.3, CH ₂	1.42 m	28.3, CH ₂	1.23 d (12.3), 2.02 dt(12.3, 3.8)
2ax 2eq	19.5, CH ₂	1.62m	17.6, CH ₂	1.84 m 1.47 m	17.6, CH ₂	1.85 m 1.55 m
3ax 3eq	41.5, CH ₂	1.23 m 1.42m	37.7, CH ₂	1.15 m 1.60 m	37.7, CH ₂	1.08 m 1.83 m
4	32.8, C	-	38.9, C	-	39.6, C	-
5	51.9, CH	1.88 d (10.6)	77.2, C	-	79.8, C	-
6	73.8, CH	4.35 dd(10.7, 6.5)	72.7, CH	4.11 bt (3.1)	73.7, CH	4.31 bt(2.8)
7a 7b	49.3, CH ₂	2.33 d (10.6), 3.30 dd(10.7, 6.5)	43.6, CH ₂	1.73 m, 2.14 dd(14.3, 3.4)	38.5, CH ₂	1.60dd(15.2, 2.8), 2.37 dd(15.2, 3.2)
8	213.1, C	-	74.7, C	-	77.2, C	-
9	107.7, C	-	49.0, CH	1.73 m	78.7, C	-
10	46.8, C	-	40.1, C	-	43.2, C	-
11ax 11eq	29.9, CH ₂	1.63 m	17.0, CH ₂	1.73 m 1.40 m	23.4, CH ₂	2.29dt(13.7, 3.9) 1.43m
12a 12b	32.0, CH ₂	1.85 m	37.8, CH ₂	1.40 m 1.59 m	31.4, CH ₂	1.29 d (13.8) 1.78 dd(13.8, 3.9)
13	43.1, C	-	36.3, C	-	35.4, C	-
14a 14b	55.3, CH ₂	2.21 d (14.2), 3.05 d (14.2)	51.1, CH ₂	1.39 m 1.43m	46.6, CH ₂	1.15dd(14.1, 2.1), 1.95d (14.1)
15	145.3, CH	6.29 dd(11.0, 17.7)	151.1, CH	5.74 dd(11.1, 17.7)	151.0, CH	5.79 dd(10.7, 17.7)
16a 16b	110.4, CH ₂	4.89 d (17.7), 5.09 d (11.1)	108.9, CH ₂	4.90dd(17.7, 1.1), 4.85 dd(11.1, 1.1)	108.9, CH ₂	4.87dd(10.7, 1.0), 4.92 dd(17.7, 1.0)
17	32.8, CH ₃	1.05 s	24.0, CH ₃	1.22 s	25.0, CH ₃	1.28 s
18	34.6, CH ₃	1.06 s	27.7, CH ₃	1.00 s	27.4, CH ₃	0.99 s
19	22.3, CH ₃	1.00 s	25.3, CH ₃	1.44 s	25.9, CH ₃	1.47 s
20	14.6, CH ₃	1.11s	18.1, CH ₃	1.42 s	19.7, CH ₃	1.54 s

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AV-6_130306150352 #1304 RT: 5.81 AV: 1 NL: 5.34E5
T: FTMS - p ESI Full ms [100.00-700.00]

Figure S1. HRESIMS value of Compound 1

Figure S2. ^1H NMR spectrum of Compound **1** (CDCl_3)

Figure S3. ^{13}C NMR spectrum of compound **1** (CDCl_3)

Figure S4. DEPT 135 spectrum of compound **1** (Chloroform-*d*)

Selected HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$) and ^1H - ^1H COSY (—) correlations

Selected NOESY (^1H ----- ^1H) correlations

Figure S5. COSY, NOESY and HMBC correlations of compound **1**

Figure S6. Comparison of β - (A) and α - (B) oriented epoxy bridge in MM2 stabilized model of Compound **1**

Figure S7. HRESIMS spectrum of Compound 2

Figure S8. ^1H NMR spectrum of Compound **2** (CDCl_3)

Figure S9. ^{13}C NMR spectrum of compound **2** (CDCl_3)

Figure S10. DEPT 135 spectrum of compound **2** (Chloroform-*d*)

Selected HMBC ($^1\text{H} \rightarrow ^{13}\text{C}$) and $^1\text{H}-^1\text{H}$ COSY (—) correlations

Selected NOESY ($^1\text{H} \rightarrow ^1\text{H}$) correlations

Figure S11. COSY, NOESY and HMBC correlations of compound **2**

Figure S12. HREIMS spectrum and value of Compound **3**

Figure S13. ^1H NMR spectrum of Compound **3** (CDCl_3)

Figure S14. ^{13}C NMR spectrum of compound **3** (CDCl_3)

Figure S15. DEPT 135 spectrum of compound **3** (Chloroform-*d*)

Figure S16. ORTEP Drawing of the X-Ray Structure Analysis of compound **3**

Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P21	
Unit cell dimensions	a = 10.9350(8) Å	$\alpha = 90^\circ$.
	b = 7.4522(6) Å	$\beta = 114.9020(10)^\circ$.
	c = 12.3825(9) Å	$\gamma = 90^\circ$.
Volume	915.24(12) Å ³	
Z	2	
Density (calculated)	1.228 g/cc	
Absorption coefficient	0.083 mm ⁻¹	
F(000)	372	
Crystal size	0.39 x 0.07 x 0.01 mm ³	
Theta range for data collection	1.81 to 24.99°.	
Index ranges	-13 ≤ h ≤ 13, -8 ≤ k ≤ 8, -14 ≤ l ≤ 14	
Reflections collected	8640	
Independent reflections	3202 [R(int) = 0.0294]	
Completeness to theta = 24.99°	99.9 %	
Max. and min. transmission	0.9994 and 0.9686	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	3202 / 1 / 225	
Goodness-of-fit on F ²	1.033	
Final R indices [I > 2σ(I)]	R1 = 0.0438, wR2 = 0.1005	
R indices (all data)	R1 = 0.0533, wR2 = 0.1075	
Absolute structure parameter	-1.4(12)	
Largest diff. peak and hole	0.320 and -0.155 e.Å ⁻³	

Table S2. Crystal data and structure refinement for compound **3**

Figure S 17. COSY, NOESY and HMBC correlations of compound **3**

Table S3. Docking Scores for D-cycloserine, compounds **2** and **8**

Compounds	Glide Score
D-cycloserine	-11.20
2	-8.50
8	-8.23

Figure S18. Binding mode of cycloserine

Figure S19. Binding mode of compound **2**

Figure S20. Binding mode of compound **8**

Table S4. The per-residue interactions observed for compound 8 with key amino acid residues in the receptor. Figures in bracket signify the quantitative values of interaction (in kcal/mol).

Compound	Per residue interactions within the receptor (kcal/mol)		
	Van der Waals	Coulombic (Electrostatic)	Hydrogen bonding
2	Val 40, Tyr 46, Trp 88, Ser 211, Ser 213, Arg 228 and Pro 229	Tyr 46, Asn 212, Ser 213, Ser 214, Arg 228 and Pro 229	Ser 213, Arg 228, and Gly 230
8	Val 40, Tyr 46, Trp 88, Ser 211, Ser 213, Arg 228 and Pro 229	Tyr 46, Asn 212, Ser 213, Ser 214, Arg 228 and Pro 229	Ser 213, Arg 228, and Gly 230