

Supplementary Materials for

Microindoline 581, an indole derivative from *Microbacterium* sp. RP581 as a novel selective antineoplastic agent to combat hepatic cancer cells: Production, optimization and structural elucidation

Roya Pournajati, Ronald Gust, Brigitte Kircher and Hamid Reza Karbalaeei-Heidari*

*To whom correspondence should be addressed. E-mail: karbalaeei@shirazu.ac.ir

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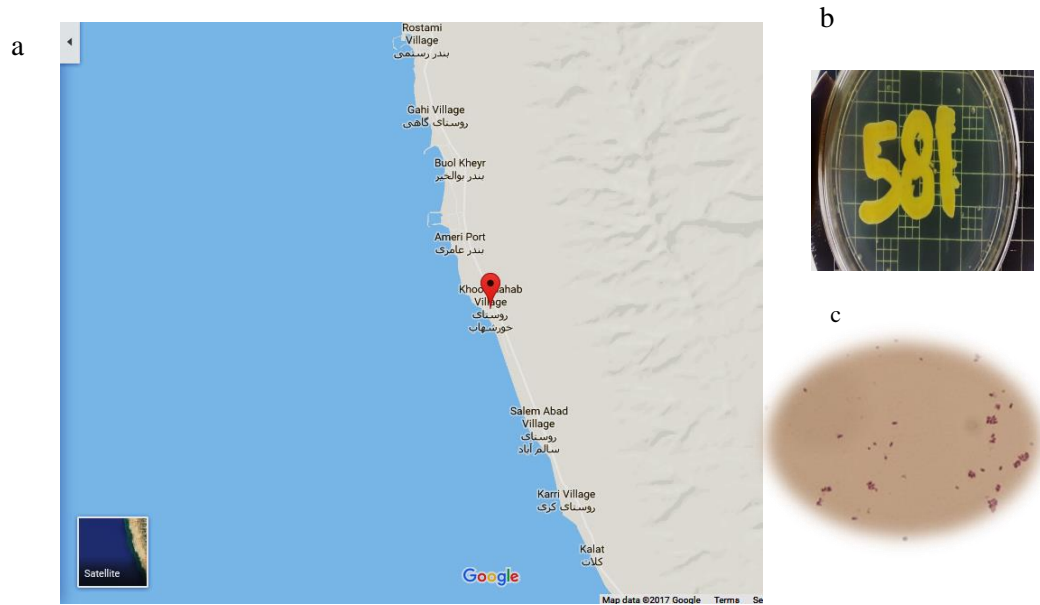


Figure S1. (a) Geographic location of sampling, (b) *Microbacterium* Sp. RP 581 colony and (c) Gram staining.

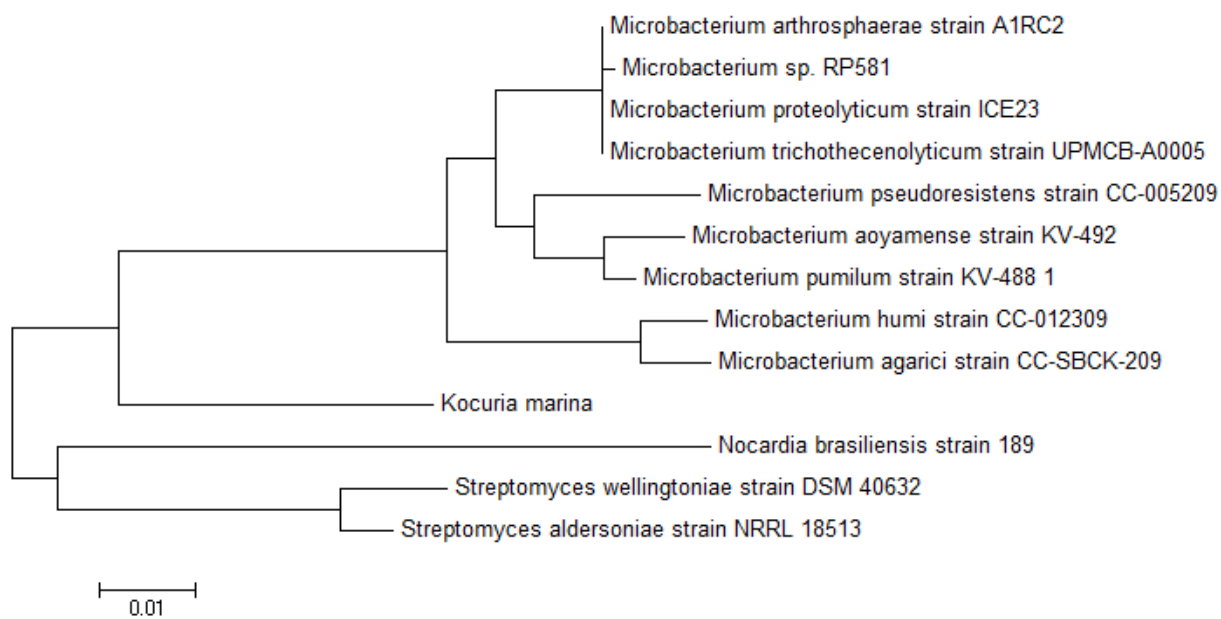
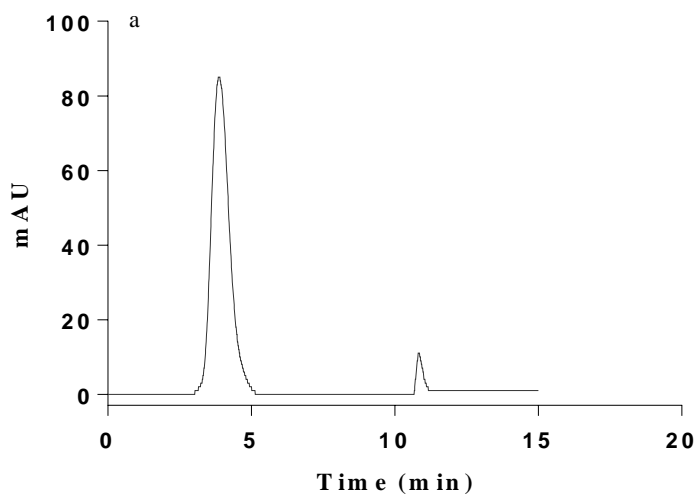
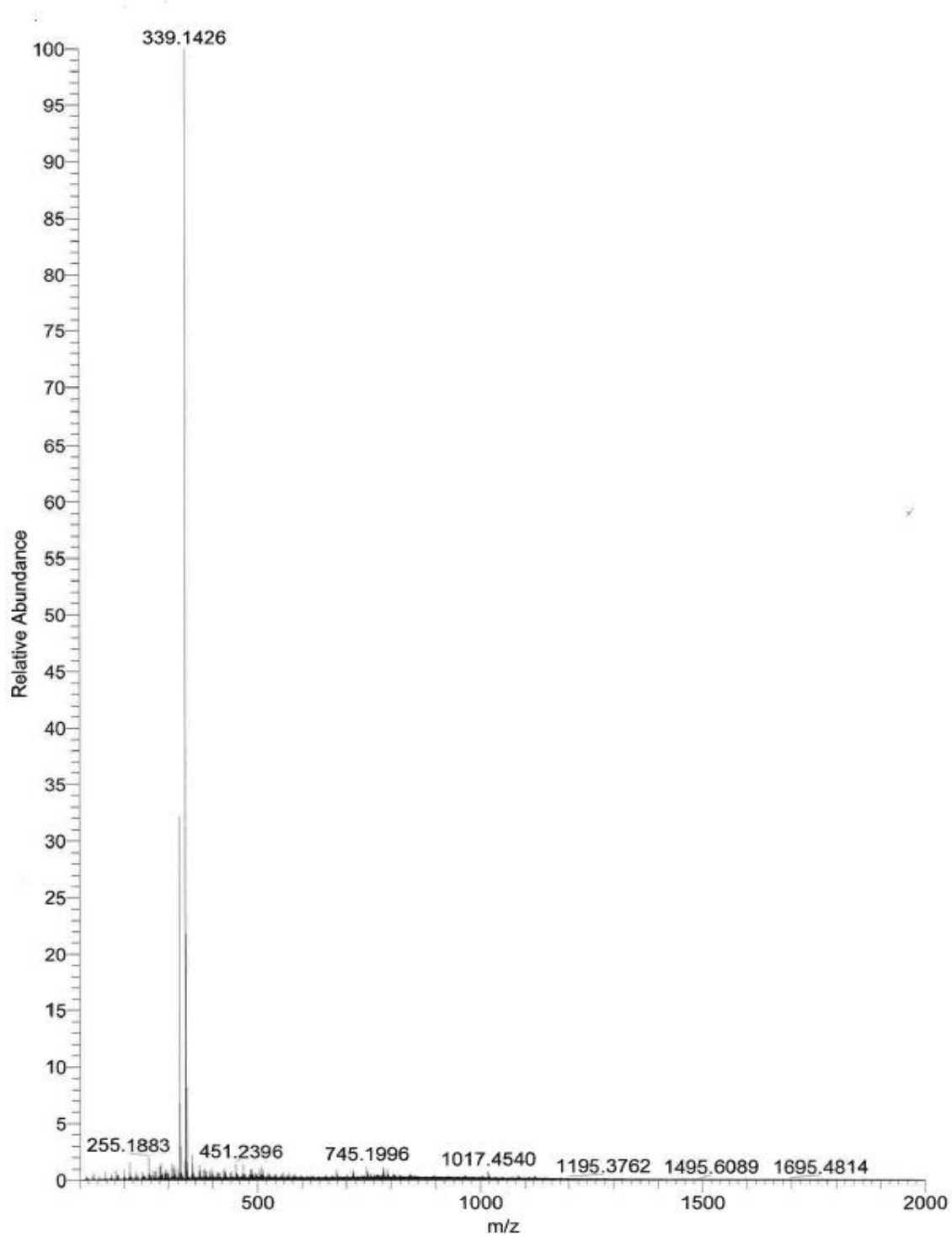


Figure S2. Phylogenetic tree of *Microbacterium* Sp. RP 581 using neighbor-joining algorithm.

Microindolin 581



b



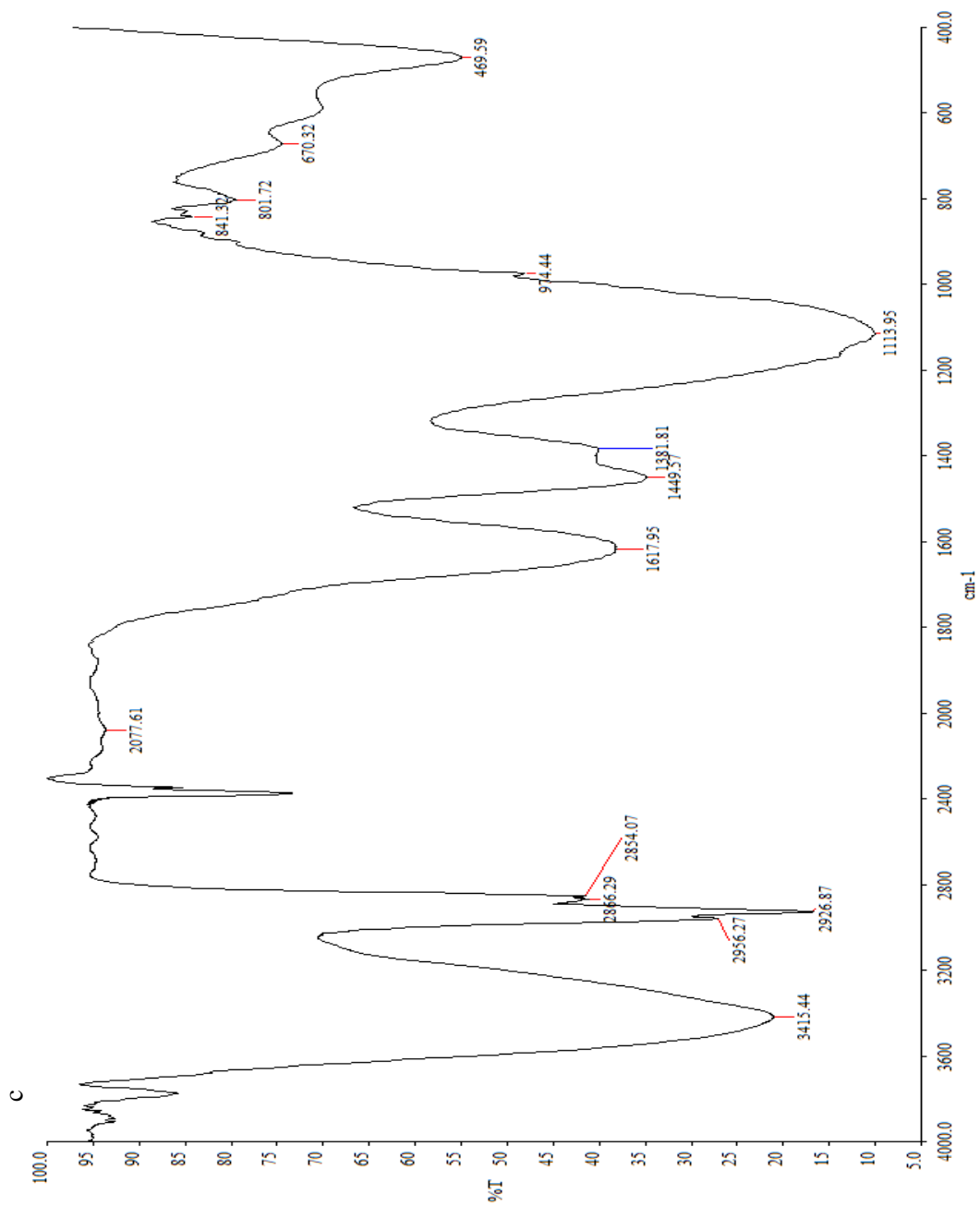


Figure S3. (a) HPLC chromatogram of the purified Microindoline 581, TSK gel C18 (7.8 × 300), MeOH: H₂O (90:10), flow rate 1mL/min, 254nm (b) Mass spectrum of Microindoline 581, negative mode and (c) FTIR spectrum.

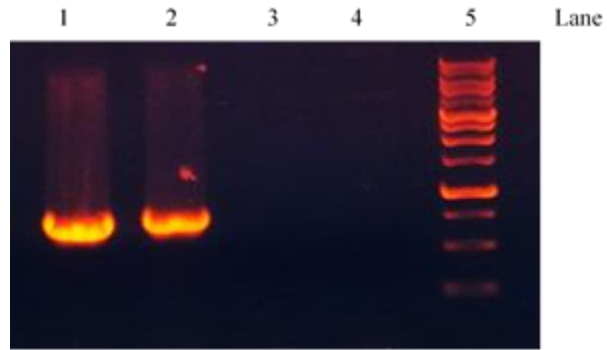


Figure S4. Gel electrophoresis of the PCR product of TtL, lanes 1-4 template DNA treated with 0 μM , 90 μM , 120 μM and 150 μM of Microindoline 581.

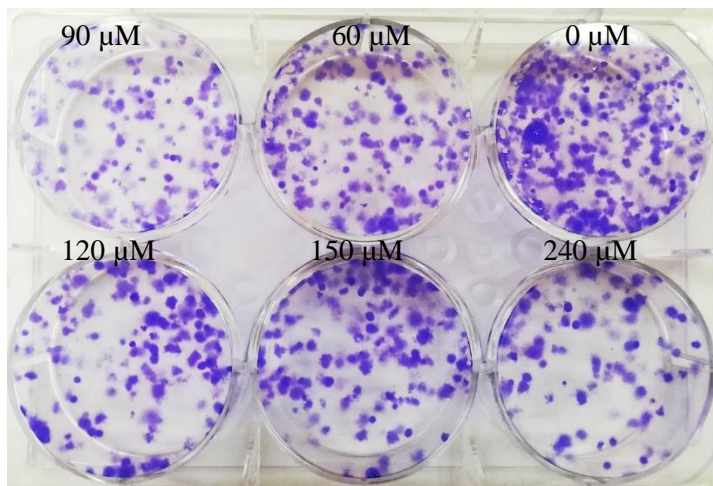


Figure S5. Colony forming assay, HePG2 cells treated with microbenzoindoline 581 for 48 h.

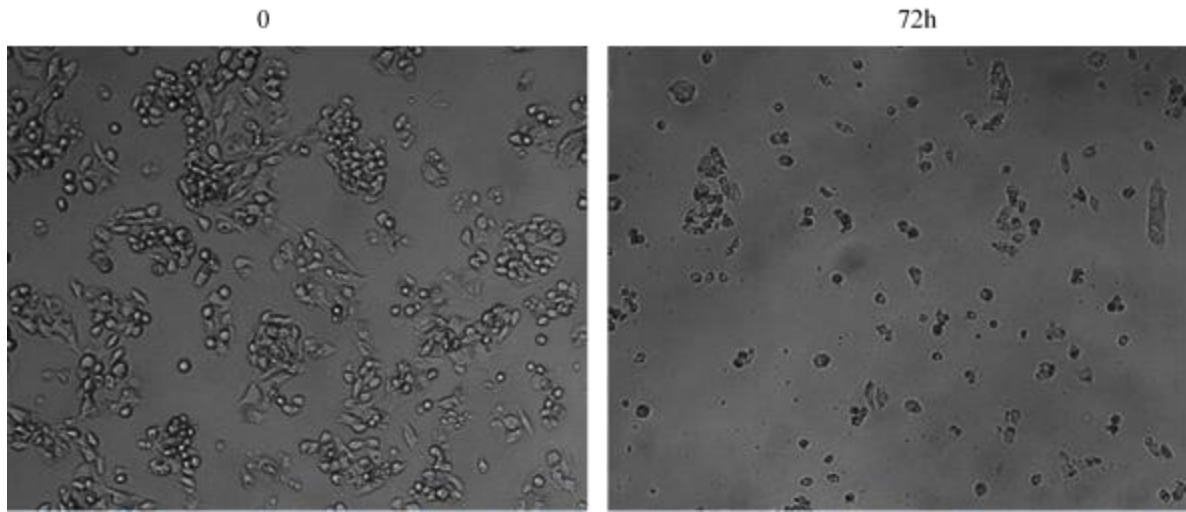


Figure S6. HepG2 cells treated with Microindoline 581 (160 μ M).

Table S1. The fractional factorial experimental design with the variables, symbol code, and experimental level of the variables.

Coded factor	-1	+1
X ₁ : carbon source (%)	0.5	1
X ₂ : Nitrogen source (%)	0.5	1
X ₃ : Sea water (%)	30	70
X ₄ : pH	6	8
X ₅ : Inoculation size (%)	1	5

Table S2. ANOVA analysis of fractional factorial experiments.

Source	Sum of square	Df	Mean square	F value	P-value	
Model	13323.35	14	951.6678	1554.235	0.0199	Significant
A-carbon source	4248.758	1	4248.758	6938.943	0.0076	Significant
B-nitrogen source	782.1811	1	782.1811	1277.434	0.0178	Significant
C-sea water	2220.53	1	2220.53	3626.502	0.0106	Significant
D-pH	2.009306	1	2.009306	3.281538	0.3211	
E-inoculation	301.9775	1	301.9775	493.1805	0.0286	Significant
AB	1261.848	1	1261.848	2060.812	0.0140	Significant
AC	496.7327	1	496.7327	811.2487	0.0223	Significant
AE	223.8764	1	223.8764	365.6282	0.0333	Significant
BC	1776.412	1	1776.412	2901.182	0.0118	Significant
BD	250.0352	1	250.0352	408.3498	0.0315	Significant
BE	385.6314	1	385.6314	629.8015	0.0254	Significant
CD	237.8535	1	237.8535	388.4551	0.0323	Significant
CE	569.8963	1	569.8963	930.7373	0.0209	Significant

Table S3. The coded independent variables used in the RSM design.

Coded factor	-1.73	-1	0	+1	+1.73
X ₁ : carbon source (%)	0.04	0.3	0.65	1	1.26
X ₂ : Nitrogen source (%)	0.04	0.3	0.65	1	1.26
X ₃ : Sea water (%)	15.36	30	50	70	84.64

Table S4. ANOVA analysis of RSM experiments.

Source	Sum of square	df	Mean square	F value	<i>P</i> -value	
Model	14631.96	9	1625.773	47.41089	< 0.0001	Significant
X ₁	650.8335	1	650.8335	18.97965	0.0014	Significant
X ₂	1104.232	1	1104.232	32.20167	0.0002	Significant
X ₃	2720.119	1	2720.119	79.32428	< 0.0001	Significant
X ₁ X ₂	161.5268	1	161.5268	4.710455	0.0551	Significant
X ₁ X ₃	684.5042	1	684.5042	19.96155	0.0012	Significant
X ₂ X ₃	844.5813	1	844.5813	24.62973	0.0006	Significant
X ₁ ²	3403.519	1	3403.519	99.25362	< 0.0001	Significant
X ₂ ²	3717.508	1	3717.508	108.4102	< 0.0001	Significant
X ₃ ²	3459.992	1	3459.992	100.9005	< 0.0001	Significant

Table S5. Colony forming assay of HePG2 cells treated with microbenzoindoline 581 for 48 h.

Dose of treatment (μM)	Colony number (Av.)	PE	Survival%
0	138 ± 2.33	0.69	100
60	83 ± 6.52	0.415	60.14
90	58 ± 4.26	0.29	42.03
120	62 ± 1.42	0.31	44.93
150	52 ± 7.81	0.26	37.68
240	36 ± 3.95	0.18	26.09