Supplemental Data

Genomics-Guided Discovery of a New and Significantly Better Source of Anticancer Natural Drug FK228

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Running title: A new and better source of FK228

Table S1. Bacterial strains and plasmids used in this study

Strains or plasmids	Description[a]	Source or reference
Burkholderia thailandensis		
MSMB43	Wild type strain, FK228-producing, Am^r	(1)
MSMB43/pBMTL3-*depR*	MSMB43 harboring pBMTL3-*depR*, FK228 production unchanged, Am^r Cm^r	This work
MSMB43/pBMTL3-*spiR*	MSMB43 harboring pBMTL3-*spiR*, FK228 production decreased, Am^r Cm^r	This work
MSMB43/pBMTL3-*tdpR*	MSMB43 harboring pBMTL3-*tdpR*, increased FK228 production, Am^r Cm^r	This work
MSMB43/pBMTL-3	Control strain, MSMB43 harboring vector pBMTL-3, FK228 production unchanged, Am^r Cm^r	This work
Chromobacterium violaceum no. 968 (=FERM BP-1968)	Wild type strain, FK228-producing, Ap^r $Thio^r$	IPOD[b]
Escherichia coli		
DH5α	General cloning host	Lab stock
S17-1	Host strain for interspecies conjugation	Lab stock
S17-1/pBMTL3-*depR*	Donor strain of pBMTL3-*depR* for conjugation	(2)
S17-1/pBMTL3-*spiR*	Donor strain of pBMTL3-*spiR* for conjugation	(3)
S17-1/pBMTL3-*tdpR*	Donor strain of pBMTL3-*tdpR* for conjugation	(4)
S17-1/pBMTL3	Donor strain of pBMTL-3 for conjugation	(2)
Plasmids		
pET29a	*E. coli* -based gene expression vector	Novagen
pBMTL-3	Cm^r, pBBR1 *ori*, broad host-range vector	(5)
pBMTL3-*depR*	Cm^r, Cv_*depR* (with RBS from pET29a) cloned into pBMTL-3	(2)
pBMTL3-*spiR*	Cm^r, *spiR* (with RBS from pET29a) cloned into pBMTL-3	(3)
pBMTL3-*tdpR*	Cm^r, *tdpR* (with RBS from pET29a) cloned into pBMTL-3	(4)

[a] Am^r, apramycin resistance; Ap^r, ampicillin resistance; Cm^r, chloramphenicol resistance; $Thio^r$, thiostrepton resistance.

[b] IPOD, International Patent Organism Depositary, Tsukuba, Japan.

Table S2. List of primers used for quantitative real-time PCR in this study.

Gene	**Primer name**[a]	**Sequence (5'→3')**	**Amplicon size**
DepA	Cv_depA-RT-F	CACCTCGGGGTCCACGCG	186 bp
	Cv_depA-RT-R	GGGAAAACCGCATTGCAAGGG	
	Bth_depA-RT-F	CGGCGACTACACCAGCAATAC	196 bp
	Bth_depA-RT-R	CAGCAGGCTCGTCAGGACTA	
DepJ	Cv_depJ-RT-F	CCTTGTTCGGGCACAGCAT	183 bp
	Cv_depJ-RT-R	TCCTCATCACCTCGTCCAGAA	
	Bth_depJ-RT-F	TGACGGAAGCCATCACGCCG	283 bp
	Bth_tdpJ-RT-R	GATCGGCAGGATCATCTTGACG	
DepR	Cv_depR-RT-F	TGATCCTGGAGGAGAACTACAC	194 bp
	Cv_depR-RT-R	CAGTTGATTGGCGGCGATG	
	Bth_depR-RT-F	AAGCGAACGCCGACAAGCT	197 bp
	Bth_depR-RT-R	CGCAACGAGGTAGTACGCATAC	
16S rDNA	16S-RT-F	GACTCCTACGGGAGGCAGC	199 bp
	16S-RT-R	GTATTACCGCGGCTGCTGGC	

[a] Cv, *C. violaceum* No. 968; Bth, *B. thailandensis* MSMB43.

Table S3. Comparison of ^{1}H- and ^{13}C-NMR data of the newly purified FK228 from *B. thailandensis* MSMB43 with the historical data of FK228 from *C. violaceum* No. 968 (6)

Position	**^{1}H NMR[a]**		**^{13}C NMR[b]**	
	Sample Source			
	Cv No. 968	*Bth* MSMB43	*Cv* No. 968	*Bth* MSMB43
1	C=O	C=O	172.5	172.1
2	C=O	C=O	172.1	171.5
3	C=O	C=O	169.9	169.5
4	C=O	C=O	168.8	168.3
5	C=O	C=O	165.6	165.4
6	quaternary C	quaternary C	131.6	130.8
7	5.80	5.64	131.5	130.6
8	5.95	5.81	130.4	129.9
9	6.21	6.10	127.8	127.6
10	5.70	5.58	71.5	71.2
11	3.96	3.85	63.0	62.2
12	4.40	4.24	59.1	58.8
13	4.68	4.52	57.7	57.1
14	3.00, 3.14	2.96, 3.08	38.8	38.3
15	2.67, 3.14	2.50, 2.99	38.4	38.1
16	3.21, 3.25	3.04, 3.17	36.1	35.7
17	2.33	2.23	32.0	31.1
18	2.70~2.59	2.55	31.0	30.5
19	2.27	2.16	29.6	28.9
20	1.10	1.03	19.5	19.5
21	1.07	1.00	19.4	19.4
22	1.02	0.94	18.9	19.0
23	0.98	0.90	18.8	19.0
24	1.66	1.59	13.4	13.8
NH-1	8.98	8.85		
NH-2	8.46	8.34		
NH-3	7.81	7.77		
NH-4	7.73	7.56		

[a] ^{1}H NMR data of our work were obtained at 500 MHz in DMSO-*d6*. The historical ^{1}H NMR data were obtained at 400 MHz in DMF-*d*7; [b] ^{13}C NMR data of our work were obtained at 125 MHz in DMSO-*d6*. The historical NMR data were obtained at 100 MHz in DMF-*d*7. The minor value differences are likely due to the use of difference instruments and solvents.

Method: The newly purified FK228 was dissolved in DMSO-*d*6 and transferred into Shigemi NMR tube. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-500 NMR spectrometer at 25^{o}C. All 2D NMR experiments were performed employing the standard Bruker Topspin 3.1.b.11 Software package.

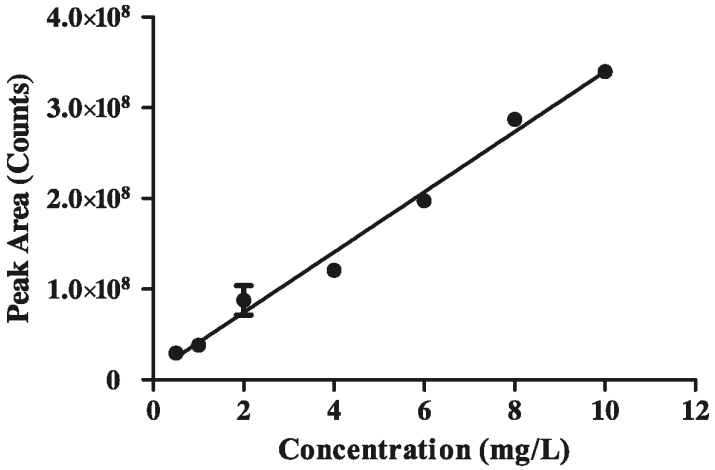

Fig. S1. Standard curve of FK228 concentrations vs. ion signal peak areas:

$Y = 3E+07X + 8E+06$ $(R^2 = 0.99)$

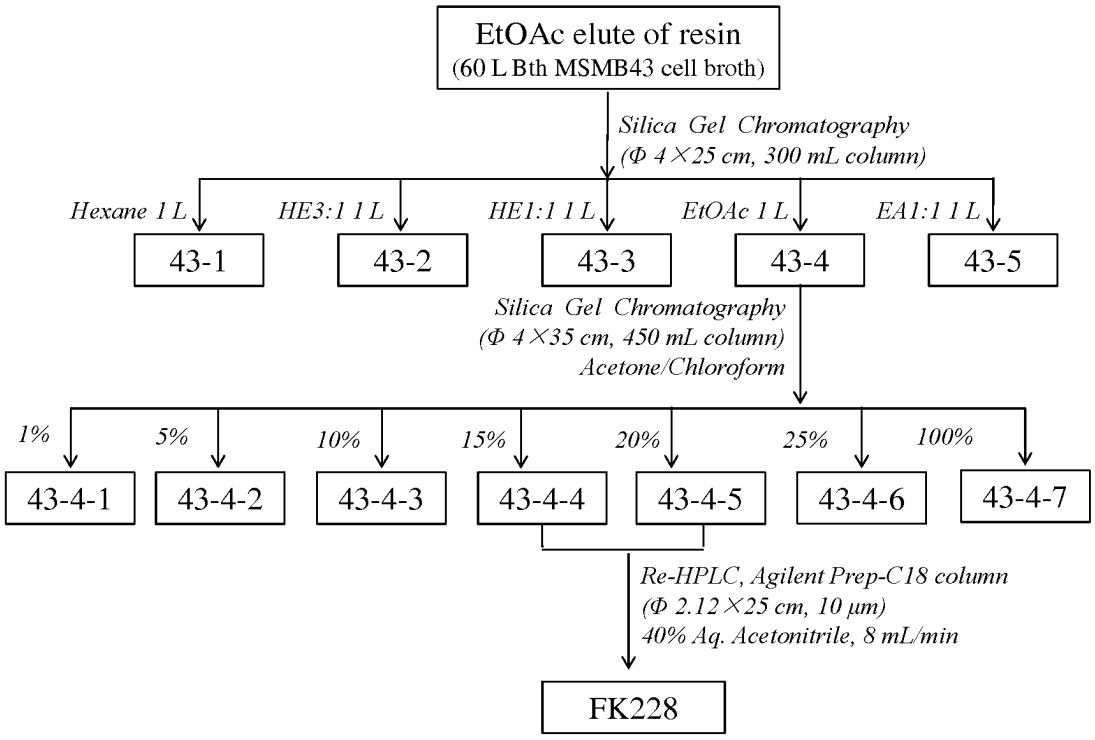

Fig. S2. Flow chart of larger scale purification of FK228 from the fermentation broth of *B. thailandensis* MSMB43.

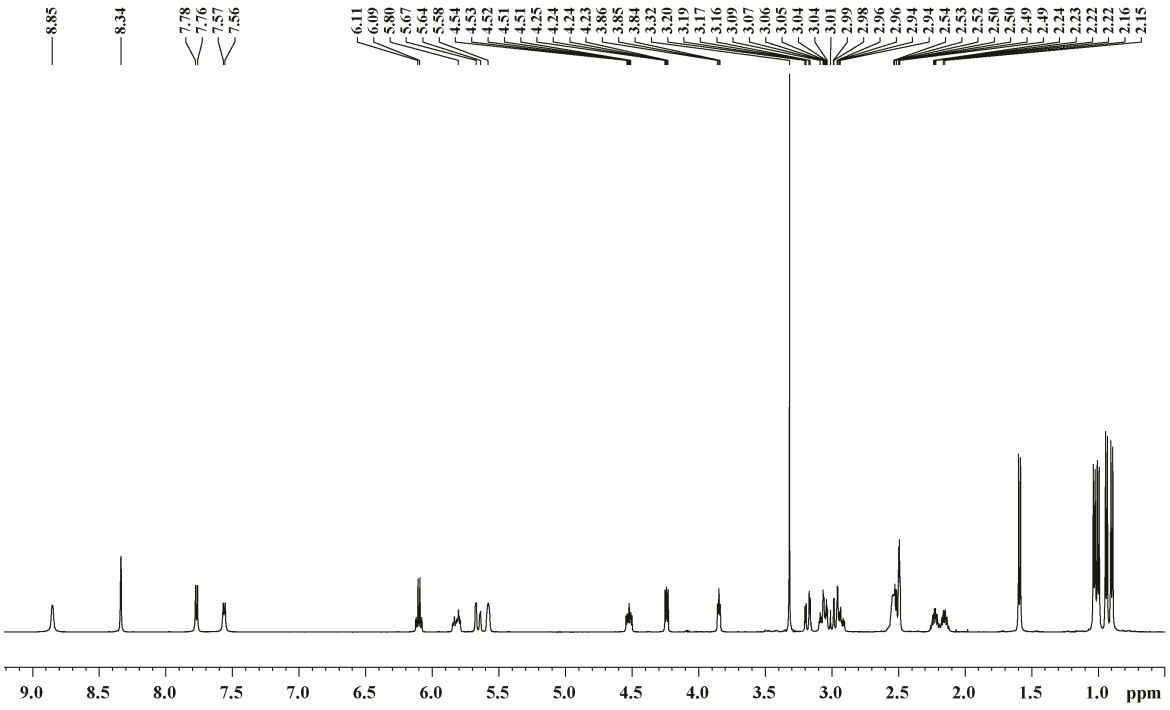

Fig. S3. Proton (^{1}H) NMR spectrum of FK228 produced by *B. thailandensis* MSMB43.

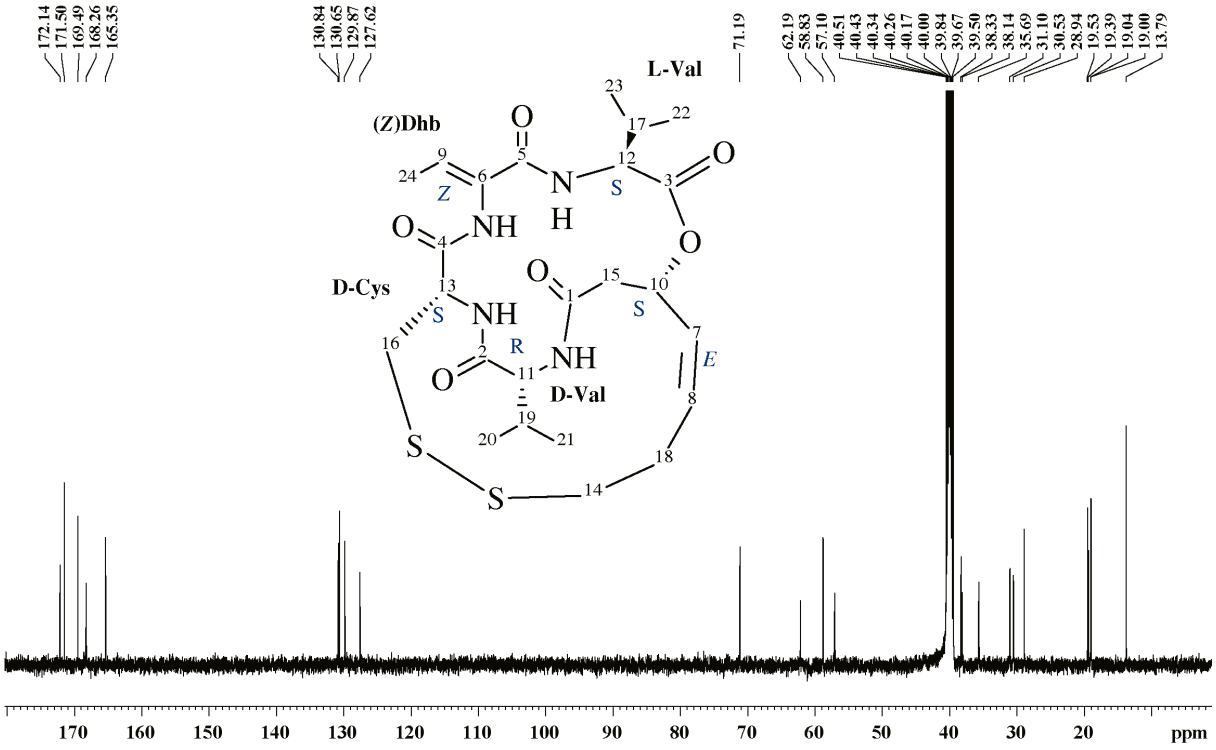

Fig. S4. Carbon (^{13}C) spectrum of FK228 produced by *B. thailandensis* MSMB43.

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