Pergamon

Tetrahedron Letters, Vol. 35, No. 27, pp. 4693-4696, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)00910-4

Fusidienol: A Novel Inhibitor of Ras Farnesyl-Protein Transferase from *Fusidium griseum*

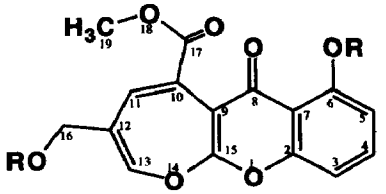
Sheo B Singh*, E. Tracy Jones, Michael A. Goetz, Gerald F. Bills, Mary Nallin-Omstead, Rosalind G. Jenkins, Russell B. Lingham, Keith C. Silverman and Jackson B. Gibbs

Merck Research Laboratories, P. O. Box 2000 Rahway, NJ 07065 and West Point, PA 19486

Abstract: Ras (p21) protein is frequently found mutated in human cancers and must be farnesylated by farnesyl protein-transferase (FPTase) to achieve cell-transforming activity. Our continued search for inhibitors of FPTase as potential cancer chemotherapeutics led to the isolation of fusidienol from extracts of the fungus *Fusidium griseum*. Fusidienol is a novel and potent oxygen-containing [7/6/6] tricyclic heterocycle.

Farnesyl-protein transferase (FPTase) catalyzes the farnesylation of proteins such as Ras (p21) on the cysteine residue near the C-terminus. For Ras, post-translational modification is required for cell-transforming activity[1]. Selective inhibitors of FPTase have the potential to be used as anticancer agents, particularly in colon and pancreatic cancers[2] where the *ras* oncogene is mutated and believed to play a major role in tumor formation. Recently, FPTase inhibitors have shown biological efficacy against *ras* dependent cell-transformation.[3]

We have recently reported the isolation of chaetomellic acids[4] as novel inhibitors of FPTase with nanomolar potency. Other microbial products which have been reported as inhibitors of FPTase are pepticinnamins,[5] (a class of peptides), gliotoxin,[6] 10'-desmethoxystreptonigrin,[7] and manumycin analogs.[8] Continued screening for potent inhibitors of FPTase resulted in the isolation of fusidienol (**1a**) from *Fusidium griseum* (Deuteromycotina, Hyphomycetes).[9] Fusidienol is a novel tricyclic oxygen-containing heterocycle with a 7/6/6 ring system that inhibited bovine brain FPTase[10] with an IC_{50} of 300 nM while being inactive against rat liver squalene synthase and bovine brain geranyl-geranyl protein transferase. Inhibition of FPTase by fusidienol is non-competitive with respect to both substrates i.e. acceptor Ras-CVLS peptide and FPP. Ki values were 1.4 and 0.5 μM with respect to Ras and FPP, respectively. Fusidienol was less active when tested against recombinant human FPTase enzyme[10] exhibiting an IC_{50} of 2.7 μM. Fusidienol diacetate (**1b**) inhibited bovine FPTase with an IC_{50} of 1 μM.



1a: R= H, Fusidienol
1b: R= $COCH_3$

Fusidium griseum, grown for 11 days on medium consisting of millet, yeast, sodium tartrate, sucrose, alfalfa and corn oil, was extracted with methyl ethyl ketone. Fusidienol (**1a**) was initially isolated by chromatography on Sephadex LH-20 followed by reversed phase HPLC. Subsequent isolations were achieved by partitioning the dried methyl ethyl ketone extract with methylene chloride and 50% aqueous methanol followed by silica gel chromatography using hexane-acetone. Crystallization from methanol afforded yellow granules (50 mg/L) of fusidienol (**1a**), mp. 168-70 °C.

STRUCTURE ELUCIDATION

Electron impact (EI) mass spectral analysis of fusidienol gave a molecular ion at m/z 316 for which the empirical formula $C_{16}H_{12}O_7$ was determined by high resolution measurement. This formula indicated that fusidienol has 11 degree of unsaturations. Fusidienol (**1a**)[11] formed a *bis*-trimethylsilyl ether (m/z 460) when reacted with BSTFA-pyridine. The UV spectrum of **1a** gave absorption bands at 230 and 327 nm, an indication of a highly conjugated system which was also apparent from the slightly yellow color of fusidienol. The infra red spectrum showed absorption bands for hydroxy (3500 cm^{-1}), ester (1724 cm^{-1}) and highly conjugated carbonyl (1651 cm^{-1}) groups. Formation of the bis-TMS derivative indicated the presence of two active hydrogens which was supported by acetylation with acetic anhydride and pyridine to give diacetate **1b**.[12] C-13 NMR analysis (Table 1) of **1a** in CD_3OD displayed 16 carbons and supported the molecular formula derived from mass spectral analysis. The APT spectrum of fusidienol revealed the following types of carbons: a methoxy carbon (δ 52.90), an oxymethylene (δ 60.72), five olefinic/aromatic methines and the remaining nine carbons were quaternaries which presented a challenge in resolving the structure. Two of the carbons were highly downfield and occupied the region of the spectrum which is normally occupied by carbonyl carbons.

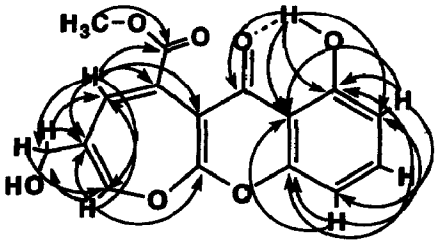


Figure 1: HMBC ($^nJ_{CH}$=7 Hz) Correlations of 1a

Examination of the ^1H NMR spectrum (Table 1) indicated the presence of a methoxy group, a 1,2,3-trisubstituted aromatic ring, two olefinic protons, and an oxymethylene which showed small couplings to both olefinic protons. The sub-structures were put together by HMBC correlations (Figure 1) using a $^nJ_{CH}$ = 7Hz. The correlation of methoxy protons with the carbonyl carbon confirmed it to be a methyl ester. Two and three bond HMBC correlations of H-11 to C-9, 10, 12, 13, 16, 17 and H-13 to C-11, 12, 15, 16 helped in assembling the left hand side of the molecule which was further corroborated by respective correlations from H-16. The right hand side of the molecule was similarly assembled with the help of HMBC correlations of H-3, 4 and 5. Unambiguous assignment of chemical shifts of C-2 *vs* C-6 and C-3 *vs* C-5 was not possible in CD_3OD due to equivocal correlations. This distinction became possible only when we made use of the HMBC correlations involving the unexchanged chelated 6-OH group. Therefore, the NMR studies were repeated in a

2:1 mixture of CD_3CN-CD_3COCD_3 (Table 1) . The HMBC experiment in this solvent gave all of the HMBC correlations which were observed in CD_3OD with additional correlations from the 6-OH. The proton at δ 12.13 gave correlations to C-5 (δ113.26), 6 (δ161.75), 7 (δ110.19) and 8 (δ183.92) thereby confirming all of the carbon chemical shift assignments. Based on these data structure **1a** was proposed for fusidienol. The mass spectral fragmentations of diacetate **1b** (Figure 2) were in complete agreement with the assigned structure.

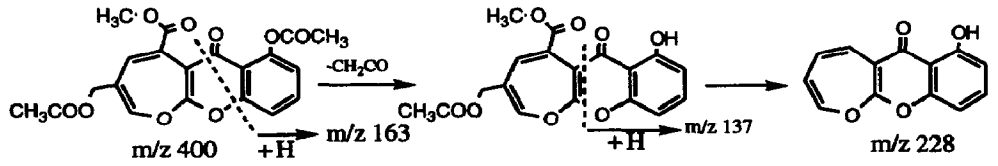


Figure 2: Mass Spectral Fragmentation of Fusidienol diacetate (1b)

Table 1: NMR Assignment of Fusidienol

Position	δC^1	δC^2	Mult	δH^1	δH^2
2	155.16	154.90	C°	---	---
3	108.11	108.23	CH	6.95, dd, 9.0, 0.5	6.95, dd, 8.4, 0.8
4	136.94	137.04	CH	7.60, t, 8.5	7.60, t, 8.4
5	113.37	113.26	CH	6.82, dd, 9.0, 0.5	6.81, dd, 8.4, 1.2
6	161.94	161.75	C°	---	---
7	110.28	110.19	C°	---	---
8	183.95	183.92	C°	---	---
9	105.80	105.72	C°	---	---
10	131.34	131.24	C°	---	---
11	134.64	134.50	CH	7.06, s	7.00, dd, 0.8, 0.4
12	131.31	131.10	C°	---	---
13	145.75	145.13	CH	6.57, t, 1.5	6.52, td, 1.2, 0.4
15	164.42	163.98	C°	---	---
16	60.72	60.63	CH_2	4.14, d, 1.5	4.13, ddd, 6.0, 1.6, 0.4
17	168.51	167.57	C°	---	---
19	52.90	52.93	CH_3	3.79, s	3.72, s
6-OH	---	---	---	---	12.13, s
16-OH	---	---	---	---	3.61, t, 6.0

[1]CD_3OD at 30 °C (500 MHz); [2]CD_3CN-CD_3COCD_3 (2:1) at 25 °C (400 MHz).

REFERENCES AND NOTES

1. (a) Gibbs, J. B. *Semin. Can. Biol.* **1992**, 3, 383. (b) Gibbs, J. B. *Cells* **1991**, 65, 1 and references cited therein. (c) Der, C. J. and Cox, A. D. *Cancer Cells* **1991**, 3, 331.(d) Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; and Brown, M. S. *Cells* **1990**, 62, 81. (e) Schaber, M. D.; O'Hara, M. B.; Garsky, V. M.; Mosser, S. D., Bergstrom, J. D.; Moores, S. L.; Marshall, M. S.; Friedman, P. A.; Dixon, R. A. F.; and Gibbs, J. B. *J. Biol. Chem.* **1990**, 265, 14701. (f) Gibbs, J. B.;

Pompliano, D. L.; Mosser, S. D.; Rands, E.; Lingham, R. B.; Singh, S. B.; Scolnick, E. M.; Kohl, N. E.; and Oliff, A. *J. Biol. Chem.* **1993**, 268, 7617.

2. (a) Barbacid, M. *Ann. Rev. Biochem.* **1987**, 56, 779. (b) Rodenhuis, S. *Semin. Can. Biol.* **1992**, 3, 241.
3. (a) Kohl, N. E.; Mosser, S. D.; deSolms, J. S.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolnick, E. M.; Oliff, A.; and Gibbs, J. B. *Science* **1993**, 260, 1934. (b) James, G. J.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; and Marsters, J. C. *Science* **1993**, 260, 1937.
4. (a) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Goetz, M. A.; Jenkins, R. G.; Nallin-Omstead, M.; Silverman, K. C.; Bills, G. F.; Mosley, R. T.; Gibbs, J. B.; Albers-Schonberg, G.; and Lingham, R. B. *Tetrahedron* **1993**, 49, 5917. (b) Ref. 1f. (c) Singh, S. B. *Tetrhedron Lett.* **1993**, 34, 6521. (d) Lingham, R. B.; Silverman, K. C.; Bills, G. F.; Cascales, C.; Sanchez, M.; Jenkins, R. G.; Gartner, S.; Martin, I.; Pelaez, F.; Mochales, S.; Kong, L.; Burg, R. W.; Meinz, M. S.; Huang, L.; Nallin-Omstead, M.; Mosser, S. D.; Schaber, M. D.; Omer, C. A.; Pompliano, D. L.; Gibbs, J. B.; and Singh, S. B. *Appl. Microbiol. Biotech.* **1993**, 40, 370.
5. (a) Omura, S.; Van Der Pyl, D.; Takahashi, Y.; and Takeshima, H. *J. Antibiotics* **1993**, 46, 222. (b) Shiomi, K.; Yang, H.; Inokoshi, J.; Van Der Pyl, D.; Nakagawa, A.; Takeshima, H.; and Omura, S. *J. Antibiotics* **1993**, 46, 229.
6. Van Der Pyl, D.; Inokoshi, J.; Shiomi, K.; Yang, H.; Takeshima, H.; and Omura, S. *J. Antibiotics* **1992**, 45, 1802.
7. Liu, W. C.; Barbacid, M.; Bulgar, M.; Clarck, J. M.; Crosswell, A. R.; Dean, L.; Doyle, T. W.; Fernandes, P. B.; Huang, S.; Manne, V.; Pirnik, D. M.; Wells, J. S.; and Meyers, E. *J. Antibiotics* **1992**, 45, 454.
8. (a) Hara, M.; Akasaka, K.; Akinaga, S.; Okabe, M.; Nakano, H.; Gomez, R.; Wood, D.; Uh, M.; and Tamanoi, F. *Proc. Natl. Acad. Sci.* USA **1993**, 90, 2281. (b) Tamanoi, F. *Trend. Biochem. Sci.* (TIBS) **1993**, 18, 349.
9. (a) *Fusidium griseum* (MF5695 = ATCC 74265) was isolated from hardwood leaf litter, near Lansing, Michigan, USA) provided by Dr. Bary Katz of MYCOsearch, North Carolina. (b) Barron, G. L. "*The Genera of Hyphomycetes from Soil*" Williams and Wilkins Co., Baltimore, **1968.** (c) Ellis, M. B.; Ellis, J. P. *Microfungi on land Plants, an Identification Handbook* " Macmillan Publishing Co. New York **1985**.
10. (a) See ref. 1f. and 4d and references cited theirin. (b) Omer, C. A.; Kral, A. M.; Diel, R. E.; Prendergast, G. C.; Powers, S.; Allen, C. M.; Gibbs, J. B.; and Kohl, N. E. *Biochemistry* **1993**, 32, 5167.
11. **1a:** mp. 168-70°C; UV: λ_{max} (CH_3OH): 230 (ε=19815), 327 (7360) nm; IR (ZnSe): 3500, 2953, 1724, 1651, 1610, 1468, 1421, 1361, 1274, 1231, 1176, 1140, 1063, 1020, 896, 816, 766 cm^{-1}; HREIMS (m/z): 316.0600 (M^+, calcd. for $C_{16}H_{12}O_7$: 316.0583).
12. **1b:** ^1H NMR (CD_3OD+$CDCl_3$, δ): 2.04 (3H, s), 2.34 (3H, s), 3.79 (3H, s), 4.67 (2H, s), 6.63 (1H, s), 7.05 (1H, d, J = 8.0 Hz), 7.22 (1H, s), 7.30 (1H, d, J = 8.0 Hz), 7.64 (1H, t, J = 8.5 Hz). HREIMS (m/z): 400.0799 (M^+, calcd. for $C_{20}H_{16}O_9$: 400.0794), 358.0693 (calcd. for $C_{18}H_{14}O_8$: 358.0689), 342.0743 (calcd. for $C_{18}H_{14}O_7$: 342.0740), 326.0430 (calcd. for $C_{17}H_{10}O_7$: 326.0427), 310.0485 (calcd. for $C_{17}H_{10}O_7$: 310.0477), 298.0484 (calcd. for $C_{16}H_{10}O_6$: 298.0477), 284.0313 (calcd. for $C_{15}H_8O_7$: 284.0321), 266.0198 (calcd. for $C_{15}H_6O_5$: 266.0215), 228.0433 (calcd. for $C_{13}H_8O_4$: 228.0423), 163.0403 (calcd. for $C_9H_7O_3$: 163.0395), 137.0241 (calcd. for $C_7H_5O_3$: 137.0239).

(*Received in USA* 18 *April* 1994; *accepted* 6 *May* 1994)