Pergamon

*Bioorganic & Medicinal Chemistry Letters*, Vol. 7, No. 2, pp. 139-144, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(96)00588-4

# SYNTHESIS AND ACTIVITY OF BREFELDIN A ANALOGS AS INDUCERS OF CANCER CELL DIFFERENTIATION AND APOPTOSIS

Ji-Wen ZHU,[a] Hitoshi HORI,[a]* Hisao NOJIRI,[b] Takahiko TSUKUDA[a], and Zenei TAIRA[c]

[a]Department of Biological Science and Technology, University of Tokushima, Minamijosan-jimacho-2, Tokushima 770, Japan ; [b]Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan; [c]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashirocho, Tokushima 770, Japan

***Abstract:*** *We designed and synthesized several brefeldin A (BFA) analogs. These compounds were evaluated for the ability to induce differentiation and apoptosis in human colonic carcinoma cell line HCT116. Diacetyl BFA (**2a**), 4-acetyl BFA (**2b**), 7-acetyl BFA (**2c**), and 10,11-epoxy BFA (**3b**) were active but tetrahydro BFA (**3a**) and other analogs could not induce the malignant cells to differentiate. The results suggested that the moiety from 1- to 4-position in BFA as well as its conformational rigidity is essential for its biological activity.*



## Introduction

Brefeldin A (BFA), a naturally occurring fungal metabolite, exhibits an extraordinarily diverse range of biological activities that includes antibiotic, antiviral, cytostatic, antimitotic, and antitumor effects.[1-3] In addition, BFA blocks the movement of proteins from the endoplasmic reticulum to the Golgi apparatus and causes redistribution of Golgi proteins into the endoplasmic reticulum in animal cells.[4-6] Furthermore, our research demonstrated that BFA induces the differentiation and apoptosis of human malignant tumor cells,[7] but not of normal cells at the same concentration. When human colonic carcinoma cell line HCT116 was treated with BFA, morphological changes showing cell differentiation were observed. DNA-ladder, which is one of the markers for apoptosis, was also observed in BFA-treated cells following such morphological changes. Recently, two major strategies are being used in the fight against cancer. The first relies on using cytotoxic agents that destroy tumor cells by targeting some of the intrinsic shortcomings of rapidly proliferating cells. The

second uses differentiation inducers that promote tumor cell differentiating and lead to apoptosis (programmed cell death ).[8] We, therefore, designed and synthesized BFA analogs and examined the actions of these compounds in HCT 116, wishing to identify the structural parts essential for the biological activity and to develop promising new drugs for differentiation and apoptosis chemotherapy.

Although BFA is a relatively small molecule (MW = 280), its structure is quite complex. It has a transoid bicyclo [11.3.0] hexadecadienoate skeleton containing five asymmetric carbon centers, two C=C double bonds, and one lactone (Figure 1). We modified this natural compound focused on four points: (1) The two hydroxyl groups were oxidized into carbonyl groups with PCC (pyridium chlorochromate) and compounds **1a** and **1b** were synthesized (Figure 1). (2) The two hydroxyl groups were acetylated or methylated to give diacetyl BFA (**2a**), 4-acetyl BFA (**2b**), 7-acetyl BFA (**2c**), and 4-methyl BFA ether (**2d**), and dimethyl BFA ether (**2e**), respectively. (3) The two C=C double bonds were hydrogenated or epoxidized to give tetrahydro BFA (**3a**) and epoxy BFA (**3b**). (4) The 13-membered ring of BFA was opened and changed into a carboxylic acid or methyl ester to give expecting derivatives (**4a, 4b, and 4c**).

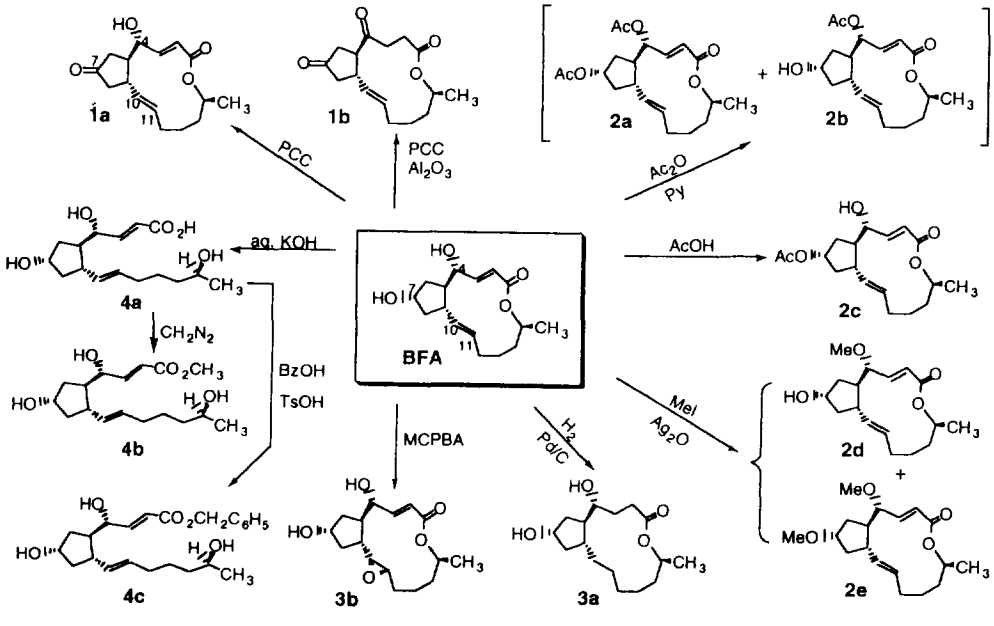


**Figure 1.** Design and synthesis of BFA analogs[9]

**Results and Discussion**

Exposure of exponentially growing HCT 116 cells to BFA analogs, screened from 0.05 μg/mL to 2.0 μg/mL, respectively, gave the results shown in Table 1. Diacetyl BFA (**2a**) (0.27 μM), 4-acetyl BFA (**2b**) (1.6 μM), 7-acetyl BFA (**2c**) (0.16 μM), and 10,11-epoxy BFA (**3b**) (4.1 μM) were potent inducers. These compounds led to the morphological changes characteristic of apoptosis: bright blue-fluorescent condensed nuclei (intact or fragmented), condensation of nuclear chromatin, nuclear fragmentation, apoptotic bodies, cell shrinkage and blebbing (data not shown).[10] Agarose gel electrophoresis of DNA[11] from cells exposed to compounds, **2a**, **2b**, **2c**, **3b**, revealed ladder patterns, indicating preferential DNA degradation at the internucleosomal linker DNA sections. In comparison, the activity of compounds **2c** and **2a** was 70% and 40% of BFA, respectively. The activity of **2b** and **3b** was 7% and 3% of BFA, much lower than **2c** and **2a** (Figure 2).[12] That is, the induction of differentiation and apoptosis: BFA > 7-acetyl BFA > diacetyl BFA > 4-acetyl BFA > epoxy BFA. These results suggest that 4-hydroxyl group is more important than 7-hydroxyl group for BFA in inducing HCT 116 to differentiate.

**Table 1.** Effects of BFA analogs on apoptotic DNA fragmentation

| BFA analog | MW | conc.(μM ; | μg /ml) | DNA fragmentation* |
|---|---|---|---|---|
| 1a | 278 | 3.6 | 1.0 | - |
| 1b | 276 | 3.6 | 1.0 | - |
| 2a | 364 | 0.27 | 0.10 | + |
| 2b | 322 | 1.6 | 0.5 | + |
| 2c | 322 | 0.16 | 0.05 | + |
| 2d | 294 | 0.85 | 0.25 | - |
| 2e | 308 | 3.2 | 1.0 | - |
| 3a | 284 | 3.5 | 1.0 | - |
| 3b | 296 | 4.1 | 1.2 | + |
| 4a | 298 | 3.4 | 1.0 | - |
| 4b | 312 | 6.4 | 2.0 | - |
| 4c | 388 | 5.2 | 2.0 | - |
| BFA | 280 | 0.11 | 0.03 | + |

*HCT 116 cells

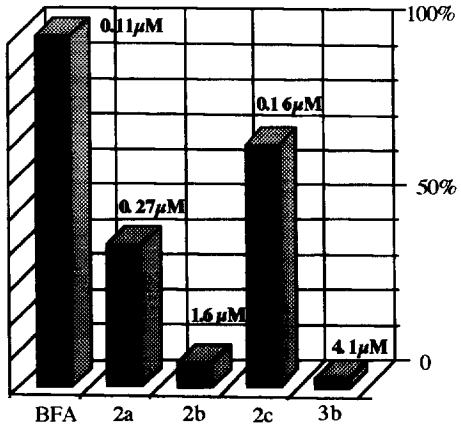


**Figure 2.** Comparison of BFA analogs with BFA for induction activity (HCT 116).

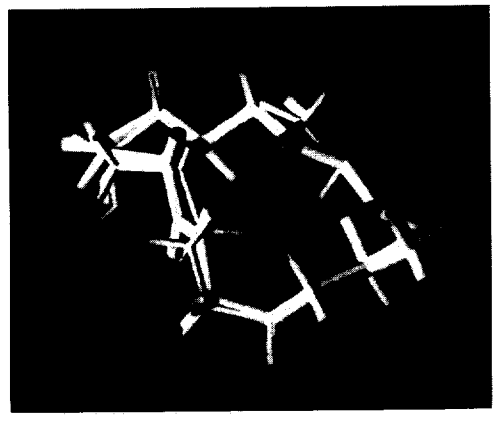

**Figure 3.** Superimposition of the crystal structures of BFA (bold) & tetrahydro BFA (thin).

Tetrahydro BFA (**3a**), without carbon-carbon double bonds, had no inducing activity. A comparison of the X-ray crystal structures of **3a** and BFA indicates that there are few conformational differences between the two compounds (Figure 3).[13, 14] Thus, we suggest that carbon-carbon double bonds participate in affinity to or interaction with receptors.

On the other hand, the ring-opened compound, **4b** and **4c**, which possess the same moiety from the 1-carbonyl group to 7-hydroxy group as BFA, also had no effect. the finding suggests that the conformational rigidity is necessary for BFA to induce differentiation and apoptosis toward cancer cells.

In conclusion, we suggest that not only the moiety of 1- to 4-position but also the conformational rigidity of BFA plays an important role in triggering apoptosis. At present, the design and synthesis of mimic BFA is underway, to confirm our notion regarding the structural requirements for BFA as an cancer cell differentiation inducer.

***Acknowledgements:*** We express our gratitude to Dr. B. Proksa, Slovak Technical University, for his kindly support. We are grateful to the staff of Analytical Instrumental Facilities of Faculty of Engineering, University of Tokushima, for spectral measurements.

**References and Notes**


1. Betina, V.; Horakova, K.; Barath, Z.*Naturwissenschaften* **1962**, *49*, 241.
2. Tamura, G.; Ando, K.; Suzuki, S.; Takatsuki, A.; Arima, K. *J. Antibiot.* **1968**, *21*, 160.
3. Betina, V. *Neoplasma* **1969**, 16, 23.
4. Misumi, Y.; Miki, K.; Takatsuki, A.; Tamura, G.; Ikehara, Y. *J. Biol. Chem.* **1986**, *261*, 11398.
5. Fujiwara, T.; Oda, K.; Yokota, S.; Takatsuki, A.; Ikehara, Y. *J. Biol. Chem.* **1988**, *263*, 18545.
6. Lippincott-Schwartz, J.; Yuan, L. C.; Bonifacino, J. S.; Klausner, R. D. *Cell* **1989**, *56*, 801.
7. Nojiri, H.; Hori, H.; Nojima, S. *Glycoconjugate J.* **1995**, *12*, 459.
8. Sartorelli A. C. *Br. J.Cancer* **1985**, *52*, 293.
9. (a) $^1$H NMR spectral data (400MHz, $CD_3OD$) of BFA were assigned by HH COSY and CH COSY experimrnts. δ 7.34 (dd, *J* = 3.2, 15.6Hz, 3-H), 5.73 (dd, *J* = 2.0, 13.6Hz, 2-H), 5.69 (ddd, *J* = 4.0, 8.4Hz, 13.2Hz, 11-H), 5.21 (dd, *J* = 9.6, 15.2Hz, 10-H), 5.14 (d, *J* = 5.6, 4-OH), 4.71 (m, 15-H), 4.53 (d, *J* = 3.6Hz, 7-OH), 4.05 (m, 7-H), 3.93 (ddd, *J* = 7.4Hz, 4-H), 2.31 (m, 9-H), 1.98 (m, 8-Ha), 1.96 (m,5- H), 1.92 (m, 13-Ha), 1.82 (m, 6-Ha), 1.80 (m, 12-Ha), 1.75 (m, 13-Hb), 1.70 (m, 14-Ha), 1.62 (m, 6-Hb), 1.50 (m, 14-Hb), 1.30 (m, 8-Hb), 1.18 (d, *J* = 6.4Hz, $CH_3$), 0.72 (m, 12-Hb).

   (b) Partial chemical shift values of some compounds ($^1$H NMR, 400MHz) :

   **2a** ($CDCl_3$): 5.12 (m, 4-H), 5.25 (m, 7-H).

   **2b** ($CDCl_3$): 5.12 (m, 4-H), 4.33 (m, 7-H).

   **2c** ($CD_3OD$): 5.08 (m, 7-H), 4.07 (m, 4-H).

   **2d** ($CD_3OD$): 4.19 (m, 7-H), 3.65 (m, 4-H).

   **2e** ($CD_3OD$): 3.80 (m, 7-H), 3.65 (m, 4-H).

   **3b** ($CD_3OD$): 5.97 (dd, *J* = 3.2, 15.6Hz, 2-H), 7.32 (dd, *J* = 2.0, 15.6Hz, 3-H).

10. Morphological Evaluation of Apoptosis. Cells cultured for 1 day were exposed to drugs in the Dulbecco's modified Eagle's miminal essential medium for 30 min at 37 °C in humidified 5% carbon dioxide / 95% air. After 1 and 2 days, the morphology of cells were evaluated by microscopy (X100).
11. Detection of Apoptotic DNA fragmentation in Agarose gels. DNA was isolated by standard means. Cells were washed with cold phosphate-buffered saline and the cell pellet was re-suspended in 200 μL of a solution containing 0.1M NaCl, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 0.5% sodium dodecyl sulfate,

and 0.1 mg/mL proteinase K. Proteolytic digestion proceeded at 50 °C for 12-16 h. The digest was extracted with phenol/chloroform/isoamyl alcohol and DNA was precipitated with 1 vol. of 7.5 M $NH_4Ac$ and 2 volumes of absolute ethanol, resuspended in 10 mM Tris-HCl / 1 mM EDTA / 0.1% SDS buffer, pH 8.0 then digested with 1 $\mu$g/mL DNase-free RNase at 37 °C overnight. The mixture was extracted and ethanol precipitated as above, then loadied onto a 2% agarose gel containing 0.1 mg/mL ethidium bromide. DNA was visualized under UV light and photographed.

12. The percentages are calculated as

$$\frac{\text{the concentration of BFA}}{\text{the concentration of BFA analog}} \times 100\%$$

13. BFA Crystal data: Weber, H. P.; Hauser, D.; Sigg, H.P. *Helv. Chim. Acta* **1971**, *54*, 2763.

14. Tetrahydro BFA Crystal data: crystal data were measured on MXC M18X diffractometer and the structure analysis was performed by Crystan-G system (MAC Science Ltd., Japan) on Silicon Graphics IRIS 4D/320GTX computer: molecular formula $C_{16}H_{28}O_4$, Mr = 285.0, orthorhombic space group $P2_12_12_1$, unit cell $a$ = 10.861 (4) Å; $b$ = 19.062 (6) Å; $c$ = 7.630 (3) Å. $V$ = 1580 (1) Å$^3$, $\mu$ (Cu K$\alpha$) = 5.98 cm$^{-1}$, $F$ (000) = 624, $Z$ = 4, $D$ obs = 1.30 g/cm$^3$, Total Reflections: 1575; Unique Reflections: 1503; Reflections used: 1444; $\Delta\rho$ in the final difference map: $-0.31 < \Delta\rho < +0.21$ eÅ$^{-3}$.

(*Received in Japan* 9 *October* 1996; *accepted* 4 *December* 1996)