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# Thelephantins D–H: five *p*-terphenyl derivatives from the inedible mushroom *Thelephora aurantiotincta*

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#### Abstract

Five *p*-terphenyl derivatives named thelephantins D–H (1–5) together with nine known compounds, thelephantins A–C (6–8), ganbajunin E (9), *p*-hydroxylbenzoic acid (10), ganbajunin C (11), thelephorin A (12), 2-*O*-methylatromentin (13) and atromentin (14), were isolated from the methanolic extract of fruit bodies of the Thelephoraceous Basidiomycete *Thelephora aurantiotincta*. Their structures were elucidated by high-resolution MS, 2D NMR, IR and UV spectroscopic analysis. © 2003 Elsevier Ltd. All rights reserved.

Keywords: Thelephora aurantiotincta; Thelephoraceae; Fungi; Basidiomycete; Thelephantin

## 1. Introduction

Occurrence of p-terphenyl derivatives in Basidiomycete fungi has been investigated by Jaegers et al. (1987) who reported the isolation of leucomelone and protoleucomelone from fruiting bodies of Boletopsis leucomelaena. Then, Takahashi et al. (1992) reported three other p-terphenyl derivatives showing an inhibitory effect on 5-lipoxygenase from the same species. Later, curtisians A–D as new free radical scavengers from Paxillus curtisii (Yun et al., 2000a); leucomentin-5 and -6 from Paxillus panuoides (Yun et al., 2000b), kynapcin-12 from Polyozellus multiplex (Lee, et al., 2000), ganbajunins A-G from Thelephora ganbajun (Hu et al., 2001; Hu and Liu, 2001), and thelephorin A from Thelephora vialis (Tsukamoto et al., 2002) were reported. In previous research for naturally occurring biologically active compounds from Badisiomycete fungi, three p-terphenyl derivatives named thelephantins A-C were isolated from Thelephora aurantiotincta (Quang et al., 2003) (Scheme 1).

In this paper, the isolation and structural elucidation of five new *p*-terphenyl derivatives named thelephantins

D-H (1–5) together with nine known related compounds (7–14) from the MeOH extract of *T. aurantiotincta* fruiting bodies are reported.

## 2. Results and discussion

The methanolic extract of fruiting bodies of *T. aurantiotincta* was subjected repeatedly to Sephadex LH-20, DIOL and SiO<sub>2</sub> column chromatography, and finally to preparative reversed phase HPLC, to give 14 compounds (1–14).

Thelephantin D (1) was obtained as a grayish solid. Its molecular formula was deduced to be  $C_{30}H_{26}O_8$  by HRFAB-MS. The IR and UV spectra of 1 indicated the absorption bands of a hydroxyl group (3401 cm<sup>-1</sup>), an ester (1749 cm<sup>-1</sup>) and a benzene (1612 and 1524 cm<sup>-1</sup>; 263 nm), respectively. The <sup>1</sup>H NMR spectrum (Table 1) of 1 showed the presence of eight aromatic protons, one phenyl, three methylenes and one methyl. The <sup>13</sup>C NMR spectrum (Table 2) of 1 revealed the presence of two ester carbonyls ( $\delta$  171.2 and 173.1), and four phenolic carbons ( $\delta$  142.5, 142.6, 158.2 and 158.2). Comparison of the <sup>1</sup>H-<sup>1</sup>H COSY, NOESY and HMBC spectra (Fig. 1) of 1 with other terphenyl derivatives (Tsukamoto et al., 2002; Quang et al., 2003) indicated

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Scheme 1. Known compounds (6–14) from *T. aurantiotincta*.

the presence of four partial structures  $\mathbf{a}$ — $\mathbf{d}$ . Among them, units  $\mathbf{a}$  and  $\mathbf{c}$  were p-substituted phenyl groups linked to C-7 and C-10, unit  $\mathbf{d}$  was butyroxyl. The NMR spectral data of  $\mathbf{1}$  were very similar to those of thelephantin A (6) (Quang et al., 2003) suggesting that compound  $\mathbf{1}$  was a p-terphenyl derivative except for the signals of unit  $\mathbf{b}$ . Unit  $\mathbf{b}$  was determined to be phenyl-

acetyloxy by the long-range coupling between H-2'/C-1', C-3', C-4' and C-8' in HMBC spectrum (Fig. 1). The linkage positions of unit **b** (phenylacetyloxy) and unit **d** (butyroxyl) to the central phenolic ring were found to be at C-8 and C-12, respectively. This was achieved by comparison of the chemical shift in the <sup>13</sup>C NMR spectrum of **1** with thelephantins A–C (6–8) (Quang et al.,

Table 1 <sup>1</sup>H -NMR spectral data for compounds **1**, **2** and **3** (600 MHz, CD<sub>3</sub>OD)

Н	1	2	3	4	5
2, 6	6.80 d (8.5)	6.71 d (8.8)	6.81 d (8.8)	6.75 d (8.8)	6.71 d (8.8)
3, 5	7.14 d (8.5)	7.20 d (8.8)	7.14 d (8.8)	7.26 d (8.8)	7.19 d (8.8)
14	7.12 d (8.8)	7.17 d (8.5)	7.12 d (8.8)	7.26 d (8.8)	
15	6.81 d (8.8)	6.82 d (8.8)	6.80 d (8.8)	6.75 d (8.8)	7.07 s
17	6.81 d (8.8)	6.82 d (8.5)	$6.80 \ d \ (8.8)$	6.75 d (8.8)	
18	7.12 d (8.8)	7.17 d (8.5)	7.12 d (8.8)	7.26 d (8.8)	7.13 s
2'	3.49 s	,	3.49 s	, ,	
3'		$7.80 \ d \ (8.5)$		7.62 d (8.8)	7.68 d (8.8)
4'	7.06 d (8.0)	7.42 dd (7.7, 8.0)	7.06 dd (1.6, 8.2)	6.62 d (8.8)	6.78 d (8.8)
5'	7.24 t (7.1)	7.60 t (7.4)	7.24 <i>d</i> (7.1)		()
6'	7.22 d (6.9)	7.42 dd (7.7, 8.0)	7.22 d (6.9)	6.62 d (8.8)	6.78 d (8.8)
7′	7.24 t (7.1)	7.80 d (8.5)	7.24 <i>d</i> (7.1)	7.62 d (8.8)	7.68 d (8.8)
8'	7.06 d (8.0)	,	7.06 dd (1.6, 8.2)		
2"	1.87 t (7.4)	3.31 s	1.67 dd (8.8, 15.1)		3.90 s
			1.92 <i>dd</i> (5.2, 15.1)		
3"	1.30 m		1.48 m	7.62 d (8.8)	
4"	0.70 t (7.4)	6.80 d (8.5)	1.29 <i>m</i>	6.62 d (8.8)	7.16 d (8.8)
5"	0.70 1 (7.1.)	6.97 t (7.4)	0.75 d (6.9)	0.02 tr (0.0)	7.08 t (7.6)
6"		7.01 <i>t</i> (7.4)	0.69 d (6.6)	6.62 d (8.8)	7.10 d (7.6)
7"		6.97 t (7.4)	0.56 d (6.9)	7.62 d (8.8)	7.08 t (7.6)
8"		6.80 d (8.5)	0.50 a (0.5)	7.02 a (0.0)	7.16 <i>d</i> (8.8)

Table 2 <sup>13</sup>C -NMR specta data for compound **1**, **2** and **3** (150MHz, CD<sub>3</sub>OD)

C	1	2	3	4	5
1	158.2 s	158.2 s	158.2 s	157.9 s	157.8 s
2, 6	116.1 d	116.1 <i>d</i>	116.1 <i>d</i>	115.9 d	116.0 d
3, 5	132.6 d	132.6 d	132.6 d	132.6 d	132.9 d
4	124.9 s	124.9 s	124.1 s	125.1 s	125.1 s
7	123.9 s	124.0 s	123.9 s	124.1 s	122.4 s
8	134.7 s	134.8 s	134.8 s	135.3 s	137.5 s
9	142.6 s	142.7 s	142.7 s	142.5 s	144.3 s
10	123.9 s	124.0 s	123.9 s	124.1 s	119.3 s
11	134.8 s	134.9 s	134.7 s	135.3 s	139.1 s
12	142.5 s	142.7 s	142.5 s	142.5 s	143.8 s
13	124.9 s	124.8 s	125.0 s	125.1 s	115.0 s
14	132.6 d	132.6 d	132.8 d	132.6 d	152.5 s
15	116.0 d	115.9 d	116.1 <i>d</i>	115.9 d	99.3 d
16	158.2 s	158.2 s	158.2 s	157.9 s	148.2 s
17	116.0 d	115.9 d	116.1 <i>d</i>	115.9 d	144.3 s
18	132.6 d	132.6 d	132.8 d	132.6 d	107.5 a
1′	171.2 s	166.1 s	171.2 s	166.6 s	166.0 s
2′	41.5 t	130.1 s	41.5 t	120.9 s	120.9 s
3′	134.8 s	131.0 d	134.8 s	133.2 d	133.5 a
4′	130.4 d	129.7 d	130.4 d	116.0 d	116.3 d
5′	129.6 d	134.8 d	129.6 d	163.8 s	164.1 s
6′	128.2 d	129.7 d	128.2 d	116.0 d	116.3 d
7′	129.6 d	131.0 d	129.6 d	133.2 d	133.5 d
8′	130.4 d		130.4 d		
1"	173.1 s	171.3 s	173.0 s	166.6 s	171.0 s
2"	36.1 t	41.3 t	39.3 t	120.9 s	41.6 t
3"	19.0 t	134.3 s	36.7 d	133.2 d	134.4 s
4"	13.7 q	130.1 d	32.7 d	116.0 d	130.3 d
5"		129.3 d	20.4 q	163.8 s	129.6 d
6"		127.9 d	18.1 q	116.0 d	128.2 d
7"		129.3 d	15.6 q	133.2 d	129.6 d
8"		130.1 d	1		130.3 d

2003) and thelephorin A (12) (Tsukamoto et al., 2002) which showed that the chemical shift of C-8, 9, 11, 12 of 1 and thelephantins A-C (6–8) and thelephorin A (12) were almost identical (with an error less than 0.3 ppm), and the NOE correlation between H-2" and C-18 in NOESY spectrum. On the basis of the above spectral data, thelephantin D (1) was determined as shown in Fig. 1.

The molecular formula of thelephantin E (2) was determined to be C<sub>33</sub>H<sub>24</sub>O<sub>8</sub> by HRFAB-MS. Its IR and UV spectra indicated the absorption bands of a hydroxyl group (3397 cm<sup>-1</sup>), a carbonyl ester (1742  $cm^{-1}$ ), and a benzene ring (1611 and 1525  $cm^{-1}$ ), and a absorption maxima at 229 and 263 nm, respectively. The <sup>1</sup>H NMR spectrum (Table 1) of 2 showed the presence of 18 aromatic protons and one methylene ( $\delta$  3.31, s). The <sup>13</sup>C NMR spectrum of 2 (Table 2) revealed the presences of 33 carbons including two ester, one methylene and four phenolic carbon atoms. The NMR spectral data of compound 2 were similar to those of thelephorin A (12) (Tsukamoto et al., 2002) except for the signals of unit b. There were correlations between (1) H-3' and H-4'; (2) H-4' and H-3', H-5'; (3) H-5' and H-4', H-6'; (4) H-6' and H-5', H-7' in the  ${}^{1}H-{}^{1}H$  COSY spectrum, and long-range correlations between H-3'/H-7' and C-1', C-2' in the HMBC spectrum suggesting that unit **b** was a benzoyl group attached to C-8 due to the NOEs between H-3' and H-3 in NOESY spectrum. Thus, thelephantin C (2) was determined as depicted in Fig. 2.

Fig. 1. Important <sup>1</sup>H–<sup>1</sup>H COSY correlations (bold line), HMBC correlations (arrows) and NOESY correlations of compound 1.

Fig. 3. Important <sup>1</sup>H–<sup>1</sup>H COSY correlations (bold lines), HMBC correlations (arrows) and NOESY correlations of compound 3.

Fig. 2. Important HMBC and NOESY correlations of compound 2.

Fig. 4. Important HMBC and NOESY correlations of compound 4.

Thelephantin F (3) was obtained as grayish solid; its molecular formula was found to be C33H32O8 by HRFAB-MS. The IR spectrum exhibited bands at 3414 cm<sup>-1</sup> (hydroxyl group), 1754 cm<sup>-1</sup> (carbonyl ester), 1612 and 1524 cm<sup>-1</sup> (aromatic). The <sup>1</sup>H NMR spectral data of 3 (Table 1) showed the presence of eight aromatic protons, one benzyl group, two methylenes, two methines and three secondary methyls ( $\delta$  0.56, 0.69 and 0.75). The <sup>13</sup>C NMR spectrum (Table 2) of 3 exhibited signals assigned to two carbonyl esters ( $\delta$  171.2 and 173.0), four phenolic carbons and three doublet methyls. The spectral data of 3 resembled those of thelephantin C (8) (Quang et al., 2003) except for signals of unit **b**. The long-range correlations between H-2'/C-1', C-3', C-4' and C-8 in HMBC spectrum and the NOEs between H-2' and H-3 (Fig. 3) indicating that unit **b** was

phenylacetyloxy linked to C-8 of central aromatic ring. Based on the above spectral data, thelephantin E (3) was identified as shown in Fig. 3 except for the absolute configuration at C-3".

The molecular formula of thelephantin F (4) was found to be  $C_{32}H_{22}O_{10}$  by HRFAB-MS. The IR spectrum of 4 showed the absorptions of a hydroxyl (3340 cm<sup>-1</sup>), an ester (1713 cm<sup>-1</sup>) and a benzene (1608 and 1514 cm<sup>-1</sup>) groups. The <sup>1</sup>H NMR spectrum (Table 1) of 4 showed the presence of 16 *ortho*-coupled aromatic protons. The <sup>13</sup>C NMR spectrum (Table 2) of 4 exhibited resonances representing 32 carbons including two ester groups ( $\delta$  166.6) and three other signals corresponding to six phenolic carbons ( $\delta$  142.5, 157.9 and 163.8). The signals observed in NMR spectral data of 4 showed a large degree of overlap, suggesting that this

compound was of a symmetrical structure. In addition, the NMR spectral data of **4** were similar to those of thelephorin A (**12**) (Tsukamoto et al., 2002) except for the signal of unit **d**. The NOEs between (1) H-3" and H-4", (2) H-6" and H-7" (Fig. 4) were observed in the NOESY spectrum and the low field shift position of C-5" ( $\delta$  163.8) pointing that unit **d** was a *p*-hydroxylbenzoyl group. Thus, thelephantin F (**4**) was determined as shown in Fig. 4.

The molecular formula of thelephantin H (5) was determined to be C<sub>33</sub>H<sub>22</sub>O<sub>10</sub> by HRFAB-MS. Its IR spectrum showed the presence of a hydroxyl (3333 cm<sup>-1</sup>), an ester (1718 cm<sup>-1</sup>) and a benzene (1608 and 1516 cm<sup>-1</sup>) group. The <sup>1</sup>H NMR spectrum (Table 1) of 5 indicated the presence of thirteen aromatic protons and two singlet aromatic protons ( $\delta$  7.07 and 7.13). The <sup>13</sup>C NMR spectrum (Table 2) of 5 showed the presence of two ester, five phenolic, and two oxygenated carbons ( $\delta$  144.3 and 152.5). The spectral data of 5 were very similar to those of ganbajunin B indicating that 5 was a p-terphenyl derivative possessing a dibenzofuran unit (from C-9 to C-14) due to the long-range correlations between (1) H-18/ C-10, C-13 and C-17; (2) H-15/ C-13, C-14, C-16 and C-17 in the HMBC spectrum (Fig. 5); and the low field position of C-14 ( $\delta$  152.5) and C-17 ( $\delta$ 144.3) (Hu et al., 2001). Unit **b** was determined to be p-hydroxylbenzoyl group due to low field position of C-5' ( $\delta$  164.1) and the HMBC correlations (Fig. 5) between (1) H-3' and H-7'/C-1'; (2) H-4' and H-6'/C-5'. Unit **d** gave rise to a set of resonances typical of a phenylacetoxyl group (Hu et al., 2001). Based on the above discussion, thelephantin H (5) was found to be as shown in Fig. 5.

In addition, nine known compounds were determined to be thelephantins A-C (6-8) (Quang et al., 2003), ganbajunin E (9) (Hu et al., 2001), p-hydroxyl benzoic

Fig. 5. Important HMBC and NOESY correlations of compound 5.

acid (10), ganbajunin C (11) (Hu et al., 2001), thelephorin A (12) (Tsukamoto et al., 2002), 2-O-methylatromentin (13) (Hu et al., 2001) and atromentin (14) (Hu et al., 2001) by spectral data, respectively.

T. aurantiotincta were collected at different locations in Japan and each component was isolated using different procedures [(the previous mushroom was fresh and extracted with EtOAc (Quang et al., 2003)). However, the chemical constituents of the previous T. aurantiotincta material (Quang et al., 2003) and the present sample were chemically identical.

## 3. Experimental

### 3.1. General

IR spectra were measured on Jasco FT/IR-5300 spectrophotometer. UV spectra were obtained on a Shimadzu UV-1650PC in MeOH. The specific optical rotations were measured on a Jasco DIP-1000 polarimeter with MeOH as solvent. NMR spectra were recorded on Varian Unity 600 (600 MHz for <sup>1</sup>H NMR and 150 MHz for <sup>13</sup>C NMR) using CD<sub>3</sub>OD. Chemical shifts are given with TMS ( $\delta$  0.00) used as internal standard ( ${}^{1}H$  NMR) and  $\delta$  49.00 (ppm) from CD<sub>3</sub>OD as a standard (13C NMR). Mass spectra including HR FAB mass spectra were recorded on a Jeol JMS AX-500 spectrometer. cc was carried out on silica gel 60 (0.2–0.5 mm, 0.04-0.063 mm, Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech, CHCl<sub>3</sub>–MeOH, 1:1). Prep. medium-pressure liquid chromatography (MPLC) was performed with a Work-21 pump (Lab-Quatec Co., Ltd) and carried out by Lobar cc chromatography (Merck). HPLC was performed on a Shimadzu liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C18-AR-II column. The spots on TLC were detected under UV 254 nm and by spraying with Godin reagent (1954), followed by heating at 120 °C.

## 3.2. Material

Fruiting bodies of *T. aurantiotincta* were collected in the Forest Park in Okayama Prefecture, Japan in August 2001 and identified by Mr. Nitaro Maekawa at Japanese mushroom center. The voucher specimen (H0208001) has been deposited in the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

#### 3.3. Extraction and isolation

Dried fruiting bodies of *T. aurantiotincta* (38.5 g) were extracted with MeOH and then evaporated to give a residue, which was partitioned between EtOAc and H<sub>2</sub>O. The EtOAc layer was evaporated to give a sticky dark residue (1.59 g) which was subjected to Sephadex

LH-20 cc using MeOH:CHCl<sub>3</sub> (1:1 v/v) to give five fractions. Fraction 1 (151.3 mg) was applied to silica gel column using EtOAc-MeOH gradient and purified by prep. HPLC with a reversed phase C-18 column using H<sub>2</sub>O: MeOH (25:65 v/v) as solvent system to afford compound 3 (5.5 mg). Fraction 2 (462.2 mg) was subjected to MPLC with a DIOL column, eluted CHCl<sub>3</sub>:H<sub>2</sub>O (10:1, v/v) to give four subfractions. Fraction 2-1 contained compound 10 (58.8 mg) as pure material. Fraction 2-2 (151.3 mg) was purified by prep. HPLC with a reversed phase C-18 column using  $H_2O:MeOH$  (35:65, v/v) to yield compound 13 (6.4 mg) and compound 9 (11.5 mg). Fraction 2-3 (139.8 mg) was applied to MPLC with a reversed phase C-18 column using H<sub>2</sub>O:MeOH (35:65, v/v) and then purified by prep. HPLC with a reversed phase column C-18 using  $H_2O:MeOH$  (40:60, v/v) to give compounds 1 (4.4 mg), 2 (10.5 mg) and **11** (44.1 mg). Fraction 2-4 (172.5 mg) was purified by the same method as mentioned above to afford compounds 6 (14.2 mg), 7 (7.6 mg), 8 (11.9 mg) and 12 (87.3 mg). Fraction 3 (153.4 mg) was applied to cc the DIOL column using CHCl<sub>3</sub>:EtOAc (2:1, v/v) to give compounds **10** (32.7 mg), **13** (11.3 mg) and a mixture which was purified by prep. HPLC with a reversed phase C-18 column, solvent system H<sub>2</sub>O:MeOH (45:55, v/v) to afford compounds 4 (10.1 mg), 6 (5.7 mg) and 12 (23.8 mg). Fraction 4 (29.8 mg) was purified on the DIOL column using CHCl<sub>3</sub>:MeOH (7:1, v/v) to give compound 5 (3.3 mg). Fraction 5 (71.1 mg) was also purified by DIOL cc using CHCl<sub>3</sub>:MeOH (10:1, v/v) to give compound **14** (2.8 mg).

# 3.3.1. *Thelephantin D* (1)

Grayish solid; Positive FAB-MS: 537 [M + Na]<sup>+</sup>; HR-FABMS m/z 537.1492 (C<sub>30</sub>H<sub>26</sub>O<sub>8</sub>Na, requires m/z 537.1525). UV  $\lambda_{\rm max}$  nm (log  $\varepsilon$ ): 262.6 (4.4). IR (KBr): 3402, 2967, 1749, 1612, 1525, 1456, 1236, 1174, 1106, 983 cm<sup>-1</sup>. For <sup>1</sup>H and <sup>13</sup>C NMR spectra, see Tables 1 and 2.

## 3.3.2. Thelephantin E(2)

Light red brown solid; Positive FAB-MS: 571  $[M+Na]^+$ ; HR-FABMS m/z 571.1350 (C<sub>33</sub>H<sub>24</sub>O<sub>8</sub>Na, requires m/z 571.1369). UV  $\lambda_{\rm max}$  nm (log  $\varepsilon$ ): 263.0 (4.3), 228.6 (4.4). IR (KBr): 3397, 1743, 1611, 1525, 1453, 1263, 1174, 1109, 974 cm<sup>-1</sup>. For <sup>1</sup>H and <sup>13</sup>C NMR spectra, see Tables 1 and 2.

# 3.3.3. Thelephantin F(3)

Grayish solid,  $[\alpha]_D^{20} + 2.93^\circ$  (c 0.82, CH<sub>3</sub>OH). Positive FAB-MS: 556 [M]<sup>+</sup>; HR-FABMS m/z 556.2098 (C<sub>33</sub>H<sub>32</sub>O<sub>8</sub>, requires m/z 556.2097). UV  $\lambda_{\text{max}}$  nm (log  $\varepsilon$ ): 260.6 (4.1). IR (KBr): 3414, 1745, 1612, 1525, 1497, 1269, 1173, 1106, 996 cm<sup>-1</sup>. For <sup>1</sup>H and <sup>13</sup>C NMR spectra see Tables 1 and 2.

#### 3.3.4. Thelephantin G(4)

Light green brown solid; Positive FAB-MS: 567  $[M+H]^+$ ; HR-FABMS m/z 567.1262  $(C_{32}H_{23}O_{10},$  requires m/z 567.1291). UV  $\lambda_{\rm max}$  nm (log  $\varepsilon$ ): 261.0 (4.5). IR (KBr): 3340, 1713, 1608, 1514, 1457, 1265, 1168, 1083, 963 cm<sup>-1</sup>. For <sup>1</sup>H and <sup>13</sup>C NMR spectra, see Tables 1 and 2.

## *3.3.5. Thelephantin H* (**5**)

Grayish solid; Positive FAB-MS: 578 [M] $^+$ ; HR-FABMS m/z 578.1207 (C<sub>33</sub>H<sub>22</sub>O<sub>10</sub>, requires m/z 578.1213). UV  $\lambda_{\rm max}$  nm (log  $\varepsilon$ ): 329.4 (4.0), 300.8 (4.0), 264.4 (4.3) and 246.4 (4.2). IR (KBr): 3333, 1718, 1608, 1516, 1472, 1263, 1167, 1086, 983 cm $^{-1}$ . For  $^{1}$ H and  $^{13}$ C NMR spectra, see Tables 1 and 2.

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