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Note

Synthesis and Inhibitory Effect on Photosynthetic Electron Transport of 1,3,5-Triazinylcarboxylic Acid Derivatives

Atsushi FUJIMORI, Yumi IKEDA, Ruiko OKANO, Masaki HIRAKI,[†] Jack J. S. van RENSEN,[††] Peter BÖGER,[†††] Hitoshi KOHNO* and Ko WAKABAYASHI

Graduate School of Agricultural Science, Tamagawa University, Tamagawa Gakuen 6–1–1, Machida-shi, Tokyo 194–8610, Japan
[†]*Center for Medical Sciences, Ibaraki Prefectural University of Health Science, 4669–2 Ami-cho, Inashiki-county, Ibaraki 300–0394, Japan*
[††]*Graduate School of Experimental Plant Science, Wageningen University, Laboratory of Plant Physiology, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands*
[†††]*Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, D-78457 Konstanz, Germany*

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This study relates to the modification of 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazine. New 1,3,5-triazine compounds with an electron-withdrawing carboxyl group, *e.g.* ester group, substituted for the trifluoromethyl group, were synthesized and assayed for activity to inhibit photosynthetic electron transport (PET) in thylakoids of spinach as well as both atrazine-resistant and wild-type *Chenopodium album*. Among the compounds with an alkylamino group, 2-ethoxycarbonyl-4-isopropylamino-6-methyl-1,3,5-triazine was the most potent PET-inhibitor, exhibiting a pI_{50} of 6.11. The inhibitory activity was generally more potent with 2-alkoxycarbonyl-4-(branched alkyl)amino-6-methyl-1,3,5-triazines than amino-analogues with straight chain alkyl groups or unsaturated alkyl groups.

Keywords: 2-ethoxycarbonyl-4-isopropylamino-6-methyl-1,3,5-triazine, PET inhibition, atrazine-resistant and wild-type *Chenopodium album*.

* To whom correspondence should be addressed.
E-mail: h-kohno@agr.tamagawa.ac.jp

INTRODUCTION

Long term use of chloro-1,3,5-triazines, such as atrazine and simazine, has led to the emergence of resistant weeds, problems with residue in soil and water, and recently, endocrine disrupting effects in animals.[1)] Thus, the use of such chloro-1,3,5-triazine herbicides in agriculture in now prohibited in developed countries.

Regarding resistance to atrazine, Kuboyama *et al.*[2)] recently found that 2-alkyl-4-benzylamino-6-trifluoromethyl-1,3,5-triazines, *e.g.* 2-(4-bromobenzylamino)-4-methyl-6-trifluoromethyl-1,3,5-triazine, exhibit strong herbicidal activity inhibiting photosynthetic electron transport (PET) against both atrazine-resistant and wild-type *Chenopodium album*.

Although benzylamino-1,3,5-triazines with a trifluoromethyl group exhibit the highest levels of herbicidal activity in pre- and post-emergence herbicidal tests, they have several halogen atoms which need to be removed in order to reduce environmental pollution.[1)]

In this paper, new 1,3,5-triazine compounds with electron-withdrawing carboxyl groups, *e.g.* ester and amide groups, substituted for the trifluoromethyl group, were synthesized and assayed for activity to inhibit PET in both atrazine-resistant and wild-type *Chenopodium album*.

MATERIALS AND METHODS

1. Chemicals

The 2-amino-1,3,5-triazinylcarboxylic acid derivatives in this study were synthesized by nucleophilic substitution reactions of corresponding trichloromethyl-1,3,5-triazines with the appropriate amines.

1.1. Synthesis of the original 2-ethoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazine

Ethyl chloroglyoxylate, $ClC(=O)C(=O)OC_2H_5$, (1.36 g, 0.01 mol) was mixed with *N*-(acetimidoyl)trichloroacetamidine[3)] (2.03 g, 0.01 mol) in dry diethyl ether (20 ml) at 0°C. After stirring at room temperature for 16 hr, the mixture was concentrated under reduced pressure. The residue was washed with water and then purified by silica gel column chromatography using ethyl acetate : hexane = 1 : 8 (v/v) to give 2-ethoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazine as an oil. The yield was 0.67 g (23.7%). IR ν_{max} (KBr) cm^{-1} : 1546 (1,3,5-triazine), 1749 (C=O). ^1H NMR δ_H ($CDCl_3$): 1.48 (3H, t, J=7.2 Hz, CH_2-C<u>H</u>$_3$), 2.96 (3H, s, C<u>H</u>$_3$), 4.57 (2H, q, J=7.2 Hz, C<u>H</u>$_2$-CH_3). MS: *m*/*z* 283(M^+), 248(M^+-Cl) and 108 (CCl_2CN). Isotope peaks for Cl_3 ; Calcd. (found) : M^+, 100% (100%) ; M^++2, 97.8 (94), M^++4, 31.9 (28.8). Isotope peaks for Cl_2 ; M^+-Cl, 100% (100%) ; M^+-Cl+2, 65.3 (67.5).

2-Methoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazine was prepared in a similar manner, starting from *N*-(acetimidoyl)trichloroacetamidine and methyl chloroglyoxylate. Yield 79.5%, viscous oil.

*1.2. Synthesis of 2-benzylamino-4-ethoxycarbonyl-6-methyl-1,3,5-triazine (**9**)*

To a solution of 2-ethoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazine (2.9 g, 0.01 mol) in 100 ml of tetrahydrofuran (THF), benzylamine (1.07 g, 0.01 mol) was added. After stirring at room temperature for 5 hr, the mixture was concentrated under reduced pressure. The residue was washed with water and then purified by column chromatography over silica gel using ethyl acetate: hexane=2 : 3 (v/v). The yield was 0.8 g (30.7%), white solid, mp. 97–99°C. IR ν_{max} (KBr) cm^{-1}: 1533 (1,3,5-triazine), 1753 (C=O). ^{1}H NMR δ_H ($CDCl_3$): 1.42 (3H, t, J=7.2 Hz, CH_2-C$\underline{H}_3$), 2.51 and 2.59 (3H in total, each s, C$\underline{H}_3$), 4.48 (2H, q, J=7.2 Hz, C$\underline{H}_2$-CH_3), 4.71 and 4.73 (2H in total, each d, J=6.0 Hz, NH-C$\underline{H}_2$), 5.86 and 6.03 (1H in total, each bs, N$\underline{H}$), 7.26–7.37 (5H, m, $C_6\underline{H}_5$).

Other 2-substituted amino-4-alkoxycarbonyl-6-methyl-1,3,5-triazines (**1**–**8**) were synthesized with a method similar to that mentioned in *1.2.* Their analytical data are cited in Table 1.

*1.3. Synthesis of 2-ethylamino-4-ethylcarbamoyl-6-methyl-1,3,5-triazine (**14**)*

An aqueous 70% ethylamine solution (1.9 g, 0.03 mol) was added to a THF (30 ml) solution of 2-ethoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazine (2.9 g, 0.01 mol), at room temperature. After stirring for 3 hr, the mixture was concentrated under reduced pressure. The residue was washed with water and then purified by column chromatography over silica gel using ethyl acetate: hexane=1 : 1 (v/v). The yield was 0.5 g (23.9%), colorless crystal, mp. 93–94°C. IR ν_{max} (KBr) cm^{-1}: 1601 (1,3,5-triazine), 1747 (C=O). ^{1}H NMR δ_H ($CDCl_3$): 1.24 (6H, m, CH_2-C$\underline{H}_3$), 2.47 and 2.50 (3H in total, each s, C$\underline{H}_3$), 3.53 (2H, m, C$\underline{H}_2$-CH_3), 5.47 (1H, bs, N$\underline{H}$), 7.87 and 7.94 (1H in total, each bs, N$\underline{H}$).

2-Methylamino-4-methylcarbamoyl-6-methyl-1,3,5-triazine (**13**) was prepared in an analogous way. Mp. 175–178°C. IR ν_{max} (KBr) cm^{-1}: 1607 (1,3,5-triazine), 1712 (C=O). ^{1}H NMR δ_H ($CDCl_3$): 2.52 and 2.59 (3H in total, each s, C$\underline{H}_3$), 4.01 (3H, s, CONH-C$\underline{H}_3$), 4.71 and 4.74 (3H in total, each d, J=6.0 Hz, NH-C$\underline{H}_3$), 5.87 and 6.08 (1H in total, each bs, N$\underline{H}$).

2. Evaluation of PET Inhibitory Activities

2.1. 50% inhibition of PET by compounds acting against spinach thylakoids

According to Böger,[4] a thylakoid suspension was prepared from *Spinacia oleracea* leaves. The inhibition of PET by the compounds assayed was determined using an oxygen electrode as in our previous paper.[5] The activity was determined by the system $H_2O \rightarrow$ potassium ferricyanide, uncoupled by NH_4Cl. The molar concentration (I_{50}) required for 50% inhibition was calculated for each compound by the probit method. The pI_{50} value was the logarithm of the reciprocal I_{50}. The pI_{50}(Sp) values for the inhibition of PET in spinach thylakoids are shown in Table 2.

2.2. 50% inhibition of PET by compounds acting against atrazine-resistant and wild-type Chenopodium album thylakoids

The cultivation of either atrazine-resistant or wild-type *Chenopodium album* and the isolation of each thylakoid was carried out according to Jansen *et al.*[6] and van Rensen *et al.*[7] Chlorophyll content was measured as described by Bruinsma,[8] and the concentration of chlorophyll adjusted to 25 μg per ml. See our previous paper[9] for details. The amount of oxygen formed was measured at 25°C, during a 20 sec illumination at 350 $\mu E/m^2$. The final concentration of solvent for the compounds assayed was kept below 1% (v/v). The pI_{50} values of PET inhibitory activity against atrazine-resistant and wild-type *Chenopodium album* thylakoids are indicated as pI_{50}(R) and pI_{50}(W), respectively, in Table 2.

RESULTS AND DISCUSSION

1. Synthetic Studies: Amination Reaction of 2-Alkoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazines

Kuboyama *et al.*[2] have synthesized many 2-methyl-4-trichloromethyl-1,3,5-triazines by the condensation of *N*-(acetimidoyl)trichloroacetamidine with corresponding acid anhydrides or acid chlorides. According to this procedure, the starting materials for a nucleophilic reaction, 2-alkoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazines, were prepared by condensation of *N*-(acetimidoyl)trichloroacetamidine with alkyl chloroglyoxylate, ClC(=O)C(=O)OR (R=CH_3 or C_2H_5).

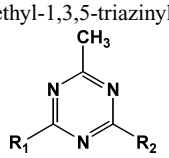
In the nucleophilic amination reaction of 2-alkoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazines, an amine: triazine ratio of 1 : 1 (mol/mol) gave only 2-amino-4-ethoxycarbonyl-6-methyl-1,3,5-triazine (A), although an excess amount of amine (amine/triazine>2) resulted in 2-amino-4-carbamoyl-6-methyl-1,3,5-triazine (B). This result indicates that the haloform reaction of the CCl_3-group with amine proceeds more rapidly than the aminolysis of the alkoxycarbonyl group with amine (see Fig. 1).

The ^{1}H-NMR spectra of 2-amino-4-methyl-1,3,5-triazinylcarboxylic acid derivatives showed a set of resonances for particular protons. For example, 2-benzylamino-4-ethoxycarbonyl-6-methyl-1,3,5-triazine (**9**) exhibited two signals at δ 2.51 and 2.59 for the 6-methyl protons, two signals at δ 4.71 and 4.73 for C$\underline{H}_2$-C_6H_5, and two signals at δ 5.87 and 6.08 for the N$\underline{H}$ proton. This finding was reported in our previous paper.[10] The π-electron on the 1,3,5-triazine ring and the lone electron pair at the (benzyl)amino nitrogen are considered to conjugate with each other to form a sort of molecular orbital, giving the bond between C-6 and nitrogen a kind of partial double bond character. Accordingly, several sets of two resonances in ^{1}H-NMR spectra can be observed due to a sort of *syn-anti* isomerism of the C-N bond occurring only in the magnetic field.

2. PET Inhibitory Activity of 2-Amino-4-methyl-1,3,5-triazinylcarboxylic Acid Derivatives against Spinach Thylakoids

The effect of all 2-amino-4-methyl-1,3,5-triazinylcarboxylic acid derivatives (**1**–**9**) synthesized in this study on PET inhibitory activity was examined using *Spinacia oleracea* thylakoids and compared with the inhibitory activity exhibited by 2-(fluorinated methyl)-4-benzylamino-6-methyl-1,3,5-triazines (**10**–**12**).[11,12] Among the derivatives with an amino group, 2-ethoxycarbonyl-4-

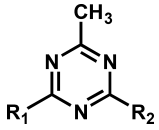
Table 1. Analytical data for new 2-substituted amino-4-methyl-1,3,5-triazinylcarboxylic acid derivatives synthesized for this study



No.	R_1	R_2	mp (°C)	Yield (%)	IR ν_{max} (KBr) cm^{-1}	^1H NMR ($CDCl_3$, TMS) ppm
1	$COOC_2H_5$	NHC_2H_5	48–52	13.6	1546, 1750	1.17 and 1.19 (3H in total, each t, J=7.2 Hz, NH-CH_2-C$\underline{H}_3$), 1.36 (3H, t, J=7.1 Hz, $COOCH_2$-C$\underline{H}_3$), 2.43 and 2.50 (3H in total, each s, C$\underline{H}_3$) 3.48 (2H, m, NH-C$\underline{H}_2$-CH_3), 4.42 (2H, q, J=7.1 Hz, $COOC\underline{H}_2$-CH_3), 5.44 and 5.60 (1H in total, each bs, N$\underline{H}$)
2	$COOC_2H_5$	NHC_3H_7-*n*	oil	62.5	1595, 1743	0.98 (3H, t, J=7.2 Hz, NH-CH_2-CH_2-C$\underline{H}_3$), 1.43 (3H, t, J=7.2 Hz, $COOCH_2$-C$\underline{H}_3$), 1.61 (2H, sextet, J=7.2 Hz, NH-CH_2-C$\underline{H}_2$-CH_3), 2.50 and 2.56 (3H in total, each s, C$\underline{H}_3$), 3.46 and 3.50 (2H in total, each q, J=7.2 Hz and J=7.2 Hz, NH-C$\underline{H}_2$-CH_2-CH_3), 4.49 (2H, q, J=7.2 Hz, $COOC\underline{H}_2$-CH_3), 5.59 and 5.75 (1H in total, each bs, N$\underline{H}$)
3	$COOC_2H_5$	NHC_3H_7-*i*	oil	22.3	1577, 1743	1.25 and 1.25 (6H in total, each d, J=6.3 Hz, NH-CH($C\underline{H}_3$)$_2$), 1.42 (3H, t, J=7.1 Hz, $COOCH_2$-C$\underline{H}_3$), 2.49 and 2.56 (3H in total, each s, C$\underline{H}_3$), 4.30 (1H, m, NH-C$\underline{H}$(CH_3)$_2$), 4.48 (2H, q, J=7.1 Hz, $COOC\underline{H}_2$-CH_3), 5.42 and 5.58 (1H in total, each bs, N$\underline{H}$)
4	$COOC_2H_5$	$NHCH_2CH{=}CH_2$	oil	54.1	1590, 1745	1.43 (3H, t, J=7.2 Hz, $COOCH_2$-C$\underline{H}_3$), 2.52 and 2.57 (3H in total, each s, C$\underline{H}_3$), 4.15 and 4.19 (2H in total, each t, J=5.9 Hz and J=5.6 Hz, NH-C$\underline{H}_2$-CH=CH_2), 4.48 (2H, q, J=7.2 Hz, $COOC\underline{H}_2$-CH_3), 5.24 and 5.28 (2H in total, each d, J=10.3 Hz and J=17.1 Hz, NH-CH_2-CH=C$\underline{H}_2$), 5.68 and 5.83 (1H in total, each bs, N$\underline{H}$), 5.89 and 5.94 (1H in total, each qui, J=5.2 Hz and J=5.6 Hz, NH-CH_2-C$\underline{H}$=CH_2)
5	$COOC_2H_5$	$NHCH_2C{\equiv}CH$	103–105	54.5	1595, 1741	1.43 (3H, t, J=6.8 Hz, $COOCH_2$-C$\underline{H}_3$), 2.27 (1H, m, NH-CH_2-C≡C$\underline{H}$), 2.54 and 2.60 (3H in total, each s, C$\underline{H}_3$), 4.31 and 4.33 (2H, dd, J=2.4 Hz, NH-C$\underline{H}_2$-C≡CH), 4.49 (2H, q, J=6.8 Hz, $COOC\underline{H}_2$-CH_3), 5.85 and 6.06 (1H in total, each bs, N$\underline{H}$)
6	$COOC_2H_5$	NHC_4H_9-*n*	oil	33.6	1580, 1750	0.95 (3H, t, J=7.3 Hz, NH-CH_2-(CH_2)$_2$-C$\underline{H}_3$), 1.42 (3H, t, J=7.2 Hz, $COOCH_2$-C$\underline{H}_3$), 1.58 (4H, m, NH-CH_2-($C\underline{H}_2$)$_2$-CH_3), 2.49 and 2.56 (3H in total, each s, C$\underline{H}_3$), 3.49 and 3.53 (2H in total, each q, J=7.2 Hz, NH-C$\underline{H}_2$-(CH_2)$_2$-CH_3), 4.48 (2H, q, J=7.2 Hz, $COOC\underline{H}_2$-CH_3), 5.56 and 5.72 (1H in total, each bs, N$\underline{H}$)
7	$COOC_2H_5$	NHC_4H_9-sec	oil	37.8	1585, 1750	0.95 (3H, t, J=7.3 Hz, NH-CH(CH_3)CH_2-C$\underline{H}_3$), 1.22 (3H, t, J=7.3 Hz, NH-CH(C$\underline{H}_3$)CH_2-CH_3), 1.42 (3H, t, J=7.2 Hz, $COOCH_2$-C$\underline{H}_3$), 1.57 (2H, qui, J=7.3 Hz, NH-CH(CH_3)C$\underline{H}_2$-CH_3), 2.49 and 2.55 (3H in total, each s, C$\underline{H}_3$), 4.15 (1H, m, NH-C$\underline{H}$(CH_3)CH_2-CH_3), 4.48 (2H, q, J=7.1 Hz, $COOC\underline{H}_2$-CH_3), 5.38 and 5.55 (1H in total, each bs, N$\underline{H}$)
8	$COOCH_3$	$NHCH_2C_6H_5$	110–112	24.4	1523, 1736	2.52 and 2.59 (total 3H, each s, C$\underline{H}_3$), 4.01 (3H, s, $COOC\underline{H}_3$), 4.71 and 4.74 (total 2H, each d, J=6.0 Hz and J=6.0 Hz, C$\underline{H}_2$), 5.87 and 6.08 (1H, each bs, N$\underline{H}$), 7.28–7.37 (5H, m, $C_6\underline{H}_5$)

Note: Physical data for compounds **10**–**12** were reported by Kuboyama *et al.*[11] and Inoue *et al.*[12] Melting points (uncorrected) were measured with a Yanagimoto-Seisakusyo melting point apparatus. IR were recorded on a JASCO FT/IR-420 spectrophotometer. ^1H NMR spectra were measured on a JEOL JNM-GX400 spectrometer at 400 MHz using tetramethylsilane (TMS) as an internal standard.

Table 2. Inhibition of PET by 2-substituted amino-4-methyl-1,3,5-triazinylcarboxylic acid derivatives, 2-(fluorinated methyl)-4-benzylamino-6-methyl-1,3,5-triazines and atrazine in spinach and *Chenopodium album* thylakoids



Compounds No.	R_1	R_2	*Chenopodium album*			Spinach
			Wild-type pI_{50}(W)	Resistant-type pI_{50}(R)	R/W	pI_{50}(Sp)
1	$COOC_2H_5$	NHC_2H_5	n.t.[a)]	n.t.	–	3.00–4.00
2	$COOC_2H_5$	NHC_3H_7-*n*	n.t.	n.t.	–	3.00–4.00
3	$COOC_2H_5$	NHC_3H_7-*i*	6.00	5.61	2.45	6.11
4	$COOC_2H_5$	$NHCH_2CH{=}CH_2$	n.t.	n.t.	–	3.00–4.00
5	$COOC_2H_5$	$NHCH_2C{\equiv}CH$	4.99	4.90	1.23	3.00–4.00
6	$COOC_2H_5$	NHC_4H_9-*n*	<4.00	n.t.	–	3.00–4.00
7	$COOC_2H_5$	NHC_4H_9-*sec*	<4.00	n.t.	–	4.37
8	$COOCH_3$	$NHCH_2C_6H_5$	n.t.	n.t.	–	4.00
9	$COOC_2H_5$	$NHCH_2C_6H_5$	4.82	4.83	0.98	4.88
10[b)]	CH_2F	$NHCH_2C_6H_5$	4.43	<4.00	–	4.98
11[b)]	CHF_2	$NHCH_2C_6H_5$	5.21	4.82	2.50	5.63
12[b)]	CF_3	$NHCH_2C_6H_5$	7.03	6.61	2.60	6.85
13	$CONHCH_3$	$NHCH_3$	n.t.	n.t.	–	<3.00
14	$CONHC_2H_5$	NHC_2H_5	n.t.	n.t.	–	<3.00
15	Atrazine		6.72	4.21	324	6.73

[a)] Not tested. [b)] From Ref. 12.

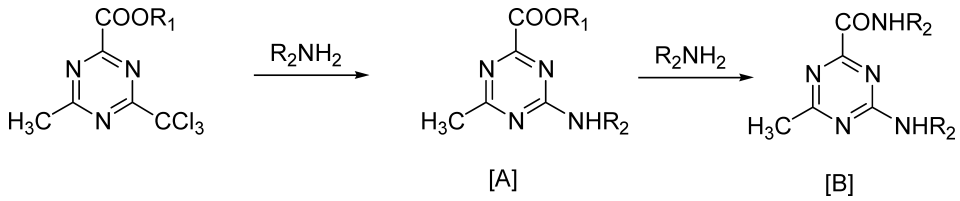


Fig. 1. Stepwise substitution reaction of 2-alkoxycarbonyl-4-methyl-6-trichloromethyl-1,3,5-triazines with amine.

isopropylamino-6-methyl-1,3,5-triazine (**3**) was the most active inhibitor with a pI_{50} of 6.11, corresponding to the inhibitory activity of the herbicidally active compounds triazine (**12**) and atrazine (**15**).

The pI_{50} values of the triazines (**13** and **14**) with an amino and carbamoyl group were lower than those of the triazines (**1**–**9**) with an amino and alkoxycarbonyl group.

3. *PET Inhibitory Activity of 2-Amino-4-ethoxycarbonyl-6-methyl-1,3,5-triazines against Chenopodium album Thylakoids*

The effect of 2-amino-4-ethoxycarbonyl-6-methyl-1,3,5-triazines (**1**–**9**) exhibiting PET inhibitory activity against spinach thylakoids was then examined using thylakoids from atrazine-resistant and wild-type *Chenopodium album*. All compounds tested were less active against thylakoids from wild-type *Chenopodium* than was atrazine. Although compound **3** was slightly less active than atrazine against wild-type *Chenopodium* thylakoids, its pI_{50}-value against atrazine-resistant *Chenopodium* thylakoids was higher than that of atrazine, indicating 15 times more inhibitory activity. The resistant ratio (R/W) obtained with 2-amino-4-ethoxycarbonyl-6-methyl-1,3,5-triazines was **1**–**3**, while that for atrazine was 324. For example, 2-ethoxycarbonyl-4-methyl-6-propargylamino-1,3,5-triazine (**5**) showed a R/W ratio of 1.23. From this finding it can be concluded, that the 1,3,5-triazines with an ethoxycarbonyl group bring about a decrease in the R/W ratio, probably contributing to the anti-resistant nature of the triazine derivatives.

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