

## Thieno [3,2-*d*] pyrimidin-4-one Derivatives as Potential Antibacterial Agents

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**ABSTRACT** Several thieno [3,2-*d*]pyrimidin-4-ones (**4a-i**) were conveniently prepared by the thio-claisen rearrangement of 5-prop-2-ynylsulfanyl pyrimidin-4-ones derivatives. The constitution of the products was supported by IR, PMR and mass spectral study. The compounds synthesized were tested in vitro against gram positive microorganisms, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and gram negative microorganism *Eseherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klabsiella pneumoniae* and *Shigella sonnei*. Standard drugs were also tested under identical conditions for comparing the results.

### INTRODUCTION

The emergence and spread of bacterial resistance represent a severe global problem (Ehlert 1999). Multidrug-resistance gram-positive pathogens, such as methicillin-resistant, *Staphylococci aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE), have become a serious problem in the medical community (Cunha 1998; Dalhoff 1994) One particular alarming sign is the acquisition of resistance to vancomycin, an antibiotic generally regarded as the agent of last resort for serious infections (File 1999 ; Witte 1999; Endtz et al. 1999). In the field of quinoline antibacterial agents, various attempts have been made to obtain potent drugs for resistant gram-positive bacteria such as torvafloxin (Gootz et al. 1992), moxifloxacin (Petersen et al.1996), gemifloxacin (Hong et al. 1997) and gatifloxacin (Hirai et al. 1990) etc, have been developed and introduced into clinical use over the past several years. Considering that the incidence of gram-positive bacterial resistance to antibacterial agents has been growing, however, antibacterial activities of these newer quinolines are not potent enough and bacteria resistant to

those agents will be problematic in the near future (Ho et al. 2000; Weiss et al. 2001). Therefore, there is an urgent need for novel chemical entities that are particularly effective against gram-positive pathogens including the multiresistant strains. In the last few years, various pyrimidinone derivatives exhibited a wide range of activity such as antitumor, antiviral, antitubercular and antifungal activities (Maria et al. 2004). In continuation of our efforts in developing heterocycles of biological interest (Bedi et al. 2004), we wish to report here the antimicrobial activities of the title thieno[3,2-*d*]pyrimidinones derivatives in order to study their antibacterial activities.

### MATERIALS AND METHODS

Melting points were determined by open capillary using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. <sup>1</sup>H NMR spectra were recorded in deuteriochloroform with Bruker AC-E 200 (200 MHz) spectrometers using TMS as internal standard. Chemical shift values are expressed as ppm downfield from TMS and J values in Hz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, q: quartet ,br: broad peak and brs: broad singlet. <sup>13</sup>C NMR spectra were also recorded on Bruker AC-200E (50.4 MHz) spectrometers in deuteriochloroform using TMS as internal standard. Mass spectra were recorded on Shimadzu GCMS-QP-2000 mass spectrometer.

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Elemental analyses were performed on Heraeus Carbon, Hydrogen, Nitrogen-Oxygen Rapid Elemental Analyzer. Column chromatography was performed on a silica gel (60–120) mesh or Harrison Research Chromatotron using 2 mm plates (Silica gel 60 PF254).

### Synthesis

The required thieno [3,2-*d*]pyrimidin-4-ones (**4a-i**) were conveniently prepared by the thio-claisen rearrangement of 5-prop-2-ynylsulfanyl pyrimidin-4-ones derivatives **3**. The pyrimidinone derivatives **3** were in turn obtained by the [4+2] cycloaddition reaction of 1,3-diazabuta-1,3-dienes **1** with prop-2-ynylsulfanyl ketene **2**, generated *in situ* from the corresponding acid and *p*-toluene sulfonyl chloride in the presence of triethylamine in dry methylene chloride at room temperature. Thermolysis of pyrimidinones **3** in refluxing chlorobenzene, resulted in the formation of thieno [3,2-*d*]pyrimidinones (**4a-i**) as crystalline solids in good yields (75-80%) (**Scheme 1**). The thieno [3,2-*d*] pyrimidinones so obtained were characterized on the basis of analytical and spectral evidences mentioned in the experimental section.

Physical and spectral data of representative compounds are as follows.

**6-Methyl-2-phenyl-3-*o*-tolyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4a)**. creamish solid, yield 77% ; m.p. 237-238<sup>o</sup>C ; Anal. Calcd for C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>OS: C, 72.26; H, 4.85; N, 8.43. Found: C, 72.35; H, 4.91; N, 8.28%;  $\nu_{max}$  (KBr): 1685 cm<sup>-1</sup>;  $d_H$  (200 MHz, CDCl<sub>3</sub>): 2.34 (s, 3H, CH<sub>3</sub>); 2.69 (s, 3H, CH<sub>3</sub>); 7.23-7.52 (m, 10H, ArH and H<sub>d</sub>);  $d_C$  (50.4 MHz, CDCl<sub>3</sub>): 16.2 (CH<sub>3</sub>), 19.2 (CH<sub>3</sub>), 114.0, 120.7, 122.8, 123.8, 127.9, 129.2, 129.9, 130.3, 134.7, 149.8, 155.7, 156.8, 157.9, and 159.5; m/z 332 (M<sup>+</sup>).

**6-Methyl-2-methylsulfanyl-3-phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4b)**. creamish solid, yield 82% ; m.p. 218-219<sup>o</sup>C ; Anal. Calcd for C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>OS<sub>2</sub>: C, 58.31; H, 4.19; N, 9.71. Found: C, 58.47; H, 4.11; N, 9.63%;  $\nu_{max}$  (KBr): 1676 cm<sup>-1</sup>;  $d_H$  (200 MHz, CDCl<sub>3</sub>): 2.70 (s, 3H, CH<sub>3</sub>); 2.89 (s, 3H, SCH<sub>3</sub>); 7.23-7.42 (m, 6H, ArH and H<sub>d</sub>);  $d_C$  (50.4 MHz, CDCl<sub>3</sub>): 16.4 (SCH<sub>3</sub>), 16.8 (CH<sub>3</sub>), 116.2, 129.5, 123.8, 127.5, 128.8, 149.3, 155.1, 157.7, 158.2 and 159.0; m/z 288 (M<sup>+</sup>).

**6-Methyl-2-methylsulfanyl-3-*p*-tolyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4c)**. white solid, yield 75% ; m.p. 212-213<sup>o</sup>C ; Anal. Calcd for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>OS<sub>2</sub>: C, 59.57; H, 4.67; N, 9.26. Found: C,

59.82; H, 4.58; N, 9.10%;  $\nu_{max}$  (KBr): 1670 cm<sup>-1</sup>;  $d_H$  (200 MHz, CDCl<sub>3</sub>): 2.34 (s, 3H, CH<sub>3</sub>); 2.67 (s, 3H, CH<sub>3</sub>); 2.90 (s, 3H, SCH<sub>3</sub>); 6.98-7.12 (m, 5H, ArH and H<sub>d</sub>);  $d_C$  (50.4 MHz, CDCl<sub>3</sub>): 16.5 (SCH<sub>3</sub>), 16.7 (CH<sub>3</sub>), 19.0 (CH<sub>3</sub>), 115.3, 119.7, 123.5, 127.9, 128.9, 148.5, 154.5, 157.2, 158.1 and 159.3; m/z 302 (M<sup>+</sup>).

**3- (4-Methoxyphenyl)-6-methyl-2-methyl sulfanyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4d)**. white solid, yield 88% ; m.p. 215-216<sup>o</sup>C ; Anal. Calcd for C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub>: C, 56.58; H, 4.43; N, 8.80. Found: C, 56.49; H, 4.68; N, 8.64%;  $\nu_{max}$  (KBr): 1668 cm<sup>-1</sup>;  $d_H$  (200 MHz, CDCl<sub>3</sub>): 2.68 (s, 3H, CH<sub>3</sub>); 2.90 (s, 3H, SCH<sub>3</sub>); 3.75 (s, 3H, OCH<sub>3</sub>); 7.05-7.27 (m, 5H, ArH and H<sub>d</sub>);  $d_C$  (50.4 MHz, CDCl<sub>3</sub>): 16.4 (SCH<sub>3</sub>), 16.7 (-CH<sub>3</sub>), 55.7 (OCH<sub>3</sub>), 114.7, 120.5, 123.5, 127.9, 130.1, 150.2, 156.4, 157.1, 157.9 and 159.0; m/z 318 (M<sup>+</sup>).

**3- (4-Chlorophenyl)-6-methyl-2-methyl sulfanyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4e)**. pale yellow solid, yield: 78% ; m.p. 202-203<sup>o</sup>C ; Anal. Calcd for C<sub>14</sub>H<sub>11</sub>ClN<sub>2</sub>OS<sub>2</sub>: C, 51.70; H, 4.03; N, 8.62. Found: C, 51.89; H, 3.95; N, 8.58%;  $\nu_{max}$  (KBr): 1668 cm<sup>-1</sup>;  $d_H$  (200 MHz, CDCl<sub>3</sub>): 2.36 (s, 3H, CH<sub>3</sub>); 2.92 (s, 3H, SCH<sub>3</sub>); 6.98-7.12 (m, 5H, ArH and H<sub>d</sub>);  $d_C$  (50.4 MHz, CDCl<sub>3</sub>): 16.6 (SCH<sub>3</sub>), 16.7 (CH<sub>3</sub>), 115.6, 119.7, 123.8, 127.7, 128.8, 148.7, 154.5, 157.4, 158.3 and 159.5; m/z 290 (M<sup>+</sup>).

**2,3-Diphenyl-6-methyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4f)**. creamish solid, yield 78% ; m.p. 223-224<sup>o</sup>C ; Anal. Calcd for C<sub>19</sub>H<sub>14</sub>N<sub>2</sub>OS: C, 71.67; H, 4.43; N, 8.80. Found: C, 71.81; H, 4.27; N, 8.82%.  $\nu_{max}$  (KBr): 1665 cm<sup>-1</sup>;  $d_H$  (200 MHz, CDCl<sub>3</sub>): 2.70 (s, 3H, CH<sub>3</sub>); 7.15-7.31 (m, 11H, ArH and H<sub>d</sub>);  $d_C$  (50.4 MHz, CDCl<sub>3</sub>): 17.2 (CH<sub>3</sub>), 116.2, 118.9, 122.3, 126.0, 128.8, 129.2, 129.9, 134.7, 149.6, 155.2, 156.8, 157.5 and 158.8; m/z 318 (M<sup>+</sup>).

**6-Methyl-2-phenyl-3-*p*-tolyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4g)**. light yellow solid, yield 78% ; m.p. 231-233<sup>o</sup>C ; Anal. Calcd for C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>OS: C, 72.26; H, 4.85; N, 8.43. Found: C, 72.18; H, 4.99; N, 8.41%;  $\nu_{max}$  (KBr): 1670 cm<sup>-1</sup>;  $d_H$  (200 MHz, CDCl<sub>3</sub>): 2.28 (s, 3H, CH<sub>3</sub>); 2.63 (s, 3H, CH<sub>3</sub>); 7.00 (d, 2H, J=8.4 Hz, ArH); 7.08 (d, 2H, J=8.4 Hz, ArH); 7.18-7.30 (m, 6H, ArH and H<sub>d</sub>);  $d_C$  (50.4 MHz, CDCl<sub>3</sub>): 16.7 (CH<sub>3</sub>), 19.6 (CH<sub>3</sub>), 114.0, 120.2, 122.8, 127.5, 128.5, 129.0, 129.7, 130.3, 136.0, 150.1, 155.7, 157.1, 157.7, and 159.0; m/z 332 (M<sup>+</sup>).

**3- (4-Methoxyphenyl)-6-methyl-2-phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (4h)**. white solid, yield 75% ; m.p. 220-221<sup>o</sup>C ; Anal. Calcd for C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>S: C, 68.94; H, 4.63; N, 8.04. Found: C, 68.73; H, 4.73; N, 8.19%;  $\nu_{max}$  (KBr): 1680 cm<sup>-1</sup>;  $d_H$  (200MHz): 2.64 (s, 3H, CH<sub>3</sub>); 3.74 (s, 3H, OCH<sub>3</sub>);

6.76 (d, 2H, J=8.8Hz, ArH); 6.99 (d, 2H, J=8.8Hz, ArH); 7.05-7.33 (m, 6H, ArH and H<sub>d</sub>); d<sub>C</sub> (50.4 MHz, CDCl<sub>3</sub>): 16.7 (CH<sub>3</sub>); 55.2 (OCH<sub>3</sub>); 114.1, 120.8, 123.5, 127.9, 128.9, 129.0, 129.9, 130.1, 135.5, 150.2, 156.6, 157.0, 157.8, 159.1: m/z 348 (M<sup>+</sup>).

**3-(4-Chlorophenyl)-6-methyl-2-phenyl-3H-thieno[3,2-d]pyrimidin-4-one (4i)**. creamish solid, yield 80% ; m.p. 239-240°C ; Anal. Calcd for C<sub>19</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>2</sub>S: C, 64.68; H, 3.71; N, 7.94. Found: C, 64.97; H, 3.58; N, 7.78%: ν<sup>max</sup> (KBr): 1680 cm<sup>-1</sup>; d<sub>H</sub> (200 MHz, CDCl<sub>3</sub>): 2.65 (s, 3H, CH<sub>3</sub>); 6.96 (d, 2H, J=8.4 Hz, ArH); 7.04 (d, 2H, J=8.4 Hz, ArH); 7.18-7.30 (m, 6H, ArH and H<sub>d</sub>); d<sub>C</sub> (50.4 MHz, CDCl<sub>3</sub>): 16.7 (CH<sub>3</sub>), 115.1, 121.0, 123.7, 127.9, 128.6, 129.2, 129.8, 130.3, 135.7, 150.0, 156.8, 157.2, 158.1 and 159.5 ; m/z 352 (M<sup>+</sup>).

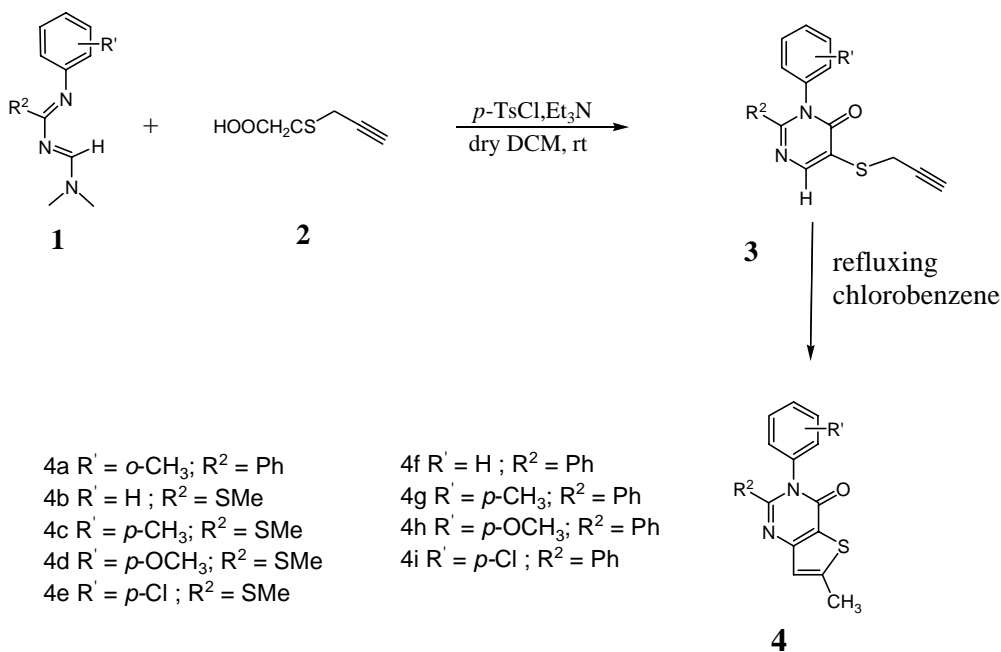
### Antibacterial Activity

The antibacterial activity of the test compounds was determined by agar well diffusion method (Aparna et al. 2005) against gram positive microorganisms, *Bacillus subtilis* MTCC 121, *Bacillus cereus* MTCC 1272, *Staphylococcus aureus* MTCC 1430, *Enterococcus faecalis* MTCC 439 and gram negative microorganism *Eseherichia coli* MTCC 42, *Pseudomonas*

*aeruginosa* MTCC 1034, *Proteus vulgaris* MTCC 744, *Klabsiella pneumoniae* MTCC 109 and *Shigella sonnei* MTCC 2957 at 1 mg/ml concentration, using dimethyl sulfoxide (DMSO) as solvent. The bacteria were subcultured on Mueller Hinton Agar Medium. Standard antibacterial ciprofloxacin was also screened under similar conditions for comparison. The Petri dishes were incubated at 37°C for 48 h to know the bacterial growth inhibition developed around the hole and was measured in millimeter for particular test solution with particular organism.

### RESULTS AND DISCUSSION

It has been observed that the compounds (**4a-4i**) when tested for the different strains of gram positive and gram negative bacteria exhibited interesting antibacterial activity, however with a degree of variation. Results as shown in Table 1,2 and Fig 1,2 revealed that compound **4c** and **4d** showed significant antibacterial activity whereas compounds **4f-4i** showed decrease in antibacterial activity against gram positive and gram negative strains as compared to the ciprofloxacin taken as standard.. However, compound **4f** did not show any activity against



Scheme 1. Synthesis of Thieno [3,2,d] pyrimidin-4-one derivatives

**Table 1:** *In vitro* antibacterial activity of compounds 4a-i (zone of inhibition in mm<sup>a</sup>) against gram positive bacteria

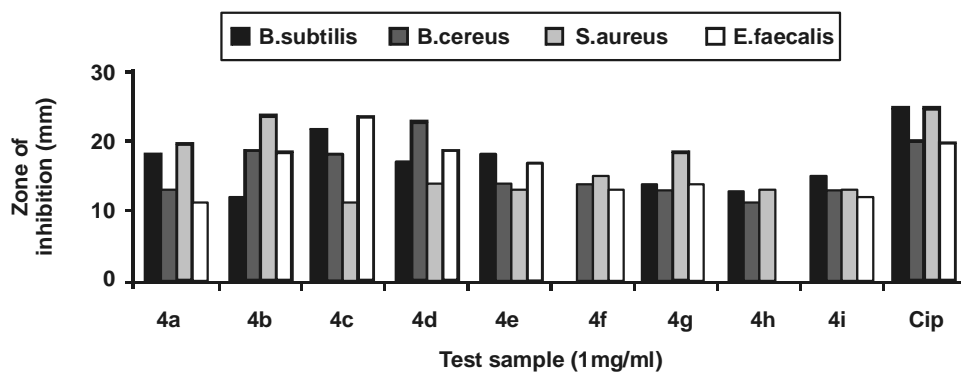
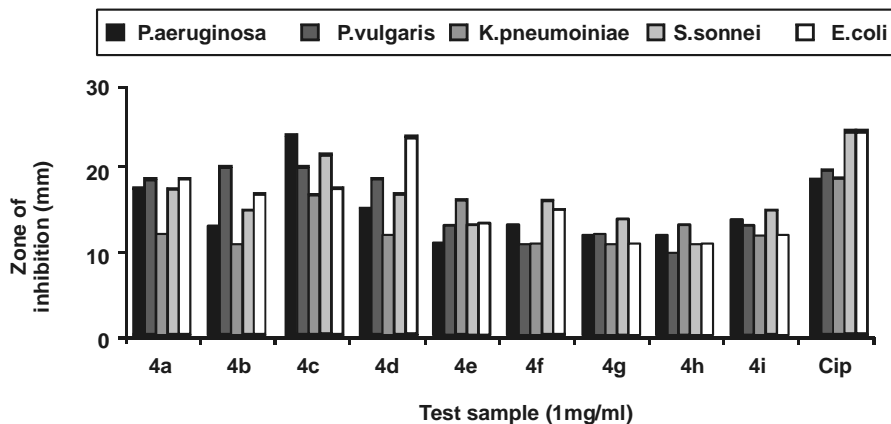
Compound	<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. faecalis</i>
4a	++	+	++	+
4b	+	++	+++	++
4c	+++	++	+	+++
4d	++	+++	+	++
4e	++	+	+	++
4f	-	+	+	+
4g	+	+	++	+
4h	+	+	+	-
4i	++	++	++	+
Cipro	+++	++	+++	++
DMSO	-	-	-	-

<sup>a</sup> + (11-15mm), ++ (16-20mm), +++ (21-25mm) and - (inactive).

**Table 2:** *In vitro* antibacterial activity of compounds 4a-i (zone of inhibition in mm<sup>a</sup>) against gram negative bacteria

Compound	<i>P. aeruginosa</i>	<i>P. vulgaris</i>	<i>K. pneumoniae</i>	<i>S. sonnei</i>	<i>E. coli</i>
4a	++	++	+	++	++
4b	+	++	+	+	++
4c	+++	++	++	+++	++
4d	+	++	+	++	+++
4e	+	+	++	+	+
4f	+	+	+	++	+
4g	+	+	+	+	+
4h	+	-	+	+	+
4i	+	+	+	+	+
Cipro	++	++	++	+++	+++
DMSO	-	-	-	-	-

<sup>a</sup> + (11-15mm), ++ (16-20mm), +++ (21-25mm) and - (inactive).

**Fig. 1.** Antibacterial activity of compds 4a-i against gram positive bacteria**Fig. 2.** Antibacterial activity of compds 4a-i against gram negative bacteria

*B. subtilis*. Similarly, compound **4h** was inactive against *E. faecalis* and *P. vulgaris*. Antibacterial data indicated that compound **4b** showed maximum activity against *P. vulgaris* and exhibited significant activity against *S. aureus* as compared to ciprofloxacin. Compound **4c** was superior in action against *E. faecalis* and *P. aeruginosa* over the standard ciprofloxacin, where as compound **4d** showed maximum activity against *B. cereus* and showed significant activity against *E. faecalis* and *E. coli* as compared to standard.

### CONCLUSION

The microbiological results of the present study show a good antibacterial profile in comparison to standard. Although, cyclic amide group (also present in  $\beta$ -lactam) is required for the antibacterial activity is present in all the tested compounds still, compounds possessing *p*-methyl (**4c**) or *p*-methoxy moieties (**4d**) are among the most active compounds in our studies. Moreover, removal of thiomethyl group, in compounds **4f-4i**, decreases its antibacterial activity against gram positive and gram-negative bacterial strains. Therefore antibacterial screening of thieno pyrimidinones revealed that the compounds having *p*-methyl group (**4c**)/*p*-methoxy group (**4d**) at C-3 and S-methyl group at C-2 positions of C-5, C-6 heterocyclic ring fused pyrimidinones showed significant activity comparable to the standard drug, against gram-positive and gram-negative bacterial strains respectively. Hence, a new class of six membered cyclic amide (pyrimidinones), analogues of  $\beta$ -lactam have been made available as potential antimicrobial agent.

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