

Molecular docking study of ginger (*Zingiber officinale*) on Immunoglobulin A for smoking cessation

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Abstract

Smoking is a big problem that can cause death throughout the world. The main ingredient in cigarettes, nicotine, is toxic to humans in several ways. Quitting smoking with the help of medication is associated with adverse side effects such as drowsiness, dry mouth, and nausea. The option of quitting smoking with herbal concoctions such as *Zingiber officinale* is the recommended choice. The active components of ginger are gingerol and shogaol, which are responsible for their pharmacological effects on immunoglobulin A, which can improve the immune system. The method used is molecular docking, which looks at the stability of human secretory immunoglobulin A when interacting with gingerol and bupropion, which are used as comparison compounds. Molecular docking findings of all herbal material samples revealed that almost all bioactive substances had lower binding energies than immunoglobulin A, especially proteins with PDB IDs 6UE7 and 6UEA. However, only a few ginger-derived bioactive compounds interacting with the 6UEA protein show binding energy values smaller than -7 ± 0.5 kcal/mol. The compounds 8-Gingerol, 8-Shogaol, 6-Shogaol, 6-Gingerol, 5-Shogaol, and 4-Shogaol were able to target the immunoglobulin A receptor protein better than the control, although not as good as the native ligand. Gingerol and Bupropion compounds have stable RMSD and RMSF values compared to human secretory immunoglobulin A without the ligand.

Keywords

Ginger, Immunoglobulin A, smoking cessation, *Zingiber officinale*

Introduction

Smoking has become a significant problem in the world that can cause death. The activity of smoking tobacco has spread to all groups of people in the world. Six million fatalities per year are thought to be attributable to tobacco smoking, which an estimated 1.1 billion people use. A further 600,000 fatalities are attributable to second-hand smoking exposure (GBD 2021; Le Foll et al. 2022).

However, tobacco smoking is a severe health risk, and according to WHO estimates, almost a billion people worldwide still use tobacco products, including the increasingly common smokeless variants (Fagerström 2012; West 2017; WHO 2022). Nicotine is perhaps the most essential and potent pharmacologically active substance in tobacco products despite its many deleterious effects on the human body (Benowitz 2009). Tobacco smoking is highly addictive because of the nicotine (FDA 2022). Several

studies have looked at how smoking affects immunoglobulin (Ig) levels (Srivastava et al. 1991). Several studies have investigated the effects of tobacco smoking on immunoglobulin (Ig) levels using various assays. Smoking is associated with decreased concentrations of IgG and IgA in serum and saliva (Giuca 2014; Tarbiah 2019). Inhibition of in vitro cytokine production by intestinal and peripheral blood mononuclear cells, as well as induction of specific allergic T and B lymphocyte subtypes, are just some of the ways nicotine can influence humoral and cellular immunity (McAllister et al. 1994; Sopori et al. 1998; Pavia and Plummer 2020).

Pharmacotherapy, such as nicotine replacement treatment, bupropion, or varenicline, may help people quit smoking. However, these medications include drowsiness, dry mouth, and nausea as adverse effects (Jiloha 2014; Howes et al. 2022; Mendelson 2022). According to many studies (Kitikannakorn et al. 2013; Dwivedi and Chopra 2015; Puttarak et al. 2018), herbal medicine may be a viable choice for smoking cessation therapy since it is more readily available with fewer adverse effects.

Ginger, commonly known as *Zingiber officinale*, is often used as a food or beverage component and herbal remedy. Ginger is well-known for its distinctive and essential medicinal properties, including its ability to be chemoprotective and have anticancer, antioxidant, anticoagulant, antibacterial, antiemetic, and antipyretic. Mahassni and Bukhari's (2019) findings showed an increase in the average number of red blood cells and haemoglobin levels in smokers and an increase in IgM levels in non-smokers after consuming ginger water extract, so the antibody response or humoral immunity is more robust against infection. The active ingredients in ginger that are responsible for its pharmacological activity were discovered to be gingerol and shogaol. Gingerol and shogaol were found to be the active compounds of ginger responsible for their pharmacological actions. Of the eight ginger elements, shogaol and ginger enone-A showed the highest scores with strong and active site residue interactions, so that they could be the most appropriate choices (Nag and Banerjee 2021). Unbranched alkyl chains with lengths and weights varying between 300 and 500 Da are present in each homologous group of ginger. For instance, 4-, 6-, 8-, 10-, and 12-gingerol and 4-, 6-, 8-, 10-, and 12-shogaol are examples of homologues of the respective compounds (Peng et al. 2023). IgA is employed as a comparison molecule in molecular docking studies using observation parameters (Pratama 2016; Syahputra et al. 2020; Harahap et al. 2021).

Materials and methods

Molecular docking

Selected target proteins' 3D structures were retrieved from the RSCB PDB database at <https://www.rcsb.org>. In contrast, ChemSketch was used to generate the 3D structures of each ligand using data in the PubChem database

(<https://www.pubchem.ncbi.nlm.nih.gov>). Additionally, the protein was created using the Discovery Studio 2019 program by eliminating water molecules, and Pyrx v.0.9.8 was used to minimize the energy of the ligands. Autodock Vina, included in Pyrx v.09.8, was used for docking (Trott and Olson 2010). The targeted docking approach and a parameter exhaustion limit of 50 were used. Using PrankWeb (<https://prankweb.cz/>) (Dávid et al. 2022), the size of the grid box was modified to the location of the amino acid residues (Table 1) based on the expected locations of the binding sites. The docking outcomes are acquired as binding affinity or affinity energy due to the compound's interaction with the protein. Furthermore, the BioVia Discovery Studio 2019 program was used to visualize the interactions between the substances and the docked proteins.

Table 1. Web Prank Prediction.

Control	PDB ID	Probabilit y	Center		Center-Dimension			Dimension
			x	y	z	x	y	
IgA	6LX3	0.007	79.9157	110.0593	108.9573	16	20	20
	6UE7	0.012	182.302	238.868	233.236	25	25	22
	6UEA	0.642	230.2669	158.9447	258.4982	30	30	30

Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) method

MMPBSA.py version 16.0 is used to compute free energy (Valdés-Tresanco et al. 2021). At a temperature of 310.15 oK, 500 total frames were examined.

Molecular Dynamics (MD) simulation method human secretory immunoglobulin a with some test compounds

Protein and ligand preparation used GROMACS 2019 (Abraham et al. 2015), consisting of topological protein preparations with pdb2gmx. The force field for proteins uses AMBER99SB (Lindorff-Larsen et al. 2010). For cold ligand topologies, acpype is used. Furthermore, combining protein and ligand topology, solvation, addition of ions, equilibration, minimization and production of MD were carried out. MD production was carried out for 50,000 ps (50 ns). Using the qtGrace program, the MD interpretation is shown as a root mean square deviation (RMSD) graph for the backbone, a root mean square fluctuation (RMSF) graph for C-alpha, and a solvent-accessible surface area (SASA) graph for protein.

Results and discussion

IgA receptors

Redocking cannot be done because the three 3D structures of the IgA receptor protein do not have native ligands/NL, so the binding site is predicted through Prank Web. The findings of molecular docking on all

samples of herbal substances revealed that practically all bioactive chemicals, particularly proteins with PDB IDs 6UE7 and 6UEA, had lower energy binding values than IgA. Only a tiny subset of ginger's bioactive chemicals, Table 2, which interact with the 6UEA protein, have energy binding values less than -7.05 kcal/mol (Trott and Olson 2010). Additionally, the top six derivative compounds— 8-Shogaol, 8-Gingerol, 6-Shogaol, 6-Gingerol, 5-Shogaol, and 4-Shogaol—from the group of 13 were chosen (based on the average binding affinity/BA ranking) and proceeded for additional investigation together with positive controls.

Table 2. IgA receptor target protein binding affinities to drug and control ligands.

Ligand	6LX3		6UE7		6UEA		Mean ranking
	Ranking	BA (Kkal/mol)	Ranking	BA (Kkal/mol)	Ranking	BA (Kkal/mol)	
IgA	13	-4.6	15	-4.5	15	-6.2	14.33
Bupropion	11	-5	7	-5.2	13	-6.4	10.33
8-Shogaol	10	-5.1	6	-5.3	2	-7.2	6.00
8-Gingerol	2	-5.3	11	-5	6	-7	6.33
6-Shogaol	5	-5.2	3	-5.7	1	-7.2	3.00
6-Gingerol	1	-5.4	9	-5.2	4	-7.1	4.67
5-Shogaol	8	-5.1	2	-5.7	3	-7.1	4.33
4-Shogaol	4	-5.2	1	-5.7	10	-6.7	5.00

Furthermore, Table 3 shows the binding site residues formed between the IgA receptor protein and the native ligand (NL), samples and controls. Hydrogen and hydrophobic bonds are the most dominant based on the type of bonds produced. The sample and control compounds retain the amino acid residue from the active site predicted by Prank Web.

The 3D visualization of the corresponding complexes of the IgA receptor protein with compound and control ligands is shown in Fig. 1 as the results. Because the grid box has been modified to the control redocking, which results in an RMSD < 2 , the binding site and each ligand are identical to the control (Trott and Olson 2010).

The herbal component ginger has a more significant interaction potential than the control but is not as excellent as the native ligand, according to molecular docking studies. It is projected that 8Gingerol, 8-Shogaol, 6-Shogaol, 6-Gingerol, 5-Shogaol, and 4-Shogaol may target IgA receptor proteins.

Results Of MMPBSA human secretory Immunoglobulin A

The free energy value of bupropion is more harmful, according to the mmPBSA findings of a molecular dynamics simulation between human secretory immunoglobulin A and Gingerol and bupropion. Human secretory immunoglobulin A and bupropion have a free energy difference of -3.42 kcal/mol. The Bupropion chemical interacts more favorably with human secretory immunoglobulin A, as shown by free energy calculations using mmPBSA. The results match those of the molecular dynamics simulation.

Table 3. Interactions of amino acid residues generated in IgA receptor proteins with compounds.

Ligand	6LX3	6UE7	6UEA
IgA	A:PHE279	F:TRP281 F:ALA289	E:SER306, E:CYS323, E:ALA325, E:THR282, E:PRO283, E:CYS266
Bupropion	A:VAL304, A:PHE279, A:THR278, A:LEU268, A:ALA325	F:PHE279, F:TRP281, F:ALA289	E:VAL304, E:LEU268, E:LEU271, E:ALA277, E:LEU246, E:CYS266, E:ALA325, E:PHE279, E:HIS327
8-Shogaol	A:THR278, A:VAL304, A:PHE279, A:TYR302, A:ALA325	F:TRP281, F:PRO283, F:ALA289, F:PHE279	E:VAL304, E:ALA325, E:LEU271, E:ALA277, E:TRP281, E:THR280, E:SER306, E:HIS327, E:CYS266, E:LEU268
8-Gingerol	A:LEU268, A:ALA325, A:THR278, A:VAL304, A:PHE279	F:PHE279, F:PRO283, F:TRP281, F:LEU308, F:ALA289	E:THR280, E:SER306, E:LEU268, E:ALA326, E:HIS327, E:CYS266, E:CYS323, E:ALA325, E:LEU271, E:ALA277, E:LEU246, E:LEU248, E:VAL304
6-Shogaol	A:PHE279, A:TYR302, A:LEU268, A:VAL304	F:TRP281, F:PRO283, F:LEU308, F:ALA289, F:PHE279	E:VAL304, E:ALA325, E:CYS266, E:CYS323, E:LEU268, E:LEU271, E:ALA277, E:HIS327
6-Gingerol	A:LEU268, A:THR278, A:VAL304, A:TYR302	F:PHE279, F:PRO283, F:TRP281, F:LEU308, F:ALA289	E:THR280, E:HIS327, E:THR324, E:LEU268, E:LEU271, E:LEU246, E:LEU248, E:CYS266, E:CYS323, E:ALA277, E:VAL304
5-Shogaol	A:TYR302, A:VAL304, A:PHE279	F:PRO283, F:LEU308, F:ALA289, F:TRP281	E:VAL304, E:ALA325, E:TRP281, E:HIS327, E:SER306, E:LEU268, E:LEU271, E:CYS266
4-Shogaol	A:TYR302, A:VAL304, A:PHE279	F:TRP281, F:PRO283, F:LEU308, F:ALA289, F:PHE279	E:VAL304, E:THR280, E:ALA325

Results of MD simulation of human secretory immunoglobulin a with some test compounds Root Mean Square Deviation (RMSD)

RMSD is a crucial indication for assessing the structural stability of a protein since it quantifies the typical departure of a protein structure from its initial shape at a particular period. To determine the stability of human secretory immunoglobulin A in its interactions with the test substances gingerol and bupropion, a molecular dynamics simulation has been run for 50,000 ps. The Native protein (human secretory immunoglobulin A) has an RMSD of around 0.8 nm, according to MD findings. Human secretory immunoglobulin A's interaction with the Bupropion test substance slightly reduced the RMSD value, which was 0.7 nm. On interaction with Gingerol, it slightly increased by 0.9 nm (Fig. 2). The interaction with Bupropion reaches equilibrium at about 15 ns. Thus, human secretory immunoglobulin A may have a stable interaction with Bupropion.

Root Mean Square Fluctuation (RMSF)

We also examined immunoglobulin A's adaptability and interactions with the test substances (Fig. 3). RMSF stands for root mean square displacement and measures how each amino acid residue in a protein differs from the typ-

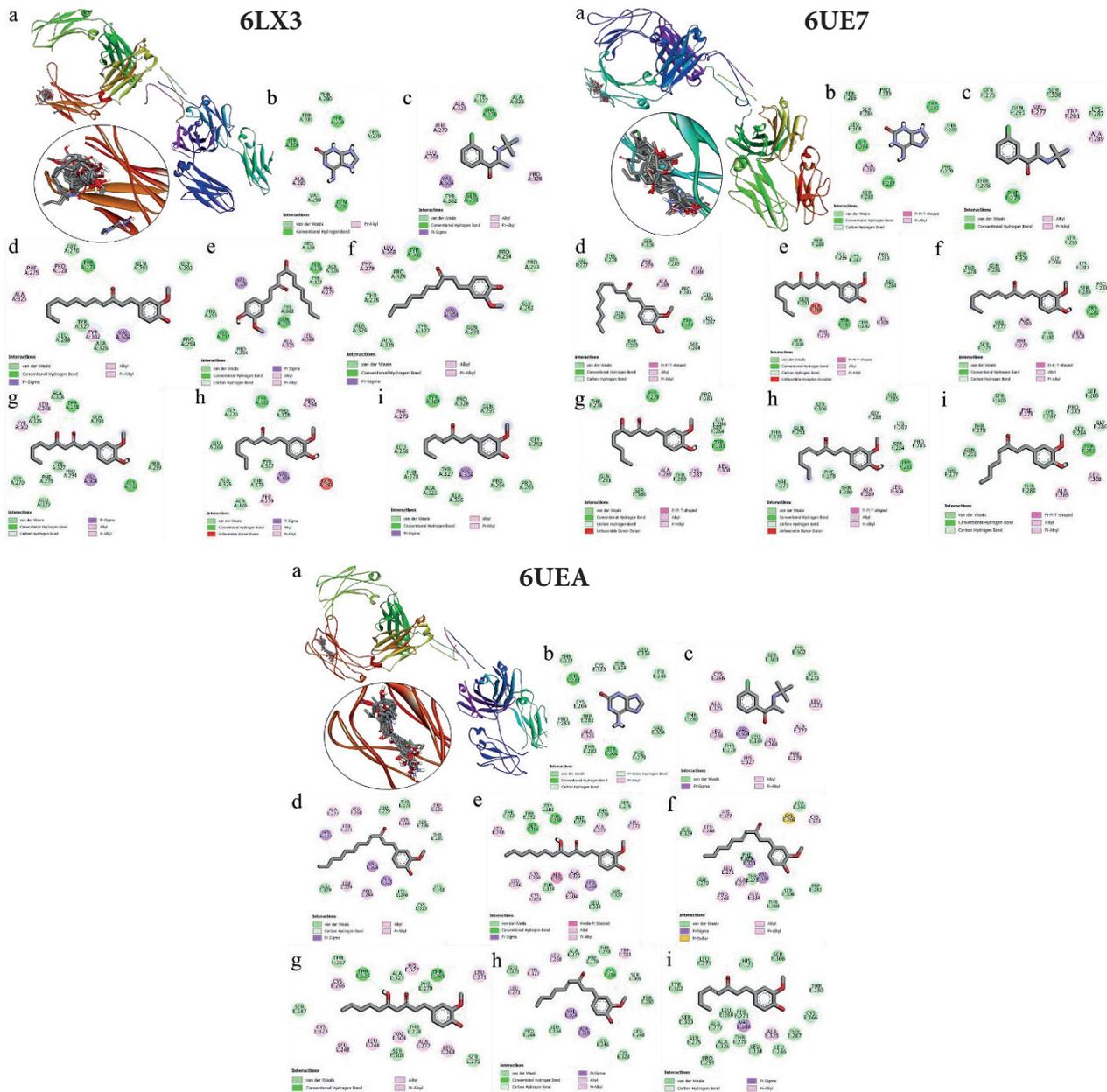


Figure 1. Visualization of docking results, **a** Ligand bond position; **b** IgA; **c** Bupropion; **d** 8Shogaol; **e** 8-Gingerol; **f** 6-Shogaol; **g** 6-Gingerol; **h** 5-Shogaol, and **i** 4-Shogaol.

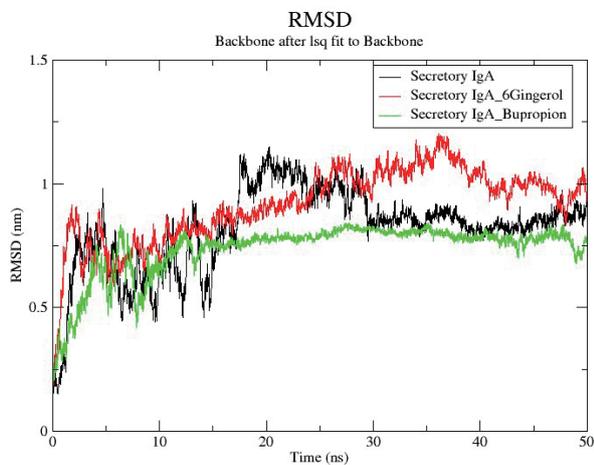


Figure 2. RMSD of human secretory immunoglobulin A.

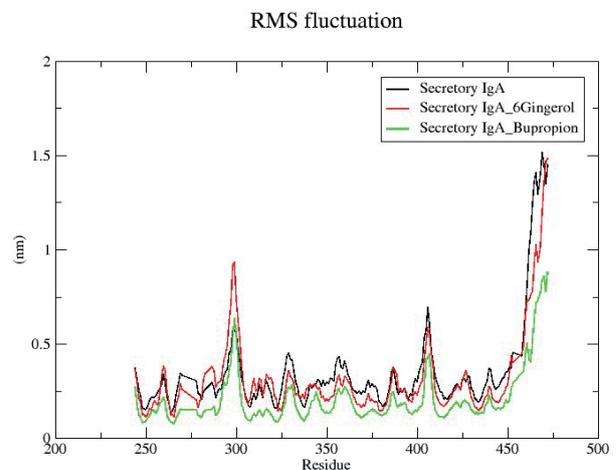


Figure 3. RMSF of human secretory immunoglobulin A.

ical conformation. According to Huang et al. 2021, the flexibility of the residue during pressure treatment increases with residual variation. The graph of RMSF human secretory immunoglobulin A when interacting with the Gingerol test compound is similar to RMSF human secretory immunoglobulin A without a ligand. Meanwhile, the RMSF value of human secretory immunoglobulin A when interacting with Bupropion decreased. This RMSF value is consistent with the RMSD value.

Solvent-Accessible Surface Area (SASA)

The protein surface area that solvents may reach is determined by SASA analysis. As SASA values rise, relative growth may be seen (Krebs and De Mesquita 2016). The SASA value for human secretory immunoglobulin A without ligand molecules was 138.15 nm². In contrast, while interacting with the test substances Gingerol and Bupropion, the SASA values of human secretory immunoglobulin A underwent a modest drop, measuring 135.06 nm² and 134.67 nm², respectively (Fig. 4).

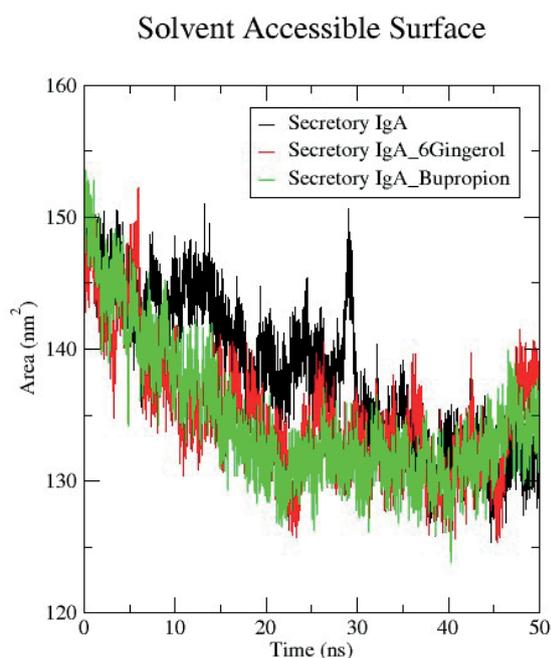


Figure 4. Solvent-Accessible Surface Area (SASA) human secretory immunoglobulin A.

Based on MD results for 50 ns, the Buprion compound has RMSD and RMSF values which are more stable compared to human secretory immunoglobulin A without the

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Table 4. Results of mmPBSA human secretory immunoglobulin A calculations.

Energy Component	Average (kcal/mol)	
	Gingerol	Bupropion
ΔBOND	0	0
ΔANGLE	0	0
ΔDIHED	0	0
ΔVDWAALS	-12.71	-14.96
ΔEEL	-6.31	-71.54
Δ1-4 VDW	0	0
Δ1-4 EEL	0	0
ΔEPB	11.99	75.05
ΔENPOLAR	-10.74	-11.9
ΔEDISPER	17.16	19.93
ΔGGAS	-19.02	-86.5
ΔGSOLV	18.41	83.08
ΔTOTAL	-0.61	-3.42

ligand. Thus, Bupropion may have the ability to bind to human secretory immunoglobulin A stably. To validate these *in silico* results, it is necessary to carry out *in vitro* and *in vivo* validation tests.

Conclusion

Molecular docking findings on all herbal material samples show that almost all bioactive compounds have lower binding energies than immunoglobulin A, especially for proteins with PDB IDs 6UE7 and 6UEA. However, only a few ginger-derived bioactive compounds interacting with the 6UEA protein show binding energy values smaller than -7 ± 0.5 kcal/mol. Herbal compounds derived from ginger have better interaction potential than controls. 8-Gingerol, 8-Shogaol, 6-Shogaol, 6-Gingerol, 5-Shogaol, and 4Shogaol can potentially interact with the immunoglobulin A receptor protein. Gingerol and Bupropion have more stable RMSD and RMSF values when human secretory immunoglobulin A is compared without ligand. Bupropion may have the ability to bind human secretory immunoglobulin A stably. However, Gingerol can bind to human secretory immunoglobulin A. *In vitro* and *in vivo* tests need to be carried out to validate the *in silico* results.

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