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Screening of *Dittrichia viscosa* (L.) Greuter metabolites as potential natural biocides for cultural heritage applications

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Biodeterioration driven by phototrophic microorganisms represents a persistent challenge in cultural heritage conservation. In this study, metabolites from *Dittrichia viscosa* (L.) Greuter was extracted, purified, and evaluated for their biocidal potential using *Raphidocelis subcapitata* as a standardized model microalga commonly employed in ecotoxicological screening. Four sesquiterpenoids—tomentosin, 11α,13-dihydrotomentosin, inuviscolide, and α-costic acid—were isolated and structurally characterized by NMR and GC–MS. Among them, tomentosin and inuviscolide exhibited significant growth-inhibitory effects, with tomentosin showing a clear dose-dependent response (IC_{50} ≈ 5–6 mg L^{-1}). Although the test organism is not a typical stone colonizer, these results provide a preliminary assessment of the phytotoxic activity of *D. viscosa* metabolites and support their further evaluation against biodeteriogenic microorganisms and heritage-relevant substrates.

Biodeterioration is one of the major threats to the long-term preservation of cultural heritage materials, particularly stone, architectural surfaces, wall paintings, and outdoor monuments. These substrates are frequently colonized by complex microbial communities—including bacteria, fungi, algae, and cyanobacteria—that form structured biofilms embedded in extracellular polymeric substances (EPS). Once established, these biofilms can induce chemical alteration, mechanical stress, pigment formation, and aesthetic degradation, ultimately compromising both the integrity and legibility of cultural artifacts[1,2].

Traditional strategies for controlling biodeteriogenic microorganisms include physical treatments (e.g., UV irradiation, microwave exposure) and chemical biocides, such as quaternary ammonium compounds and other synthetic formulations[3–5]. While often effective, these approaches present several limitations, including restricted applicability *in situ*, potential toxicity to operators and the environment, and the risk of promoting microbial resistance. These concerns have stimulated growing interest in alternative, more sustainable approaches based on naturally derived antimicrobial compounds[6].

Higher plants constitute a rich source of bioactive secondary metabolites evolved for chemical defense, allelopathy, and protection against microbial attack. In recent years, plant-derived compounds—particularly terpenoids, phenolics, and essential oil constituents—have been investigated as potential "green biocides" in cultural heritage research[6]. Their appeal lies in their renewable origin, chemical diversity, and often favorable toxicological profiles. However, before any application on heritage materials can be envisaged, systematic biological screening and careful evaluation of selectivity are required.

Dittrichia viscosa (L.) Greuter (*Asteraceae*), a perennial Mediterranean species commonly regarded as a weed, has attracted attention due to its rich phytochemical profile and reported antimicrobial, antifungal, and allelopathic activities[7]. Previous studies have identified sesquiterpene lactones, flavonoids, and phenolic acids in *D. viscosa*, some of which exhibit inhibitory effects against microorganisms and phototrophic species[7–11]. Despite this growing body of phytochemical knowledge, the biological activity of individual *D. viscosa* metabolites remains insufficiently explored in a conservation-oriented context.

In the present study, we report the extraction, purification, and structural identification of four sesquiterpenoid metabolites from *D. viscosa* and assess their inhibitory activity using *Raphidocelis subcapitata* as a standardized model microalga. Although *R. subcapitata* is not a typical stone-colonizing species, it is widely used in ecotoxicological and phytotoxicity assays due to its high sensitivity, reproducibility, and well-characterized physiological responses (OECD Test Guideline 201)[12,13]. Its use here is intended as a first-tier screening tool to identify biologically active compounds prior to more targeted testing against biodeteriogenic microorganisms and heritage-relevant substrates.

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Accordingly, this work does not aim to demonstrate direct applicability to cultural heritage materials, but rather to provide a preliminary evaluation of the biocidal potential of *D. viscosa* metabolites, thereby laying the groundwork for future conservation-focused studies.

Methods

Plant material

Whole aerial parts of *Dittrichia viscosa* (L.) Greuter were collected in late spring–early summer from naturally infested flowerbeds at the *Complesso Universitario di Monte Sant'Angelo*, University of Naples Federico II (Naples, Italy), prior to flowering. Plant identification was performed by Prof. Antonino Pollio, and a voucher specimen was deposited in the departmental collection.

Extraction, purification, and identification of metabolites

Plant material (10 g) was extracted (1 × 200 mL) by H_2O/MeOH (1/1, *v*/*v*) under stirred conditions at room temperature for 24 h, and the suspension was filtered under vacuum. The supernatant was extracted by *n*-hexane (3 × 200 mL) and the resulting organic extract was dried under reduced pressure to yield brown oil (110.2 mg). The organic extract was further purified by column chromatography on silica gel (Kieselgel 60, 0.063–0.200 mm; Merck, Darmstadt, Germany) eluted with $CHCl_3$/*i*-PrOH (98/2, *v*/*v*), yielding seven groups of homogeneous fractions. The residue of the second fraction resulted to be a pure oily compound identified as tomentosin (58.2 mg). The residues of third and fourth fractions (13.1 mg) were combined and further purified by analytical TLC (Kieselgel 60, F_{254}, 0.25 mm; Merck, Darmstadt, Germany) using as eluent $CHCl_3$/*i*-PrOH (98/2, *v*/*v*), yielding two oily compounds identified as 11α,13-dihydrotomentosin and inuviscolide (6.2 mg and 1.1 mg, respectively). The residue (20.1 mg) of the fifth fraction was purified by preparative TLC (Kieselgel 60, F_{254}, 0.5 mm) using EtOAc/*n*-hexane (1/1, *v*/*v*), affording α -costic acid (18.3 mg) as a yellow oil. The spots were visualized on the TLC by exposure to UV radiation, or by spraying with 10% H_2SO_4 in MeOH followed by heating at 120 °C for 10 min. ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$ by Bruker spectrometers (Karlsruhe, Germany), and optical rotation was measured in $CHCl_3$ solution by a P-1010 digital polarimeter (Jasco, Tokyo, Japan) to identify the four isolated compounds.

GC-MS analysis

Analysis, characterization, and quantification of tomentosin in the extract was conducted using an Agilent GC 7890B coupled with a 5977A MS detector. Quantification was conducted by means of a calibration line constructed with tomentosin standards purified. Extract was solubilized in hexane, and 1 µl of solution was injected. Each analysis was conducted in triplicate.

The carrier gas used in all the methods was helium. A method involving a mass range of 30–600 Da, a solvent delay of 3 min, was used. The initial temperature is 50 °C, for about 2 min, then reaching a temperature of 230 °C with an increase of 20 °C/min, 240 °C, and 270 °C[14].

Biological *in vitro* assays

Biological assays were conducted in accordance with OECD Test Guideline 201 (Freshwater Algae and *Cyanobacteria*, Growth Inhibition Test)[12], employing the unicellular freshwater chlorophyte *Raphidocelis subcapitata* as the standard test organism, due to its well-characterized and reproducible stress response profile[13]. The assay was initiated using unialgal batch cultures of *R. subcapitata*, kindly supplied by the Algal Collection of the University of Naples Federico II (A.C.U.F., https://www.acuf.net/). Stock cultures were maintained in Bold's Basal Medium (BBM) under controlled conditions (24 ± 0.1 °C, 280 rpm orbital shaking, continuous light at 20 µmol photons m^{-2} s^{-1}) to ensure exponential-phase growth at the time of exposure.

Crude extract and pure constituents isolated from *Dittrichia viscosa* were solubilized in an aqueous 5% DMSO vehicle. For each tested compound or extract, a gradient of five to six concentrations in geometric series was prepared and inoculated. Six biological replicates were prepared for each of the tested concentrations, consisting of 920 µl of actively growing BBM algal culture; the inoculum was standardized to 80 µl. Control groups —including a sterile water inoculum, 5% Water/DMSO vehicle, and the positive control (3% Biotin T)—were likewise set up in six biological replicates. All exposures were conducted in 4 × 6 multiwell plates, incubated under the same culture conditions described above for 72 h. For each of the tested compounds/extracts, the stock algal culture was homogeneously selected with OD ranging from 0.1 to 0.25, measuring cell density daily for three days before the test to ascertain the start of exponential growth.

At the end of the exposure period, key physiological parameters were recorded: cell density and some of the spectrometric peaks connected to cells' pigment content (chlorophyll *a*, chlorophyll *b*, and total carotenoids) were obtained spectrophotometrically using a Victor plate reader (PerkinElmer, Inc., Shelton, CT, USA) at 750 nm, 450 nm, 490 nm, and 531 nm respectively, following 30 s of agitation. Fluorescence measurements of chlorophyll *a*, at 450 and 485 nm, were additionally recorded using the spectrofluorometric function of the instrument. All measurements were performed in technical triplicate.

Statistical analyses

The half-maximal inhibitory concentration (IC_{50}) of each tested compound on algal growth was determined from optical density data using a four-parameter logistic (4PL) regression model. Mean values and standard deviations from six biological replicates (each measured in triplicate) were imported and merged in RStudio (v.2024.12.0, R 4.4.1) using the tidyverse and minpack.lm packages. Untreated suspensions (Negative Control) served as baseline for normalization, computed as:

$$I = 1 - \frac{A_T}{A_C} \tag{1}$$

where At and Ac denote the mean optical densities of treated and control samples, respectively. Standard errors were propagated for a total of $n = 18$ replicates and combined by inverse-variance weighting to account for heteroscedasticity among concentrations.

Normalized inhibition data were fitted to a 4PL dose–response function:

$$y = bottom + \frac{(top - bottom)}{1 + (\frac{x}{IC_{50}})^{slope}} \tag{2}$$

using the Levenberg–Marquardt optimization (nlsLM()). Parameter bounds ($0 \le$ bottom ≤ 0.6; $0.7 \le$ top ≤ 1.0; $-10 \le$ slope ≤ -0.01) and concentration limits were applied to ensure stable convergence. Initial estimates were automatically inferred from the observed inhibition range, and model adequacy was verified by residual inspection and covariance analysis.

Two parameters were reported: the model-based IC_{50}_model, corresponding to the curve's inflection point, and the absolute IC_{50}_abs, interpolated at 50% inhibition when within range (via uniroot()). Deviations below 20% between the two estimates were considered within expected experimental variability. Dose–response curves were visualized with ggplot2, displaying observed inhibition values (±SEM), fitted 4PL lines, and reference markers for characteristic doses and IC_{50}. Numerical outputs included bottom, top, IC_{50}_model, IC_{50}_abs, and the p-value of the log-linear regression.

Results

Extraction of the plant material

The extraction of metabolites from the aerial part of *D. viscosa* was optimized, as reported in detail in the material and methods section. Briefly, the plant was macerated in a solution of H_2O/MeOH (1/1, *v*/*v*), and the filtrate was extracted with *n*-hexane. This latter extract was tested *in vitro* on *Raphidocelis subcapitata*.

Preliminary biological *in vitro* assay (*n*-hexane extract)

The exposure of *Raphidocelis subcapitata* cultures to crude *n*-hexane extract of *Dittrichia viscosa* elicited a heterogeneous toxicological response (Fig. 1). A statistically significant reduction in key physiological indicators (cell density, chlorophyll, carotenoids) was observed when compared to negative controls. However, no evident dose–response relationship was established, suggesting an early onset of maximal effect at low exposure levels. This plateau phenomenon may reflect either rapid saturation of toxic targets or antagonistic interference among bioactive and inactive constituents within the crude matrices.

Purification of the *n*-hexane extract and identification of secondary metabolites

Preliminary investigation of the bioactive extract obtained from *D. viscosa* by GC-MS and ^{1}H NMR highlighted the presence of two main metabolites. In particular, the GC-MS chromatogram (Fig. S1) showed two main picks at 22 and 26 min while the ^{1}H NMR spectrum (Fig. S2) exhibited characteristic chemical shifts and coupling patterns consistent with the presence of sesquiterpenoids[15].

Thus, the extract was purified as reported in detail in the material and methods section, obtaining these two main metabolites, which were identified as tomentosin and α-costic acid (Fig. 1) by the comparison of their spectroscopic (^{1}H NMR) and optical methods with those reported in literature[16–18].

In particular, the ^{1}H NMR of tomentosin (Fig. S3) showed three olefinic protons, two of them typical of an exocyclic methylene, a proton on an oxygenated carbon, an acetyl group, a methyl, and methylene and methine protons. The ^{1}H NMR of α-costic acid (Fig. S4) showed three olefinic protons, two methyl groups, methylene, and methine protons. The identification was supported by the GC-MS spectra of tomentosin (Fig. S5) and α-costic acid (Fig. S6) and their fragments reported in Tables S1 and S2[19]. The value of the specific optical rotations $[\alpha]_D^{25} + 25.4$ (c 0.2 in $CHCl_3$) for tomentosin[17] and α-costic acid $[\alpha]_D^{25} + 10.1$ (c 0.2 in $CHCl_3$)[18] confirmed the stereochemistry of the two compounds.

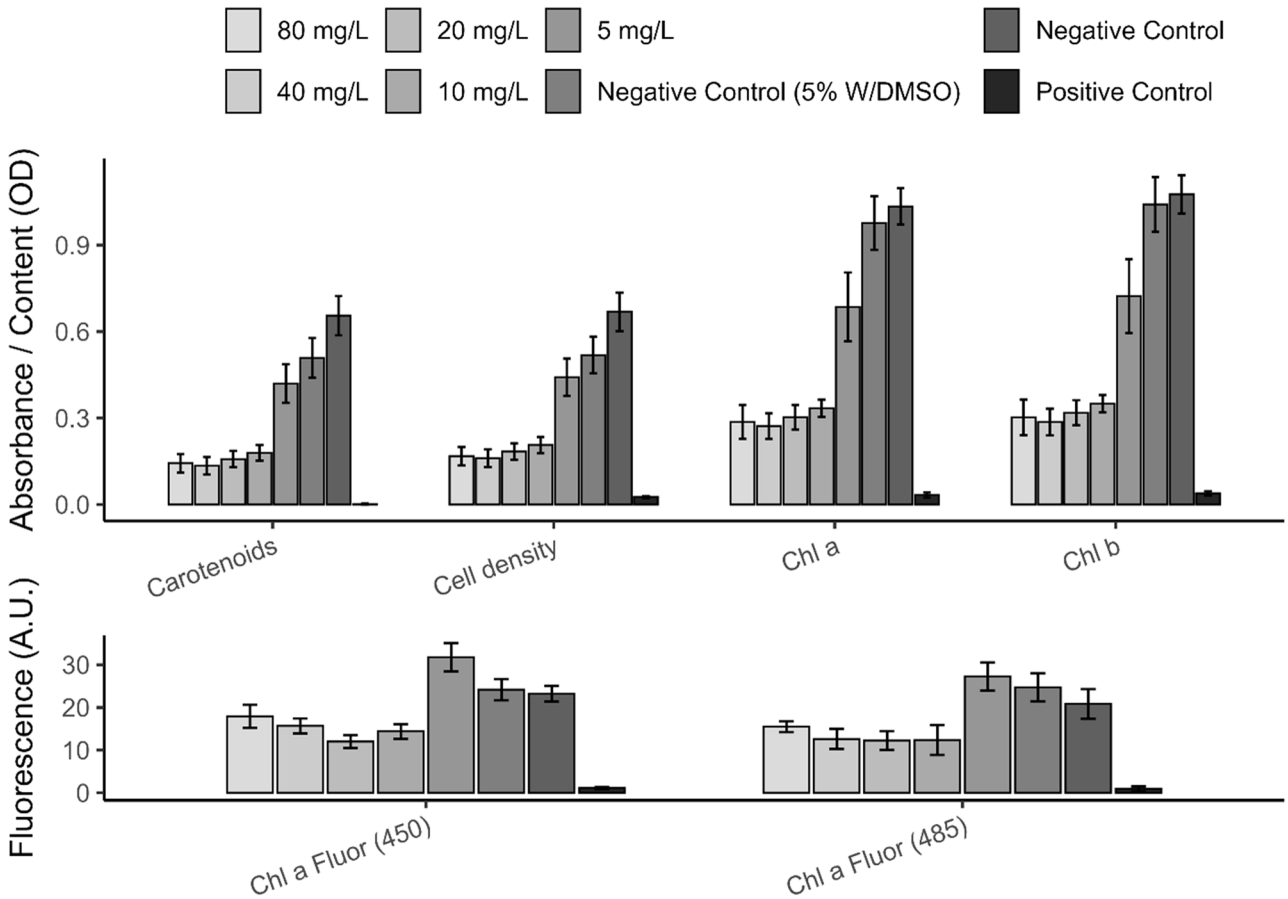

Fig. 1 | *n*-Hexane extract in vitro tests results. Measured ODs for the selected biological variables (above) and fluorescence of Chlorophyll *a* (below) for both treatment concentrations and controls. A saturation effect at higher concentrations is visible.

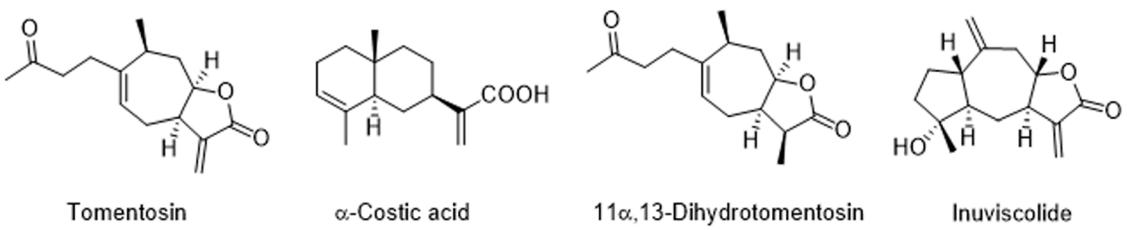

Fig. 2 | Chemical structures of the compounds isolated from the extract of *D. viscosa*.

Further purification of the extract allowed us to isolate other two minor metabolites, which were identified as 11α,13-dihydrotomentosin and inuviscolide (Fig. 2), comparing the spectroscopic (^{1}H NMR) and optical data with those reported in literature[17].

Biological in vitro assays (isolated compounds)

Among the isolated metabolites, α-costic acid and 11α,13-dihydrotomentosin exhibited limited or negligible inhibitory effects on algal growth (Figs. S7 and S8). In contrast, tomentosin and inuviscolide induced marked reductions in cell density and photosynthetic pigment content (Figs. S9 and S10). The activity assays were compared with those performed on other biocides[14].

Tomentosin displayed a steep and reproducible dose-dependent response, with near-complete inhibition observed at concentrations ≥10 mg L^{-1}. The fitted four-parameter logistic model yielded IC_{50} values in the low mg L^{-1} range, indicating high phytotoxic potency (Fig. 3). Inuviscolide showed a weaker but statistically significant inhibitory effect, characterized by a shallower dose–response curve and incomplete saturation within the tested concentration range (Fig. 4).

The observed effects are consistent with previous reports on sesquiterpene lactones, which are known to interfere with cellular redox balance, membrane integrity, and photosynthetic processes[20–22]. Although the precise mode of action was not investigated in this study, the consistency of the biological responses supports a genuine inhibit.

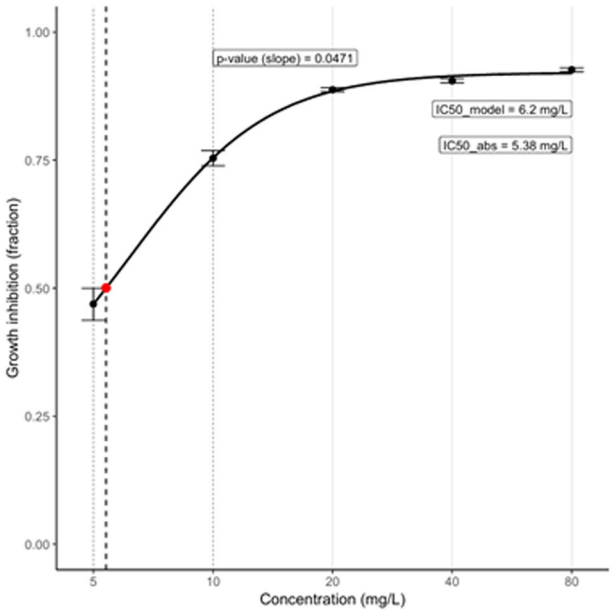

Fig. 3 | Tomentosin fitted four-parameters logistic (4PL) dose-response curve, with measured IC50 = 5.38 mg/L. The values shown at any given point represent growth inhibition, obtained by normalizing cell density OD values with the ones from negative control (relative growth).

Discussion

This study provides a preliminary evaluation of the biocidal potential of secondary metabolites isolated from *Dittrichia viscosa* (L.) Greuter using a standardized model phototrophic microorganism. Among the four identified sesquiterpenoids, tomentosin emerged as the most biologically active compound, displaying a clear and reproducible dose-dependent inhibition of algal growth at relatively low concentrations. Inuviscolide exhibited a weaker but significant inhibitory effect, while α-costic acid and 11α,13-dihydrotomentosin showed minimal activity under the tested conditions. Although the experimental model employed does not directly replicate biodeterioration processes affecting cultural heritage materials, the results highlight *D. viscosa* as a promising source of bioactive compounds worthy of further investigation. Future studies should address biodeteriogenic microorganisms, material compatibility, formulation strategies, and comparative efficacy against established biocides before any conservation-oriented application can be considered.

It is important to emphasize that *Raphidocelis subcapitata* was employed as a standardized screening organism and does not represent a typical biodeteriogenic species colonizing cultural heritage materials. Consequently, the present findings should be interpreted as an initial assessment of biological activity rather than direct evidence of efficacy against heritage-related biofilms.

No interactions with cultural heritage substrates were evaluated. This aspect represents an essential preliminary step for future research and is a prerequisite for any practical application in conservation contexts. The present study should therefore be regarded as a preparatory step aimed at identifying promising candidate compounds for further investigation.

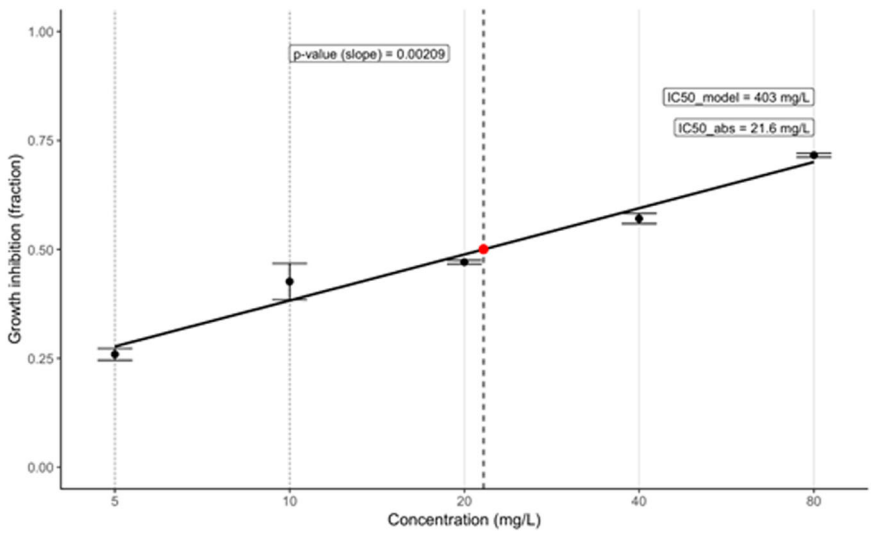

Fig. 4 | Inuviscolide fitted four-parameters logistic (4PL) dose-response curve, with IC50 = 21.6 mg/L. The values shown at any given point represent growth inhibition, obtained by normalizing cell density OD values with the ones from negative control (relative growth).

Data availability

Data are available upon reasonable request to the corresponding author.

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Author contributions

Conceptualization, A.Ca. and M.Ma.; supervision, A.Ca., M.Ma., A.Ci., A.P., and P.C.; extraction, M.Ma, A.Ci., G.M.S.; GC-MS analysis, M.Mo. and G.M.S; NMR analysis, M.Ma, A.Ci., G.M.S; biological assay, A.D.R., A.P., and P.C.; data curation, M.Mo., A.D.R., G.M.S., and M.Ma.; project administration, A.Ca.; writing—original draft preparation, A.Ca.; writing—review and editing, M.Mo., M.Ma., and A.D.R. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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