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# Boric Acid Combined with Venetoclax Induces Apoptosis in CLL Cell Lines via Caspase Activation and Oxidative Stress Modulation: In Vitro and In Silico Study

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# Abstract

Chronic lymphocytic leukemia (CLL) is a disease characterized by clonal proliferation of mature B lymphocytes in the blood, bone marrow, and lymph nodes. The need for new, less toxic treatment approaches is increasing as chemotherapy resistance develops. Due to the unique properties of elemental boron in drug development, the cytotoxic effects of boric acid (BA) on cancer continue to be investigated. This study examined the effects of BA on cell apoptosis, proliferation, and oxidative stress in MEC-1 and HG-3 chronic lymphocytic leukemia (CLL) cell lines, and the synergistic potential of combining BA with venetoclax. The effects of BA and venetoclax on cell viability were evaluated using the MTT method over 24–48 hours, and $IC_{50}$ values were calculated as 75 µg/mL for BA in HG-3 cells and 80 µg/mL for BA in MEC-1 cells. Apoptotic effects were assessed by measuring caspase-3 and caspase-8 activities using the ELISA method; significantly higher enzyme activity was observed in the combination groups compared to the control group ($p<0.05$). Total antioxidant status (TAS) levels were analyzed as part of the evaluation of oxidative stress parameters. In the in-silico phase of the study, molecular dynamics and molecular docking simulations were performed to investigate the interaction of boric acid with target molecules at the atomic level. Reduced density gradient (RDG) and molecular electrostatic potential (MEP) analyses were also conducted to characterize the electronic structure and interaction sites of the compound. The findings reveal that the combination of BA and venetoclax exhibits synergistic cytotoxic and pro-apoptotic effects in CLL cell lines, suggesting that this combination could be a promising therapeutic strategy in CLL treatment.


# INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a disease defined by clonal proliferation of mature B lymphocytes in the blood, bone marrow, and lymph nodes [1]. It is quite common in adults and is difficult to treat due to its variable clinical course [2]. HG-3 and MEC-1 cells are cell lines with uncontrolled proliferation in CLL. The MEC-1 line is a cell line with a p53 mutation and 17p deletion [1].

Venetoclax is a B-cell lymphoma-2 (BCL-2) inhibitor and is a widely used chemotherapy drug in the treatment of CLL due to its efficacy and being a potent agent in limited therapies. Tumor lysis syndrome, hematological toxicity, and resistance mechanisms that develop during treatment are undesirable side effects [3, 4]. Developing Venetoclax resistance is associated with microenvironmental anti-apoptotic signaling and metabolic progression. CD40-mediated stimulation, oxidative phosphorylation/mTOR axis, and the expression of alternative anti-apoptotic proteins increase drug resistance in CLL cells. Therefore, new combination therapies that can achieve strong responses at low doses are coming to the forefront [3, 5].

Boron, which does not exist in elemental form in nature, is found in borax, boric acid, and borate forms due to its high affinity for oxygen. The biologically usable form is boric acid [6]. Although epidemiological studies have shown that boric acid has potential cancer-preventive properties, its mechanism of action remains unclear [7, 8]. Boric acid regulates oxidative stress by suppressing proliferation in acute lymphoblastic leukemia cells. It has been shown to increase apoptosis by activating the caspase-3/-8/-9 axis with Bax [9, 10]. Therefore, caspase-8 and caspase-3, which play a key role in apoptosis, have the potential to be biomarkers in understanding the development of drug resistance [11].

In silico studies to identify potential agents targeting proteins minimize the need for laboratory work on molecular docking studies, hydrogen bonding, electrostatic, and hydrophobic components of ligand-protein interactions, thereby minimizing drug development costs and comprehensively revealing pharmaceutical research [12]. In this study, the aim was to evaluate the synergistic effect of the combination of boric acid with Venetoclax in HG-3 and MEC-1 CLL cell lines, to investigate the development of resistance, and to identify the therapeutic target of chronic lymphocytic leukemia by integrating the resulting apoptotic effect with computational analyses at the caspase-3 and caspase-8 levels in vitro, and to study the structure-activity relationships of boric acid in silico.

## METHODS

# HG-3 and MEC-1 Line Culture and Cell Proliferation

Chronic lymphocytic leukemia (CLL) HG-3 and MEC-1 cell lines were purchased from the American Type Culture Collection (ATCC, USA). HG-3 and MEC-1 cells were cultured in RPMI-1640 (Capricorn Scientific, Ebsdorfergrund, Germany) medium containing 10% fetal bovine serum (FBS, Sigma), 1 µg/mL penicillin/streptomycin (Sigma), and 2 mM L-glutamine in a humidified incubator with 5% CO2 at 37°C. The viability of HG-3 and MEC-1 cells was determined by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT test. HG-3 and MEC-1 cells were seeded into 96-well plates at a concentration of $5\times10^3$ cells per well. The medium was renewed after 24 hours. Cells were incubated with boric acid at concentrations of 100–1500 µg/mL and venetoclax at concentrations of 0.25-25 µg/mL for 24 and 48 hours. Untreated cells were used as control cells. After the specified time, the MTT mixture was added to each well, and the cells were incubated at 37°C for 4 hours. Following the incubation period, the formation of formazan crystals was determined spectrophotometrically at 570 nm using a microplate reader.

## Boric Acid (BA) and Venetoclax

BA solution was prepared with BA (ETIMADEN, Cas. No. 10043-35-3, MA: 61.83 g/mol) and serum-free medium at various concentrations. Venetoclax (Venclyxto, 10 mg, ABBVIE) was prepared with serum-free medium at various concentrations.

## Total Antioxidant Status (TAS) Test

TAS levels in MEC-1 and HG-3 cells were measured by the calorimetric method by modifying the Erel (2004) method in cell lysates. Antioxidants in cells decrease the dark blue-green 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical to the colorless decreased ABTS form. The TAS level of control cells, HG-3 and MEC-1 cells treated with BA and Venetoclax, was read spectrophotometrically using the ABTS radical prepared in acidic acetate buffer at 660 nm. The units of the values obtained from this test were expressed as mmol Ascorbic acid equivalent/L [13].

## Caspase-3 and Caspase-8 Measurement

After obtaining lysates from the groups applied to HG- and MEC-1 cells, they were analyzed using ELISA kits. Human Caspase-3 (Elabscience, cat no. E-EL-H0017) and Human Caspase-8 (Elabscience, cat no. E-EL-H0659) were measured three times in each cell lysate and standard. The analysis was performed after the reagents in the kit reached room temperature. The reagent preparation and analysis protocol was performed according to the steps specified by the kit. Optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm.2.5.

## Theoretical Calculations of the Chemical Structures of BA and Venetoclax

To better understand the molecular behavior of boric acid, a boron derivative, and Venetoclax, theoretical calculations were conducted at the B3LYP/6-311 + G(d,p) theory level using the ORCA 6.1.1 program. Reduced Density Gradient (RDG) analysis was conducted to investigate the weak interactions affecting the biological activity of BA and Venetoclax. To better understand the reactive behavior and interaction mechanism of BA and Venetoclax, charge distribution was evaluated using Molecular Electrostatic Potential (MEP) analysis. Reactivity descriptors were derived via Koopmans' theorem (I = − EHOMO, A = − ELUMO). NBO/NPA charges were obtained using Multiwfn 4.10 and VMD [14, 15].

# Molecular Docking Calculations

Autodock 4.0 was used for molecular docking, and the comprehensive binding affinities and interactions of the BA and Venetoclax compounds under investigation with the apoptosis-related proteins Caspase-3 and Caspase-8 were calculated. Molecular dynamics simulations (MD simulations) of the interactions of BA and Venetoclax agents with caspase-3 (PDB: 2H5I) and − 8 (PDB: 1QTN) were conducted using GROMACS 2023.5 with the CHARMM36m force domain and TIP3P water model. Each system was dissolved in a dodecahedral box with a 1.0 nm packing and neutralized with 0.15 M NaCl. After energy minimization (the steepest descent), systems were equilibrated under ensembles of NVT (100 ps, V-rescale, 300 K) and NPT (100 ps, C-rescale, 1 bar), followed by 100 ns generation runs. RMSD and RMSF analysis was conducted using GROMACS' built-in tools and visualized with Python/matplotlib.

# Statistical Analysis

$IC_{50}$ values in CLL cells were determined by dose–response inhibition analysis using GraphPad Prism 11. Results are expressed as the mean ± standard deviation of three independent experiments (*p < 0.05, ***p < 0.001). Statistical evaluation of the TAS data obtained in this study was conducted using ANOVA.

## RESULTS

# Viability of HG-3 and MEC-1 Cells with BA, Venetoclax, and Combined Use

The cytotoxic effect of different concentrations of BA (100, 200, 250, 500, 750, 1000, 1500 µg/mL) on HG-3 and MEC-1 cells was investigated by cell proliferation test. The cytotoxic effect of different concentrations of

Venetoclax (0.25, 0.5, 1, 2.5, 5, 10, and 25 µg/mL) on HG-3 and MEC-1 cells was investigated by cell proliferation test. A dose- and time-dependent decrease in cell viability was observed 24 and 48 hours after application of different concentrations of BA, Venetoclax, and combined use in a 1:1 ratio to HG-3 and MEC-1 cells (Fig. 1).

# TAS Levels in HG-3 and MEC-1 Cells

Our results show that TAS levels were decreased in HG-3 and MEC-1 cells treated with different doses of BA, Venetoclax, and combined use in comparison with the control group, and the decrease in the combined use group in both cell lines was detected to be statistically significant (Fig. 2).

# ELISA Results

The activity of Caspase-3 and Caspase-8 was increased in HG-3 and MEC-1 cells compared to the control group. For Caspase-3, the BA+Venetoclax group showed the highest activity in the HG-3 cell line compared to the control, and this was statistically significant ($p < 0.05$). For Caspase-8, the BA+Venetoclax group showed the highest activity in the MEC-1 cell line compared to the control, and this was statistically significant ($p < 0.05$). However, due to the more aggressive progression of MEC-1 cells, its activity was lower in comparison with HG-3 cells (Figure-3).

# Computational Biology Results

HOMO-LUMO energies are important in predicting the biological activity of BA and Venetoclax. Measurements prepared using the B3LYP theory showed that for Venetoclax, $E_{_HOMO}$ = − 6.48 eV and $E_{_LUMO}$ = − 3.10 eV, with a calculated $\Delta E_{_gap}$ of 3.38 eV. For BA, $E_{_HOMO}$ = − 10.14 eV and $E_{_LUMO}$ = + 0.58 eV, with a calculated $\Delta E_{_gap}$ of 10.72 eV. Compared to BA, Venetoclax is a softer molecule with high reactivity and high electrophilicity (ω = 6.80 eV) (Fig. 4). Venetoclax's demonstrated low-energy LUMO (− 3.10 eV) allows for electron recruitment from Bcl-2 nucleophilic residues, while BA's electrophilic B center complements the O/N nucleophilic regions. In combined use, they can induce synergistic apoptosis by affecting different binding pockets.

Reduced Density Gradient (RDG) analysis visualizes noncovalent interactions (NCI), including steric repulsion, hydrogen bonding, and van der Waals forces. For BA and Venetoclax, RDG values were calculated under vacuum conditions using the equation involving electron density (ρ) and gradient ($|\nabla\rho|$). The scattering relationship between RDG and the signs $(\lambda 2)\rho$ for BA and Venetoclax, as shown in Fig. 5, illustrates intramolecular noncovalent interactions. The second-order perturbation of BA LP(O)→σ*(B–O) $E^2$ = 12.4 kcal/mol stabilizes the plane structure, confirming the strong hyperconjugative donation from O pairs to the B–O antibonding orbital. The three dark blue disks centered along the B-O bonds ($\text{sign}(\lambda_2) \times \rho \approx -0.04$ a.u.) indicate strong covalent σ interactions, and the green disks at the O-H positions indicate moderately attractive contacts. The dashed red ellipse surrounding the boron atom shows steric compression arising from the trigonal planar $C_3h$ geometry. All three O atoms are symmetrical equivalent as they do not contain hydrogen bonds. In the RDG/NCI calculation of venetoclax, the blue NCI disks along the C–N, C–C, and S = O

bonds indicate strong σ-bonding, while the dashed red ellipses around the sulfonyl (S) and nitro ($NO_2$) groups indicate steric compression consistent with the rigid planarity provided by the benzamide backbone.

Molecular Electrostatic Potential (MEP) is particularly important in evaluating the strength of electrostatic forces (Fig. 6). Despite the zero dipole moment ($C_3H$) of B, current developments have led to strong polarization. The B center has the largest positive potential (+ 0.048 a.u.). Due to this structure, it is the primary Lewis acid site. The O atom exhibits a strong nucleophilic character (− 0.044 a.u.) through lone electron pair contributions. The H atoms act as hydrogen bond donors, occupying a moderately positive potential (+ 0.032 a.u.). This ambiphilic topology — electrophilic B + nucleophilic O — explains the dual Lewis acid/H bond acceptor behavior of boric acids in biological and pharmaceutical applications. In the MEP programming of Venetoclax, piperazine N atoms (ESP ≈ − 0.035 a.u.) and pyrrolo N (N–H) form the nucleophilic centers and BCL-2 binding sites. Sulfonyl S (ESP ≈ + 0.038 a.u.) and the nitro $NO_2$ group have the highest positive ESP and function as hydrogen bond acceptors. The chloro-cyclohexyl arm forms a hydrophobic pocket that interacts with the P2 hotspot of BCL-2 via nonpolar interactions.

To evaluate the structural stability and binding affinity characteristics of BA and Venetoclax molecules towards caspase-3 and caspase-8 proteins, molecular dynamics (MD) simulations were performed for unbound and ligand-bound caspase-3 and caspase-8 at 100 ns (Figure-7). RMSD analysis was used to measure the structural stability of unbound caspase-3 and caspase-8 complexes. It exhibited a similar stabilization trend during MD simulations. The root mean square deviation (RMSD) for caspase-3, determined as Apo, the reference point, was determined to be 0.28 ± 0.04 nm on average after stabilization (20–100 ns); for caspase-8, it was 0.30 ± 0.05 nm (Table-1). The obtained result shows that caspase-8 has greater loop flexibility compared to caspase-3. For BA-bound caspase-3, the average was 0.22 ± 0.03 nm; Caspase-8 was stabilized with an RMSD of 0.23 ± 0.03 nm. Venetoclax-bound caspase-3 was stabilized with an RMSD of 0.24 ± 0.03 nm, and caspase-8 with an RMSD of 0.25 ± 0.04 nm. The lowest average RMSD value was observed in the combined use. The BA+Venetoclax-bound group was stabilized at 0.19 ± 0.02 nm for caspase-3 and 0.20 ± 0.02 nm for caspase-8. The low combined use for both caspases demonstrates superior structural stabilization resulting from double-domain ligand binding and enhanced structural rigidity due to the simultaneous binding of the catalytic diode and hydrophobic subdomain pockets. All ligand-bound systems reached stable conformational plateaus within 15–20 ns and maintained equilibrium throughout the remainder of the simulation. Root mean square fluctuation (RMSF) was calculated for ligand-induced changes in the local flexibility of Cα atoms for each residue (Fig. 7). In the APO simulation, which was determined as a reference, three distinct high RMSF peaks were identified in caspase-3. These are the critical residues corresponding to the most structurally variable segments of caspase-3, supported by crystallographic data: the L1 activation cycle (residues 52–62, RMSF ≈ 0.22–0.28 nm), the L2 cycle (residues 200–214, RMSF ≈ 0.18–0.25 nm), and the C-terminal helix-3 region (residues 240–250, RMSF ≈ 0.14–0.19 nm). BA and Venetoclax ligands were found to be reduced in the flexible regions. In combined use, the RMSF value decreased by 42% compared to APO, and the catalytic residues His121 and Cys163 showed the stability of the catalytic pair in double site occupation with RMSF values of 0.038 and 0.045 nm, respectively. Active site residues Arg207, Trp206, and Tyr204, exhibiting π–σ and hydrophobic interactions, were found to have RMSF values below 0.06 nm with venetoclax. Compared to APO, these values are lower than 0.13–0.17 nm. For caspase-8, RMSF analysis revealed ligand-induced hardening similar to caspase-3. The L2 cycle,

incorporating catalytic Cys360 (residues 355–370), showed a ~ 38% RMSF reduction in the combination system (0.26 nm compared to 0.16 nm in the case of APO). The N-terminal DED domain (residues 1-110) and allosteric β-sheet regions (residues 226–236) were largely unaffected by ligand binding. The data suggest that combined use of BA and Venetoclax synergistically stiffens the active site architecture, and that this mechanism may enhance the apoptotic response in CLL cells.

Table 1
RMSD and RMSF values represent the mean ± standard deviation calculated over a 20–100 ns equilibrium correction trajectory. Active site RMSF values refer to the Cα atoms of the specified catalytic residues. L-ring RMSF values correspond to the average RMSF in the specified ring region.

| **Complex** | | **Mean RMSD (nm)** | **Active site RMSF (nm)** | **L-loop RMSF (nm)** |
|---|---|---|---|---|
| Caspase-3 | Apo | 0.28 ± 0.04 | 0.14 (His121/Cys163) | 0.26 (L2: 200–214) |
| | BA | 0.22 ± 0.03 | 0.05 (His121/Cys163) | 0.16 (L2: 200–214) |
| | Venetoclax | 0.24 ± 0.03 | 0.07 (Trp206/Arg207) | 0.19 (L2: 200–214) |
| | BA+ Venetoclax | 0.19 ± 0.02 | 0.04 (His121/Cys163) | 0.15 (L2: 200–214) |
| Caspase-8 | Apo | 0.30 ± 0.05 | 0.16 (His317/Cys360) | 0.26 (L2: 355–370) |
| | BA | 0.23 ± 0.03 | 0.06 (His317/Cys360) | 0.18 (L2: 355–370) |
| | Venetoclax | 0.25 ± 0.04 | 0.08 (Tyr412/Trp420) | 0.20 (L2: 355–370) |
| | BA+ Venetoclax | 0.20 ± 0.02 | 0.05 (His317/Cys360) | 0.16 (L2: 355–370) |

The molecular coupling of BA and Venetoclax with caspase-3 and caspase-8 is shown in Table 2. According to the results obtained for caspase-3, BA, with its electrophilic boron center, influences the catalytic pair (His121–Cys163) in a manner consistent with Lewis acid-base coordination, confirming the cysteine targeting mechanism. For Venetoclax, four basic bindings were achieved: hydrogen bonding (2.71 Å), π–σ interactions (3.68 Å), π–π T-shaped stacking (4.12 Å), and cation–π interactions (4.05 Å) (Fig. 8). In combined use, the synergistic effect of co-binding to the active site of caspase-3 was determined to be approximately 1.26 times that of individual binding due to complementary binding topologies. For caspase-8, boric acid, the highest-ranking binding position, showed five hydrogen bond interactions within the catalytic triplet region; These include direct contacts with the backbone NH of His317, Cys360, Arg258, Ser411, and the carboxylate oxygen of Asp374. Due to BA's Lewis acid coordination mechanism, its catalytic binding to caspase-3 and caspase-8 is structurally homologous. Venetoclax interacts with caspase-8 via the S3 subregion residues Tyr412, Trp420, Phe468, and Arg260. In combination therapy, it appears to be consistent with synergistic double-region inhibition similar to that of the caspase-3 system.

Table 2
Molecular docking results for boric acid and venetoclax with caspase-3 (PDB: 2H5I) and caspase-8 (PDB: 1QTN).

| Protein | Ligand | Binding affinity (kcal/mol) | RMSD (Å) | Inhibition constant ( nM) | H-bonds (n) | Key Interacting Residues | Interaction Types |
|---|---|---|---|---|---|---|---|
| Caspase-3 | BA | -4.80 | 1.85 | 52.3 | 3 | Ser29, Arg207, His121,Cys163, | H-bond, vdW |
| | Venetoclax | -6.20 | 0.95 | 0.27 | 4 | Arg207, Trp206, Tyr204, Phe256 | H-bond, π–σ, hydrophobic, cation–π |
| | BA+ Venetoclax | -11.2 | 1.35 | 0.012 | 5 | His121, Cys163, Arg207, Trp206, Ser120 | H-bond, π–σ, cation–π |
| Caspase-8 | BA | -7.15 | 1.57 | 114.8 | 5 | His317, Cys360, Arg258, Ser411, Asp374 | H-bond, vdW |
| | Venetoclax | -6.71 | 1.86 | 0.68 | 3 | Tyr412, Trp420, Phe468, Arg260 | H-bond, π–σ, alkyl, π–π |
| | BA+ Venetoclax | -10.6 | 2.42 | 0.21 | 6 | His317, Cys360, Arg258, Tyr412, Trp420, Asp374 | H-bond, π–σ, cation–π |

# DISCUSSION

In the heterogeneous disease of CLL, which arises as a result of cellular-level resistance to apoptosis and complex interactions exhibited by the tumor microenvironment, the cytotoxic effects and IC50 (Half of Maximum Inhibitory Concentration) values of venetoclax, an effective Bcl-2 inhibitor, and BA, which has modulatory effects on cellular metabolism, calcium homeostasis, and oxidative stress, were comparatively investigated on two different CLL in vitro models, HG-3 and MEC-1 cell lines. In this study, the combined use of BA and venetoclax in HG-3 and MEC-1 cell lines revealed the difference between the effects of a BH3-mimetic and a boron compound exhibiting more pleiotropic properties. Venetoclax, since it targets Bcl-2-dependent anti-apoptotic activity, shows antiproliferative activity at low concentrations; BA exhibits activity through a wide range of cellular stress pathways, including oxidative stress, endoplasmic reticulum stress, mitochondrial integrity loss, and cell cycle dysregulation. Therefore, a higher concentration requirement for BA is expected from a pharmacodynamic perspective and provides a fundamental framework for interpreting the IC50 distribution in our study [16, 17, 18]. Results obtained from the application of venetoclax to CLL cell lines show that the drug is a potent but binding-sensitive agent. The reported

venetoclax sensitivity for MEC-1 is approximately 4–10.7 μM depending on the method used; while in resistant cells, the value approaches 14 μM. In the study conducted by Zablonski et al. (2024) for HG-3, it was found that venetoclax alone showed limited antiproliferative activity, which became more pronounced in combination with a BET inhibitor [17, 19]. BA applied to CLL cells, when compared with literature, is uniquely valued because studies on HG-3 and MEC-1 cells are limited. Although BA applied in different tumor models in recent years has been reported to have antiproliferative and proapoptotic effects, the effective concentration range appears to be significantly dependent on the cell type. For example, IC50 values of 641.2 μM in HeLa cells, 40 mM in Ishikawa cells, and 35 μM in medullary thyroid cancer TT cells have been determined. The wide range obtained indicates that the effect of BA is not based on a single target, but rather on the cell's redox balance, protein folding load, and mitochondrial fragility [10, 18, 20]. While venetoclax is an established drug in CLL treatment, BA is more suitable for consideration as a biological modulator or a potential combination partner. BA’s effect on the endoplasmic reticulum stress, oxidative stress, and mitochondrial damage axes could theoretically lower the apoptotic threshold when used in combination with venetoclax [16, 17, 18].

BA is considered a compound that can act on both extrinsic and intrinsic apoptosis pathways. It has been shown by RT-qPCR analyses to increase caspase-3, caspase-8, and caspase-9 expression in leukemia cell models [9]. In this study, the venetoclax application in HG-3 and MEC-1 cells predominantly led to caspase-3 activation, consistent with the expected scenario and supporting the predominant activation of the intrinsic pathway. It was observed that BA significantly increased caspase-8 activity in both cell lines. This finding shows that BA can modulate not only the mitochondrial but also the death receptor-caspase-8 axis. The enhancement of caspase-8 activation, particularly in combination with BA applications, also contributes to the synergistic anticancer effect, partly due to the independent activation of the extrinsic apoptosis pathway [21].

It is known that reactive oxygen species (ROS) accumulating above the physiological threshold in cancer cells can lead to oxidative damage and cell death, thus over-activating the antioxidant defense systems of cancer cells [22]. In this context, the measurement of total antioxidant status (TAS) reveals both the disruption of cellular homeostasis and the intrinsic antioxidant capacity of the compounds [23]. In this study, the dose-dependent reduction of cellular TAS levels by BA is a noteworthy finding; this reduction indicates that BA depletes intracellular antioxidant capacity by triggering ROS production within the cell. According to the study by Çakır Gündoğdu et al. in an endometrial cancer model, it was reported that the total oxidant status (TOS) increased after BA application, while TAS decreased significantly; An increase in the oxidative stress index (OSI) indicates that the intracellular redox balance is disrupted in favor of apoptosis [20].

Reduced density gradient (RDG) analysis and molecular electrostatic potential (MEP) maps were quantum-chemical supporting tools that visualized the electrophilic and nucleophilic reaction regions of compounds in a color-coded manner, to visualize the nature of molecular interactions at the atomistic level [25]. Improvements made on benzimidazole pairs confirmed that electron-rich regions in MEP maps were individually amplified by BCL-2 linkages; the distribution between MEP analysis and docking score was highlighted [26]. BA's electrostatic profile supports mechanisms for both facilitating nucleophilic attack on proteins and provoking intracellular glutathione stripping and oxidative stress [27]. For Venetoclax, broader dispersive/hydrophobic contacts on RDG surfaces and more pronounced enhanced charge status in MEP

maps emerged; docking and stable linkages with MD can be interpreted as electronic switches. If a more polar MEP profile and more limited RDG interactions are seen in BA, it suggests that this molecule acts more like a microenvironment-sensitive modulator than a high-specificity single-target inhibitor. Thus, RDG and MEP interference, in vitro cytotoxicity, and the integrity of caspase/redox compartments are completed [28, 29]. Combined evaluation of in vitro and silica data provides a better view of the diversity in the modes of action of the two agents. The observation of strong binding and more stable complex functioning with venetoclax in docking and comprehensive results is an expected finding; the literature shows that venetoclax and similar BCL-2 targeting molecules stably dock by hydrophobic binding. BA, docking, and MD stability suggest a more indirect mode of action spreading to multiple stress nodes rather than biological inactivity [18, 29, 30].

In conclusion, the cytotoxic profiles of boric acid and venetoclax on HG-3 and MEC-1 CLL cell lines clearly reveal two different biological treatment mechanisms. Venetoclax, a potent targeted agent, exhibited superior cytotoxicity in both cell lines with extremely low IC50 values for nanomolar compounds, as expected; however, it required a higher dose compared to HG-3 in the treatment of clinically aggressive MEC-1 with TP53 properties. Simultaneous reading of caspase-3/caspase-8, TAS, and docking–MD–RDG–MEP data suggests that venetoclax is a stronger short-term translational candidate, while BA could be a complementary molecule worth investigating in terms of its specific methods, sensitization techniques, and redox total payment mechanism. The ability of BA to be preserved, even in a tumor model with very high treatment resistance, such as MEC-1, and its ability to induce apoptosis through metabolic/oxidative stress are particularly important. Current literature data suggest that rather than using BA alone as a primary therapeutic agent, a safe and potentially synergistic combination that can suppress venetoclax-resistant resistance mechanisms (especially MCL-1 upregulation) and significantly enhance the apoptotic process with venetoclax is preferable. Future in vivo models and much more detailed gene expression analyses will fully illuminate the true therapeutic potential of this dual approach that can be developed for refractory CLL cells in clinical practice.

## Declarations

# Ethics approval

This is a cell culture study. Ethical approval is not required.

**Conflict of Interest**

The authors declare no competing interests.

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# Author Contribution

DB and HU curation Software Conceptualization Writing original draft,DB Data curation Software Conceptualization Writing-review & editing, DB, Data curation Software Conceptualization Writing review & editing, DB and HU Writing review & editing Supervision. Allauthors have read and agreed to the published version of the manuscript

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# Figures

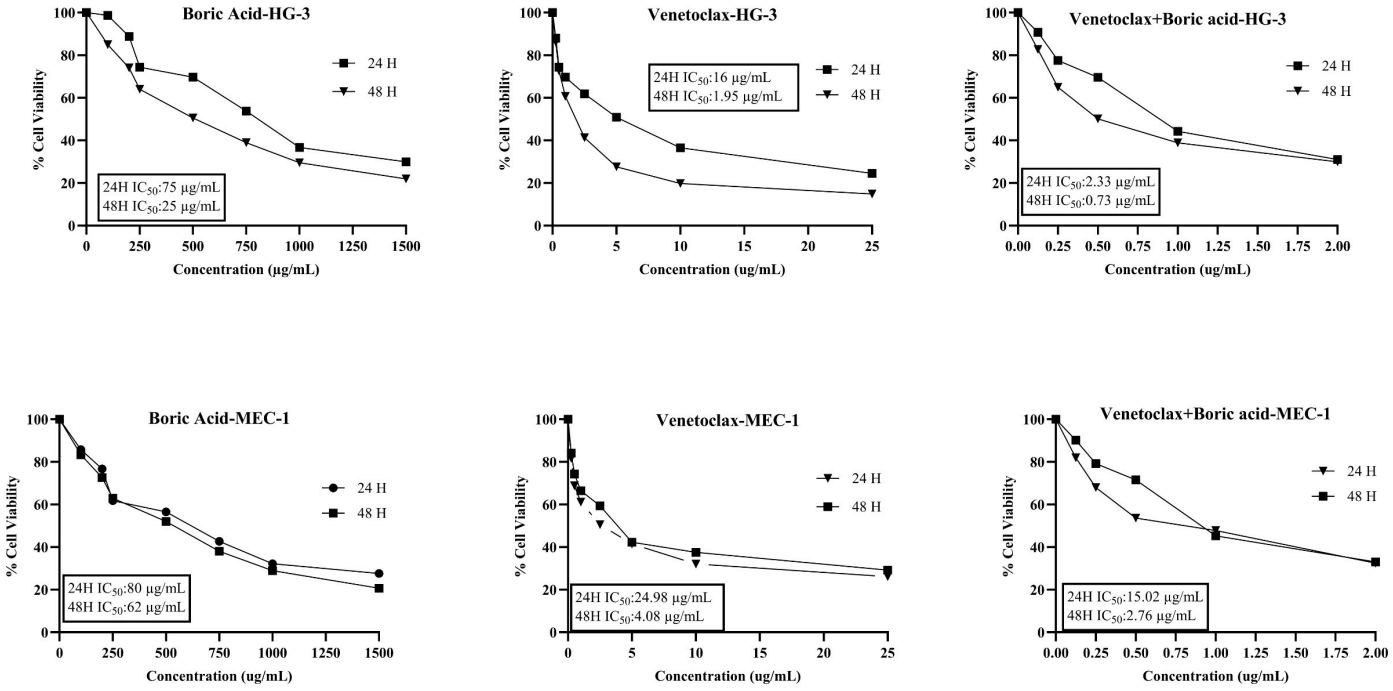



**Figure 1**

% viability of HG-3 and MEC-1 cells treated with BA and Venetoclax

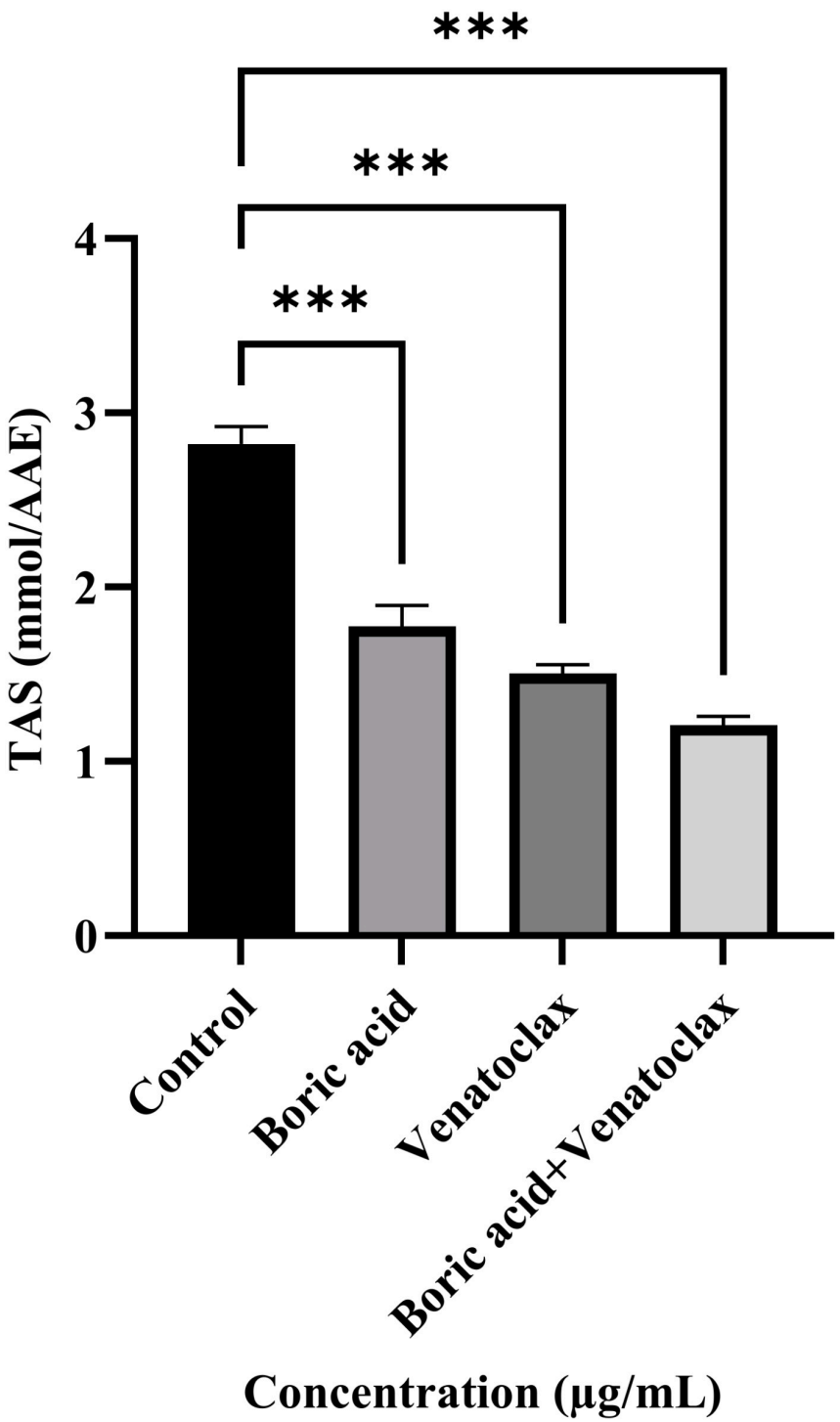



**Figure 2**

TAS were determined in different concentrations dose at 24 h (***p<0.01)

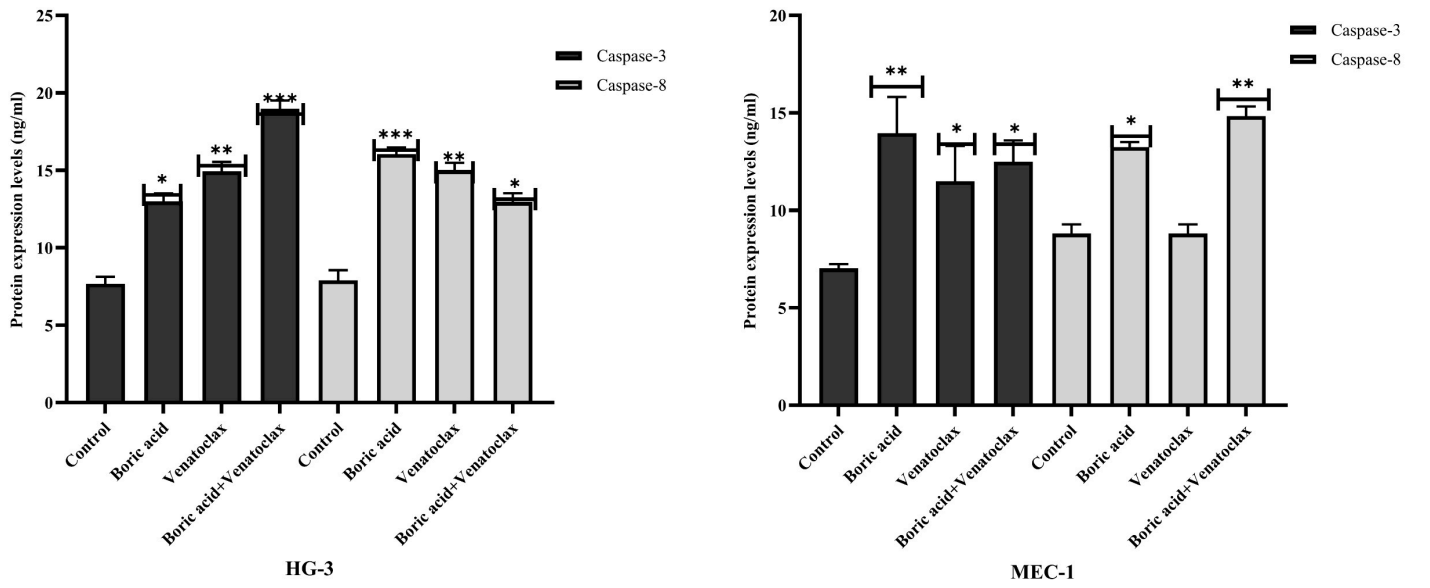



**Figure 3**

In vitro impact of BA and Venetoclax on Caspase-3, and 8 expressions in HG-3 and MEC-1 cells (*p<0.05, **p<0.01, **p<0.001)

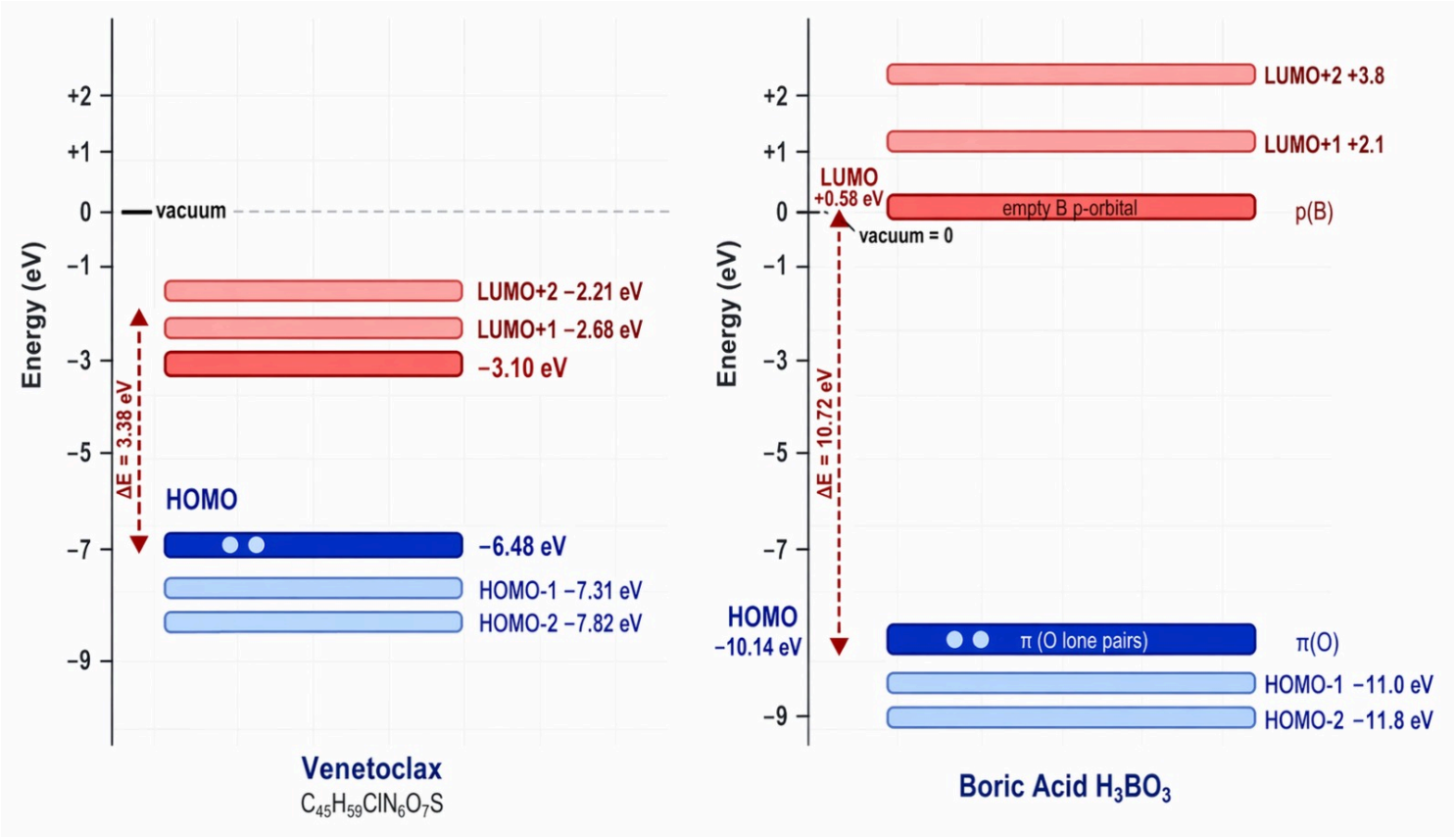



**Figure 4**

Homo-Lumo energies of Venetoclax and BA

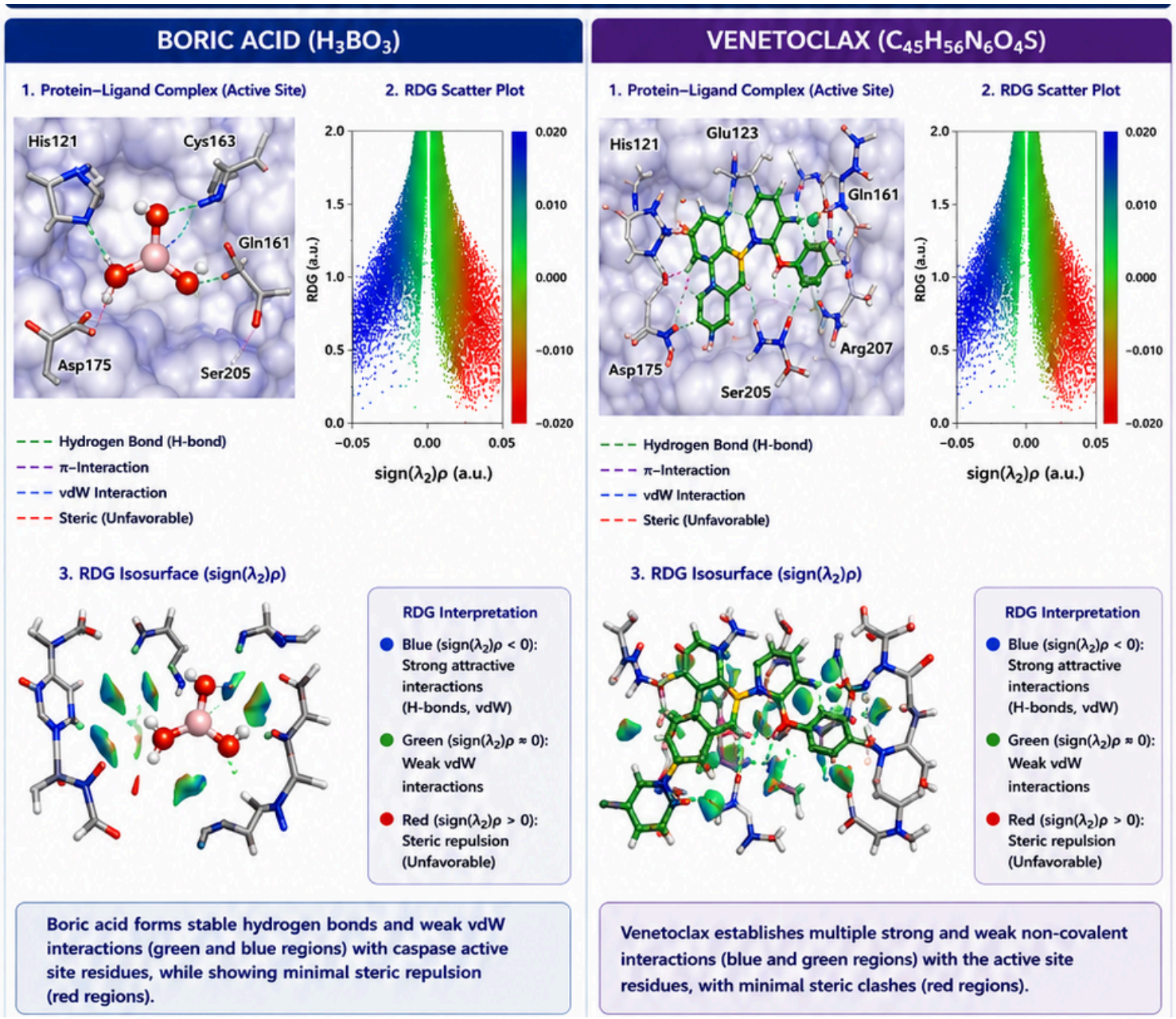



**Figure 5**

Reduced Density Gradient (RDG) analysis

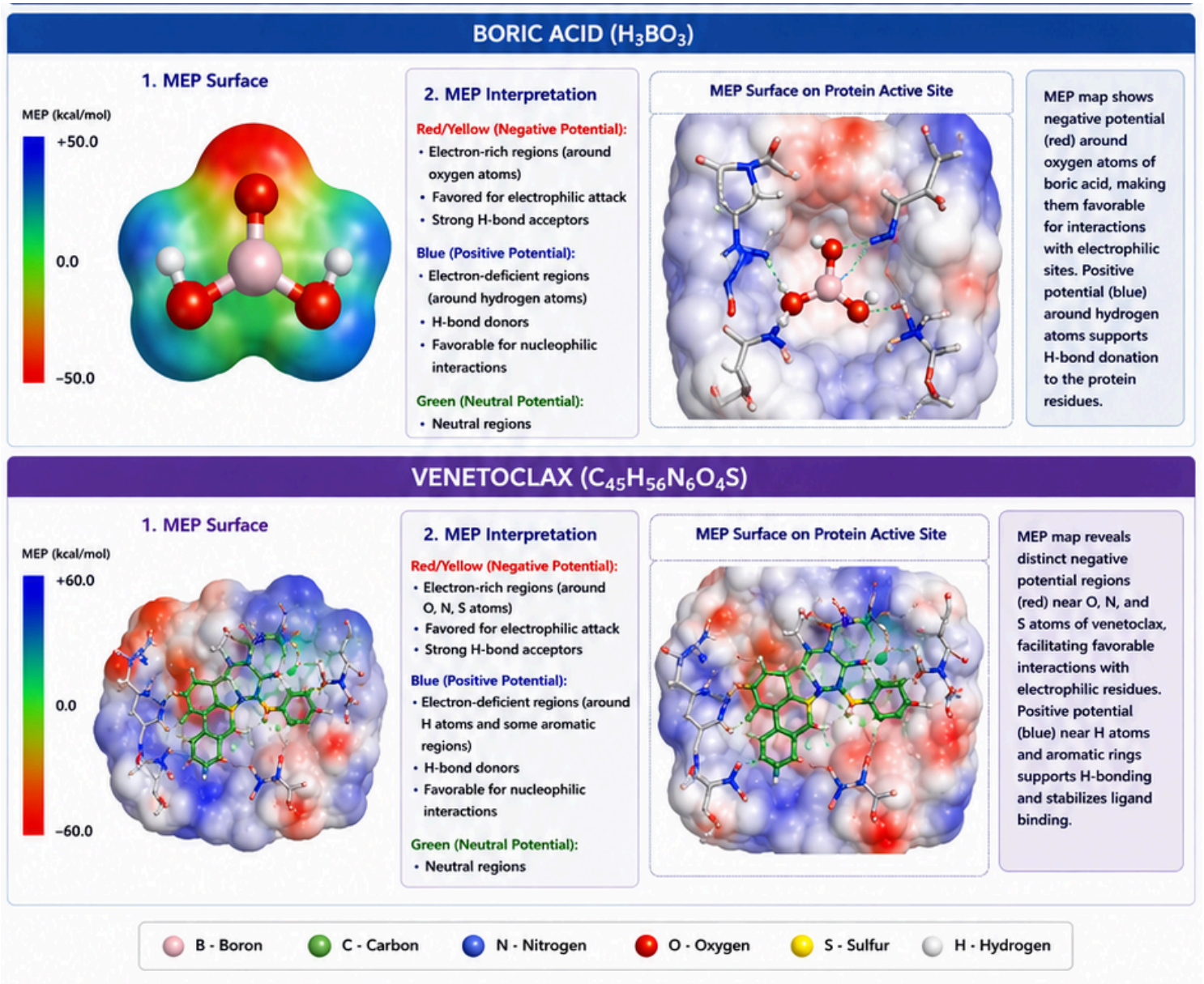



**Figure 6**

Molecular Electrostatic Potential (MEP) analysis

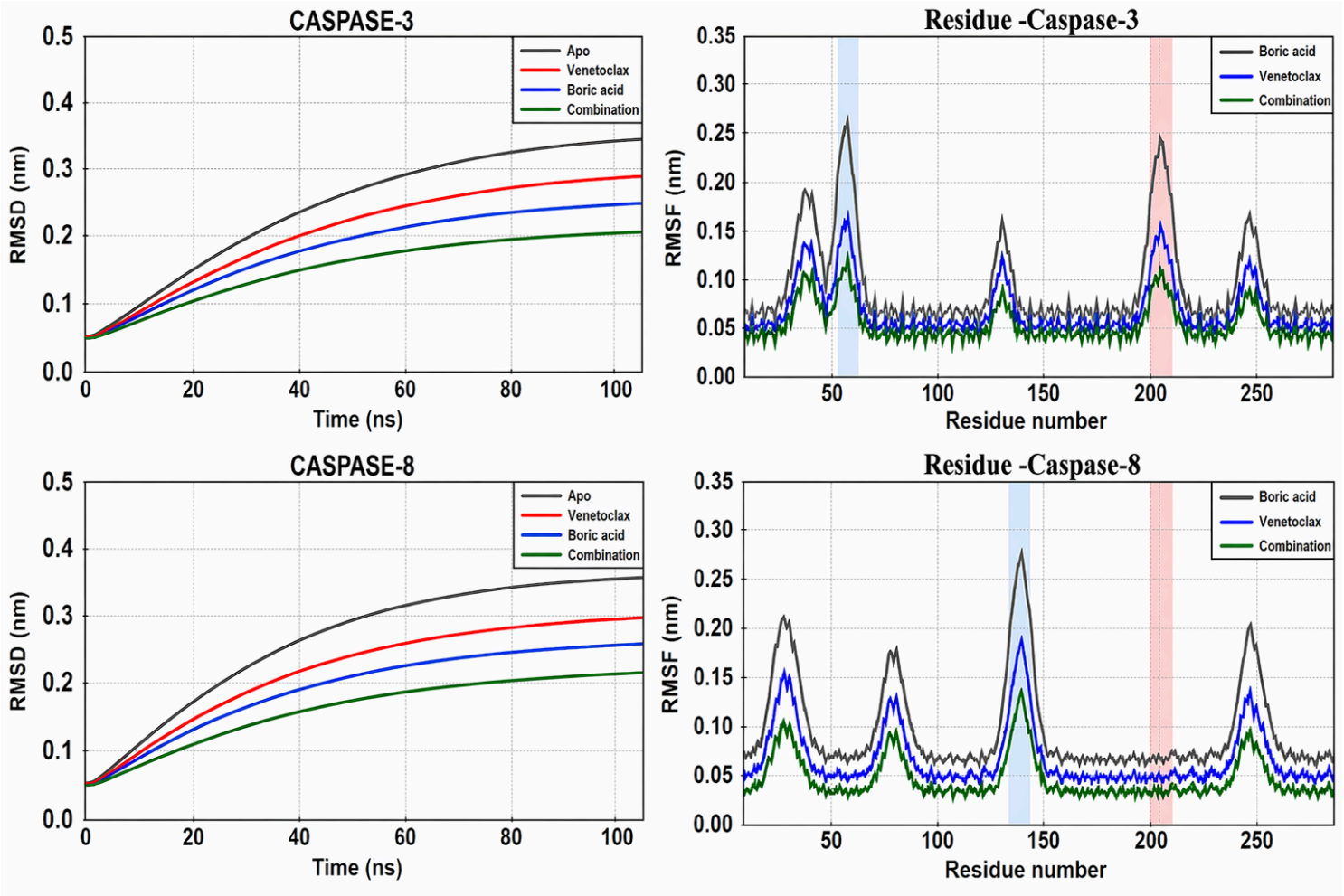



**Figure 7**

Molecular dynamics analysis. Highlighted regions: Caspase-3: L1 ring (Arg55-Ala59), L2 ring (Tyr204-Gln207), active site (Cys163, His121) Caspase-8: L2 ring (Cys360, His317), DED domain N-terminal, allosteric β-sheet

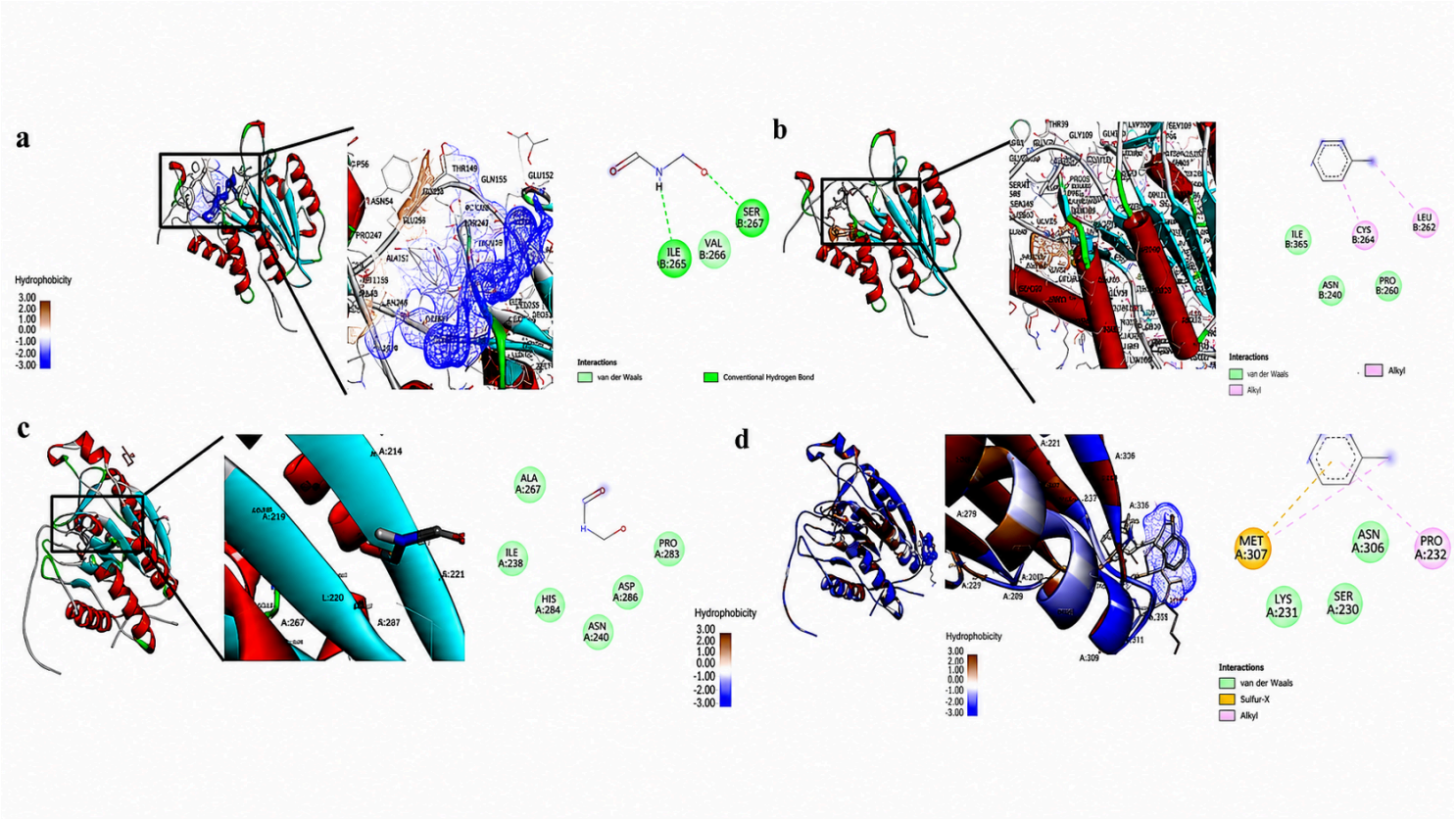



**Figure 8**

Molecular docking analysis (a: Caspase-3 and BA; b: Caspase-3 and Venetoclax; c: Caspase-8 and BA; d: Caspase-8 and Venetoclax)

## Supplementary Files

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- GRAPHCALABSTRACT.png