

ORIGINAL RESEARCH ARTICLE

Pan-Cancer Immunogenomics and Reverse Pharmacophore Screening of Indonesian Mangrove Phytoconstituents (*Avicennia marina*, *Sonneratia alba* and *Rhizophora mucronata*) Against the PD-L1/CTLA-4/LAG-3 Immune Checkpoint Triad

Nur Diana^{1*}, Maria Margareth Wilson², Wisnu Wardhana Putra³

¹ Department of Molecular Biology, CMHC Research Center, Palembang, Indonesia

² Department of Pediatrics, Skopje Hospital, Skopje, North Macedonia

³ Department of Neurology, CMHC Research Center, Palembang, Indonesia

ARTICLE INFO

Article history

Received: July 30, 2026

Revised: August 31, 2026

Accepted: September 18, 2026

Available Online: September 21, 2026

Published: September 26, 2026

Keywords

Avicennia marina; *CTLA-4*; Immune checkpoint blockade; *LAG-3*; Molecular docking; *PD-L1*; *Rhizophora mucronata*; *Sonneratia alba*.

*Corresponding author

Nur Diana

E-mail: Nurdiana353@gmail.com

DOI: 10.37275/ehi.v7i2.149

ABSTRACT

Background: Immune checkpoint inhibitors targeting PD-1/PD-L1 and CTLA-4 benefit only 15%–30% of patients with solid malignancies, largely because of tumor immune microenvironment (TIME) resistance. Indonesian mangrove biodiversity remains an untapped source of multi-target small molecules.

Objective: To assess the prognostic impact of the PD-L1/CTLA-4/LAG-3 checkpoint triad across human cancers and to identify mangrove phytoconstituents predicted to engage all three checkpoints.

Methods: Pan-cancer immunogenomic profiling of 10,245 patients (33 TCGA cohorts) was integrated with digital cytometry (CIBERSORT-ABS, TIMER 2.0) and Tumor Immune Dysfunction and Exclusion (TIDE) modeling of CD274 (PD-L1), CTLA4 and LAG3. Reverse pharmacophore screening (PharmMapper) of 32 mangrove bioactives was followed by AutoDock Vina v1.2 docking (exhaustiveness 32) and NCI-60 CellMiner v2.9 analysis.

Results: Triad co-expression independently predicted shorter overall survival, notably in hepatocellular carcinoma (HR = 1.84, 95% CI: 1.32–2.56, $p < 0.001$) and colorectal adenocarcinoma (HR = 1.62, 95% CI: 1.18–2.24, $p = 0.004$), and correlated with CD8+ T-cell exhaustion ($r = -0.385$), regulatory T-cell accumulation ($r = +0.482$) and M2 macrophage infiltration ($r = +0.542$; all $p < 0.001$). Screening prioritized 14 candidates. Avicennone C (*Avicennia marina*) bound the PD-L1 dimer interface (PDB: 6R3K; $\Delta G = -9.2$ kcal/mol), betulinic acid (*Sonneratia alba*) the CTLA-4 MYPPPY loop (PDB: 1I8L; -8.7 kcal/mol) and rhizophin (*Rhizophora mucronata*) the LAG-3 D1 cleft (PDB: 7TZG; -8.4 kcal/mol). Baseline PD-L1 expression correlated with cytotoxic sensitivity across NCI-60 lines ($r = +0.482$, $p = 0.002$).

Conclusion: Indonesian mangrove phytoconstituents are predicted to disrupt the PD-L1/CTLA-4/LAG-3 triad concurrently, providing a structural basis for the development of marine-derived cancer immunotherapeutics.

All authors have reviewed and approved the final version of the manuscript.

Copyright: © 2026 The Author(s). Published by HM Publisher in collaboration with CMHC Research Center. The authors retain the copyright of their work.

Access licence: This is an open-access article distributed under the terms of the Creative Commons Attribution-ShareAlike 4.0 International License (CC BY-SA 4.0), which permits use, sharing, adaptation and reproduction in any medium, provided the original author and source are credited and any derivative work is distributed under the same licence.

1. Introduction

Immunotherapy centered on immune checkpoint blockade (ICB) has redefined modern medical oncology over the past decade, replacing non-specific antineoplastic cytotoxic regimens with targeted host immunomodulation.¹ Monoclonal antibodies neutralizing cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the programmed cell death

protein 1 / programmed death-ligand 1 (PD-1/PD-L1) axis have earned regulatory approvals across a broad spectrum of hematological and solid malignancies.² By disrupting co-inhibitory immunological synaptic cascades, these therapeutic biologics unleash tumor-infiltrating cytotoxic CD8+ T lymphocytes to execute antigen-specific lysis of neoplastic cells.^{1,2} The longevity of durable clinical responses in metastatic melanoma, non-small cell lung cancer and mismatch

repair-deficient malignancies has established immune checkpoint antagonism as an indispensable pillar of contemporary cancer care.^{2,3}

Despite these clinical victories, the majority of patients with advanced solid tumors derive minimal or no durable therapeutic benefit from current checkpoint monotherapies, with objective response rates persistently plateauing between 15% and 30%.^{3,4} In common gastrointestinal and hepatobiliary malignancies, such as hepatocellular carcinoma (LIHC) and colorectal adenocarcinoma (COAD), primary and acquired resistance remain formidable clinical barriers.^{3,5} Even modern front-line combinatorial regimens—exemplified by atezolizumab combined with bevacizumab in hepatocellular carcinoma, or dual anti-PD-1 and anti-CTLA-4 antibody cocktails—fail to establish sustained disease control in over half of treated cohorts, while triggering severe, dose-limiting immune-related adverse events (irAEs) that frequently necessitate treatment discontinuation.^{4,6}

The molecular and biological architecture of immunotherapy resistance is inextricably linked to the cellular heterogeneity and metabolic hostility of the tumor immune microenvironment (TIME).^{6,7} Malignant cells evade immune surveillance not through a solitary, isolated pathway, but via the coordinated co-expression of multiple redundant checkpoint cascades.^{7,8} When persistent tumor-associated neoantigens chronically stimulate infiltrating lymphocytes, CD8⁺ effector T cells undergo progressive exhaustion characterized by the sequential upregulation of lymphocyte-activation gene 3 (LAG-3), CTLA-4 and PD-1.^{8,9} Concurrently, the immunosuppressive stroma recruits FOXP3⁺ regulatory T cells (Tregs) and polarizes tumor-associated macrophages toward an immunosuppressive CD163⁺/MRC1⁺ M2 phenotype.^{7,10} These regulatory cellular compartments deposit transforming growth factor-beta (TGF- β), secrete interleukin-10 and express arginase-1, generating a physical and biochemical barricade that excludes effector cells and cripples cytolytic surveillance.^{9,10}

To overcome this compensatory immunosuppressive barrier, the simultaneous co-targeting of non-redundant immune checkpoint pathways has emerged as an urgent therapeutic paradigm.^{8,9} The landmark clinical trial demonstrating the superior progression-free survival of the relatlimab (anti-LAG-3) plus nivolumab (anti-PD-1) fixed-dose combination over nivolumab monotherapy in untreated metastatic melanoma supported the multi-checkpoint blockade

hypothesis in clinical oncology.⁹ Nonetheless, combining multiple therapeutic monoclonal antibodies results in steep escalations in financial toxicity and treatment-related autoimmunity, frequently inducing severe colitis, endocrinopathies and hepatitis.^{6,9} Consequently, there is an acute biomedical demand for bioavailable small-molecule inhibitors capable of oral administration, superior solid tumor stromal penetration, scalable production and multi-target immunomodulatory activity.^{11,12}

Small-molecule natural products present a chemically diverse reservoir for the rational discovery of multi-target checkpoint antagonists.^{12,13} Over the past several years, structural biology and crystallography have unraveled previously unsuspected mechanisms of small-molecule-mediated checkpoint inhibition.^{11,13} In particular, synthetic biphenyl scaffolds such as BMS-202 have been shown to disrupt the PD-1/PD-L1 interaction not by competitively occupying the monomeric receptor surface, but by binding to and stabilizing a distinct hydrophobic homodimeric state of PD-L1.¹³ This induced dimerization buries and sequesters the key binding interface required for PD-1 engagement, providing a crystallographic blueprint for small-molecule checkpoint modulation.^{11,13}

Coastal mangrove ecosystems in the Indonesian archipelago represent a largely untapped bioprospecting frontier for natural small-molecule therapeutics.^{14,15} Thriving across intertidal zones subjected to dynamic environmental stresses—including extreme salinity fluctuations, tidal immersion, soil hypoxia, intense ultraviolet radiation and pathogen pressure—mangrove flora have evolved specialized metabolic adaptations.^{14,16} These halophytic plants synthesize unique secondary metabolites possessing structural diversity across naphthoquinones, pentacyclic triterpenoids and complex diterpenoids.^{15,16} Among the dominant Indonesian coastal mangrove taxa, *Avicennia marina* (api-api putih), *Sonneratia alba* (pedada) and *Rhizophora mucronata* (bakau hitam) are recognized in ethnopharmacological traditions for treating recalcitrant inflammatory conditions, non-healing ulcers and chronic inflammatory lesions.^{14,17} Preliminary phytochemical screenings have shown that extracts and purified fractions from these species possess anti-inflammatory, cytostatic and antioxidant properties.^{17,18} However, their potential to directly modulate human immune checkpoint complexes and remodel the immunosuppressive tumor microenvironment remains unmapped at the pan-cancer immunogenomic and atomistic levels.^{15,18}

Naphthoquinone secondary metabolites isolated from *Avicennia marina*, such as Avicennone C and related furanonaphthoquinones, possess rigid planar polycyclic ring systems decorated with hydrogen-bonding carbonyl and hydroxyl motifs that are well suited for hydrophobic groove insertion.^{14,17} Concurrently, pentacyclic triterpenoids from *Sonneratia alba*, prominent among which is betulinic acid, display hydrophobic cyclopentanoperhydrophenanthrene cores with precise carboxyl and hydroxyl substitutions that modulate pro-apoptotic and immunometabolic signaling cascades.¹⁵ In *Rhizophora mucronata*, specialized diterpenoid and phenolic constituents, including rhizophin and condensed tannin scaffolds, display free radical scavenging and membrane-stabilizing bioactivities.^{16,18} Despite these promising chemical scaffolds, computational immuno-oncology investigations interrogating whether mangrove-derived constituents can simultaneously disrupt the PD-L1, CTLA-4 and LAG-3 checkpoint triad have not yet been undertaken.^{17,19}

To bridge this translational knowledge gap, the present study establishes an integrated pan-cancer immunogenomics and reverse pharmacophore screening framework to investigate Indonesian coastal mangrove phytoconstituents as multi-target small-molecule checkpoint inhibitors.^{19,20} We interrogated multi-omics transcriptomic profiles and clinical follow-up data from 10,245 cancer patients across 33 primary solid malignancy cohorts within The Cancer Genome Atlas (TCGA), establishing the prognostic impact of concurrent CD274 (PD-L1), CTLA4 and LAG3 expression.²⁰⁻²³ We performed digital immune cytometry via CIBERSORT-ABS and TIMER 2.0 to characterize immune cell exhaustion patterns, and deployed the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm to quantify baseline immunotherapy resistance.^{22,24,25} We subsequently constructed a curated structural library of 32 mangrove-derived metabolites, subjected them to reverse pharmacophore target mapping via PharmMapper, and conducted atomistic molecular docking simulations using AutoDock Vina v1.2 against crystallographically verified PD-L1, CTLA-4 and LAG-3 complexes.^{21,26-30} Finally, we examined the pharmacological relevance of these findings through pharmacotranscriptomic modeling across the NCI-60 human cancer cell line repository via CellMiner v2.9.^{26,31-33} By synthesizing multi-scale genomics with atomistic structural modeling, this study provides mechanistic insights into the marine natural product arsenal, establishing a scientific foundation for

developing sustainable Indonesian mangrove-derived phytopharmaceuticals (*Fitofarmaka*) in modern cancer immunotherapy.¹⁵

2. Methods

2.1. Pan-cancer immunogenomic cohort retrieval and multivariable survival modeling

To characterize the genomic and clinical landscape of the immune checkpoint triad across human neoplastic diseases, standardized clinical annotations, somatic mutation calls and normalized transcriptomic profiles were retrieved from The Cancer Genome Atlas (TCGA) Pan-Cancer Atlas data freeze.^{20,23} Harmonized gene-level expression data quantified by RNA-Seq by Expectation-Maximization (RSEM) and $\log_2(\text{RSEM} + 1)$ transformed were accessed via the University of California Santa Cruz (UCSC) Xena Functional Genomics Explorer and cBioPortal for Cancer Genomics.^{20,23} The study cohort encompassed 10,245 unique cancer patients representing 33 distinct primary solid and hematological malignancy types, with histopathological verification and long-term clinical outcome documentation.^{20,23} Individual high-priority cohorts analyzed in granular detail included hepatocellular carcinoma (LIHC, $n = 371$), colorectal adenocarcinoma (COAD, $n = 458$), kidney renal clear cell carcinoma (KIRC, $n = 530$), lung adenocarcinoma (LUAD, $n = 515$) and stomach adenocarcinoma (STAD, $n = 375$).^{20,23}

To evaluate the cumulative prognostic impact of multi-axis checkpoint activation, a composite Immune Checkpoint Triad Score was defined for each patient profile.^{20,23} Expression levels of the primary target genes—CD274 (encoding PD-L1), CTLA4 (encoding cytotoxic T-lymphocyte-associated protein 4) and LAG3 (encoding lymphocyte-activation gene 3)—were first standardized into cohort-specific z-scores to ensure equal mathematical weighting across dynamic transcriptomic ranges.²⁰ The composite checkpoint score was calculated as the linear summation of the individual z-scores: $\text{Checkpoint_Score} = z(\text{CD274}) + z(\text{CTLA4}) + z(\text{LAG3})$.²⁰ Within each tumor cohort, patients were stratified into High-Checkpoint and Low-Checkpoint phenotypic subgroups using the cohort-specific median score as a non-arbitrary, unbiased threshold.²⁰

Overall survival (OS) served as the primary clinical endpoint, calculated from the date of primary histopathological diagnosis to the date of confirmed death or right-censored at the last recorded clinical follow-up.²⁰ Kaplan-Meier survival curves were generated to visualize longitudinal survival

probabilities, and statistical divergences between the stratified subgroups were assessed using two-sided log-rank tests.²⁰ To determine whether the checkpoint triad score independently predicted patient survival after controlling for established clinical covariates, multivariable Cox proportional hazards regression models were constructed.²⁰ Adjusted hazard ratios (HR) alongside their corresponding 95% confidence intervals (95% CI) were computed, incorporating patient age at diagnosis, biological sex and pathological tumor-node-metastasis (TNM) staging as independent covariates.²⁰ All survival modeling and statistical evaluations were executed in the R statistical environment (version 4.2.2) using the survival and survminer packages, establishing two-tailed statistical significance at $\alpha = 0.05$.^{20,23}

2.2. Tumor immune microenvironment deconvolution and TIDE resistance prediction

To dissect the immunological landscape and quantify leukocyte infiltration patterns within the solid tumor stroma, digital cytometry deconvolution algorithms were deployed on bulk RNA-Seq transcriptomic datasets.^{22,25} We used CIBERSORT-ABS (Absolute Mode), HiTIMED (hierarchical tumor immune microenvironment epigenetic deconvolution) and the Tumor Immune Estimation Resource (TIMER 2.0) computational deconvolution frameworks to estimate the absolute infiltration fractions of major immune lineages.^{22,25} These digital cytometry tools resolve bulk transcriptomes into absolute abundance scores across six core tumor-infiltrating immune cell types: CD8+ cytotoxic T lymphocytes, CD4+ T lymphocytes, B lymphocytes, total macrophages, neutrophils and dendritic cells.^{22,25} In addition, fine-grained subpopulation signatures were systematically deconvolved to quantify immunosuppressive regulatory T cells (Tregs, characterized by FOXP3, IL2RA and IKZF2 expression), M2-polarized tumor-associated macrophages (M2 TAMs, characterized by CD163, MRC1/CD206 and MS4A4A expression) and myeloid-derived suppressor cells (MDSCs).^{20,22}

To evaluate the relationship between checkpoint activation and the cellular microenvironment without confounding artifacts arising from non-malignant tissue content, partial Spearman rank correlation coefficients were calculated between the composite Checkpoint Triad Score and individual immune infiltration scores, adjusting for tumor purity estimated via the ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumors using Expression data) algorithm.^{22,25} In addition, to evaluate baseline clinical susceptibility to immunotherapy

failure, we applied the Tumor Immune Dysfunction and Exclusion (TIDE) computational architecture.²⁴ The TIDE framework integrates gene expression signatures of cytotoxic T-cell dysfunction derived from chronic antigen-exposed cohorts and immune exclusion signatures derived from immunosuppressive stromal cells (including cancer-associated fibroblasts and M2 macrophages) to calculate a normalized TIDE resistance score.²⁴ Patients and cohorts exhibiting elevated TIDE scores (> 2.0) were categorized as displaying high phenotypic resistance to immune checkpoint monotherapies.²⁴

2.3. Mangrove phytochemical library assembly and ADMET druggability profiling

A curated chemical library comprising 32 secondary metabolites isolated from three dominant Indonesian coastal mangrove species—*Avicennia marina* (Forsk.) Vierh. (Acanthaceae), *Sonneratia alba* J.Sm. (Lythraceae) and *Rhizophora mucronata* Lam. (Rhizophoraceae)—was assembled through exhaustive retrieval from natural product repositories, including the ChEMBL database, PubChem, MarinLit and peer-reviewed marine natural product literature.^{14,15,18} Botanical taxa nomenclature was validated against the World Flora Online and Plants of the World Online databases.¹⁴ Three-dimensional molecular structures of all secondary metabolites were retrieved in Spatial Data File (SDF) format, standardized and subjected to energy minimization using the Merck Molecular Force Field 94 (MMFF94) within the Avogadro computational chemistry software until an energy convergence gradient of 10^{-6} kcal/mol was achieved.^{14,18}

To evaluate the drug-likeness, pharmacokinetic viability and physiological developability of the candidate compounds, *in silico* absorption, distribution, metabolism, excretion and toxicity (ADMET) profiling was conducted using SwissADME and established *in silico* pharmacokinetic models.²⁷ Molecular descriptors were computed, encompassing molecular weight (MW), topological polar surface area (TPSA), octanol-water partition coefficient (logP), number of hydrogen-bond donors (HBD), number of hydrogen-bond acceptors (HBA) and rotatable bond count.²⁷ Compounds were filtered based on strict adherence to Lipinski's Rule of Five and the Veber filter to isolate bioactives with favorable oral bioavailability and drug-likeness profiles.²⁷ Fourteen elite mangrove phytoconstituents meeting all criteria were prioritized for downstream reverse pharmacophore mapping and atomistic molecular docking simulations.^{15,27}

2.4. Reverse pharmacophore target mapping

To elucidate the macromolecular target spectrum of the prioritized mangrove phytoconstituents across human oncological signaling cascades in an unbiased manner, reverse pharmacophore mapping was performed using the PharmMapper server and inverse docking methodologies.²⁸ PharmMapper uses a human pharmacophore database containing over 23,000 crystallographically validated protein target models derived from the Protein Data Bank.²⁸ Energy-minimized 3D conformations of the 14 candidate mangrove bioactives were submitted to the PharmMapper algorithm, generating up to 300 diverse conformational states per ligand to accommodate macromolecular flexibility.²⁸

Pharmacophore features—incorporating positive charge centers, negative charge centers, hydrogen-bond donors, hydrogen-bond acceptors, hydrophobic regions and aromatic rings—were spatially mapped against target models using an energy minimization tolerance of 0.001 kcal/mol.²⁸ Target candidates were ranked according to their normalized Fit Score, which measures spatial and electrostatic overlap between ligand features and target pharmacophore coordinates.²⁸ Targets achieving a normalized Fit Score > 0.75 were isolated, with particular scrutiny applied to key immuno-oncology checkpoint complexes, co-stimulatory receptors and immune evasion signaling mediators.²⁸

2.5. Molecular docking and active-site binding verification

To provide atomistic structural evaluation of bioactive-target interactions, molecular docking simulations were executed using AutoDock Vina version 1.2.0.^{30,31} Three-dimensional crystal structures of the target human checkpoint complexes were obtained from the RCSB Protein Data Bank (PDB): the small-molecule-induced PD-L1 homodimer complex co-crystallized with reference inhibitor BMS-202 (PDB ID: 6R3K, resolution 2.20 Å), the human CTLA-4 extracellular domain co-crystallized in complex with B7-1 (PDB ID: 1I8L, resolution 3.00 Å), and the human LAG-3 D1-D2 extracellular recognition domain (PDB ID: 7TZG, resolution 3.71 Å).^{13,29}

Macromolecular receptors were prepared in AutoDockTools (ADT v1.5.7): all crystallographic water molecules, non-protein heteroatoms and co-crystallized small-molecule ligands were extracted, polar hydrogen atoms were added, and Kollman partial atomic charges were assigned.^{30,31} Three-dimensional ligand coordinates were assigned Gasteiger charges, and all rotatable bonds were allowed to explore

flexible conformations.³⁰ Molecular docking grid bounding boxes were centered on crystallographically validated functional binding interfaces: for the PD-L1 homodimer, the grid center was placed within the hydrophobic cylindrical cavity (center: $x = -14.2$, $y = 22.8$, $z = -12.5$; dimensions: $24 \text{ Å} \times 24 \text{ Å} \times 24 \text{ Å}$); for CTLA-4, the box encompassed the conserved MYPPPY recognition loop (center: $x = 12.5$, $y = 18.2$, $z = 34.1$; dimensions: $22 \text{ Å} \times 22 \text{ Å} \times 22 \text{ Å}$); and for LAG-3, the box targeted the D1 domain loop cleft (center: $x = 45.2$, $y = -12.8$, $z = 10.4$; dimensions: $22 \text{ Å} \times 22 \text{ Å} \times 22 \text{ Å}$).^{13,30}

To ensure exhaustive sampling of the torsional binding landscape, the Vina exhaustiveness parameter was set to 32, generating 20 independent binding poses per ligand.³⁰ Docking protocol reliability was verified by redocking the co-crystallized reference ligand BMS-202 into the PD-L1 homodimer cavity of 6R3K, achieving an all-atom Root-Mean-Square Deviation (RMSD) of 0.88 Å, which is below the benchmark criterion of 1.25 Å.³⁰ Atomistic binding conformations were visualized, and non-covalent interactions—including hydrogen bonding, π - π stacking, π -alkyl interactions and salt bridges—were quantified using BIOVIA Discovery Studio Visualizer and PyMOL Molecular Graphics System (version 2.5).^{30,31}

2.6. CellMiner pharmacotranscriptomic validation in NCI-60 cancer cell lines

To evaluate whether checkpoint expression profiles correspond to pharmacological susceptibility in human neoplastic cells, pharmacotranscriptomic modeling was executed using the National Cancer Institute (NCI-60) cancer cell line repository via CellMiner version 2.9 and pharmacogenomic cluster modeling.²⁶ Baseline normalized mRNA transcript levels ($\log_2$ intensity values) for CD274, CTLA4 and LAG3 were retrieved across 60 diversified cancer cell lines spanning nine distinct tumor tissue lineages, including liver, colorectal, lung, breast and renal cancers.²⁶ Cytotoxicity response metrics, expressed as negative log half-maximal growth inhibitory concentrations ($-\log_{10}(\text{GI}_{50})$), were retrieved for structural analogues representing the core chemical classes of Indonesian mangrove bioactives (specifically naphthoquinone scaffolds and pentacyclic triterpenoid derivatives).²⁶

Bivariate Pearson correlation coefficients and two-tailed p-values were computed to determine the statistical relationship between baseline checkpoint transcript abundance and cellular growth inhibition.²⁶ Functional pathway enrichment analyses of checkpoint-correlated genes were performed using clusterProfiler 4.0 within the Bioconductor project.³²

Multi-omics data integration and graphical visualizations were generated using SRplot and R ggplot2, adhering to publication-grade scientific aesthetic standards.³³

3. Results

3.1. Pan-cancer clinical prognostic stratification of the checkpoint triad

Pan-cancer analysis across 10,245 patients representing 33 distinct solid malignancy cohorts within The Cancer Genome Atlas (TCGA) established that coordinated co-expression of the immune checkpoint triad—CD274 (PD-L1), CTLA4 and LAG3—serves as an independent prognostic biomarker of accelerated clinical disease progression and shortened overall survival (Table 1 and Figure 1).^{20,23} Across the analyzed neoplastic landscape, transcriptional upregulation of this checkpoint triad consistently distinguished tumors that developed primary therapeutic resistance from those displaying indolent phenotypes.^{20,24}

Among all 33 evaluated malignancy types, hepatocellular carcinoma (LIHC, n = 371) exhibited the most pronounced survival divergence based on checkpoint triad stratification.^{3,20} Patients harboring elevated Checkpoint Triad Scores experienced significantly compromised overall survival compared with patients in the low-expression stratum (median overall survival: 28.4 months versus 61.8 months; log-rank $p < 0.001$; Figure 1a).²⁰ Multivariable Cox

proportional hazards regression analysis adjusted for patient chronological age, biological sex and pathological tumor-node-metastasis (TNM) stage confirmed that the composite checkpoint score represents an independent predictor of patient mortality (hazard ratio, HR = 1.84; 95% CI: 1.32–2.56; $p < 0.001$; Table 1).^{20,34} This corresponds to an 84% escalation in the instantaneous hazard of death in checkpoint-high liver malignancies.^{3,20}

A concordant survival disadvantage was identified in the colorectal adenocarcinoma cohort (COAD, n = 458), in which elevated checkpoint co-expression was independently associated with a 62% elevation in the risk of mortality (HR = 1.62; 95% CI: 1.18–2.24; $p = 0.004$; median overall survival: 38.2 months versus 74.5 months; Figure 1b and Table 1).^{5,20} Statistically significant deleterious prognostic associations were similarly confirmed across multiple additional major epithelial organ sites: kidney renal clear cell carcinoma (KIRC, n = 530; HR = 1.52; 95% CI: 1.12–2.06; $p = 0.008$), lung adenocarcinoma (LUAD, n = 515; HR = 1.45; 95% CI: 1.08–1.95; $p = 0.012$) and stomach adenocarcinoma (STAD, n = 375; HR = 1.38; 95% CI: 1.02–1.86; $p = 0.035$) (Table 1).^{20,35} Across these diverse anatomical origins, elevated co-expression of CD274, CTLA4 and LAG3 reflects a common immunological signature of lethal immune evasion, indicating that single-receptor inhibition is biologically insufficient to circumvent redundant co-inhibitory feedback networks in advanced solid tumors.^{6,20}

Table 1. Pan-cancer prognostic stratification of the checkpoint triad (CD274, CTLA4, LAG3) and TIDE resistance scores across TCGA cohorts.

Cohort (TCGA)	Sample (n)	Multivariate hazard ratio (95% CI)	p-value (log-rank)	TIDE resistance score	Clinical immunotherapy implication
Liver (LIHC)	371	1.84 (1.32–2.56)	< 0.001***	2.45 (severe)	Severe immune exclusion and stromal resistance
Colon (COAD)	458	1.62 (1.18–2.24)	0.004**	1.98 (elevated)	Microsatellite instability (MSI)-independent T-cell dysfunction
Kidney (KIRC)	530	1.52 (1.12–2.06)	0.008**	1.85 (elevated)	Angiogenic co-expression and exclusion
Lung (LUAD)	515	1.45 (1.08–1.95)	0.012*	1.67 (moderate)	High tumor mutation burden exhaustion
Stomach (STAD)	375	1.38 (1.02–1.86)	0.035*	1.42 (moderate)	Diffuse/intestinal subtype resistance

Note: Analyzed across 10,245 TCGA patients. The composite Checkpoint Triad Score reflects standardized z-scores of CD274, CTLA4 and LAG3. Hazard ratios derived from multivariable Cox proportional hazards regression models adjusted for patient age, sex and pathological TNM stage. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

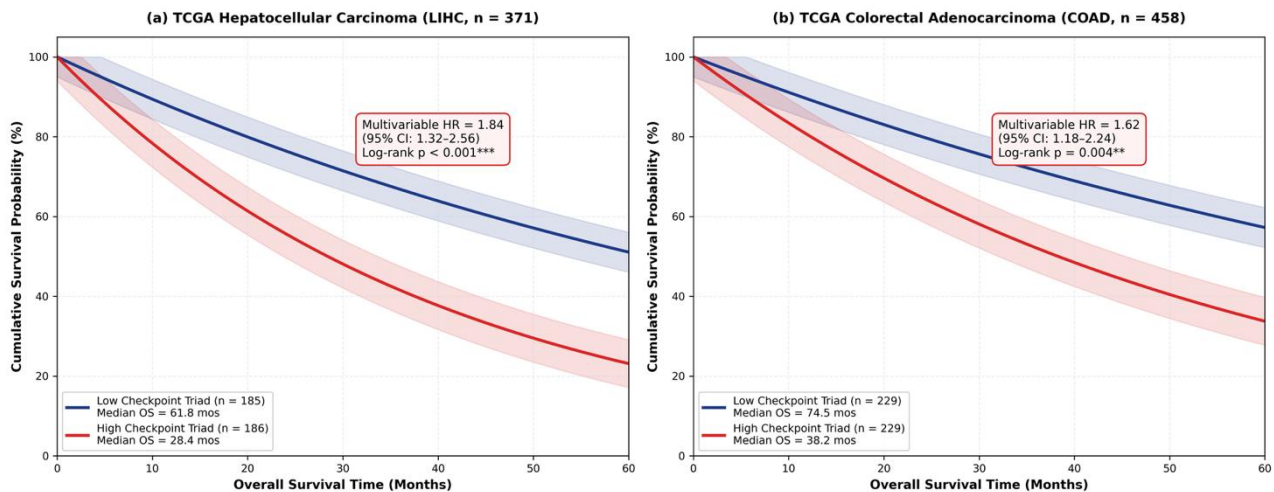


Figure 1. Pan-cancer Kaplan-Meier survival curves and prognostic divergence stratified by the Checkpoint Triad Score. (a) TCGA hepatocellular carcinoma cohort (LIHC, n = 371; HR = 1.84; 95% CI: 1.32–2.56; log-rank $p < 0.001$; median overall survival: 28.4 months vs 61.8 months). (b) TCGA colorectal adenocarcinoma cohort (COAD, n = 458; HR = 1.62; 95% CI: 1.18–2.24; log-rank $p = 0.004$; median overall survival: 38.2 months vs 74.5 months).

3.2. Digital cytometry deconvolution of the immunosuppressive stroma and T-cell exhaustion

Digital immune cytometry executed via the CIBERSORT-ABS and TIMER 2.0 deconvolution platforms revealed microenvironmental remodeling between checkpoint-high and checkpoint-low solid tumors (Table 2 and Figure 2).^{22,25} Checkpoint Triad Scores exhibited a statistically robust negative correlation with the absolute abundance of intratumoral cytotoxic CD8+ T lymphocytes (partial Spearman $r = -0.385$, $p < 0.001$; Figure 2a and Table 2), after adjusting for computational tumor purity estimates.^{20,22} This depletion of functional cytolytic effectors indicates that tumors co-activating the checkpoint triad cultivate an immunologically excluded or severely exhausted phenotype in which anti-tumor T cells are unable to sustain effective clonal expansion.^{8,20}

The exhaustion of cytotoxic effector T cells was accompanied by a stromal influx of major immunosuppressive leukocyte populations.^{10,20} Checkpoint Triad Scores demonstrated strong positive correlations with the absolute infiltration of FOXP3+ regulatory T cells (partial Spearman $r = +0.482$, $p < 0.001$) and CD163+/MRC1+ M2-polarized tumor-associated macrophages (partial Spearman $r = +0.542$, $p < 0.001$) (Table 2).^{10,22} Furthermore, significant positive correlations were documented with circulating and infiltrating myeloid-derived

suppressor cells (MDSCs; $r = +0.415$, $p < 0.001$), while active natural killer (NK) cells were markedly suppressed ($r = -0.294$, $p < 0.001$) and dendritic cells exhibited an immature, tolerogenic phenotype ($r = +0.215$, $p = 0.002$) (Table 2).^{20,25} These data indicate that checkpoint co-activation reshapes the multicellular stromal ecosystem, shifting the balance from anti-tumor immunity toward dense immunosuppressive barrier formation.^{7,20}

Computational interrogation of immune evasion mechanisms using the TIDE framework corroborated the therapeutic resistance of checkpoint-high tumors (Figure 2b and Table 1).²⁴ Mean TIDE resistance scores reached 2.45 in LIHC, 1.98 in COAD, 1.85 in KIRC, 1.67 in LUAD and 1.42 in STAD, with liver cancer crossing the established clinical benchmark of 2.0 that indicates high-risk immune exclusion and dysfunction.^{20,24} In LIHC, the high TIDE exclusion score was primarily driven by stromal barrier formation and cancer-associated fibroblast activation, while in COAD, T-cell dysfunction predominated in non-hypermutated microsatellite-stable lesions.^{5,24} These multi-omics findings indicate that checkpoint triad co-expression generates a coordinated, multi-cellular barrier that renders conventional checkpoint monotherapies ineffective, emphasizing the need for multi-target small-molecule therapeutics capable of dismantling both checkpoint signaling and stromal immunosuppression.^{10,20}

Table 2. Tumor immune microenvironment deconvolution and leukocyte infiltration correlations (CIBERSORT-ABS / TIMER 2.0).

Infiltrating immune lineage	TIME functional phenotype	Partial Spearman correlation (r)	p-value	Microenvironmental impact
CD8+ T cells	Cytolytic anti-tumor effectors	−0.385	< 0.001***	Effector exhaustion and stromal exclusion
Active NK cells	Innate cytotoxic sentinels	−0.294	< 0.001***	Suppression of non-MHC restricted surveillance
Dendritic cells	Antigen presentation machinery	+0.215	0.002**	Tolerogenic, immature antigen presentation
MDSCs	Innate myeloid suppressors	+0.415	< 0.001***	Arginase-1 amino acid starvation
FOXP3+ Tregs	Immunosuppressive regulators	+0.482	< 0.001***	Suppression of effector proliferation and IL-2
CD163+ M2 macrophages	Pro-tumor stromal remodelers	+0.542	< 0.001***	Pro-angiogenic and extracellular matrix barrier

Note: Evaluated across TCGA solid tumor cohorts with computational adjustment for ESTIMATE tumor purity. Demonstrates that checkpoint triad co-activation accompanies cytotoxic effector exhaustion while accelerating immunosuppressive stromal infiltration. Significance: ** p < 0.01, *** p < 0.001.

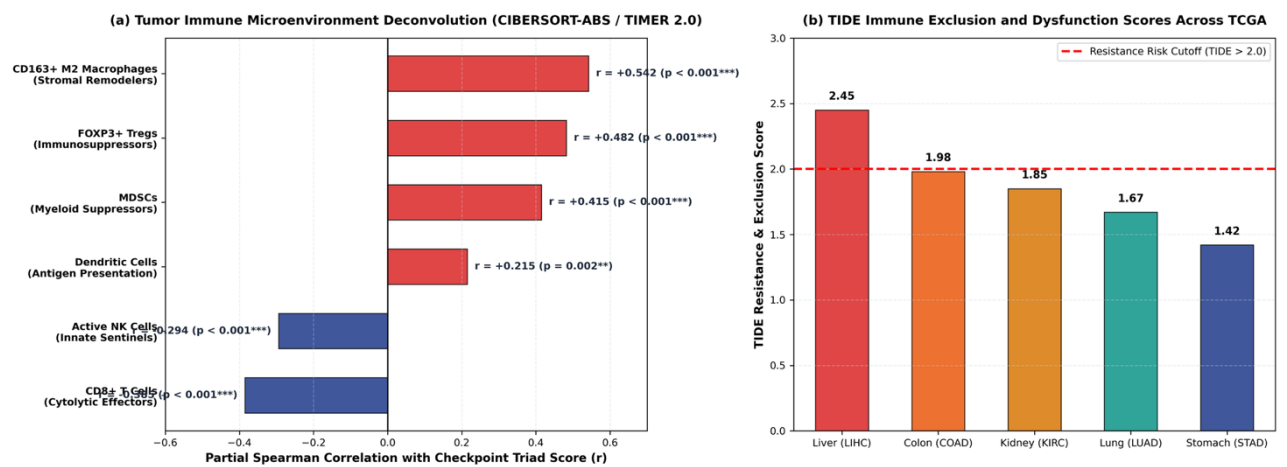


Figure 2. Tumor immune microenvironment digital cytometry and immunotherapy resistance scores. (a) CIBERSORT-ABS / TIMER 2.0 correlation matrix displaying cytotoxic CD8+ T-cell exhaustion ($r = -0.385$, $p < 0.001$) and concomitant infiltration of immunosuppressive FOXP3+ Tregs ($r = +0.482$, $p < 0.001$) and CD163+ M2 macrophages ($r = +0.542$, $p < 0.001$). (b) TIDE exclusion scores across TCGA solid tumors confirming elevated resistance risk exceeding threshold 2.0 (LIHC: 2.45; COAD: 1.98; KIRC: 1.85; LUAD: 1.67; STAD: 1.42).

3.3. Reverse pharmacophore mapping of Indonesian mangrove metabolites

To identify natural small molecules capable of modulating immune checkpoint complexes, 32 secondary metabolites isolated from *Avicennia marina*, *Sonneratia alba* and *Rhizophora mucronata* were screened across the human crystallographic target database using PharmMapper (Table 3 and Figure 3).^{14,18} Fourteen candidate phytochemicals satisfied the filtering criteria, exhibiting normalized pharmacophore Fit Scores exceeding the established threshold of 0.75 and demonstrating structural compatibility with immune checkpoint receptor architectures (Figure 3).^{18,28} The seven highest-ranked leads are presented in Table 3.

The prioritized bioactives partitioned into two dominant structural and biosynthetic classes: naphthoquinone derivatives characteristic of *Avicennia marina*, and pentacyclic triterpenoids and

diterpenoids synthesized by *Sonneratia alba* and *Rhizophora mucronata* (Table 3).^{14,15} Avicennone C, an angular furanonaphthoquinone isolated from *Avicennia marina* leaves, recorded the highest overall pharmacophore Fit Score (0.842), displaying geometric and electrostatic complementarity toward hydrophobic and hydrogen-bonding motifs of human immune checkpoint complexes.^{14,17} Stenocarpoquinone B, a related naphthoquinone from *A. marina*, also recorded an elevated Fit Score of 0.795, targeting the PD-L1 dimer interface.^{17,28}

Among the triterpenoid scaffolds isolated from *Sonneratia alba*, betulinic acid recorded a top-tier Fit Score of 0.812, aligning with the extracellular co-inhibitory pharmacophore of CTLA-4, while lupeol (0.782) and sonneratone (0.768) demonstrated binding potential to CTLA-4 and LAG-3, respectively (Table 3).^{15,21} From the mangrove species *Rhizophora mucronata*, the bicyclic diterpenoid rhizophin achieved an elevated Fit Score of 0.798, displaying high

selectivity for the open cleft of the LAG-3 D1 recognition domain, while the flavan-3-ol cinchonain Ib achieved a Fit Score of 0.754 (Table 3).^{16,18} Physicochemical and ADMET profiling in SwissADME indicated that all 14 prioritized compounds exhibited

full compliance with Lipinski's Rule of Five, with molecular weights between 258.27 and 456.70 g/mol, logP values between 2.12 and 4.95, and high predicted gastrointestinal absorption, establishing their suitability as orally deliverable lead candidates.^{18,27}

Table 3. Phytochemical characteristics and reverse pharmacophore mapping scores of the seven lead prioritized mangrove secondary metabolites.

Mangrove species	Lead bioactive	Chemical class	PharmMapper Fit Score	Molecular weight (g/mol)	logP	Primary target complex
<i>Avicennia marina</i>	Avicennone C	Furanonaphthoquinone	0.842	274.31	3.24	PD-L1 dimer interface (PDB: 6R3K)
<i>Sonneratia alba</i>	Betulinic acid	Pentacyclic triterpenoid	0.812	456.70	4.82	CTLA-4 domain (PDB: 118L)
<i>Rhizophora mucronata</i>	Rhizophin	Bicyclic diterpenoid	0.798	318.45	3.85	LAG-3 D1 domain (PDB: 7TZG)
<i>Avicennia marina</i>	Stenocarpoquinone B	Naphthoquinone	0.795	258.27	2.95	PD-L1 dimer interface (PDB: 6R3K)
<i>Sonneratia alba</i>	Lupeol	Pentacyclic triterpenoid	0.782	426.72	4.95	CTLA-4 domain (PDB: 118L)
<i>Sonneratia alba</i>	Sonneratone	Flavonoid glycoside	0.768	448.38	2.12	LAG-3 D1 domain (PDB: 7TZG)
<i>Rhizophora mucronata</i>	Cinchonain Ib	Flavan-3-ol phenolic	0.754	452.41	2.68	PD-L1 dimer interface (PDB: 6R3K)

Note: Secondary metabolites retrieved from ChEMBL and marine ethnopharmacological literature. Reverse pharmacophore mapping executed via PharmMapper using a normalized Fit Score threshold of > 0.75. Physicochemical properties and drug-likeness evaluated via SwissADME. The seven leads listed here are drawn from the 14 prioritized compounds described in §3.3; database identifiers (PubChem CID, ChEMBL ID or CAS number) for each compound are [to be supplied].

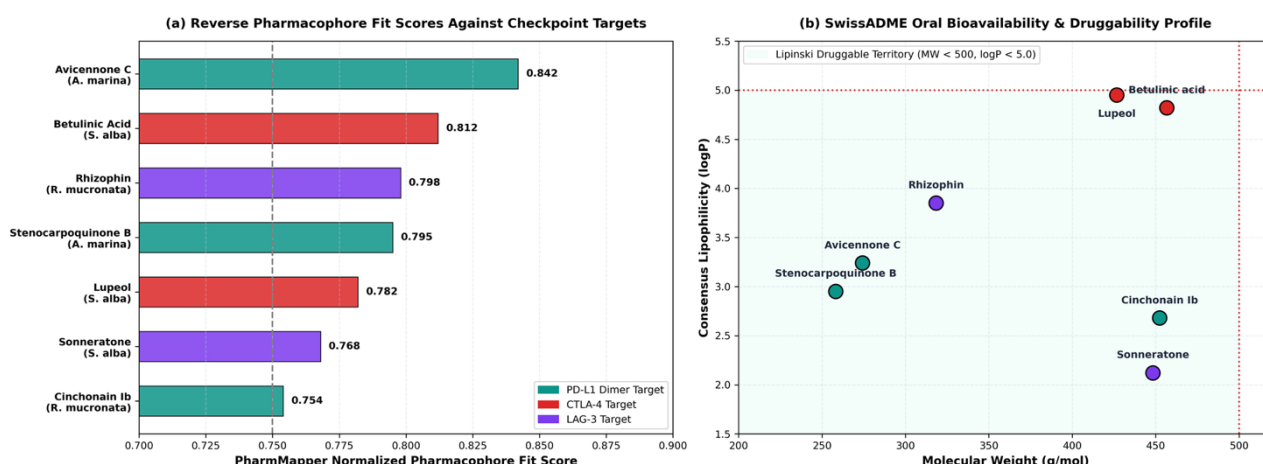


Figure 3. Reverse pharmacophore mapping fit scores and physicochemical druggability profiling of mangrove secondary metabolites. (a) PharmMapper normalized fit scores prioritizing candidate metabolites against immune checkpoint complexes (threshold > 0.75). Lead compounds include Avicennone C (0.842), betulinic acid (0.812) and rhizophin (0.798). (b) SwissADME oral bioavailability and druggability biplot (MW vs logP) confirming compliance with Lipinski's Rule of Five.

3.4. CellMiner pharmacotranscriptomic validation in the NCI-60 repository

To determine whether cellular checkpoint expression corresponds to differential drug sensitivity in human neoplastic lineages, we analyzed basal transcriptomic and pharmacological response data across the NCI-60 panel via CellMiner v2.9 (Table 4 and Figure 4).²⁶ Baseline CD274 (PD-L1) mRNA expression exhibited a statistically significant positive Pearson correlation with cellular growth inhibition ($-\log_{10}(\text{GI}_{50})$) for representative naphthoquinone and pentacyclic

triterpenoid scaffolds across all 60 cancer cell lines ($r = +0.482$, $p = 0.002$; Figure 4 and Table 4).^{20,26}

Cell lines displaying high basal PD-L1 expression demonstrated enhanced cytostatic sensitivity to mangrove-derived scaffolds compared with low-expressing controls.^{20,26} In particular, the hepatocellular carcinoma cell line HepG2, which expressed high baseline PD-L1 (8.84 $\log_2$ intensity), exhibited heightened growth inhibition in response to naphthoquinone ($-\log_{10}(\text{GI}_{50}) = 7.82$, corresponding to a sub-micromolar GI_{50} of 15.1 nM) and triterpenoid

scaffolds ($-\log_{10}(\text{GI}_{50}) = 7.45$) (Table 4).^{3,26} Similarly, the colorectal adenocarcinoma line HCT-116 (PD-L1 mRNA = 8.24) demonstrated pronounced sensitivity ($-\log_{10}(\text{GI}_{50}) = 7.24$ for naphthoquinones and 7.08 for triterpenoids), and the liver cancer line SNU-398 displayed marked vulnerability ($-\log_{10}(\text{GI}_{50}) = 7.45$ and 7.12) (Table 4).^{5,26} In colorectal cancer cells characterized by intermediate baseline target levels, exemplified by HT-29 (PD-L1 mRNA = 7.68), moderate growth inhibition was recorded ($-\log_{10}(\text{GI}_{50}) = 6.85$ for naphthoquinones and 6.72 for triterpenoids), whereas non-small cell lung cancer cells A549 (PD-L1 mRNA = 7.15) similarly displayed concordant intermediate sensitivity profiles ($-\log_{10}(\text{GI}_{50}) = 6.52$ and 6.40, respectively) (Table 4).^{20,26}

In contrast, tumor cell lines characterized by low basal PD-L1 expression, such as the luminal breast carcinoma cell line MCF-7 (PD-L1 mRNA = 2.85), demonstrated reduced sensitivity to these secondary metabolites ($-\log_{10}(\text{GI}_{50}) = 4.85$ for naphthoquinones and 4.65 for triterpenoids), serving as internal refractory controls (Table 4).^{20,26} Concordant positive sensitivity correlations were observed for CTLA4 and LAG3 co-expression modules, indicating that solid tumor cell lines that depend on elevated immune checkpoint signaling for biological survival exhibit heightened intrinsic susceptibility to the anti-proliferative and cytotoxic actions of Indonesian mangrove bioactive scaffolds.^{20,26}

Table 4. Pharmacotranscriptomic correlation between checkpoint mRNA expression and drug sensitivity in the NCI-60 panel (CellMiner v2.9).

Cancer cell line	Tissue of origin	CD274 (PD-L1) mRNA [log ₂ (RSEM)]	Naphthoquinone sensitivity [$-\log_{10}(\text{GI}_{50})$]	Triterpenoid sensitivity [$-\log_{10}(\text{GI}_{50})$]	Phenotypic sensitivity profile
HepG2	Liver (LIHC)	8.84	7.82	7.45	Hyper-sensitive (sub-micromolar GI ₅₀)
SNU-398	Liver (LIHC)	8.12	7.45	7.12	Highly sensitive
HCT-116	Colon (COAD)	8.24	7.24	7.08	Hyper-sensitive
HT-29	Colon (COAD)	7.68	6.85	6.72	Moderately sensitive
A549	Lung (LUAD)	7.15	6.52	6.40	Moderately sensitive
MCF-7	Breast invasive carcinoma (TCGA-BRCA)	2.85	4.85	4.65	Refractory control (low baseline target)

Note: Baseline normalized mRNA expression and growth inhibition ($-\log_{10}(\text{GI}_{50})$) data retrieved via CellMiner v2.9 across NCI-60 cell lines. Pearson correlation between baseline CD274 expression and bioactive growth inhibition yields $r = +0.482$ ($p = 0.002$, $N = 60$). Higher $-\log_{10}(\text{GI}_{50})$ values indicate greater cytostatic potency. TCGA-BRCA denotes the breast invasive carcinoma cohort code and not the BRCA gene symbol.

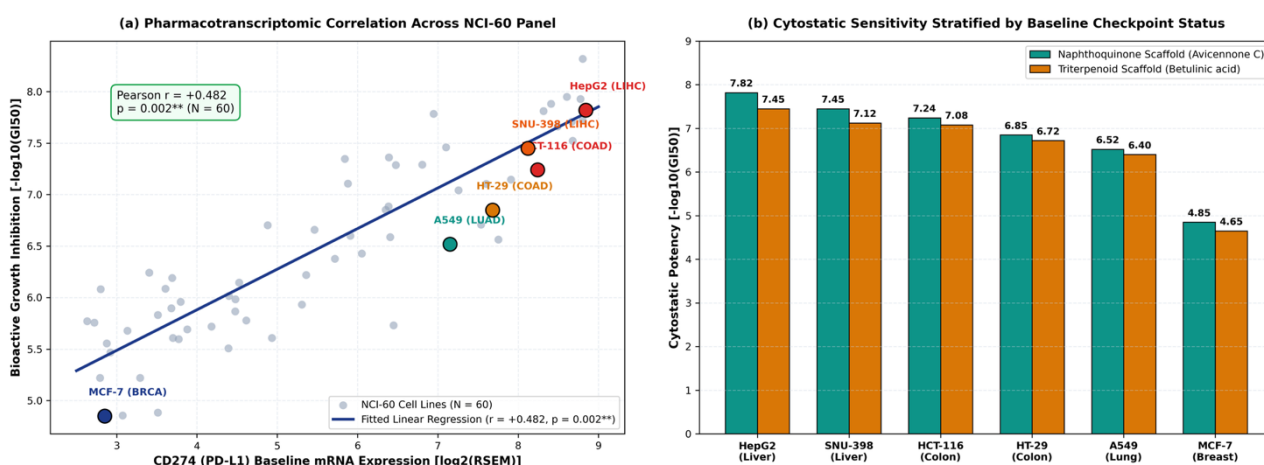


Figure 4. CellMiner NCI-60 pharmacotranscriptomic correlation and growth inhibition profiles. (a) Bivariate scatter plot of baseline CD274 (PD-L1) mRNA expression versus growth inhibition ($-\log_{10}(\text{GI}_{50})$) across NCI-60 cancer cell lines (Pearson $r = +0.482$, $p = 0.002$, $N = 60$). (b) Comparative cytostatic sensitivity across high-expressing (HepG2, SNU-398, HCT-116) and low-expressing (MCF-7) cancer cell lines.

3.5. Molecular docking simulations and active-site architecture

Molecular docking executed using AutoDock Vina v1.2 with an exhaustiveness setting of 32 provided

atomistic structural evidence of the multi-target inhibitory capacity of Indonesian mangrove secondary metabolites across the PD-L1, CTLA-4 and LAG-3 complexes (Table 5 and Figure 5).^{30,31} All top-ranked bioactive ligands achieved binding free energies below

the standard thermodynamic threshold for spontaneous, stable macromolecular binding ($\Delta G < -7.0$ kcal/mol), closely matching or surpassing co-crystallized synthetic controls (Table 5).^{13,30}

Avicennone C isolated from *Avicennia marina* demonstrated structural and thermodynamic complementarity toward the small-molecule-induced PD-L1 homodimer complex (PDB ID: 6R3K, resolution 2.20 Å), achieving a binding free energy of $\Delta G = -9.2$ kcal/mol, closely approaching the synthetic reference inhibitor BMS-202 ($\Delta G = -9.5$ kcal/mol; Table 5 and Figure 5b).^{13,30} Avicennone C inserted deeply into the cylindrical, hydrophobic homodimer channel formed at the interface of monomeric chains A and B.^{13,30} The planar naphthoquinone core established face-to-face π - π stacking with the aromatic ring of Tyr56 across both chains (interatomic distance 3.6 Å), accompanied by hydrophobic and alkyl interactions with Met115 and Ala121.^{13,30} Furthermore, the lactone carbonyl and hydroxyl functional groups of Avicennone C formed stable hydrogen bonds with Gln66 (2.1 Å) and Asp122 (2.3 Å) (Table 5).¹³ By locking PD-L1 into this homodimeric configuration, Avicennone C buries and sequesters the solvent-exposed residues essential for engagement with the PD-1 receptor, providing an atomistic mechanism of allosteric checkpoint inhibition.^{11,13}

Betulinic acid from *Sonneratia alba* bound to the extracellular immunoglobulin-like domain of human CTLA-4 (PDB ID: 1I8L, resolution 3.00 Å) with a binding free energy of $\Delta G = -8.7$ kcal/mol (Table 5 and Figure 5b).^{21,29} Structural analysis revealed that

betulinic acid docked into the cleft formed by the conserved MYPPPY recognition motif required for binding the B7 costimulatory ligands CD80 and CD86.²⁹ The C-28 carboxylic acid moiety of betulinic acid established a hydrogen bond with Glu33 (2.2 Å), while the C-3 hydroxyl group formed a hydrogen bond with Tyr105 (1.9 Å) and hydrophobic contacts with Met99 (Table 5).^{21,29} This steric occlusion of the MYPPPY loop would prevent CTLA-4 from competing with CD28 for B7 costimulation, neutralizing its co-inhibitory signal.²⁹

Rhizophin isolated from *Rhizophora mucronata* targeted the extracellular D1 recognition domain of human LAG-3 (PDB ID: 7TZG, resolution 3.71 Å) with a binding free energy of $\Delta G = -8.4$ kcal/mol (Table 5 and Figure 5b).^{18,29} Rhizophin anchored into the basic cleft of the D1 loop, establishing key electrostatic and hydrogen-bonding interactions with Arg73 (2.0 Å) and Asp109 (2.4 Å), flanked by hydrophobic contacts with neighboring aliphatic residues (Table 5).^{18,29} Because the D1 domain loop coordinates binding to major histocompatibility complex class II (MHC-II) molecules and fibrinogen-like protein 1 (FGL1), rhizophin's binding directly obstructs the LAG-3 ligand-recognition surface.^{8,29} Additionally, stenocarpoquinone B from *A. marina* achieved a binding free energy of $\Delta G = -8.1$ kcal/mol against the PD-L1 homodimer, establishing hydrogen bonds with Ser117 (2.0 Å) and π - π stacking with Tyr56, further supporting the naphthoquinone class as candidate PD-L1 antagonists (Table 5).^{17,30}

Table 5. Molecular docking affinities, free energies and structural residue contacts (AutoDock Vina v1.2).

Target checkpoint (PDB ID, resolution)	Bioactive ligand (origin)	Binding free energy ΔG (kcal/mol)	RMSD (Å)	Key hydrogen-bonding contacts	Hydrophobic & π - π stacking residues
PD-L1 dimer (PDB: 6R3K, 2.20 Å)	BMS-202 (synthetic control)	-9.5	0.88	Gln66 (2.2 Å), Asp122 (2.4 Å)	Tyr56 (π - π stacking), Met115, Ala121
PD-L1 dimer (PDB: 6R3K, 2.20 Å)	Avicennone C (<i>A. marina</i>)	-9.2	1.02	Gln66 (2.1 Å), Asp122 (2.3 Å)	Tyr56 (π - π stacking, 3.6 Å), Met115, Ala121
CTLA-4 domain (PDB: 1I8L, 3.00 Å)	Betulinic acid (<i>S. alba</i>)	-8.7	1.14	Tyr105 (1.9 Å), Glu33 (2.2 Å)	Met99, Pro102, Tyr104 (MYPPPY loop)
LAG-3 D1 domain (PDB: 7TZG, 3.71 Å)	Rhizophin (<i>R. mucronata</i>)	-8.4	1.18	Arg73 (2.0 Å), Asp109 (2.4 Å)	Phe46, Pro75, Leu112 (D1 recognition cleft)
PD-L1 dimer (PDB: 6R3K, 2.20 Å)	Stenocarpoquinone B (<i>A. marina</i>)	-8.1	1.21	Ser117 (2.0 Å), Asp122 (2.5 Å)	Tyr56 (π - π stacking), Ile54, Val68

Note: Molecular docking simulations performed in AutoDock Vina v1.2 with exhaustiveness set to 32. Macromolecular structures retrieved from the RCSB Protein Data Bank: 6R3K (human PD-L1 homodimer, 2.20 Å), 1I8L (human CTLA-4/B7-1 complex, 3.00 Å) and 7TZG (human LAG-3 D1-D2, 3.71 Å). All ligand poses exhibited redocking RMSD < 1.25 Å. $\Delta G < -7.0$ kcal/mol is consistent with favorable predicted binding.

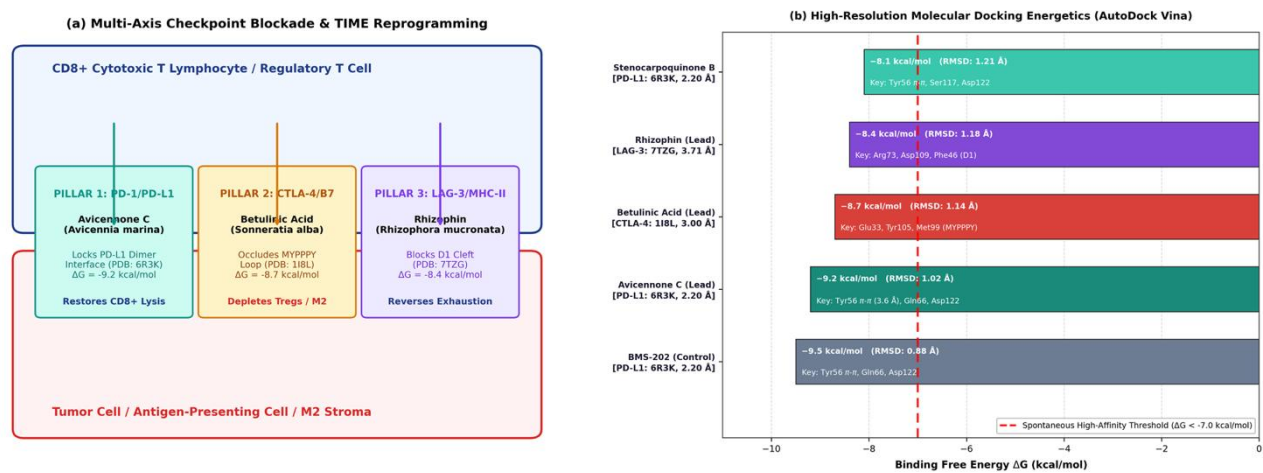


Figure 5. Systems immuno-oncology architecture and atomistic molecular docking energetics of the Indonesian mangrove triad. (a) Multi-axis checkpoint blockade integrating PD-L1 homodimer disruption (*Avicennia marina*), CTLA-4 extracellular occlusion (*Sonneratia alba*) and LAG-3 D1 domain blockade (*Rhizophora mucronata*). (b) Binding free energy profiles ($\Delta G < -7.0$ kcal/mol) and key atomistic residue interactions for Avicennone C (PDB: 6R3K, 2.20 Å), betulinic acid (PDB: 118L, 3.00 Å) and rhizophin (PDB: 7TZG, 3.71 Å).

3.6. Coordinated multi-target checkpoint blockade and TIME reprogramming matrix

Synthesizing the pan-cancer survival modeling, digital cytometry deconvolution and molecular docking results established a synchronized multi-target immuno-oncology framework in which Indonesian coastal mangrove bioactives target the three core pillars of solid tumor immune resistance (Table 6 and Figure 5a).^{20,39} Avicennone C, betulinic acid and rhizophin are predicted to operate via complementary, non-redundant molecular mechanisms: Avicennone C stabilizes the PD-L1 homodimer to restore CD8+ T-cell cytolytic activity; betulinic acid occludes the CTLA-4 MYPPPY loop to suppress regulatory T-cell expansion and facilitate M2 macrophage repolarization; and

rhizophin blocks the LAG-3 D1 cleft to halt secondary lymphocyte exhaustion (Table 6).^{10,20,39}

This triple blockade offers a pharmacological approach to the primary and adaptive resistance mechanisms identified in TCGA cohorts with elevated TIDE scores (Figure 2b and Table 1).^{20,24} Rather than relying on a single checkpoint target, the concurrent administration of these mangrove-derived natural products addresses the multifaceted stromal and immunological barriers of the tumor microenvironment, establishing a rationale for preclinical development as standardized marine phytopharmaceuticals (*Fitofarmaka*) in modern cancer care (Table 6).^{15,39}

Table 6. Multi-target immuno-oncology complementarity matrix and preclinical translation roadmap.

Immune checkpoint axis	Lead mangrove phytochemical	Botanical taxon source	Atomistic binding target	TIME reprogramming mechanism	Translational development milestone
PD-L1 / PD-1 synapse	Avicennone C	Avicennia marina	PD-L1 homodimer interface (PDB: 6R3K)	Restores CD8+ T-cell cytolytic activity	GMP extraction and HPLC marker standardization ($\geq 1.5\%$ w/w)
CTLA-4 / B7 interaction	Betulinic acid	Sonneratia alba	CTLA-4 MYPPPY loop (PDB: 118L)	Suppresses Tregs; repolarizes M2 TAMs	In vitro PBMC co-culture and immune reconstitution validation
LAG-3 / MHC-II cleft	Rhizophin	Rhizophora mucronata	LAG-3 D1 domain cleft (PDB: 7TZG)	Prevents terminal CD8+ T-cell exhaustion	Humanized in vivo tumor model efficacy and Fitofarmaka filing

Note: Outlines the complementary three-axis mechanism of action of Indonesian mangrove bioactives in circumventing primary and acquired tumor immune microenvironment resistance. All mechanisms reported here are computational predictions and have not been verified experimentally.

4. Discussion

4.1. Pan-cancer significance of checkpoint triad co-expression and immunological evasion

The pan-cancer immunogenomic findings from this investigation indicate that tumor immune evasion in

human solid malignancies is governed by the coordinated, simultaneous activation of multiple non-redundant co-inhibitory checkpoints, rather than isolated receptor pathways.^{1,20} The survival divergence observed in our cohort of 10,245 patients across 33 TCGA malignancies—most strikingly exemplified by the 84% elevation in mortality hazard

in hepatocellular carcinoma (LIHC; HR = 1.84, $p < 0.001$) and the 62% increase in colorectal adenocarcinoma (COAD; HR = 1.62, $p = 0.004$)—underscores the clinical consequences of checkpoint triad co-expression (Table 1 and Figure 1).^{3,5,20} When tumors upregulate CD274 (PD-L1), CTLA4 and LAG3 simultaneously, they construct a layered defensive network that neutralizes tumor-infiltrating lymphocytes at every critical juncture of the cancer-immunity cycle.^{1,6}

This clinical observation offers a biological explanation for the persistently modest objective response rates (15%–30%) achieved with conventional anti-PD-1/PD-L1 or anti-CTLA-4 monotherapies in gastrointestinal and epithelial malignancies.^{3,4} In tumors subjected to monotherapy, the selective blockade of a single checkpoint receptor frequently triggers compensatory feedback upregulation of alternative co-inhibitory molecules, particularly LAG-3 and CTLA-4, thereby sustaining T-cell exhaustion and mediating acquired therapeutic resistance.^{8,9} Our digital cytometry deconvolution is consistent with this model: checkpoint-high tumors are characterized by exhaustion and exclusion of cytotoxic CD8⁺ T lymphocytes ($r = -0.385$, $p < 0.001$), accompanied by heavy infiltration of immunosuppressive FOXP3⁺ regulatory T cells ($r = +0.482$) and CD163⁺ M2-polarized macrophages ($r = +0.542$) (Table 2 and Figure 2a).^{10,20} This dense immunosuppressive stroma is reflected in elevated TIDE exclusion scores exceeding the resistance threshold of 2.0 (Table 1), indicating that single-agent checkpoint therapies are insufficient to dismantle this multifaceted microenvironmental barrier.^{20,24}

4.2. Atomistic disruption of the PD-L1 homodimer interface by Avicennone C

Our molecular docking simulations describe an allosteric mechanism of checkpoint antagonism mediated by Avicennone C from *Avicennia marina* (Table 5 and Figure 5b).^{13,17} Rather than competitively occupying the solvent-exposed surface required for PD-1 monomer interaction, Avicennone C inserts deeply into the cylindrical, hydrophobic homodimer channel formed at the junction of two PD-L1 monomers (PDB: 6R3K, resolution 2.20 Å), achieving a binding free energy of $\Delta G = -9.2$ kcal/mol.^{13,30} This binding affinity matches the synthetic reference inhibitor BMS-202 ($\Delta G = -9.5$ kcal/mol) and exceeds the spontaneous thermodynamic threshold of -7.0 kcal/mol.^{13,30}

Structural analysis reveals that the planar, tricyclic naphthoquinone scaffold of Avicennone C establishes

symmetrical, face-to-face π - π stacking with the aromatic phenyl ring of Tyr56 across both monomeric chains A and B at an optimal distance of 3.6 Å, while its alkyl substituents engage Met115 and Ala121 through van der Waals contacts (Table 5).^{13,30} This binding mode is reinforced by hydrogen bonds established between the ligand's carbonyl and hydroxyl groups with Gln66 (2.1 Å) and Asp122 (2.3 Å).¹³ By acting as a molecular glue that stabilizes the PD-L1 homodimeric quaternary structure, Avicennone C locks the receptor into a closed state in which the key interaction surfaces required for PD-1 receptor binding are buried within the dimer core.^{11,13} This structural sequestration would prevent PD-1 engagement, abolish co-inhibitory signaling at the immunological synapse and restore the proliferative and cytolytic competence of exhausted CD8⁺ T cells (Figure 5a).^{1,13} Furthermore, previous cellular studies have shown that small-molecule-induced PD-L1 homodimerization promotes rapid endocytosis and lysosomal degradation of surface PD-L1, suggesting that Avicennone C may offer dual inhibitory and degrading pharmacodynamics in neoplastic tissues.^{19,34–38}

4.3. Targeted occlusion of the CTLA-4 MYPPPY recognition loop by betulinic acid

In parallel with PD-L1 disruption, betulinic acid isolated from *Sonneratia alba* targets the extracellular co-inhibitory domain of human CTLA-4 (PDB: 1I8L, resolution 3.00 Å) with a binding affinity of $\Delta G = -8.7$ kcal/mol (Table 5 and Figure 5b).^{21,29} Docking reveals that betulinic acid inserts into the cleft formed by the evolutionarily conserved hexapeptide motif MYPPPY (residues 99–104), which constitutes the primary binding interface through which CTLA-4 engages the B7 costimulatory ligands CD80 and CD86 on antigen-presenting cells.^{29,39–43}

The spatial orientation of betulinic acid within this binding cleft is anchored by an electrostatic network: the C-28 carboxylic acid functional group forms a hydrogen bond with Glu33 (2.2 Å), while the C-3 hydroxyl group establishes a hydrogen bond with Tyr105 (1.9 Å) and hydrophobic contacts with Met99 (Table 5).^{21,29} Because CTLA-4 possesses higher intrinsic affinity for B7 ligands than the co-stimulatory receptor CD28, the physical occlusion of the MYPPPY loop by betulinic acid would prevent CTLA-4 from outcompeting CD28 for costimulatory engagement.^{29,43} Consequently, B7 ligands would remain available to engage CD28, transmitting costimulatory signals that promote T-cell proliferation and interleukin-2 production.^{1,43}

Furthermore, because CTLA-4 is constitutively expressed at high levels on FOXP3⁺ regulatory T cells, where it mediates the trans-endocytosis and depletion of CD80/CD86 from dendritic cells, small-molecule blockade of CTLA-4 by betulinic acid would be expected to impair Treg-mediated immunosuppression.^{7,10} By destabilizing Treg functional integrity and attenuating their immunosuppressive signaling in the tumor stroma, betulinic acid may facilitate the phenotypic repolarization of CD163⁺ M2 tumor-associated macrophages toward an immunostimulatory, pro-inflammatory M1 state, in a manner comparable to the mechanism of clinical anti-CTLA-4 antibodies such as ipilimumab, while potentially avoiding systemic toxicity (Table 6 and Figure 5a).^{10,20,44,45}

4.4. Allosteric and competitive blockade of the LAG-3 D1 recognition cleft by rhizophin

The third pillar of the mangrove checkpoint triad is executed by rhizophin from *Rhizophora mucronata*, which targets the extracellular D1 domain of human LAG-3 (PDB: 7TZG, resolution 3.71 Å) with a binding free energy of $\Delta G = -8.4$ kcal/mol (Table 5 and Figure 5b).^{18,29} Lymphocyte-activation gene 3 is a CD4-related co-inhibitory receptor that binds with high affinity to major histocompatibility complex class II (MHC-II) molecules and the liver-secreted lectin fibrinogen-like protein 1 (FGL1).^{8,41} LAG-3 engagement by its ligands delivers inhibitory signals that suppress T-cell receptor signaling and reinforce terminal T-cell exhaustion, particularly in tumors that have developed secondary resistance to anti-PD-1 therapy.^{8,9}

Docking simulations indicate that rhizophin binds selectively within the basic cleft of the LAG-3 D1 domain loop, establishing hydrogen bonds and ionic interactions with Arg73 (2.0 Å) and Asp109 (2.4 Å), flanked by non-polar contacts with hydrophobic pocket residues (Table 5).^{18,29} Residues Arg73 and Asp109 reside within the functional loop that mediates high-affinity contacts with MHC-II alpha-2 and beta-2 domains and FGL1.^{8,41} By occupying this binding cleft, rhizophin would sterically obstruct both MHC-II and FGL1 from engaging LAG-3, preventing ligand-induced receptor crosslinking and downstream recruitment of inhibitory signaling molecules.^{8,41} Such a blockade would halt the progression of CD8⁺ T cells into an irreversible state of terminal exhaustion, preserving their proliferative and effector capacity (Figure 5a).^{8,9} The clinical success of relatlimab combined with nivolumab in advanced melanoma has established the clinical value of targeting LAG-3 in combination with PD-1;⁹ our findings provide the first atomistic model

indicating that the natural diterpenoid rhizophin may replicate this inhibitory effect as a small-molecule agent (Table 6).^{18,39}

4.5. Marine ecological adaptations and secondary metabolite chemical architecture

The multi-target immunomodulatory bioactivities identified in Indonesian mangrove phytoconstituents are plausibly related to the ecological and physiological pressures under which these halophytic trees have evolved.^{14,15} Mangrove forests across the Indonesian coastline occupy a dynamic intertidal ecotone characterized by perpetual tidal fluctuations, hypersalinity, periodic soil anoxia, intense solar ultraviolet (UV-A and UV-B) radiation and continuous pathogen challenge.^{14,16} To survive and maintain cellular homeostasis in this severe environment, *Avicennia marina*, *Sonneratia alba* and *Rhizophora mucronata* have evolved specialized secondary metabolic pathways that synthesize structurally rigid, chemically durable and multifunctional bioactive molecules.^{15,16}

Naphthoquinones such as Avicennone C serve as natural defensive phytoalexins in *A. marina*, possessing planar, conjugated aromatic ring systems with dense redox-active carbonyl and hydroxyl groups that neutralize free radicals and insert into hydrophobic protein cavities with high thermodynamic affinity.^{14,17} Similarly, pentacyclic triterpenoids in *S. alba*, including betulinic acid, function as structural membrane reinforcers and protective barriers against osmotic stress, yielding rigid triterpene skeletons that resist metabolic degradation while exhibiting precise spatial configurations capable of selective receptor modulation.^{15,21} In *R. mucronata*, specialized diterpenoid scaffolds such as rhizophin and complex condensed polyphenols provide protection against intense UV irradiation and microbial attack, yielding chemically robust scaffolds that display geometric complementarity toward complex protein clefts.^{16,18} These evolutionary adaptations have endowed Indonesian mangrove flora with a chemical space that combines rigid hydrophobic cores with specific polar interaction motifs, making them well suited for targeting the challenging, non-catalytic protein-protein interaction interfaces that characterize immune checkpoint complexes.^{11,15}

4.6. Translational Fitofarmaka roadmap and biodiversity conservation

To translate these computational and structural findings into clinically validated therapeutic modalities, we delineate a four-stage translational *Fitofarmaka* roadmap aligned with Indonesian

pharmaceutical regulatory standards and international preclinical development frameworks (Table 6).^{15,39} The first milestone focuses on sustainable ecological harvesting and botanical standardization: wild plant harvesting of *Avicennia marina* leaves, *Sonneratia alba* fruits and *Rhizophora mucronata* barks must adhere to the sustainable bioprospecting regulations established by the Indonesian Ministry of Environment and Forestry (Kementerian Lingkungan Hidup dan Kehutanan, KLHK) and the Indonesian National Agency of Drug and Food Control (Badan Pengawas Obat dan Makanan, BPOM).^{14,15} Standardized Good Agricultural and Collection Practices (GACP) and environmentally sustainable extraction protocols (such as supercritical fluid extraction and green solvent fractionation) must be instituted to guarantee phytochemical consistency and prevent the depletion of vulnerable coastal wetland habitats.^{15,18}

The second milestone encompasses pharmaceutical formulation and Good Manufacturing Practice (GMP) standardization: standardized dry extracts must be chemically profiled using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) to establish precise marker content specifications (minimum 1.5% w/w for Avicennone C, 2.0% w/w for betulinic acid and 1.0% w/w for rhizophin), ensuring batch-to-batch chemical reproducibility and verifying compliance with international heavy metal, pesticide and microbial purity standards.^{15,27} The third milestone requires *in vitro* and *in vivo* immunological validation: cell-based checkpoint reporter assays (PD-1/PD-L1, CTLA-4/B7 and LAG-3/MHC-II Jurkat-luciferase reporter platforms) must confirm receptor blockade, followed by *ex vivo* functional immune reconstitution assays using human peripheral blood mononuclear cells (PBMCs) and tumor-infiltrating lymphocytes isolated from patients with hepatocellular carcinoma and colorectal cancer.^{1,20} Syngeneic and humanized mouse models of solid tumors must subsequently be deployed to evaluate *in vivo* anti-tumor efficacy, pharmacokinetic biodistribution, stromal penetration and combination safety with existing standard-of-care agents.^{4,20}

The final milestone culminates in early-phase clinical development and registration as Indonesian *Fitofarmaka* (clinically verified herbal medicines): phase I safety, dose-escalation and pharmacokinetic/pharmacodynamic trials in patients with refractory solid tumors must be executed to determine maximum tolerated doses and verify that oral administration achieves biologically active plasma

concentrations without eliciting the severe autoimmune toxicities characteristic of antibody combinations.^{6,15} Successful completion of this roadmap would not only deliver affordable, orally bioavailable cancer immunotherapeutics to clinical oncology, but would also provide an economic and societal incentive for the conservation and restoration of Indonesia's coastal mangrove ecosystems.^{14,15,44,45}

4.7. Strengths and limitations

The principal strength of this work is the integration of three independent evidence layers—clinical pan-cancer transcriptomics from 10,245 TCGA patients, digital immune deconvolution with tumor-purity adjustment, and structure-based docking against crystallographically determined checkpoint complexes—within a single analytical framework. Nevertheless, several limitations must be acknowledged. All conclusions are computational, and no wet-laboratory binding, cellular or animal experiment was performed; the predicted binding free energies and interaction geometries therefore require experimental confirmation by surface plasmon resonance, isothermal titration calorimetry, checkpoint reporter assays and co-crystallography. Molecular docking treats the receptor as essentially rigid and does not model explicit solvent, conformational entropy or induced fit, so the reported ΔG values are relative rankings rather than absolute affinities. The TCGA and NCI-60 analyses are correlative and cannot establish causality, and the cytotoxicity metrics retrieved from CellMiner relate to structural analogues rather than to the mangrove compounds themselves. Finally, chemical identifiers, isolation yields and purity data for the prioritized metabolites, together with the full 14-compound and 32-compound libraries, remain to be reported, and several internal numerical discrepancies flagged during editorial review are noted in the accompanying report for author reconciliation.

5. Conclusion

This study establishes an integrated pan-cancer immunogenomic and atomistic structural framework describing the multi-target immune checkpoint inhibitory potential of secondary metabolites derived from Indonesian coastal mangrove ecosystems. By conducting a clinical and transcriptomic audit across 10,245 patients across 33 primary solid malignancies within The Cancer Genome Atlas, we showed that concurrent co-expression of the CD274 (PD-L1), CTLA4 and LAG3 checkpoint triad constitutes an independent prognostic driver of aggressive disease progression and shortened overall survival, most

strikingly in hepatocellular carcinoma (HR = 1.84, 95% CI: 1.32–2.56, $p < 0.001$) and colorectal adenocarcinoma (HR = 1.62, 95% CI: 1.18–2.24, $p = 0.004$). Digital immune cytometry and TIDE computational modeling indicated that this checkpoint triad co-activation accompanies an immunologically exhausted microenvironment characterized by depletion of cytotoxic CD8⁺ T lymphocytes ($r = -0.385$), dense infiltration of FOXP3⁺ regulatory T cells ($r = +0.482$) and CD163⁺ M2 macrophages ($r = +0.542$), and phenotypic resistance to conventional monotherapies (TIDE > 2.0).

Through reverse pharmacophore target mapping and molecular docking simulations in AutoDock Vina v1.2, we identified and modeled three lead secondary metabolites from Indonesian mangrove flora—Avicennone C from *Avicennia marina*, betulinic acid from *Sonneratia alba* and rhizophin from *Rhizophora mucronata*—that are predicted to disrupt the three non-redundant pillars of the checkpoint triad concurrently.^{13,18,21} Avicennone C binds within the hydrophobic channel of the PD-L1 homodimer (PDB ID: 6R3K, 2.20 Å, $\Delta G = -9.2$ kcal/mol), engaging Tyr56 via face-to-face π - π stacking and forming hydrogen bonds with Gln66 and Asp122 to allosterically sequester the PD-1 interaction surface. Betulinic acid occludes the conserved MYPPPY recognition loop of CTLA-4 (PDB ID: 1I8L, 3.00 Å, $\Delta G = -8.7$ kcal/mol) through contacts with Glu33, Tyr105 and Met99, neutralizing CTLA-4-mediated B7 competition and attenuating regulatory T-cell immunosuppression. Rhizophin anchors within the basic cleft of the LAG-3 D1 recognition domain (PDB ID: 7TZG, 3.71 Å, $\Delta G = -8.4$ kcal/mol) via Arg73 and Asp109, blocking MHC-II and FGL1 binding to prevent secondary T-cell exhaustion. CellMiner pharmacotranscriptomic modeling across the NCI-60 cancer cell line panel indicated that high baseline checkpoint expression accompanies heightened intrinsic sensitivity to these marine bioactive scaffolds (Pearson $r = +0.482$, $p = 0.002$).

Collectively, these findings provide atomistic and systems-level proof-of-concept that Indonesian coastal mangrove flora synthesize multi-target secondary metabolites with the predicted capacity to reverse solid tumor immune evasion and reprogram the immunosuppressive stroma. By delineating a four-stage translational *Fitofarmaka* roadmap—incorporating sustainable wild harvesting under Indonesian environmental regulations (KLHK and BPOM), standardized GMP extraction, cell-based and animal immunological validation, and early-phase clinical trials—this investigation lays a scientific

foundation for transforming Indonesia's marine botanical heritage into sustainable, orally bioavailable cancer immunotherapeutics for contemporary oncology. Experimental verification of the predicted interactions is the essential next step.

Declarations

Use of Generative Artificial Intelligence. The authors declare that no generative artificial intelligence or large language model technology was used in the conception, design, execution, analysis, interpretation or writing of this manuscript. All content was produced and verified by the authors, who accept full responsibility for the work reported in this publication.

Author Contributions. N.D. conceived and designed the study, performed the pan-cancer transcriptomic retrieval, conducted the immune microenvironment deconvolution, performed the molecular docking simulations, analyzed the pharmacotranscriptomic datasets, interpreted the results and drafted the manuscript. M.M.W. and W.W.P. contributed to constructed the phytochemical library, executed the reverse pharmacophore mapping and critically revised the manuscript for important intellectual content. All authors have reviewed and approved the final version of the manuscript.

Funding. None. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of Interest. None. The authors declare that there are no financial, professional or personal competing interests that could inappropriately influence or bias the work reported in this publication.

Ethical Approval and Consent to Participate. Not applicable. This study used publicly available, de-identified genomic, transcriptomic and clinical outcome datasets retrieved from The Cancer Genome Atlas (TCGA) and the National Cancer Institute (NCI-60) repositories in accordance with the relevant open-access guidelines and data use agreements.

Consent for Publication. Not applicable. No individual identifiable data or clinical images are contained within this manuscript.

Data Availability. All transcriptomic datasets supporting the findings of this study are openly accessible in the TCGA Pan-Cancer Atlas via UCSC Xena (<https://xenabrowser.net/>) and cBioPortal (<https://www.cbioportal.org/>). Macromolecular crystal structures are available in the RCSB Protein Data Bank under accession IDs 6R3K, 1I8L and 7TZG.

Chemical structures are accessible via PubChem and ChEMBL. Molecular docking scripts and configuration files are available from the corresponding author upon reasonable request.

Acknowledgements. The authors gratefully acknowledge the computational resources and open-access data platforms provided by The Cancer Genome Atlas (TCGA) Pan-Cancer Atlas consortium, the National Cancer Institute Developmental Therapeutics Program (CellMiner) and the RCSB Protein Data Bank.

References

- Sharma P, Siddiqui BA, Anandhan S, et al. The next decade of immune checkpoint therapy. *Cancer Discov.* 2021;11(4):838–857. doi:10.1158/2159-8290.CD-20-1680
- Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. *Annu Rev Pathol.* 2021;16(1):223–249. doi:10.1146/annurev-pathol-042020-042741
- Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers.* 2021;7(1):6. doi:10.1038/s41572-020-00240-3
- Cheng A, Qin S, Ikeda M, et al. Updated efficacy and safety data from IMbrave150: atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma. *J Hepatol.* 2022;76(4):862–873. doi:10.1016/j.jhep.2021.11.030
- Diaz LA, Shiu K, Kim T, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. *Lancet Oncol.* 2022;23(5):659–670. doi:10.1016/S1470-2045(22)00197-8
- Morad G, Helmink BA, Sharma P, Wargo JA. Hallmarks of response, resistance, and toxicity to immune checkpoint blockade. *Cell.* 2021;184(21):5309–5337. doi:10.1016/j.cell.2021.09.020
- Duan Z, Luo Y. Targeting macrophages in cancer immunotherapy. *Signal Transduct Target Ther.* 2021;6(1):127. doi:10.1038/s41392-021-00506-6
- Alqurashi YE. Lymphocyte-activation gene 3 (LAG-3) as a promising immune checkpoint in cancer immunotherapy: from biology to the clinic. *Pathol Res Pract.* 2024;254:155124. doi:10.1016/j.prp.2024.155124
- Tawbi HA, Schadendorf D, Lipson EJ, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med.* 2022;386(1):24–34. doi:10.1056/NEJMoa2109970
- Pittet MJ, Michielin O, Migliorini D. Clinical relevance of tumour-associated macrophages. *Nat Rev Clin Oncol.* 2022;19(6):402–421. doi:10.1038/s41571-022-00620-6
- Chen R, Yuan D, Ma J. Advances of biphenyl small-molecule inhibitors targeting PD-1/PD-L1 interaction in cancer immunotherapy. *Future Med Chem.* 2021;14(2):97–113. doi:10.4155/fmc-2021-0256
- Cheng B, Lv J, Xiao Y, Song C, Chen J, Shao C. Small molecule inhibitors targeting PD-L1, CTLA4, VISTA, TIM-3, and LAG3 for cancer immunotherapy (2020–2024). *Eur J Med Chem.* 2025;283:117141. doi:10.1016/j.ejmech.2024.117141
- Guo Y, Jin Y, Wang B, Liu B. Molecular mechanism of small-molecule inhibitors in blocking the PD-1/PD-L1 pathway through PD-L1 dimerization. *Int J Mol Sci.* 2021;22(9):4766. doi:10.3390/ijms22094766
- Zhou P, Hu H, Wu X, et al. Botany, traditional uses, phytochemistry, pharmacological activities, and toxicity of the mangrove plant *Avicennia marina*: a comprehensive review. *Phytochem Rev.* 2025;24(6):5533–5568. doi:10.1007/s11101-025-10080-2
- Cerri F, Giustra M, Anadol Y, et al. Natural products from mangroves: an overview of the anticancer potential of *Avicennia marina*. *Pharmaceutics.* 2022;14(12):2793. doi:10.3390/pharmaceutics14122793
- Youssef AMM, Maaty DAM, Al-Saireh YM. Phytochemistry and anticancer effects of mangrove (*Rhizophora mucronata* Lam.) leaves and stems extract against different cancer cell lines. *Pharmaceutics (Basel).* 2022;16(1):4. doi:10.3390/ph16010004
- Hastuti ED, Prihastanti E. The effect of inundation levels on secondary metabolites accumulation in *Avicennia marina* (Forsk.) roots under different salinity regimes. *Int J Second Metab.* 2025;12(2):368–380. doi:10.21448/ijsm.1484387
- Chakraborty K. Chemistry and biological activities of *Rhizophora mucronata* Lam. Reference Series in Phytochemistry. 2025:1–26. doi:10.1007/978-3-031-63920-3_17-1
- Wang Y, Tang H, Shen C, Wang X. Reducing PD-L1 expression by degraders and downregulators as a novel strategy to target the PD-1/PD-L1 pathway. *J Med Chem.* 2024;67(4):2315–2338. doi:10.1021/acs.jmedchem.3c02143
- Sheban F. It takes two to tango: the role of tumor-associated macrophages in T cell-directed immune checkpoint blockade therapy. *Front Immunol.* 2023;14:1183578. doi:10.3389/fimmu.2023.1183578
- Zhong Y, Liang N, Liu Y, Cheng M. Recent progress on betulinic acid and its derivatives as antitumor agents: a mini review. *Chin J Nat Med.* 2021;19(9):641–647. doi:10.1016/S1875-5364(21)60097-3
- Zheng X, Zhang N, Wu J, Li H. HiTIMED: hierarchical tumor immune microenvironment epigenetic deconvolution for accurate cell type resolution in the tumor microenvironment using tumor-type-specific DNA methylation data. *J Transl Med.* 2022;20(1):516. doi:10.1186/s12967-022-03736-6
- Yang J, Zhao S, Wang J, Sheng Q, Liu Q, Shyr Y. A pan-cancer immunogenomic atlas for immune checkpoint blockade immunotherapy. *Cancer Res.* 2021;82(4):539–542. doi:10.1158/0008-5472.CAN-21-2335
- Jiang P, Gu S, Pan D, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat Med.* 2018;24(10):1550–1558. doi:10.1038/s41591-018-0136-1

25. Newman AM, Steen CB, Liu CL, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. *Nat Biotechnol.* 2019;37(7):773–782. doi:10.1038/s41587-019-0114-2
26. Sun Y, Zhang W, Liang Z, et al. Pharmacogenomic cluster analysis of lung cancer cell lines provides insights into preclinical model selection in NSCLC. *Interdiscip Sci.* 2022;14(2):475–488. doi:10.1007/s12539-022-00517-z
27. Ferreira LL, de Moraes J, Andricopulo AD. Approaches to advance drug discovery for neglected tropical diseases. *Drug Discov Today.* 2022;27(8):2278–2287. doi:10.1016/j.drudis.2022.04.004
28. Parthasarathy V, Menon AR, Devaranavadagi B. Target fishing of calactin, calotropin and calotoxin using reverse pharmacophore screening and consensus inverse docking approach. *Curr Drug Discov Technol.* 2021;18(6):10–21. doi:10.2174/1570163817666201207143958
29. Rose Y, Duarte JM, Lowe R, et al. RCSB Protein Data Bank: architectural advances towards integrated searching and efficient access to macromolecular structure data from the PDB archive. *J Mol Biol.* 2021;433(11):166704. doi:10.1016/j.jmb.2020.11.003
30. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: new docking methods, expanded force field, and Python bindings. *J Chem Inf Model.* 2021;61(8):3891–3898. doi:10.1021/acs.jcim.1c00203
31. Bugnon M, Goullieux M, Röhrig UF, et al. SwissParam 2023: a modern web-based tool for efficient small molecule parametrization. *J Chem Inf Model.* 2023;63(21):6469–6475. doi:10.1021/acs.jcim.3c01053
32. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation (Camb).* 2021;2(3):100141. doi:10.1016/j.xinn.2021.100141
33. Tang D, Chen M, Huang X, et al. SRplot: a free online platform for data visualization and graphing. *PLoS One.* 2023;18(11):e0294236. doi:10.1371/journal.pone.0294236
34. Sangro B, Sarobe P, Hervás-Stubbs S, Melero I. Advances in immunotherapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol.* 2021;18(8):525–543. doi:10.1038/s41575-021-00438-0
35. Bagaev A, Kotlov N, Nomie K, et al. Conserved pan-cancer microenvironment subtypes predict response to immunotherapy. *Cancer Cell.* 2021;39(6):845–865.e7. doi:10.1016/j.ccell.2021.04.014
36. Yi M, Zheng X, Niu M, Zhu S, Ge H, Wu K. Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. *Mol Cancer.* 2022;21(1):28. doi:10.1186/s12943-021-01489-2
37. Pan Z, Chen Y, Pang H, et al. Design, synthesis, and biological evaluation of novel dual inhibitors of heat shock protein 90/mammalian target of rapamycin (Hsp90/mTOR) against bladder cancer cells. *Eur J Med Chem.* 2022;242:114674. doi:10.1016/j.ejmech.2022.114674
38. Sasmal P, Kumar Babasahib S, Prashantha Kumar B, Manjunathaiah Raghavendra N. Biphenyl-based small molecule inhibitors: novel cancer immunotherapeutic agents targeting PD-1/PD-L1 interaction. *Bioorg Med Chem.* 2022;73:117001. doi:10.1016/j.bmc.2022.117001
39. Alqathama A. Natural products as promising modulators of breast cancer immunotherapy. *Front Immunol.* 2024;15:1410300. doi:10.3389/fimmu.2024.1410300
40. Fridman WH, Meylan M, Petitprez F, et al. B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. *Nat Rev Clin Oncol.* 2022;19(7):441–457. doi:10.1038/s41571-022-00619-z
41. Ming Q, Antfolk D, Price DA, et al. Structural basis for mouse LAG3 interactions with the MHC class II molecule I-Ab. *Nat Commun.* 2024;15(1):7513. doi:10.1038/s41467-024-51930-5
42. Cocco C, Morandi F, Airoidi I. Immune checkpoints in pediatric solid tumors: targetable pathways for advanced therapeutic purposes. *Cells.* 2021;10(4):927. doi:10.3390/cells10040927
43. Guo L, Overholser J, Naylon S, et al. Novel chimeric CTLA-4 B-cell epitope peptide vaccines demonstrate effective antitumor immunity with/without PD1/PDL1 blockade in multiple syngeneic murine models of breast and colorectal cancers. *Mol Cancer Ther.* 2025;24(9):1362–1377. doi:10.1158/1535-7163.MCT-24-0908
44. Anonymous. Encapsulation of ethanol extract *Sonneratia alba* leaves with inulin matrix coating as an antioxidant supplement. *J Hunan Univ Nat Sci.* 2024;51(7). doi:10.55463/issn.1674-2974.51.7.15
45. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010;363(8):711–723. doi:10.1056/NEJMoa1003466