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Original Article

EXPLORING PROTEIN TARGETS OF TRITERPENE SAPONINS ($C_{54}H_{86}O_{24}$): A BIOINFORMATICS APPROACH TO NOVEL THERAPEUTIC DEVELOPMENT

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ABSTRACT

Objective: The exploration of triterpene saponins as novel therapeutic agents is essential due to the rising prevalence of chronic diseases and limitations of current treatments. These compounds exhibit diverse biological activities, particularly anti-inflammatory and anticancer effects; however, their systematic identification and protein target interactions remain underexplored.

Methods: Triterpene saponin compounds were identified using the KNApSAcK database, prioritized via the ChEMBL database, and further analyzed using SuperPred to predict high-confidence protein interactions. A protein interaction matrix was constructed to map therapeutic targets and clinical indications. Furthermore, glycosidic linkage variations among the compounds were examined using a Structure–Activity Relationship (SAR) approach based on valence bond theory, providing a comprehensive bioinformatics-based framework for therapeutic potential assessment.

Results: Seven compounds classified as triterpene saponins ($C_{54}H_{86}O_{24}$) were selected. Structural variations were observed in sugar moieties, side chains, functional groups, stereochemistry, and glycosidic linkages, contributing to their chemical diversity and potential specificity in protein interactions.

Conclusion: Our study highlights the potential of triterpene saponins ($C_{54}H_{86}O_{24}$) as promising drug candidates targeting key proteins, including APEX1, TLR-4, and KinaseP110.

Keywords: Chronic diseases, Biological targets, Protein interactions

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INTRODUCTION

The increasing prevalence of chronic diseases and the limitations of current pharmacological treatments necessitate the exploration of novel therapeutic agents [1]. Among these, triterpene saponins have garnered attention for their diverse biological activities, including anti-inflammatory, anticancer, and immunomodulatory effects [2]. However, despite their potential, the systematic identification and characterization of these compounds remain underexplored [3], particularly in relation to their interactions with specific biological targets [4, 5].

Previous research has made strides in identifying various bioactive compounds and their therapeutic applications [6, 7]. For instance, studies have utilized databases such as KNApSAcK and ChEMBL to catalog natural products and predict their biological activities. These efforts have highlighted the importance of triterpene saponins in traditional medicine and their potential as modern therapeutics [8]. However, many existing studies focus on isolated compounds without thoroughly investigating the broader spectrum of triterpene saponins or their specific interactions with target proteins [2, 7].

Our study systematically explores the structural diversity of triterpene saponins and their interactions with key protein targets, thereby providing a foundation for future research into their therapeutic applications.

MATERIALS AND METHODS

Compound identification and selection

The study started with the identification of triterpene saponins using the KNApSAcK database (http://www.knapsackfamily.com/knapsack_core/top.php). For further investigation, we prioritized the chemical $C_{54}H_{86}O_{24}$ in the ChEMBL database (https://www.ebi.ac.uk/chembl).

Protein target prediction

A total of seven compounds classified as triterpene saponins ($C_{54}H_{86}O_{24}$) were identified. The canonical Simplified Molecular Input Line Entry System (SMILES) data obtained from the derivatives were investigated using SuperPred (https://prediction.charite.de/subpages/target_prediction.php) to predict interactions with proteins cataloged in the database [9]. Proteins with probability scores and model accuracy above 80% were shortlisted, where the probability represents the likelihood that the input structure binds to a specific target based on a trained machine learning model.

Development of protein interaction matrix

A protein interaction matrix was developed to summarize the therapeutic potential, including target proteins and clinical indications, while mapping the probability and accuracy performance across all compounds.

Structure–activity relationship (SAR) and glycosidic linkage analysis

Following the development of the protein interaction matrix, we further analyzed the differences in glycosidic linkages among the triterpene saponin structures. This was conducted using a Structure-Activity Relationship (SAR) approach based on valence bond theory, aiming to evaluate how variations in stereochemistry and glycosidic configuration might influence the predicted molecular interactions.

RESULTS AND DISCUSSION

Structural diversity of selected triterpene saponins from the ChEMBL database

Utilizing the ChEMBL database, we selected seven compounds classified as triterpene saponins ($C_{54}H_{86}O_{24}$). These compounds exhibit distinct chemical structures, characterized by variations in functional groups, side chains, sugars, stereochemistry, and glycosidic linkages (Fig. 1).

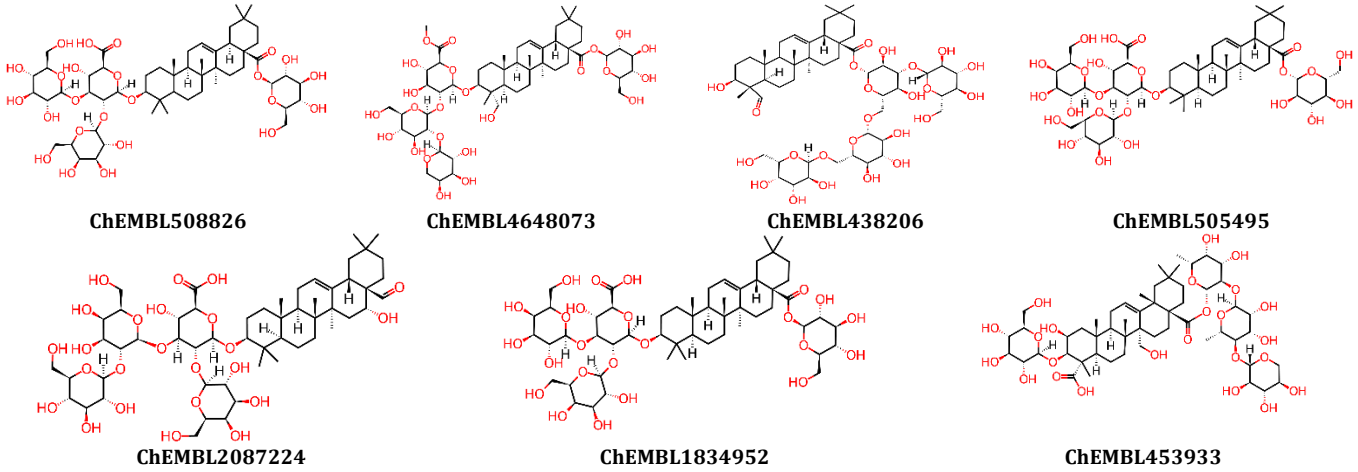

Fig. 1: Identification of triterpene saponins ($C_{54}H_{86}O_{24}$) in the ChEMBL database

Triterpenoid saponins such as ChEMBL-438206, ChEMBL453933, ChEMBL505495, and ChEMBL4648073 are classified as monodesmosidic compounds, as they exhibit glycosidic attachment exclusively at the C-3 position of the aglycone [10–12]. These compounds exhibit varying sugar chain complexity. ChEMBL438206 and ChEMBL453933 present linear β-O-glycosidic linkages, with the latter characterized by a saturated glycoside moiety and an unmodified aldehyde at C-28 [10]. In contrast, ChEMBL505495 and ChEMBL4648073 display more extensive glycosylation, with up to 12 oxygen atoms involved and evident branching, indicating increased hydrophilicity and potential for membrane interaction [13]. These monoglycosylated saponins are structurally simpler and potentially more stable, but may differ in biological targeting due to their lower valency [10, 11].

On the other hand, bides compounds like ChEMBL508826, ChEMBL1834952, and ChEMBL2087224 are categorized as bidesmosidic saponins due to the presence of glycosidic linkages at both the C-3 and C-28 positions of their triterpene cores [10]. This dual attachment results in a more complex valency profile, with sugar units often showing branching patterns. ChEMBL1834952 and ChEMBL508826 demonstrate similar configurations with elongated chains at C-3 and shorter glycosidic esters at C-28, forming amphipathic structures known to enhance bioactivity [10, 11]. ChEMBL2087224 further diversifies this group with a symmetric branching pattern, which may influence receptor binding specificity due to spatial distribution of hydrogen-bond donors and acceptors [14]. The overall oxygen atom count in these bidesmosidic saponins reflects not only the sugar content but also the complexity of functionalization around the core structure [15].

Predicted protein targets and therapeutic indications of triterpene saponins

TLR-4 shows broad potential as a target protein, tested for various clinical indications, including allergy, (type 1 diabetes) T1D, hepatitis B virus (HBV), melanoma, non-hodgkin lymphoma (NHL), prostate cancer, sepsis, and solid tumors. Probability predictions vary from 82% to 86%, while accuracy remains consistent at around 92%. For indications like melanoma and solid tumors, accuracy peaks at 97% and 99%, respectively. APEX1 emerges as a relevant target for indications such as glioma, melanoma, ocular cancer, and solid tumors. APEX1's probability predictions range from 94% to 99%, with consistent accuracy at 91%. CTX1 was evaluated exclusively for hypertension, with a probability prediction of 88% and exceptionally high accuracy of 99%. KinaseP110 is relevant solely for breast cancer, with a probability prediction of 88% and accuracy of 94% (Table 1).

Table 1: Probability and model accuracy scores of triterpene saponins on DNA-apurinic, toll-like receptor 4, cathepsin-D, and kinase P110

No.	Therapeutic approach		Probability (Prob.) and model accuracy (Acc.) scores (%)													
	Protein target	Clinical indication	ChEMBL 508826		ChEMBL 453933		ChEMBL 1834952		ChEMBL 505495		ChEMBL 438206		ChEMBL 2087224		ChEMBL 4648073	
			Prob.	Acc.	Prob.	Acc.	Prob.	Acc.	Prob.	Acc.	Prob.	Acc.	Prob.	Acc.	Prob.	Acc.
1.	APEX1	Glioma	97	91	99	91	97	91	97	91	98	91	94	91	94	91
2.	APEX1	Melanoma	97	91	99	91	97	91	97	91	98	91	94	91	94	91
3.	APEX1	Ocular cancer	97	91	99	91	97	91	97	91	98	91	94	91	94	91
4.	APEX1	Solid tumor	97	91	99	91	97	91	97	91	98	91	94	91	94	91
5.	TLR-4	Allergy	86	92	82	92	86	92	86	92	85	92	NI	NI	NI	NI
6.	TLR-4	T1D	86	92	82	92	86	92	86	92	85	92	NI	NI	NI	NI
7.	TLR-4	HBV	86	92	82	92	86	92	86	92	85	92	NI	NI	NI	NI
8.	TLR-4	Melanoma	86	92	82	92	86	92	86	92	85	97	NI	NI	NI	NI
9.	TLR-4	NHL	86	92	82	92	86	92	86	92	85	92	NI	NI	NI	NI
10.	TLR-4	Prostate cancer	86	92	82	92	86	92	86	92	85	92	NI	NI	NI	NI
11.	TLR-4	Sepsis	86	92	82	92	86	92	86	92	85	92	NI	NI	NI	NI
12.	TLR-4	Solid Tumor	86	92	82	92	86	92	86	92	85	99	NI	NI	NI	NI
13.	CTX1	Hypertension	88	99	88	99	88	89	88	99	NI	NI	NI	NI	NI	NI
14.	KinaseP110	Breast Cancer	NI	NI	NI	NI	NI	NI	NI	NI	84	94	81	94	88	94

Abbreviations: No Interaction (NI), DNA-apurinic (APEX1), Toll-like receptor 4 (TLR4), Cathepsin-D (CTX1), Type-1 Diabetes (TID), Hepatitis-B Virus (HBV), Non Hodgkin Lymphoma (NHL)

The variable activity of triterpene saponins ($C_{54}H_{86}O_{24}$) underscores their therapeutic potential across various conditions in developing drugs with specific therapeutic targets. APEX1's crucial role in regulating genes related to DNA repair and purine/pyrimidine metabolism presents new therapeutic opportunities. The multifaceted roles of TLR4 in inflammation, cancer, and immune

regulation highlight its potential as a therapeutic target in various pathological contexts. Additionally, targeting HER2 and P110β offers promising treatment options for aggressive breast cancers.

Biological dysfunctions associated with APEX1 have been linked to various types of cancer and neurodegenerative diseases [16]. In glioma, APEX1 plays a critical role in resistance to alkylating agents by repairing DNA at abasic sites, as demonstrated in studies on SNB19 glioma cells, which exhibited reduced resistance after APEX1 depletion using antisense oligonucleotides [17]. APEX1 also contributes to cell proliferation and migration in melanoma through the activation of transcription factors [18, 19]. In solid tumors, APEX1 polymorphisms influence DNA repair capabilities [19], while cytoplasmic APEX1 expression serves as a potential diagnostic biomarker for clear cell renal cell carcinoma (ccRCC) [20], hepatocellular carcinoma (HCC) [21], and distal cholangiocarcinoma (CC) [20], as well as a predictor of recurrence [20]. Furthermore, research on ocular cancer reveals that APEX1 regulates genes involved in DNA repair and purine/pyrimidine metabolism, offering new therapeutic avenues for ocular neovascularization treatment [22].

Toll-Like Receptor 4 (TLR4) plays a pivotal role in various pathological conditions. In allergies, TLR4 contributes to inflammation by recognizing specific allergens and influencing the severity of allergic rhinitis, making it a potential therapeutic target. In autoimmune diabetes, TLR4 signaling regulates CD4+T-cell activation and autoantigen proliferation, reducing insulitis and preventing diabetes onset [23]. For viral hepatitis, TLR4 expression modulates immune responses to HBV through cytokine production and PAMP recognition, impacting virus persistence and intrauterine transmission [24]. In melanoma, TLR4 activation promotes cell survival, while its inhibition hampers cancer progression [18, 25]. Similarly, in prostate cancer, TLR4 signaling alters tumor microenvironments, potentially driving growth and metastasis [26]. Although no direct link between TLR4 and non-Hodgkin lymphoma has been established, its expression is crucial for intratumoral therapies. In sepsis, blocking TLR4 protects against Jarisch-Herxheimer reactions, highlighting its role in managing antibiotic-induced shock [27, 28]. Additionally, in solid tumors like neuroblastoma, TLR4's interaction with pathways such as EGFR and cathepsin D expression influences chemoresistance and prognosis [29].

The P110 kinase subunit and cathepsin D exhibit significant roles in disease pathogenesis and prognosis. In breast cancer, P110α expression correlates with hormone receptor positivity but does not impact overall survival, while P110β expression is linked to worse outcomes due to associations with HER2 overexpression, younger onset, higher disease grades, lymph node involvement, and distant metastases [30]. Meanwhile, cathepsin D levels are positively associated with hypertension, a major cardiovascular risk factor [31], but current cross-sectional studies cannot confirm its role as a predictive marker for atherosclerosis [32].

Glycosidic linkage as a determinant of bioactivity

Triterpenoid glycosides exhibit strong binding potential to protein targets such as APEX1, a key enzyme in DNA repair and transcriptional regulation [33]. Their polar nature and complex glycosidic structures enhance target specificity [34]. Structural variations-especially glycosidic substitutions on ring A and D-significantly influence APEX1 affinity [22, 34].

ChEMBL508826 and ChEMBL453933 show high predicted interaction with APEX1, likely due to their polar and extended glycosidic chains [34]. ChEMBL438206 exhibits antitumor potential [33], while ChEMBL2087224 and ChEMBL4648073 display moderate affinity for TLR4 and PI3K/P110 [35], relevant to inflammation and breast cancer.

ChEMBL508826, ChEMBL1834952, and ChEMBL505495 are predicted to target TLR4, a receptor involved in melanoma [36], NHL [37], and prostate cancer. Their glycosidic patterns-three substitutions on ring A and one on ring D-differ from ChEMBL453933, possibly explaining their target specificity [22, 34].

ChEMBL4648073 shows the strongest interaction with Kinase P110 [33], attributed to two serially linked glycosides on ring A. This linear configuration likely enhances binding compared to compounds with parallel glycoside attachments [34].

Limitations and further research

Our study presents an integrated in silico prediction of potential protein targets for triterpene saponin ($C_{54}H_{86}O_{24}$). Nonetheless, it is important to recognize the methodological limitations. The use of SuperPred, although a robust tool for early hypothesis generation, is inherently limited by algorithmic bias due to its dependence on ligand similarity and curated databases [9, 38]. Moreover, it lacks the capacity to simulate the dynamic and conformational flexibility of protein-ligand interactions under physiological conditions [39–41]. Therefore, the current findings should be interpreted as predictive rather than conclusive.

Future studies should incorporate molecular docking simulations [22, 23] following in silico target prediction to model the binding orientation and affinity of drug candidates toward their protein targets [44, 45]. To confirm the binding specificity and kinetics, surface plasmon resonance (SPR) [46] or isothermal titration calorimetry (ITC) [47, 48] experiments are recommended. Additionally, cellular assays such as Western blotting, qPCR, or cytotoxicity tests are crucial to validate the biological impact of these compounds at the cellular level [49, 50].

CONCLUSION

Our study highlights the potential of triterpene saponins ($C_{54}H_{86}O_{24}$) as promising drug candidates targeting key proteins, including APEX1, TLR-4, and KinaseP110. APEX1 is implicated in the progression of several cancers, such as glioma, melanoma, ocular cancer, and solid tumors. TLR-4 plays a critical role in immune responses and infectious diseases, being associated with conditions like allergies, type 1 diabetes, hepatitis B virus (HBV) infection, as well as various cancers, including melanoma, non-Hodgkin lymphoma, and prostate cancer. Additionally, TLR-4 is involved in sepsis and solid tumors. KinaseP110 is linked to breast cancer, while CTX1 demonstrates potential in managing hypertension. These findings suggest that triterpene saponins could offer therapeutic benefits across a range of diseases. Therefore, these results serve as predictions that require further validation.

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AUTHORS CONTRIBUTIONS

Setiyo Budi Santoso; Conceptualization, methodology development, manuscript writing, clinical relevance analysis. Alfian Syarifuddin: Compound identification and selection, biological interpretation. Arief Kusuma Wardani: Structural analysis, chemical validation, annotation of side chains and glycosidic linkages. Vian Putri Widiastuti and May Fahtun Ninda: Cross-comparison of predicted targets therapeutic indications, data synthesis.

CONFLICT OF INTERESTS

Declared

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