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DISCOVERY OF POTENT COX-2 BINDING TRIAZOLO/TETRAZOLOQUINAZOLINE DERIVATIVES AS NOVEL VENOTONIC CANDIDATES: MOLECULAR DOCKING AND STRUCTURE-ACTIVITY RELATIONSHIPS

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Abstract. Chronic venous insufficiency (CVI) is a progressive pathological condition characterized by inadequate venous return of blood from the lower extremities. The pathophysiology involves venous hypertension, valvular insufficiency, and subsequent activation of an inflammatory cascade, leading to endothelial dysfunction, increased capillary permeability, and tissue hypoxia. Current therapeutic approaches remain limited, as existing venotonics demonstrate modest efficacy and require long-term use to achieve sustained clinical benefits. This study investigates novel triazolo/tetrazoloquinazoline derivatives as potential venotonic drug candidates using molecular docking assays against one of the key inflammatory targets implicated in the pathophysiology of CVI. Our computational approach reveals promising binding profiles for several compounds, particularly against cyclooxygenase-2 (COX-2), with binding affinities superior to or comparable to established reference compounds. These results provide a basis for further pharmacological and toxicological evaluations aimed at developing more effective and targeted therapies.

Introduction. The clinical manifestations of venous insufficiency (CVI) include leg pain, edema, skin changes, and in advanced cases, venous ulceration [1]. The progressive nature of the disease significantly impacts patient quality of life and

represents a considerable healthcare burden requiring effective therapeutic interventions. The correlation between cardiovascular activity and venotropic effects represents a fundamental relationship in cardiovascular physiology, where venotropic agents directly influence cardiac performance [2] through their actions on the venous system. Under steady-state conditions, venous return must equal cardiac output when averaged over time because the cardiovascular system is essentially a closed loop. This fundamental principle establishes that any venotropic intervention, that alters venous return will correspondingly affect cardiac output, creating a direct correlation between venous function and cardiovascular performance.

Current venotonic agents demonstrate varying degrees of clinical efficacy and distinct mechanisms of action. Flavonoid-based compounds represent one major class, with diosmin (Fig. 1) [3] being the most extensively studied member of this group. These compounds demonstrate moderate efficacy in reducing venous insufficiency symptoms through mechanisms involving capillary permeability reduction and anti-inflammatory effects.

Saponin-based venotonics, exemplified by horse chestnut seed extract, demonstrate superior direct effects through calcium-dependent venous smooth muscle contraction mechanisms. Escin (Fig. 1) exhibits dose-dependent contractile effects on isolated venous preparations, with sustained action and clear calcium-dependent mechanisms of action [4].

Comparative analysis reveals, that certain venotonic agents demonstrate superior therapeutic efficacy compared to conventional treatments. For, instance, pycnogenol pine, (*Pinus maritima*) bark extract, demonstrates enhanced therapeutic effects across multiple clinical domains [5]. Current research indicates, that its treatment produces significantly greater reductions in leg volume, ambulatory venous pressure, and clinical symptom scores compared to standard treatment regimens [6].

Pycnogenol's enhanced potency appears attributable to its multi-target mechanisms, including superior antioxidant capacity and anti-inflammatory activity. The extract demonstrates inhibition of cyclooxygenase-2 (COX-2) enzyme generation and reduction in matrix metalloproteinase-9 (MMP-9) release, providing comprehensive anti-inflammatory effects [7, 8]. Still its mixture of active compounds, where between 65% and 75% are procyanidins comprising of catechin and epicatechin subunits with varying chain lengths [9, 10].

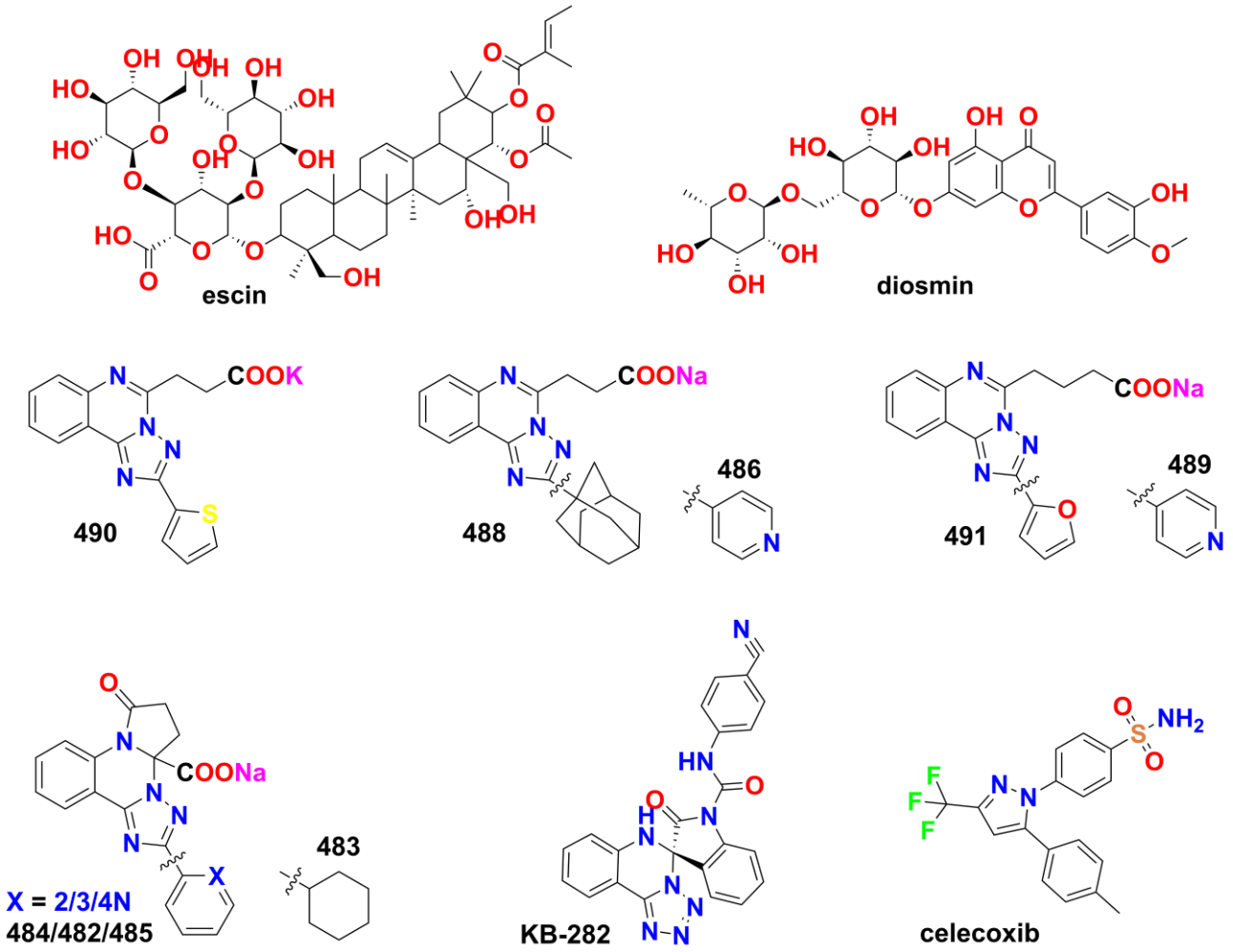

Figure 1. Molecular structures of studied substances.

So, diosmin and escin (Fig. 1) were selected as the primary reference compounds for this study as they are individual substances, as well as due to their established clinical presence and well-documented pharmacological profile in chronic venous insufficiency treatment.

The diosmin represents the most studied flavonoid-based venotonic, with clinical evidence demonstrating improvements in venous hemodynamics [3]. Its mechanism involves prolonging the vasoconstrictor effect of norepinephrine on the vein wall, increasing venous tone, and reducing venous capacitance, distensibility, and stasis [11]. Diosmin exerts effects on the *in vitro* metabolism of noradrenaline by varicose veins, potentially benefitting vascular health [3]. Network pharmacology studies have identified molecular targets demonstrating stable binding affinity with diosmin, including CASP3, ANXA5, MMP9, and HSP90AA1 [12, 13].

Escin (Fig. 1) was selected as the secondary reference compound, due to its superior direct venotonic mechanisms and well-defined calcium-dependent contractile effects [4]. Unlike diosmin's indirect mechanisms, escin demonstrates clear dose-dependent venous contractility enhancement through calcium sensitization pathways, providing a mechanistically distinct reference for molecular docking comparisons.

Escin's molecular docking studies reveal strong binding activities with multiple protein targets, with binding energies ranging from -5.0 to -8.8 kcal/mol [14].

Particularly strong binding interactions were observed with PTGS2 (COX-2, -8.8 kcal/mol), SRC (-8.7 kcal/mol), MMP9 (-8.5 kcal/mol), and MAPK1 (-8.0 kcal/mol), indicating multi-target therapeutic potential. Clinical validation demonstrates that escin produces statistically significant improvements in venous function, with meta-analyses showing significant leg volume reductions after treatment [15]. The compound's superior clinical validation establishes escin as an essential reference for evaluating novel venotonic candidates.

Cyclooxygenase-2 (COX-2) represents one of the key inflammatory targets with significant implications for venous inflammation modulation [1]. Its expression increases during inflammatory responses, contributing to prostaglandin production and perpetuation of inflammatory cascades in chronic venous disease. The rationale for targeting COX-2 in venous disease extends beyond simple anti-inflammatory effects. Its upregulation in venous pathology contributes to prostaglandin-mediated vasodilation and increased vascular permeability — two key pathophysiological mechanisms in CVI. Furthermore, its inhibition has demonstrated potential to modulate endothelial dysfunction and reduce matrix metalloproteinase expression, thereby preserving venous wall integrity. This multi-faceted impact of COX-2 inhibition on venous function provides a compelling rationale for exploring novel selective COX-2 inhibitors as venotonic agents.

This relationship between COX-2 inhibition and improved venous function suggests potential for developing dual-acting compounds with both venotonic and anti-inflammatory properties. The triazolo/tetrazoloquinazoline scaffold represents a promising chemical platform for developing such dual-acting compounds, combining potential anti-inflammatory properties with direct venotonic effects.

Materials and methods. The CB-Dock2 [16], a protein-ligand autoblind docking tool, that has the curvature-based cavity detection procedure with AutoDock Vina, was used for calculations of 10 triazolo/tetrazoloquinazoline derivatives' affinity towards cyclooxygenase-2 (RCSB PDB ID: 3LN1) [17], and 2 against matrix metalloproteinase-9 (MMP-9) (1GKC) [18], downloaded from RCSB Protein Data Bank in pdb format. Reference compounds included diosmin (CAS: 520-27-4), escin (CAS: 6805-41-0), and celecoxib (CAS: 169590-42-5), selected based on their established clinical relevance in chronic venous insufficiency treatment and COX-2 inhibition, respectively.

CB-Dock2 employs a curvature-based cavity detection algorithm, that identifies potential binding pockets across the entire protein surface. This template-based blind docking approach utilizes the crystallographic structure of celecoxib bound to COX-2 as a reference to guide the docking process, while simultaneously exploring alternative binding sites. The perfect reproduction of the known celecoxib binding pose (RMSD = 0) validates the docking protocol's accuracy. For each 13 compounds, binding affinities were calculated across five distinct cavities to provide comprehensive assessment of binding site preferences and structure-activity relationships.

Results and discussion. So, the molecular docking analysis of hetaryl/cycloalkyl/spiro[1,2,4]triazolo[1,5-*c*]quinazoline carboxylic acids' salts (**482**-**486**, **488**-**491**), and *N*-(4-cyanophenyl)-2-oxo-6'*H*-spiro[indoline-3,5'-tetrazolo[1,5-

c]quinazoline]-1-carboxamide (**KB-282**) against COX-2 revealed promising binding affinities across multiple binding sites, with several compounds demonstrating superior performance compared to established reference compounds (Table 1).

Table 1

Binding score analysis of studied compounds and reference ligands against cyclooxygenase-2 (COX-2, RCSB PDB: 3LN1) binding sites ranked by affinity strength in each of 5 favorable cavities.

24422 Å³	kcal/ mol	3967 Å³	kcal/ mol	4186 Å³	kcal/ mol	4220 Å³	kcal/ mol	4435 Å³	kcal/ mol
KB-282	-11.9	**Cele-coxib**	-11.2	**Cele-coxib**	-11.2	**Dios-min**	-10.8	**Dios-min**	-10.4
Dios-min	-11.2	**489**	-10.3	**486**	-10.1	**488**	-10.4	**488**	-10.3
488	-10.0	**488**	-10.1	**491**	-10.0	**482**	-10.2	**KB-282**	-10.3
482	-9.7	**491**	-10.1	**488**	-9.9	**485**	-10.1	**Cele-coxib**	-10.0
484	-9.6	**486**	-9.8	**489**	-9.7	**KB-282**	-10.1	**486**	-10.0
485	-9.6	**490**	-9.8	**490**	-9.6	**486**	-10.0	**491**	-9.7
Cele-coxib	-9.4	**Escin**	-7.5	**Dios-min**	-9.1	**489**	-9.9	**489**	-9.5
483	-9.1	**KB-282**	-7.5	**Escin**	-8.5	**Cele-coxib**	-9.8	**490**	-9.5
489	-9.0	**482**	-7.1	**KB-282**	-8.4	**490**	-9.6	**482**	-9.1
Escin	-8.9	**483**	-7.0	**482**	-7.4	**491**	-9.2	**484**	-8.9
491	-8.9	**485**	-7.0	**483**	-7.4	**484**	-8.7	**485**	-8.9
486	-8.7	**Dios-min**	-6.7	**485**	-7.3	**483**	-8.7	**Escin**	-8.8
490	-8.3	**484**	-6.5	**484**	-7.2	**Escin**	-7.9	**483**	-8.7

The comprehensive evaluation across five distinct binding cavities provides valuable insights into the ***structure-activity relationships*** and therapeutic potential of this compound series (Table 1).

Reference compounds. Celecoxib exhibits remarkable selectivity for the smaller cavities (4186 Å³ and 3967 Å³), achieving -11.2 kcal/mol in both sites. The observed binding profile of celecoxib aligns with its clinically established mechanism as a selective COX-2 inhibitor. The cavity 3967 Å³ identified as the optimal binding pocket corresponds to the catalytic site of COX-2, where celecoxib exerts its therapeutic action. The perfect RMSD value (0) confirms that our docking protocol accurately reproduces the crystallographically observed binding mode. This validation strengthens the reliability of our binding predictions for the novel compounds, particularly those demonstrating comparable or superior binding affinities to celecoxib.

Diosmin shows strong performance in larger cavities, but weaker binding in smaller sites, while escin demonstrates generally moderate affinity across all binding sites.

Spiroindoline derivatives. KB-**282**, featuring a spiroindoline-tetrazoloquinazoline scaffold with a cyanophenyl substituent, demonstrates exceptional binding affinity in the largest cavity (24422 Å³) with a score of -11.9 kcal/mol, establishing it as the most potent compound in this binding site. The rigid spiro-configuration and extended aromatic system likely contribute to favorable π-π stacking interactions and optimal geometric complementarity within this cavity. However, **KB-282** shows diminished performance in smaller cavities, suggesting size-dependent selectivity.

Pyrrolotriazoloquinazoline series. The pyrrolotriazoloquinazoline derivatives (**482**-**485**) exhibit variable binding profiles dependent on pyridine substitution patterns. Compound **482** (pyridin-3-yl) demonstrates superior performance compared to its positional isomers **484** (pyridin-2-yl) and **485** (pyridin-4-yl), particularly in the 4220 Å³ cavity (-10.2 kcal/mol). This suggests, that the meta-pyridine configuration provides optimal spatial orientation for receptor interactions. The cyclohexyl derivative **483** shows consistently weaker binding across all cavities, indicating that the aromatic pyridine moiety is critical for maintaining high affinity.

Triazoloquinazoline propanoate and butanoate derivatives. Compounds **486**, **488**, and **489** represent structural variations in the triazoloquinazoline core with different linker lengths and substituents. The adamantyl derivative **488** exhibits robust binding performance across multiple cavities, with particularly strong affinity in the 4220 Å³ site (-10.4 kcal/mol). The bulky, lipophilic adamantyl group likely enhances hydrophobic interactions within the binding pocket. In contrast, the pyridin-4-yl derivatives **486** and **489** show moderate binding, with the butanoate linker in **489** providing slightly enhanced affinity in smaller cavities compared to the propanoate in **486.**

Heterocyclic variations. The thiophene derivative **490** and furan derivative **491** demonstrate the impact of heteroaromatic substitution. Both compounds show intermediate binding affinities, with **491** generally outperforming **490**, suggesting that the electronic properties of the five-membered heterocycle influence binding interactions.

Hence, SAR analysis reveals that optimal COX-2 binding requires consideration of both molecular size and electronic properties. Rigid, extended aromatic systems favor binding in larger cavities, while compact, electronically diverse structures show enhanced affinity for smaller binding sites. The pyridine substitution pattern significantly influences binding affinity, with meta-substitution proving superior to ortho- or para-configurations. Additionally, the incorporation of bulky lipophilic groups such as adamantyl enhances binding through favorable hydrophobic interactions, while maintaining appropriate molecular flexibility appears crucial for multi-cavity binding capability.

The superior binding affinities exhibited by compounds **KB-282** and **488** suggest potential for enhanced COX-2 inhibitory activity compared to reference compounds. However, their distinct binding site preferences, — with **KB-282** favoring larger cavities and **488** demonstrating versatile binding across multiple sites, may translate to

different pharmacodynamic profiles and selectivity patterns. These differential binding characteristics could manifest as distinct efficacy and safety profiles, highlighting the importance of subsequent experimental validation through enzymatic assays and cellular models.

From a toxicological perspective, selective COX-2 inhibition offers potential advantages over non-selective NSAIDs in terms of gastrointestinal safety; however, cardiovascular risk remains a critical consideration for novel COX-2 inhibitors. The binding site selectivity patterns observed in our study may inform structure-based optimization strategies aimed at maximizing therapeutic efficacy while minimizing off-target effects and potential toxicities. Correlation between our computational predictions and future experimental toxicity data will be essential for advancing lead compounds with optimal benefit-risk profiles.

Limitations of the present study include the requirement for experimental validation of predicted binding affinities through enzymatic assays and the need for additional molecular dynamics simulations to assess binding stability under physiological conditions. The multi-target approach demonstrated here enables development of compounds with balanced activity profiles addressing multiple pathophysiological mechanisms underlying chronic venous disease.

Future studies. For further studies, a comprehensive evaluation of these compounds against multiple targets involved in venous pathophysiology is planned:

Matrix metalloproteinase-9 (MMP-9) emerges as a critical target for venous wall integrity modulation and inflammatory process regulation [18]. Our preliminary molecular docking studies have already demonstrated promising results, with tested compound **KB-282** (-8.3 kcal/mol) and **489** (-9.4 kcal/mol) showing affinity to MMP-9 (RCSB PDB: 1GKC) comparable to diosmin (-10.2 kcal/mol) and superior to escin (-6.9 kcal/mol).

Alpha-1 adrenergic receptors, which mediate sympathetic nervous system responses including vascular smooth muscle contraction [19] through Gq-protein coupling and the IP3 signal transduction pathway [20], represent also an important target. Additionally, endothelial nitric oxide synthase (eNOS) will be investigated as a critical molecular target for venous endothelial function modulation. Vascular nitric oxide [21], synthesized by eNOS from L-arginine with tetrahydrobiopterin (BH4) as an essential cofactor, plays a fundamental role in maintaining endothelial function, making eNOS a rational therapeutic target in chronic venous disease.

A systematic toxicological evaluation will complement these target-based studies, with particular emphasis on:

- *Acute and subchronic toxicity* in appropriate animal models to establish safety margins and identify target organs of toxicity.
- *Cardiovascular safety assessment*, including thorough QT studies and hemodynamic evaluations, given the known cardiovascular risks associated with some COX-2 inhibitors.
- *Gastrointestinal safety profiles*, with the hypothesis that selective COX-2 inhibitors may demonstrate reduced GI toxicity compared to non-selective NSAIDs.

- *COX-1/COX-2 selectivity ratios* determined through enzymatic assays to correlate with in silico predictions and inform safety profiles.
- *Hepatic and renal safety evaluations* to assess potential off-target toxicities common to many small-molecule therapeutics.

This integrated approach, combining computational predictions, target engagement studies, and systematic toxicological evaluation, will guide the optimization of lead compounds and establish a robust preclinical data package for the most promising candidates.

Conclusions. The investigation of hetaryl/cycloalkyl/spiro[1,2,4]triazolo[1,5-*c*]-quinazoline carboxylic acids' salts and *N*-(4-cyanophenyl)-2-oxo-6'*H*-spiro[indoline-3,5'-tetrazolo[1,5-*c*]quinazoline]-1-carboxamide as novel venotonic agents represents a rational approach to addressing limitations of current therapies. The selection of diosmin, escin, and celecoxib as reference compounds provides comprehensive benchmarking against both flavonoid-based and saponin-based mechanisms, with demonstrated binding affinities and clinical validation supporting their use as comparative standards.

Our computational approach identifies several promising candidates, particularly compounds **KB-282** and **488**, which demonstrate superior or comparable COX-2 binding compared to established references. The differential binding site preferences observed among these compounds suggest potential for optimizing both efficacy and safety profiles through targeted structural modifications. This systematic structure-based approach provides a foundation for advancing novel venotonic therapeutics that could significantly improve treatment outcomes for chronic venous insufficiency patients.

Further investigation is warranted to validate computational predictions through experimental studies, analyze toxicity profiles, and optimize lead compounds for clinical development. The integration of rigorous toxicological evaluations with target-based pharmacological assessments will be essential for translating these promising computational findings into clinically viable therapeutics with improved efficacy and safety profiles compared to existing therapies.

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