

α -Carotene does not occupy the galectin-3 carbohydrate-recognition site in a blind docking screen benchmarked against olitigaltin

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Abstract

Galectin-3 is a β -galactoside-binding lectin implicated in inflammation, fibrosis and adverse ventricular remodelling after myocardial infarction, and dietary phytochemicals are frequently screened against it by molecular docking. Such screens rarely include a reference inhibitor, and rarely verify where the predicted ligand actually binds. We docked eight phytochemicals reported from *Vaccinium corymbosum* L. (blueberry), together with the clinical-stage galectin-3 inhibitor olitigaltin (TD139), against the human galectin-3 carbohydrate-recognition domain (PDB 4BM8, 0.96 Å). A single grid enclosing the entire protein was used, with identical parameters for all nine ligands (AutoDock Vina via PyRx, exhaustiveness 32). Olitigaltin returned the most favourable score (−7.9 kcal/mol) and, without any site restraint, located the canonical carbohydrate-recognition site, forming hydrogen bonds with Asn160, Arg144 and Gly238 and π -interactions with His158, Arg162 and Trp181. α -Carotene ranked highest among the phytochemicals (−6.1 kcal/mol) but occupied an adjacent hydrophobic surface, contacting Pro117, Ile115, Tyr118 and Ala146 exclusively through alkyl and π -alkyl interactions and forming no hydrogen bonds. α -Carotene therefore does not engage the residues that determine galectin-3 carbohydrate binding, and its docking rank should not be read as evidence of target engagement. Blind docking screens of phytochemicals should report a reference inhibitor and the identity of the contacted residues, not scores alone.

Keywords Galectin-3; Blind molecular docking; α -Carotene; *Vaccinium corymbosum*; Olitigaltin; Binding-site specificity

1. Introduction

Myocardial infarction remains a leading cause of death worldwide, and the ventricular remodelling that follows ischaemic injury determines much of its long-term morbidity (Reed et al., 2017; World Health Organization, 2025). Galectin-3, a β -galactoside-binding lectin, is released by activated macrophages in the infarcted myocardium and promotes fibroblast activation, collagen deposition and adverse remodelling; genetic and pharmacological inhibition attenuates these processes in animal models (de Boer et al., 2010; Frangogiannis, 2014; Meijers et al., 2015; Wang et al., 2023; Yu et al., 2013). It is therefore an attractive target for small-molecule intervention.

Galectin-3 recognises its ligands through a shallow groove on the carbohydrate-recognition domain, in which a conserved network of residues — Arg144, His158, Asn160, Arg162, Asn174, Trp181 and Glu184 — forms hydrogen bonds and stacking contacts with the galactose ring. Inhibitor development has been directed almost exclusively at this groove, and the thiodigalactoside olitigaltin (TD139, GB0139) binds it with nanomolar affinity and has reached clinical evaluation (Hsieh et al., 2016). Because galectin-3 function depends on carbohydrate binding, a compound that does not occupy this groove has no evident route to inhibiting the protein.

Dietary phytochemicals are widely screened against galectin-3 and comparable targets by molecular docking,

and carotenoids in particular are reported to have antioxidant and anti-inflammatory properties relevant to cardiovascular protection (Fiedor & Burda, 2014; Kaulmann & Bohn, 2014; Zhuang et al., 2022). Two weaknesses recur in these studies. First, scores are reported without a reference inhibitor, so the reader cannot judge whether a given value is meaningful. Second, when the search grid encloses the whole protein — a common and otherwise reasonable choice when the site is not assumed in advance — the reported pose is the global optimum anywhere on the surface, which need not be the functional site; the contacted residues are seldom examined. We therefore screened eight phytochemicals reported from *Vaccinium corymbosum* L. against galectin-3 under unbiased whole-protein conditions, with olitigaltin included as an internal reference under identical settings, and asked not only which compound scored best but where it bound.

2. Materials and methods

2.1 Protein and ligand preparation

The crystal structure of the human galectin-3 carbohydrate-recognition domain was obtained from the RCSB Protein Data Bank (Berman et al., 2000), entry 4BM8, solved by X-ray diffraction at 0.96 Å resolution (Noresson et al., 2014). Chain A was retained; water molecules and the co-crystallised ligand were removed, polar hydrogens were added and the structure was energy-minimised in BIOVIA Discovery Studio before conversion to PDBQT format.

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Eight cardioprotective phytochemicals reported from *Vaccinium corymbosum* L. were identified using Dr. Duke's Phytochemical and Ethnobotanical Database. Their three-dimensional structures, and that of the reference inhibitor olitigaltin, were retrieved from PubChem (Kim et al., 2023), energy-minimised in PyRx using the universal force field and converted to PDBQT format (Dallakyan & Olson, 2015).

2.2 Blind molecular docking

Docking was performed with AutoDock Vina 1.2.0 through the PyRx interface (Eberhardt et al., 2021; Trott & Olson, 2010). No binding site was assumed. A single search grid enclosing the entire protein was defined, centred at $x = -12.32$, $y = 0.91$, $z = 5.34$ with dimensions $41.55 \times 45.15 \times 40.31$ Å, and the same grid centre, grid dimensions and search settings were applied to all nine ligands, including the reference inhibitor. The resulting search volume of approximately 7.6×10^4 Å³ exceeds the 2.7×10^4 Å³ threshold above which AutoDock Vina advises raising the exhaustiveness setting to compensate for the larger search; exhaustiveness was therefore set to 32, four times the program default. For each ligand the pose with the lowest predicted binding energy was retained and taken forward for interaction analysis.

2.3 Interaction and ADMET analysis

Protein–ligand contacts in the top-ranked poses were identified and classified in Discovery Studio Visualizer, and residue identities and contact distances were recorded. Physicochemical and pharmacokinetic descriptors were predicted using SwissADME (Daina et al., 2017) and acute oral toxicity using ProTox-3.0 (Banerjee et al., 2024).

3. Results and discussion

3.1 The reference inhibitor outscored every phytochemical

Predicted binding affinities are given in Table 1. Olitigaltin returned the most favourable value of the nine ligands, -7.9 kcal/mol. Among the phytochemicals, α -carotene ranked highest at -6.1 kcal/mol, followed by ferulic acid (-5.1 kcal/mol), with the remaining compounds between -4.4 and -3.1 kcal/mol. The 1.8 kcal/mol separation between olitigaltin and the best phytochemical is consistent with expectation: olitigaltin is the product of structure-guided optimisation against this groove, whereas the phytochemicals are dietary constituents under no selective pressure to bind it.

Molecular mass should be kept in view when reading this ranking. α -Carotene is the largest phytochemical in the set at 536.9 g/mol, and AutoDock Vina scores, which are not normalised for ligand size, tend to favour larger molecules through accumulated contact terms. Part of its advantage over the smaller phytochemicals, several of which are fragments of 74 – 194 g/mol, is therefore attributable to size rather than to complementarity.

3.2 Only the reference inhibitor occupied the carbohydrate-recognition site

Because no site was specified, the position of each top-ranked pose is itself a result. Olitigaltin converged on the canonical carbohydrate-recognition groove (Figure 1A). Its contacts (Figure 2A) comprise conventional hydrogen bonds to Asn160 (2.85 Å), Arg144 (2.83 Å) and Gly238 (1.95 and 2.53 Å), a carbon–hydrogen bond to Asp239 (3.28 Å), π -cation interactions with His158 (3.82 Å)

Table 1. Identity and predicted binding affinity of the eight *Vaccinium corymbosum* phytochemicals and the reference inhibitor olitigaltin, ranked by docking score. All ligands were docked against PDB 4BM8 using an identical whole-protein grid.

Compound	PubChem CID	Molecular formula	Molecular weight (g/mol)	Binding affinity (kcal/mol)
Olitigaltin (reference)	73774610	C ₂₈ H ₃₀ F ₂ N ₆ O ₈ S	648.64	-7.9
α -Carotene	6419725	C ₄₀ H ₅₆	536.87	-6.1
Ferulic acid	445858	C ₁₀ H ₁₀ O ₄	194.18	-5.1
Niacin	938	C ₆ H ₅ NO ₂	123.11	-4.4
Arginine	6322	C ₆ H ₁₄ N ₄ O ₂	174.20	-4.2
Nerol	643820	C ₁₀ H ₁₈ O	154.25	-4.2
Benzaldehyde	240	C ₇ H ₆ O	106.12	-4.0
Alanine	5950	C ₃ H ₇ NO ₂	89.09	-3.7
Butan-1-ol	263	C ₄ H ₁₀ O	74.12	-3.1

Arg162 (3.67 Å), a π – π stacked interaction with Trp181 (5.45 Å), and halogen contacts to Asn174 (2.16 Å) and Glu184. These are the residues that define β -galactoside recognition in galectin-3, and their recovery from an unbiased whole-protein search, with distances typical of the thiodigalactoside series, is a stringent check that the docking protocol was able to identify the functional site when a true ligand was supplied. Its top-ranked pose lay along an adjacent hydrophobic surface (Figure 1B), and its contacts (Figure 2B) consisted exclusively of alkyl and π -alkyl interactions with Pro117 (4.07 and 5.11 Å), Ile115

(4.37 and 4.86 Å), Tyr118 (4.45 and 4.60 Å) and Ala146 (4.42 Å), together with long-range contacts to Trp181 (4.74 – 7.01 Å) and His158 (4.72 Å). No hydrogen bond was formed. This is the expected consequence of its chemistry: α -carotene is a C₄₀ hydrocarbon with no hydrogen-bond donors or acceptors and no polar surface, and is constitutionally incapable of reproducing the hydrogen-bonding pattern through which galectin-3 recognises galactosides. Of the nine residues contacted by olitigaltin, α -carotene shares only two, Trp181 and His158, and does so at 4.7 Å or more, which is peripheral rather than

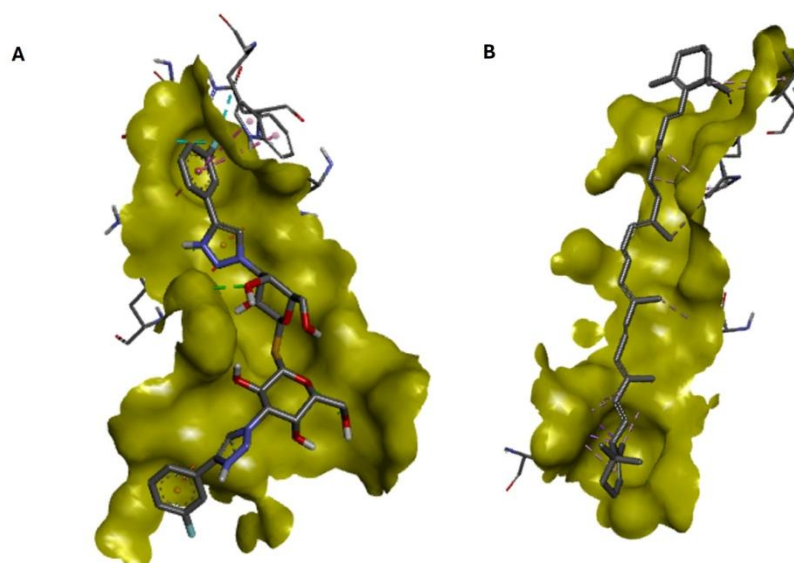


Figure 1. Top-ranked docked poses on the galectin-3 surface. (A) Olitigaltin, occupying the carbohydrate-recognition groove. (B) α -Carotene, extended along an adjacent hydrophobic surface. Both poses were obtained with an identical whole-protein search grid.

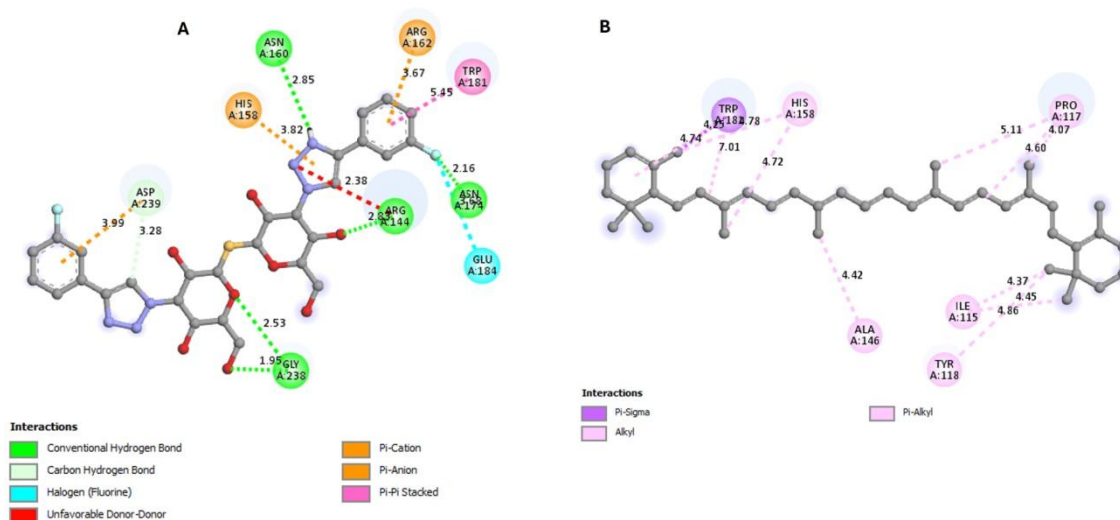


Figure 2. Two-dimensional interaction profiles of the top-ranked poses. (A) Olitigaltin forms hydrogen bonds and π -interactions with Arg144, His158, Asn160, Arg162, Asn174, Trp181, Glu184, Gly238 and Asp239. (B) α -Carotene forms only alkyl and π -alkyl contacts, with Pro117, Ile115, Tyr118 and Ala146, and no hydrogen bonds.

Table 2. Predicted physicochemical and pharmacokinetic descriptors (SwissADME). HBD, hydrogen-bond donors; HBA, hydrogen-bond acceptors; Log Kp, skin permeation coefficient; CYP, cytochrome P450 isoform predicted to be inhibited.

Compound	HBD	HBA	Consensus LogP	Log Kp (cm/s)	Aromatic heavy atoms	CYP inhibition
Olitigaltin (reference)	6	14	2.24	-10.09	22	CYP3A4
α -Carotene	0	0	8.27	0.12	0	None
Ferulic acid	2	4	1.41	-6.41	6	None
Niacin	1	3	0.47	-6.80	6	None
Arginine	4	4	-2.99	-10.34	0	None
Nerol	1	1	2.92	-4.71	0	None
Benzaldehyde	0	1	1.43	-5.90	6	CYP1A2
Alanine	2	3	0.42	-8.95	0	None
Butan-1-ol	1	1	1.69	-6.13	0	None

engaged. The two ligands are therefore not competing for the same site in any meaningful sense. Since galectin-3 exerts its profibrotic effects through carbohydrate-dependent cross-linking of glycoconjugates, a ligand that lies outside the recognition groove has no established mechanism by which to interfere with that function, whatever its docking score. The ranking in Table 1 and the binding-site assignment in Figure 2 thus support different conclusions, and the second is the more informative.

3.3 Predicted physicochemical and toxicity profiles

Predicted descriptors are summarised in Table 2. α -Carotene returned a consensus LogP of 8.27 with no hydrogen-bond donors or acceptors, a profile that lies far outside conventional oral drug-likeness criteria. This is consistent with the known behaviour of dietary carotenoids, whose absorption is low, highly variable and strongly dependent on food matrix and lipid co-ingestion (Nagao, 2014). In the BOILED-Egg analysis one compound fell outside the plotted range, and the remaining phytochemicals distributed across the regions associated with passive gastrointestinal absorption. ProTox-3.0 assigned α -carotene a predicted oral LD₅₀ of 1510 mg/kg and olitigaltin 1000 mg/kg, both corresponding to toxicity class IV; the prediction for α -carotene rested on a higher average structural similarity to the training set (71.45%) than that for olitigaltin (36.83%). These descriptors are secondary to the binding-site result. A favourable predicted toxicity class carries no weight if the compound does not engage the target, and the lipophilicity of α -carotene would in any case present a formulation problem before any pharmacological question could be asked.

3.4 Implications and limitations

The practical message is methodological. A screen reporting only that α -carotene achieved -6.1 kcal/mol against galectin-3 would have supported a claim of target engagement that the same data refute once the contacted residues are examined. Including a reference inhibitor

4. Conclusion

Under unbiased whole-protein docking with a reference inhibitor included at identical settings, olitigaltin located the galectin-3 carbohydrate-recognition site and reproduced its established contacts, whereas α -carotene — the best-scoring of eight *Vaccinium corymbosum* phytochemicals — bound an adjacent hydrophobic surface and formed no hydrogen bonds. α -Carotene is therefore not a galectin-3 carbohydrate-site ligand, and its rank in the screen reflects size and hydrophobic surface complementarity rather than target engagement. Docking screens of dietary compounds should report a reference inhibitor and the identity of contacted residues; scores alone can support conclusions that the underlying poses do not.

Acknowledgements

The authors thank Ms. Tamilbharathi, Program Lead, OriGene Biosolution, Salem, Tamil Nadu, India, for her guidance on the docking validation strategy, in particular the inclusion of a reference inhibitor docked under identical conditions, which substantially strengthened the interpretation of the results.

Author contributions

Anjali T: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. Kavitha D: Conceptualization, Supervision, Validation, Writing – review & editing, Resources.

Funding

This work received no external funding.

Data availability

Receptor and ligand PDBQT files, the docking configuration file and the output logs are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

Use of generative AI

Generative AI tools were not used in the design, analysis or interpretation of this study.

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Received : 3 September 2026

Revised : 20 October 2026

Accepted : 21 October 2026

Published : 24 October 2026

Cite this article: Anjali, T., and Kavitha, D. (2026). α -Carotene does not occupy the galectin-3 carbohydrate-recognition site in a blind docking screen benchmarked against olitigaltin. *International Journal of Advances in Interdisciplinary Research*, 6(2), e006. <https://doi.org/10.29191/ijaidr.2026.06.e006>.

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