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SUPPLEMENTARY MATERIAL TO

Theoretical evaluation of pectin therapeutic potential in relation to degree of methylation

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Abstract: Pectin is the focus of scientific interest due to both its physicochemical and biochemical properties, as well as its non-toxic nature. Methylation of pectin is a natural process that exists as part of the cell wall defence system against various pathogens. In this study, docking analysis was conducted to predict if methylation and to what extent affects the anticancer and antimicrobial properties of pectin. Four pectin derivatives with varying degrees of methylation and two sets of biomolecules were used. The first set included enzymes responsible for anticancer activity (HMGR, the AGE Receptors, p53 cancer mutant, and Oncogenic Phosphatase SHP2), while the second set included those for antimicrobial activity (Salmonella Typhi TtsA, Pseudomonas aeruginosa Earp, Streptococcus mutans MetE, and Staphylococcus aureus Cas9). The results indicated that the degree of methylation does not play a decisive role in the mentioned activities because all bind to the same sites with similar binding energies. Additionally, it was shown that pectin derivatives have a higher binding affinity towards DNA than towards enzymes. Only the fully methylated derivative exhibited different behaviour, binding to a different binding site in the case of Streptococcus mutans MetE.

SM1. DOCKING STUDY ON HMG-COA REDUCTASE

Coenzyme A, serving as the control compound, was tested in its neutral state, considering its neutrality under gastrointestinal tract conditions (pKa_1 = 4.0 (adenine NH_3^+) and pKa_2 = 9.6 (thiol)). The pH gradient in the gastrointestinal tract gradually rises from pH 6 in the small intestine to approximately pH 7.4 in the terminal ileum. Subsequently, the pH decreases to 5.7 in the caecum, followed by a gradual increase to pH 6.7 in the rectum. Dissolved pectin exhibits a negative charge at neutral pH, approaching neutrality at low pH, with a pKa value of

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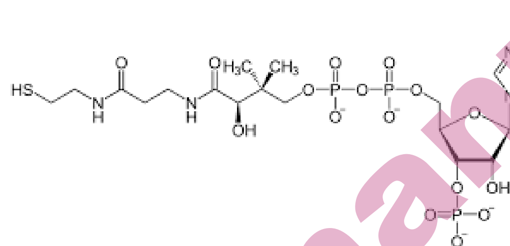
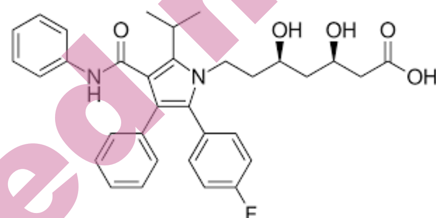
approximately 3.5. Atorvastatin, belonging to the class of medications known as HMG-CoA reductase inhibitors (statins), functions by inhibiting the synthesis of cholesterol in the body, thereby reducing the accumulation of cholesterol on arterial walls and mitigating the risk of arterial blockage, particularly in vital organs such as the heart and brain. In the gastrointestinal tract, atorvastatin bears a negative charge due to its pKa value of 4.46.



Coenzyme A



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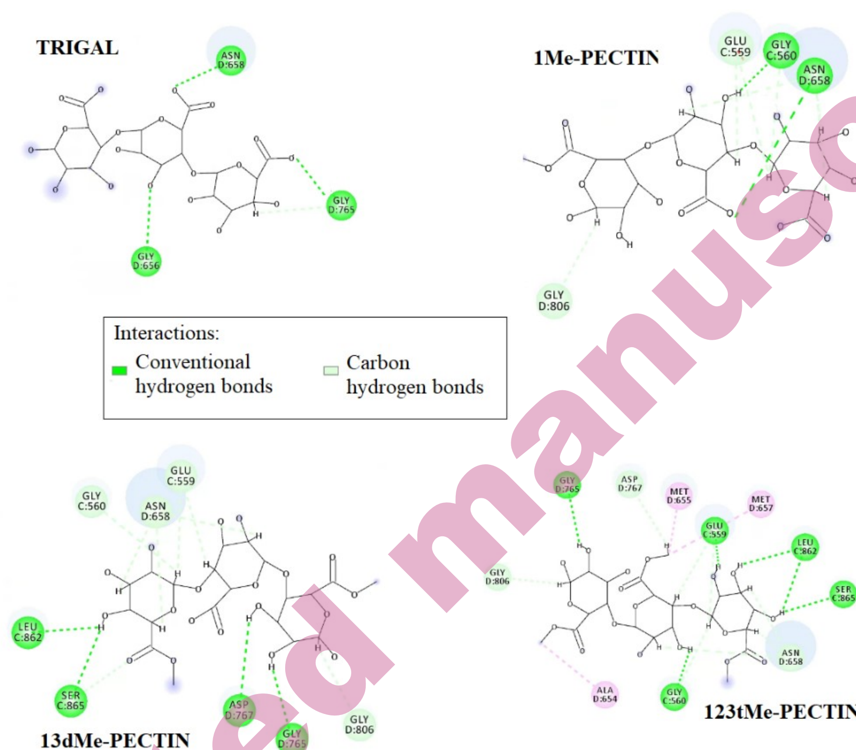

Fig. S1. Amino acid environment of the investigated pectin derivatives for HMG-CoA Reductase, in the binding site with the highest binding energy.

SM2. DOCKING STUDY ON AGE RECEPTORS (RAGE)

The binding of ligands to the extracellular domain of RAGE initiates a multifaceted intracellular signaling cascade, triggering the generation of reactive oxygen species (ROS), immunoinflammatory responses, cellular proliferation, or apoptosis, along with the upregulation of RAGE itself. Human RAGE is composed of three main domains: an extracellular region (residues 23–342), a hydrophobic transmembrane region (residues 343–363), and a cytoplasmic region (residues 364–404). The extracellular structure of RAGE comprises three immunoglobulin-like domains: a variable (V) domain (residues 23–116), and two constant C1 (residues 124–221) and C2 (residues 227–317) domains. Within the V domain, eight strands (A′, B, C, C′, D, E, F, and G) are connected by six loops, forming two β-sheets linked by a disulfide bridge between *Cys38* and *Cys99.*[23] Specifically, residues showing chemical shift perturbations (CSPs) in the V domain correspond to crucial residues in the binding surface, such as *Glu50, Ly52, Arg98, Gln100, Ala101, Lys110,* and *Asn112* (which are key ligand interaction residues for sites 1 and 2). Further analysis of this structure has revealed details about interactions in

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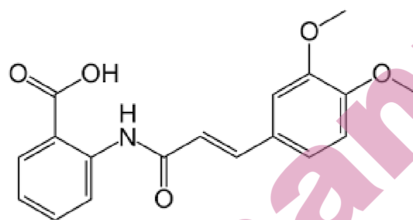
a newly identified "site 3," including a strong interaction with *Lys39* and an additional contact with *Tyr113*. These residues are of particular interest, as some initial hit fragments caused CSPs in these residues, suggesting their potential as starting points for incorporating the new site 3 into future inhibitor designs.[11] Tranilast, anti-allergic drug molecule that significantly inhibits the binding interactions of V-domain of RAGE, was used as a control compound.



Tranilast

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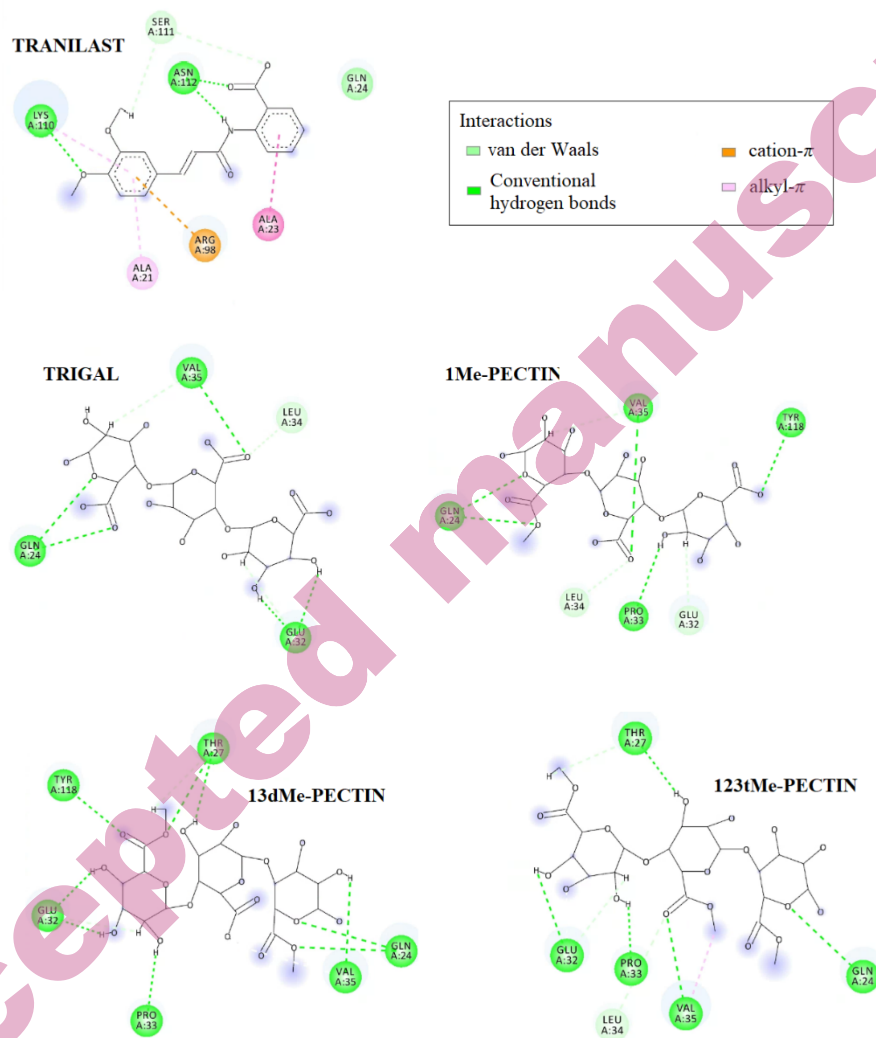

Fig. S2. Amino acid environment of drug tranilast and the investigated pectin derivatives for the AGE receptors (RAGE), in the binding site with the highest binding energy.

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SM3. DOCKING STUDY ON TUMOR PROTEIN P53

The p53 tumor suppressor gene plays a pivotal role in triggering apoptosis and is commonly known as the guardian of the genome.[SM1] In approximately half of human cancers, mutations in the p53 tumor suppressor gene are observed, often manifesting as missense substitutions in its core domain, specifically the DNA-binding core domain (CD) (residues 94–312). The p53-Y220C mutant serves as a notable model for developing drugs intended to stabilize mutant p53 proteins.[SM1] Mutations affecting DNA contacts within the core DNA-binding domain can result in the loss of function of the p53 tumor suppressor protein. Nevertheless, this functionality can be reinstated through second-site suppressor or rescue mutations.[SM2] Six residues within p53 are identified as hotspots for tumorigenic mutations, all localized within the DNA-binding substructure. These mutations may disrupt DNA contacts or induce structural alterations, leading to local conformational instability or global denaturation. Four "structural" hotspots (*Arg175, Gly245, Arg249,* and *Arg282*) predominantly exhibit a single substitution, while two "DNA-contact" hotspots, *Arg248* and *Arg273*, are distinguished.[SM3]

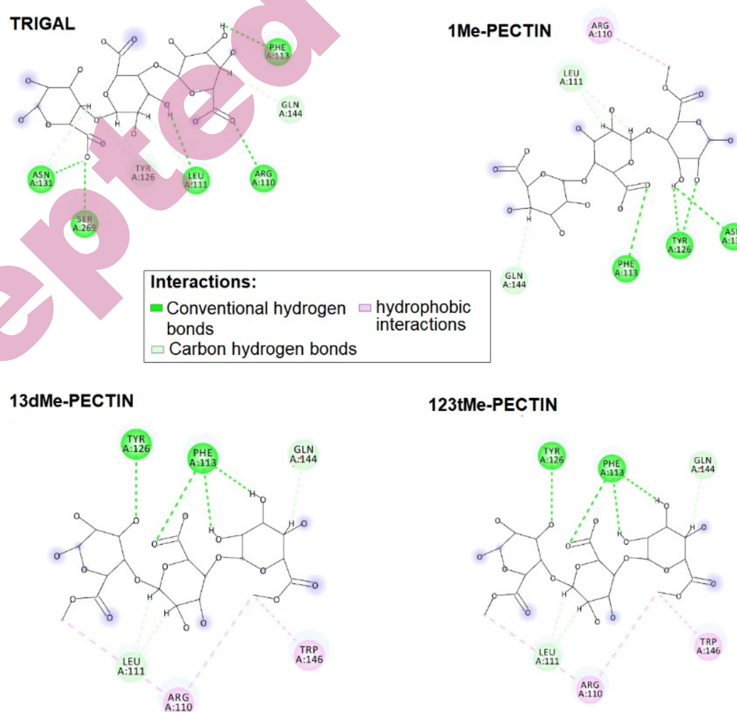

Fig. S3. Amino acid environment of investigated pectin derivatives for the p53 cancer mutant, in the binding site with the highest binding energy.

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SM4. DOCKING STUDY ON THE ONCOGENIC PHOSPHATASE SHP2

SHP2 is a 68 kDa protein comprising 593 amino acid residues, which include two tandem SH2 domains (N-SH2 and C-SH2) spanning residues 1–120, a catalytic protein tyrosine phosphatase domain (PTP domain residues 237-525), and a disordered C-terminal tail (residues 526–593) containing phosphorylation sites at *Tyr542* and *Tyr580*.[SM4] In its inactive state, SHP2 is autoinhibited by residues located on the catalytic surface of the PTP domain and the N-SH2 domain, thereby suppressing the protein's activity and restricting substrate access to its catalytic site. Activation by growth factors induces intramolecular "dissociation" upon phosphopeptide binding, releasing the N-SH2 domain from the PTP domain. In case of catalytic site obstruction, the N-terminal SH2 domain promptly obstructs its active site.

Traditionally, inhibition of SHP2 catalytic activity would necessitate targeting the PTP domain. Historically, various selective SHP2 inhibitors have been investigated in the context of leukemia-associated SHP2 mutants; however, many of these compounds still face challenges related to low bioavailability or permeability.[28] Targeting the PTP active site requires the development of highly selective molecules, and efforts to discover compounds with specific binding to this site are ongoing. Moreover, the positively charged environment of the PTP catalytic pocket poses unique challenges for drug discovery, as most catalytic site inhibitors require multiple ionizable functional groups to inhibit the enzyme. Key residues within the PTP catalytic cleft include *Cys459*, serving as the catalytic nucleophile; *Arg465*, contributing to the "phosphate binding cradle"; and *Asp425*, acting as the general acid in catalysis.[SM5]

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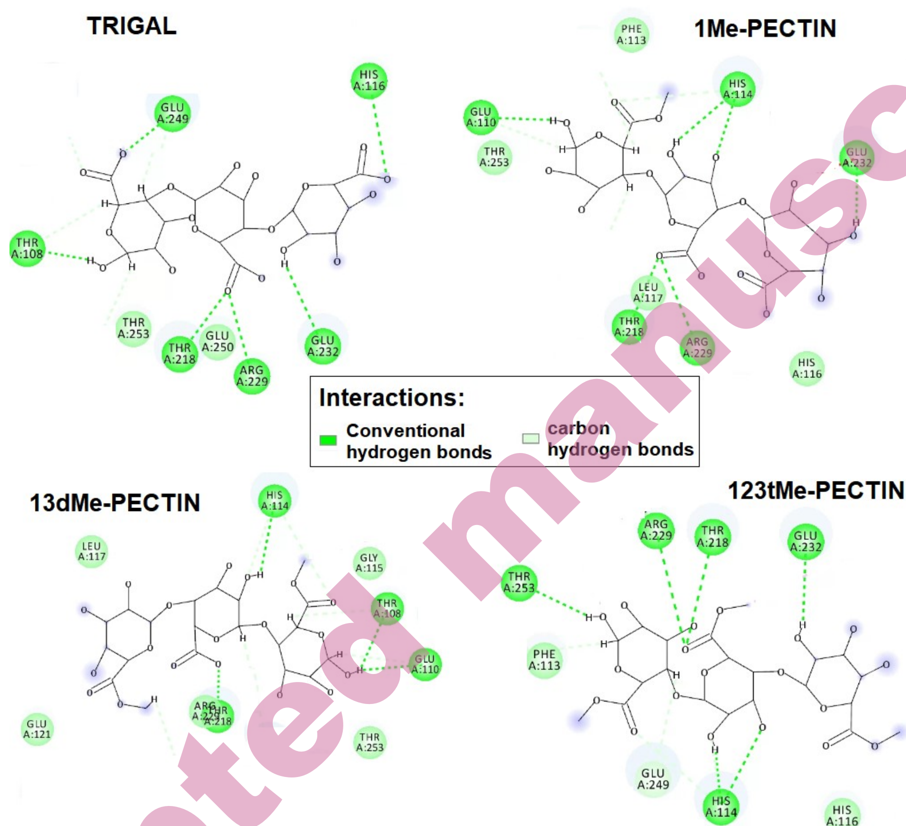

Fig. S4. Amino acid environment of the investigated pectin derivatives for the Oncogenic Phosphatase SHP2, at the binding site with the highest binding energy.

SM5. DOCKING STUDY ON THE *SALMONELLA TYPHI* TTSA

Salmonella Typhi TtsA -This enzyme, represented by PDB code 6V40 with four chains (A, B, C, D), exhibits muramidase activity vital for traversing the peptidoglycan layer. Muramidases like TtsA catalyze the hydrolysis of bonds in peptidoglycan, facilitating the passage of toxin-containing vesicles through the cell wall and contributing to bacterial cell lysis. The enzyme contains from 4 chains A, B, C, D. In the protein's structure, the amino terminal domain hosts the lysozyme-like catalytic triad positioned beneath a 'flap' structure formed by a loop. This loop is tethered to the domain through interactions with three short helices. Conversely, the carboxy-terminal substrate-binding domain comprises six anti-parallel α-helices that assemble into a helix bundle arrangement. This helix bundle serves as a scaffold for a platform housing a central groove, which, in conjunction

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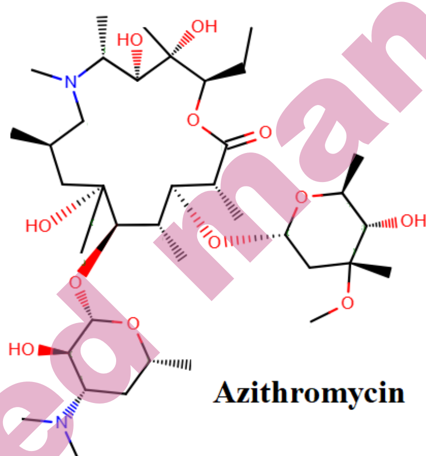
with the catalytic triad-containing flap, adopts a ring-like configuration. For the purposes of the docking study, only A chain was taken as the target.

Presently, ceftriaxone, ciprofloxacin, and azithromycin have garnered recommendation as primary antibiotics for the clinical management of Salmonella infection, particularly those precipitated by S. Typhimurium.[29] Azithromycin [9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin] is a part of the azalide subclass of macrolides. Azithromycin functions by impeding bacterial proliferation, thereby augmenting the host immune response against infection.



Azithromycin

This pharmaceutical agent is frequently prescribed to address an assortment of bacterial afflictions,[29] including but not limited to respiratory conditions such as bronchitis, pneumonia, and sinusitis. Additionally, azithromycin finds utility in treating certain sexually transmitted infections (STIs) such as chlamydia and gonorrhea, alongside other bacterial infections like dermatological and otological conditions.[SM6]

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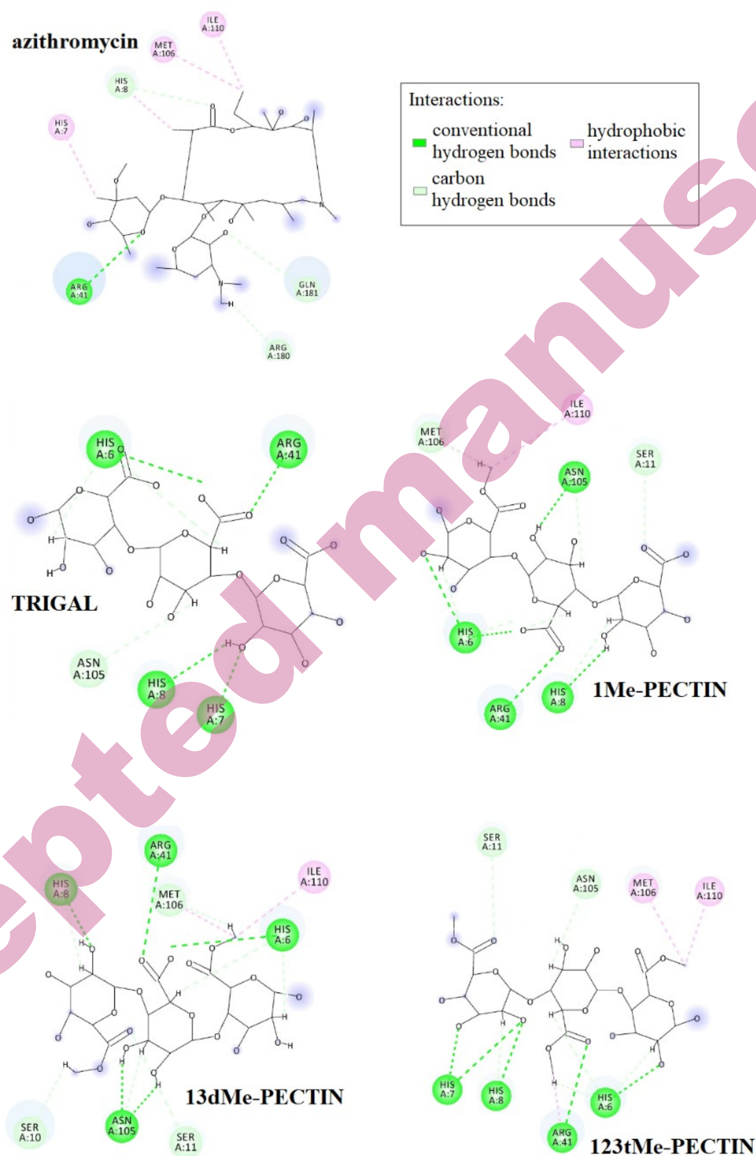

Figure S5. Amino acid environment of the investigated pectin derivatives for *Salmonella Typhi* TtsA, at the binding site with the highest binding energy.

SM6. DOCKING STUDY OF *PSEUDOMONAS AERUGINOSA* EARP

Protein glycosylation, the most widespread posttranslational modification in nature, significantly influences protein structure and function. Initially thought to be exclusive to eukaryotes, it's now evident that bacteria, including pathogens,

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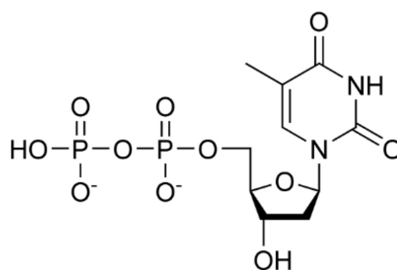
possess both O- and N-linked glycosylation pathways, exhibiting similarities and unexpected variations compared to their eukaryotic counterparts.[30] N-linked protein glycosylation typically targets Asn residues within Asn-X-Ser/Thr sequences, with X representing any amino acid except proline. Notably, the type III secretion effector NleB from attaching/effacing pathogens has been found to catalyze rare N-glycosylation of arginine residues on host proteins with N-acetylglucosamine (GlcNAc), thereby inhibiting antibacterial and inflammatory host responses. Pseudomonas aeruginosa EarP exhibits a distinctive structure consisting of two Rossmann-like domains separated by a deep cleft. The N-terminal domain (NTD features a four-stranded parallel β-sheet at its core flanked by α-hairpins, surrounded by helices.[30] In contrast, the C-terminal domain (CTD) comprises a five-stranded parallel β-sheet with an antiparallel strand on one side, encircled by helices. Interdomain contacts are maintained by a C-terminal helix extending from the CTD and connecting loops between the domains. The active site is located in a cavity in the cleft between the NTD and the CTD (Figure 1B). TDP-Rha" stands for "thymidine diphosphate-L-rhamnose." It is a nucleotide sugar molecule consisting of thymidine diphosphate (TDP) bound to L-rhamnose. TDP-Rha serves as a substrate in various biological processes, particularly in glycosylation reactions, where the rhamnose moiety is transferred to acceptor molecules, such as proteins, lipids, or other carbohydrates, by glycosyltransferase enzymes.[30] This modification plays critical roles in various cellular functions, including cell wall biosynthesis, protein glycosylation, and the biosynthesis of secondary metabolites. Docking Study was obtained using crystal structure of Pseudomonas aeruginosa Earp (pdb code: 6J7L). For control compound we docked the cleared enzyme with thymidine-5'-diphosphate (TDP).



thymidine-5'-diphosphate (TDP)

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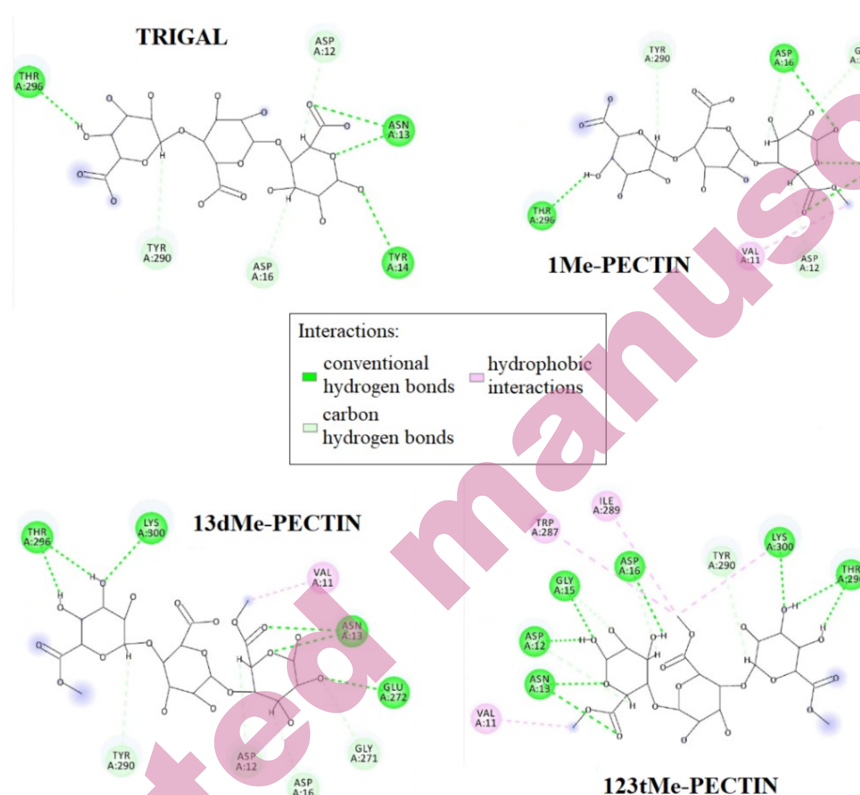

Figure S6. Amino acid environment of the investigated pectin derivatives for *Pseudomonas aeruginosa* Earp, at the binding site with the highest binding energy.

SM7. DOCKING RESULTS ON *STREPTOCOCCUS MUTANS* METE

Streptococcus mutans MetE is an enzyme found in the bacterium *Streptococcus mutans*. Specifically, MetE is involved in the biosynthesis of methionine, an essential amino acid. In S. mutans, methionine biosynthesis is crucial for various cellular processes, including protein synthesis and metabolism.[SM7] *S. mutans* is primarily known for its role in dental caries (tooth decay) and dental plaque formation. However, it can also cause other oral health issues such as gingivitis and periodontitis when conditions favor its growth and colonization in the oral cavity. Additionally, *S. mutans* has been implicated in infective endocarditis, a serious infection of the heart valves, particularly in individuals with underlying heart conditions or compromised immune systems. MetE has two domains, N and C, The N-domain is made up of residues 1–359, while the C-domain contains residues 398–745. each folded into (βα)8 barrels, joined by a helix linker.[31] The active site, housing a zinc atom, lies at their interface. The two domains are positioned with the two barrels facing each other

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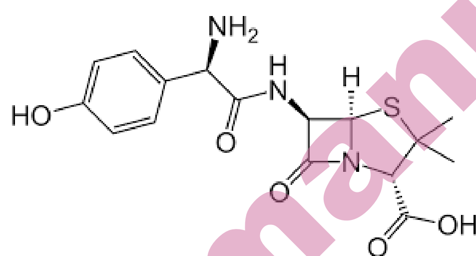
in a head-to-head alignment. A deep groove is formed with the active site of MetE at the domain interface. The deep groove between the two domains also provides a solvent-accessible path leading to the active site. The zinc atom is situated at the active site figure 1 and regulates enzyme function and can impact cellular processes. Amoxicillin (AMX) is one of the most prescribed antibiotics globally. The high absorption rate of AMX into the bloodstream reduces side effects and increases serum concentrations, making it desirable as an oral treatment.



Amoxicillin

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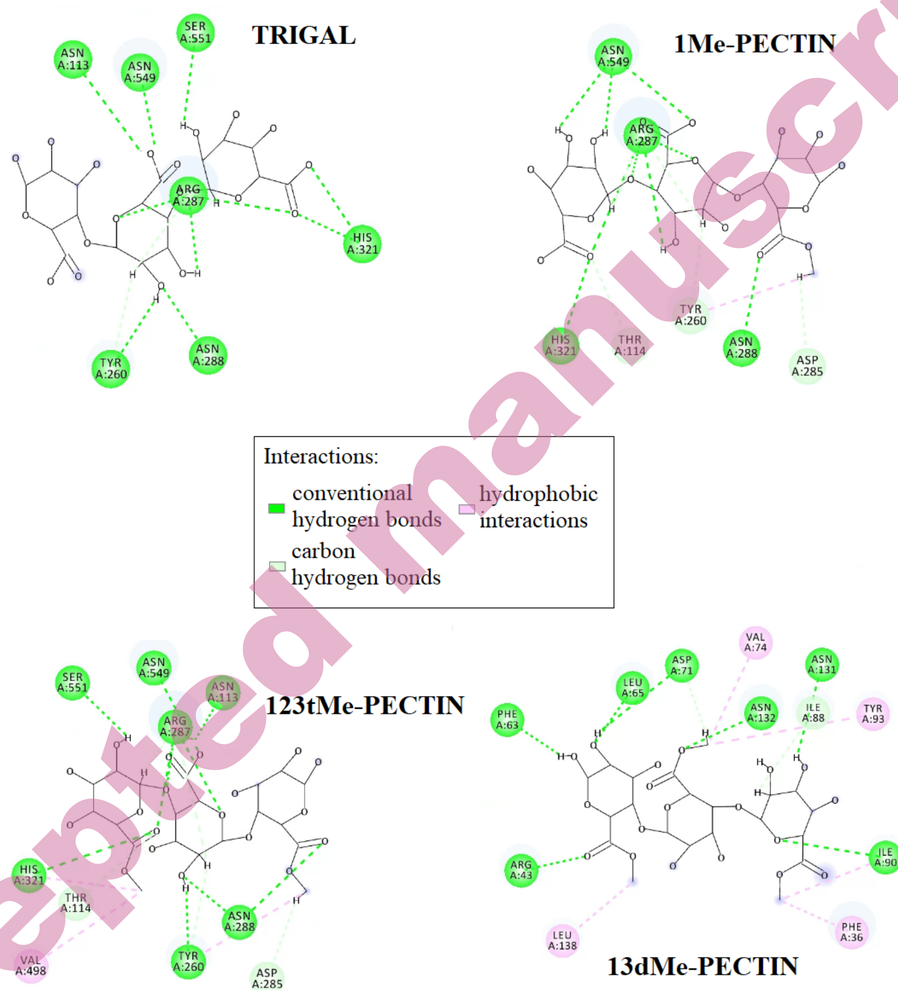

Figure S7. Amino acid environment of the investigated pectin derivatives for *Streptococcus mutans* MetE, at the binding site with the highest binding energy.

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