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ORIGINAL ARTICLE

Inhibitory effects and molecular interaction analysis of emergency cardiac drugs on human serum paraoxonase 1: an integrated *in vitro* and *in silico* approach

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ABSTRACT

Context: Paraoxonase 1 (PON1) is a crucial antioxidant enzyme involved in the hydrolysis of organophosphates and the prevention of oxidative damage to lipoproteins. **Objective**: This study aimed to purify PON1 using a newly synthesised hydrophobic interaction chromatography gel and to investigate the inhibitory effects of selected emergency cardiac drugs on PON1 activity through in vitro and in silico approaches. **Materials and methods**: PON1 was purified using a Sepharose-4B-L-tyrosine-6-aminochrysene hydrophobic interaction chromatography gel. The inhibitory effects of deslanoside, digitoxin, esmolol, and adenosine were evaluated via kinetic inhibition assays, molecular docking, molecular dynamics simulations, and MMPBSA calculations. **Results**: Among the tested compounds, esmolol exhibited the strongest inhibition of PON1 activity (IC_{50} = 0.131 ± 0.071 μM, K_i = 0.044 ± 0.009 μM) via a competitive mechanism. Molecular docking revealed strong binding affinity of esmolol to the PON1 active site, which was further supported by molecular dynamics simulations over 150 ns. **Discussion and conclusion**: The findings indicate a potential interaction between commonly used emergency cardiac drugs and PON1, highlighting the importance of evaluating off-target effects on critical metabolic enzymes in cardiovascular therapy.

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Paraoxonase 1; purification; inhibition; docking; MD simulation

Introduction

Paraoxonase 1 (PON1) is a calcium-dependent esterase primarily bound to high-density lipoprotein (HDL) in human serum. It plays a multifaceted role in human physiology through its enzymatic hydrolysis of organophosphates, oxidised phospholipids, and homocysteine thiolactone—molecules implicated in the pathogenesis of atherosclerosis, oxidative stress, and thrombosis (Harel et al., 2004; Rodrigo et al., 2001). Notably, PON1 exhibits phospholipase A2 activity and contributes to HDL's antioxidant capacity, providing protection against LDL oxidation and supporting reverse cholesterol transport (Ahmed et al., 2001).

Structurally, PON1 possesses a hydrophobic N-terminal domain, which resembles a signal peptide and is critical for its interaction with HDL particles. The active site contains two calcium ions, one responsible for maintaining structural stability and the other for catalytic function. These unique features render PON1 not only crucial in lipid metabolism but also an important modulator in inflammatory and oxidative processes.

Recent studies have emphasised the broad substrate specificity of PON1, extending beyond lipids to include various exogenous and endogenous xenobiotics. This has led to increased recognition of its role in drug metabolism and detoxification processes (Camps et al., 2023; Costa et al., 2020). Moreover, PON1 activity is influenced by genetic polymorphisms, environmental exposures, pathological states, and possibly pharmacological agents. Individuals with reduced PON1 activity are more susceptible to cardiovascular diseases, diabetes, cancer, autoimmune disorders, and neurodegenerative conditions (Gökçe, 2023; Shokri et al., 2020).

Despite this growing body of knowledge, the interaction between PON1 and widely used therapeutic agents remains insufficiently studied. Various reports suggest that drugs may modulate PON1 activity directly or indirectly, thereby altering its physiological

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roles (Alim & Beydemir, 2016; Argan et al., 2022). Yet, the potential of emergency cardiac drugs—administered intravenously at high doses in acute care settings—to affect PON1 activity has not been systematically explored. These include esmolol, a short-acting beta-1 adrenergic blocker; adenosine, a purinergic receptor agonist; and the cardiac glycosides digitoxin and deslanoside (Supplementary Figure 1). While these agents are life-saving in cardiovascular emergencies, they may also exert unintended off-target effects on metabolic enzymes such as PON1.

Given the essential protective functions of PON1, it is clinically relevant to investigate how such drugs interact with the enzyme. Understanding these interactions is crucial not only for enzyme-based drug development but also for anticipating adverse reactions that may arise from enzyme inhibition during emergency therapy.

In this study, we first purified PON1 from human serum using a novel hydrophobic interaction chromatography method based on a Sepharose-4B-L-tyrosin e-6-aminochrysene gel synthesised using a new method. We then evaluated the inhibitory effects of esmolol, adenosine, digitoxin, and deslanoside on PON1 activity through *in vitro* kinetic analysis. Furthermore, molecular docking, molecular dynamics simulations, and MMPBSA free energy calculations were employed to characterise the binding behaviour and stability of drug–enzyme interactions at the molecular level. By integrating biochemical and computational approaches, this study provides new insights into the potential modulation of PON1 by emergency cardiac medications and highlights implications for cardiovascular drug safety.

Materials and methods

Purification of paraoxonase 1 by sepharose-4B-L-tyrosine-6-aminochrysene gel

Human serum paraoxonase 1 (PON1) was purified using a two-step procedure involving ammonium sulphate precipitation followed by hydrophobic interaction chromatography with a Sepharose-4B-L-tyrosin e-6-aminochrysene gel synthesised using a new method.

Initially, human serum was subjected to 60–80% ammonium sulphate saturation to precipitate proteins. The resulting pellet was dissolved in 0.1 M Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0) and applied to a hydrophobic interaction column (10 × 2 cm) equilibrated with 0.1 M Na_2HPO_4 buffer (pH 8.0) containing 1 M $(NH_4)_2SO_4$.

The hydrophobic gel was synthesised as follows: L-tyrosine was covalently bound to CNBr-activated Sepharose-4B via its amine group, forming a stable amide linkage. Subsequently, diazotised 6-aminochrysene was coupled to the meta position of the tyrosine aromatic ring. L-tyrosine served as a spacer arm to reduce steric hindrance between the support matrix and the hydrophobic ligand, enhancing accessibility to the enzyme binding sites. This strategy has been employed in previous studies to improve ligand presentation and protein-ligand interaction efficiency by Sinan et al. (2006).

Protein elution was monitored by absorbance at 280 nm (Manchester, 1996), and protein quantification was performed using the Bradford assay with bovine serum albumin as the standard (Kruger, 2009). Specific activity was calculated based on enzyme activity per milligram of protein. Paraoxonase activity was determined spectrophotometrically using paraoxon (diethyl p-nitrophenyl phosphate) as a substrate, following the procedure previously described in our earlier study (Gökçe & Muhammed, 2023; Arslan et al., 2023) and originally based on the Gan method (Gan et al., 1991), with minor modifications. The increase in absorbance at 412 nm due to the formation of p-nitrophenol was monitored in 50 mM Tris-HCl buffer (pH 8.0) containing 1 mM $CaCl_2$ at 25 °C. One unit of enzyme activity was defined as the amount of enzyme hydrolysing 1 µmol of paraoxon per minute under these conditions. The purified enzyme was stored at +4 °C for short-term use. All purification experiments were performed in triplicate to ensure reproducibility.

In vitro *inhibition kinetic studies*

The inhibitory effects of four emergency cardiac drugs (esmolol, adenosine, digitoxin, and deslanoside) on purified human serum PON1 activity were evaluated. Enzyme assays were conducted in the absence (control) and presence of each inhibitor at five different concentrations. IC_{50} values (the concentration of inhibitor causing 50% inhibition) were determined from dose–response curves. For each inhibitor, five different concentrations were initially tested to construct % activity–inhibitor concentration plots. Based on these plots, appropriate inhibitor concentrations were selected for subsequent kinetic analyses and determination of Ki values using Lineweaver–Burk double reciprocal plots.

For esmolol, the mode of inhibition was characterised using Lineweaver–Burk double reciprocal plots at varying paraoxon concentrations (Lineweaver & Burk, 1934),

allowing determination of the inhibition constant (K_i). All measurements were performed in triplicate, and data were analysed using GraphPad Prism 8.0. Results are presented as mean ± standard error of the mean (SEM), and $p < 0.05$ was considered.

Molecular docking

The three-dimensional crystal structure of human serum paraoxonase 1 (PON1) complexed with 2-hydroxyquinoline (PDB ID: 3SRG) was retrieved from the Protein Data Bank (Ben-David et al., 2012). The missing N-terminal region was modelled using the homology modelling tools available in PyMol to complete the structure before docking.

The ligands (diltiazem, digoxin, esmolol, and adenosine) were obtained from the PubChem database (Kim et al., 2021) in SDF format. Ligand preparation involved the addition of polar hydrogen atoms and the assignment of Gasteiger charges. Similarly, the protein structure was prepared by removing water molecules, adding polar hydrogens, and calculating Gasteiger charges (Muhammed et al., 2022).

Molecular docking simulations were performed using AutoDock Vina (Trott & Olson, 2010). A grid box encompassing the active site was defined with dimensions of $20 \times 20 \times 20$ Å^3, centred on the binding pocket of the co-crystallized ligand. The exhaustiveness parameter was set to 8 to ensure adequate conformational sampling. Following docking, the binding poses were visualised and analysed using Biovia Discovery Studio Visualiser. Binding affinities, hydrogen bonds, and interaction types (e.g. hydrophobic, electrostatic) were evaluated for each compound.

Molecular dynamics simulation

Molecular dynamics (MD) simulations were performed using GROMACS 2021 software (Abraham et al., 2015) to investigate the dynamic behaviour and stability of the PON1–drug complexes. The CHARMM36 force field was applied to describe the protein topology. Ligand topologies were generated using the CGenFF server (Lindahl et al., 2010).

Each complex was placed in a triclinic simulation box and solvated using the TIP3P water model. The systems were neutralised by adding an appropriate number of Na^+ counterions to counterbalance the total charge. Energy minimisation was performed using the steepest descent algorithm for 50,000 steps to remove unfavourable contacts and relax the system. Following energy minimisation, equilibration was carried out in two phases. A 100 ps NVT ensemble simulation at 300 K to stabilise the temperature. A 100 ps NPT ensemble simulation at 1 bar pressure to stabilise the density.

Temperature was maintained using the V-rescale thermostat, and pressure was controlled using the Parrinello–Rahman barostat. The integration time step was set to 2 femtoseconds (fs), and the LINCS algorithm was used to constrain all bond lengths involving hydrogen atoms.

Production MD simulations were subsequently conducted for 150 nanoseconds (ns) under periodic boundary conditions. Trajectories were saved every 2 ps for further analysis. Root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and hydrogen bond formation analyses were performed using built-in GROMACS tools. Graphs were plotted using QtGrace software to visualise the dynamic behaviour of the complexes.

MMPBSA calculations

Binding free energy calculations for the PON1–drug complexes were performed using the MMPBSA.py module from the AmberTools20 package (Kumari et al. 2014) .

The molecular dynamics (MD) trajectories generated by GROMACS were converted into the AMBER format using the gmx_MMPBSA tool to facilitate compatibility. Before the energy calculations, all water molecules and counterions were removed from the systems. Snapshots were evenly extracted from the last 150 ns of the MD simulations, resulting in a total of 1000 frames for the MMPBSA analysis. The total binding free energy ($\Delta G\_$ bind) was decomposed into van der Waals energy, electrostatic energy, polar solvation energy, and non-polar solvation energy components. The final binding free energy values were obtained by averaging the results across all snapshots. These calculations provided a more comprehensive insight into the strength and stability of the ligand binding to the PON1 enzyme.

Results and discussion

Paraoxonase enzyme purification

PON1, one of the most powerful antioxidant enzymes, has become the subject of intense research. However, despite many efforts, there is still a need to better elucidate the structure and mechanism of action of PON1. For this reason, since the early 90s, researchers have tried to purify the enzyme by different procedures to evaluate it in kinetic studies and enzyme-drug

interaction studies (Furlong et al., 1991; Gan et al., 1991; Gil et al., 1993).

Sinan et al. (2006) succeeded in purifying PON1 from human serum using a two-step procedure, involving ammonium sulphate precipitation followed by hydrophobic interaction chromatography with a Sepharose-4B-L-tyrosine-1-naphthylamine gel, achieving 227-fold purification and 72.54% yield. This simple hydrophobic interaction chromatographic protocol involving a two-step procedure has been used by many researchers to purify and characterise paraoxonase enzyme from different sources. Several modified hydrophobic interaction chromatography methods using different extension arms and pre-purification processes are available in the literature (Colak & Gençer, 2012; Demir et al., 2016; Gençer & Yavuz, 2017). In recent years, researchers have focused on the effects of hydrophobic interaction chromatography gels prepared using novel ligands with different apolarities on PON1 purification. These ligands, targeted for alternative gels; amino anthracene (Demir et al., 2016). and amino phenanthrene (Gençer & Yavuz, 2017) have three cyclic aromatic rings and are more hydrophobic than naphthylamine (Sinan et al., 2006). Thus, it was thought that the PON1 enzyme binds more tightly to the column than 1-naphthylamine since it has more hydrophobic characteristics. In the study in which 9-aminophenanthrene was used as a ligand; the enzyme was purified 526 times with a very high degree of purification, but the yield was found to be lower 39.8% (Colak & Gençer, 2012). This situation; in the hydrophobic interaction chromatography method; as the ligand has greater hydrophobicity, this can be a disadvantage; interpreted that the tightly bound enzyme may also be more difficult to separate from the column. However, although the 6-aminochrysene compound has a larger hydrophobic group in our Sepharose-4B-L-tyrosine-6-aminochrysene gel synthesised using a new method, purification was successfully carried out under mild conditions. From this study, 329.42-fold purification degree and 66.17% yield of PON1 enzyme from human serum were obtained (Table 1). The specific activity was also found to be 243.77 U/mg (Table 1). The results showed that further studies on enzyme purification are needed. As shown in Figure 1, the SDS-PAGE analysis of the purified PON1 revealed a single band corresponding to ~46 kDa, indicating a high degree of purity.

In vitro *inhibition kinetic studies*

Enzymes have the ability to catalyse almost all chemical reactions in living organisms. Many chemicals can affect metabolic processes even at very low concentrations by either reducing or enhancing normal enzyme activity, particularly by inhibiting vital enzymes, thereby making them important drug targets.

Several studies have been conducted by our research group on the interactions between various drugs and enzymes (Kurt et al., 2016; Rifati-Nixha et al., 2019). Common enzyme families studied as multi-drug targets include the carbonic anhydrase isoenzyme family (Alım et al., 2022; Cakmak et al., 2021; Tokalı et al., 2023), acetylcholinesterase (Alım et al., 2022; Yamali et al., 2018), and glucose-6-phosphate dehydrogenase (Çalışkan et al., 2022; Yıldız et al., 2022).

However, there are relatively few studies in the literature investigating the effects of medicinal drugs on PON1 activity (Alim & Beydemir, 2016; Argan et al., 2022; Ekinci et al., 2010), and no studies have been reported regarding the effects of the emergency cardiac drugs deslanoside, digitoxin, esmolol, and adenosine on PON1. Four emergency cardiac drugs—deslanoside, digitoxin, esmolol, and adenosine—were evaluated for their inhibitory effects on purified PON1 activity. All drugs effectively inhibited PON1 at varying micromolar concentrations (Table 2, Figure 2).

In vitro assays revealed that these compounds reduced hPON1 activity in a dose-dependent manner, with IC_{50} values of $0.131 \pm 0.71\,\mu M$ for esmolol, $74.792 \pm 1.13\,\mu M$ for adenosine, $96.721 \pm 2.82\,\mu M$ for digitoxin, and $173.329 \pm 3.45\,\mu M$ for deslanoside, respectively. Corresponding K_i values were calculated as $0.044 \pm 0.09\,\mu M$ (esmolol), $65.453 \pm 2.07\,\mu M$ (adenosine), $88.54 \pm 1.15\,\mu M$ (digitoxin), and $157.407 \pm 4.56\,\mu M$ (deslanoside). The inhibitory potency of these compounds against PON1 followed the order: esmolol > adenosine > digitoxin > deslanoside. Different

Table 1. Purification steps and results for human serum paraoxonase 1 using hydrophobic interaction chromatography.

Purification steps	Volume (ml)	Activity (U/ml)	Total activity (U/ml)	Protein (mg/ml)	Total protein (mg)	Specific activity (U/mg)	% Yield	Purification fold
Serum sample	25	17.24	431	23.71	580.25	0.74	100	–
Ammonium sulphate precipitation (60–80%)	11	33.60	369.60	15.24	167.64	2.20	85.75	2.97
Hydrophobic interaction chromatography	3	95.07	285.21	0.39	1.17	243.77	66.17	329.42

modes of inhibition were observed: adenosine, digitoxin, and deslanoside acted as non-competitive inhibitors, while esmolol exhibited a competitive inhibition mechanism. Non-competitive inhibitors, by definition, bind to sites distinct from the active site of the enzyme, causing conformational changes that impair enzymatic activity without directly blocking substrate binding (Figure 3). PON1 contains two calcium ions with different roles at its active centre: one ion interacts with amino acid residues to stabilise the structure, while the catalytic calcium ion is directly involved in chemical binding and enzymatic activity.

Based on these results, the competitive inhibition exhibited by esmolol suggests that it interacts directly with amino acid residues at the enzyme's active site. This interaction was further supported by molecular docking and molecular dynamics simulation studies, which revealed that esmolol forms hydrogen bonds, pi-cation, and pi-pi interactions with specific residues within the active site. Notably, Asp183 was observed to form a hydrogen bond with the nitrogen atom of esmolol. The binding mode predicted through docking studies indicates a strong binding affinity of esmolol towards PON1.

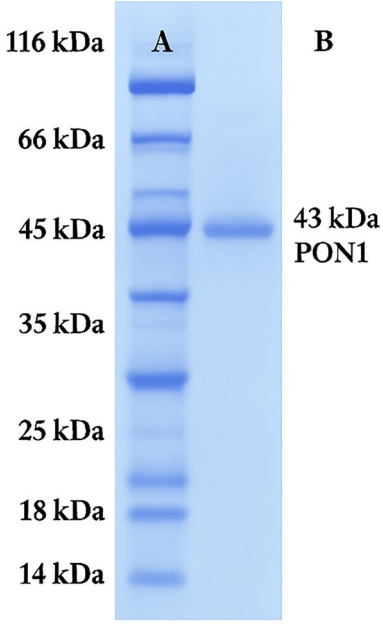

Figure 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of purified human serum paraoxonase 1 (hPON1). Column A: standard proteins. Column B: SDS-PAGE bands of PON1 purified from human serum.

Among the tested compounds, esmolol demonstrated the strongest inhibitory potential, with an IC_{50} value of 0.131 ± 0.71 µM and a K_i value of 0.044 ± 0.09 µM. In contrast, deslanoside exhibited the weakest inhibitory effect, with an IC_{50} value of 173.329 ± 3.45 µM and a K_i value of 157.407 ± 4.56 µM. These *in vitro* findings consistently indicated that esmolol acts as the most potent inhibitor of PON1 activity compared to the other tested cardiac emergency drugs (Figure 3). These results showed that it is important to better understand the structure-activity relationship (SAR) established on the basis of different substituents and their positions in the compounds.

Molecular docking

The binding modes of the most active compound and the bound ligand with PON1 were assessed through molecular docking first. Prior to docking, the missing region of the PON1 structure retrieved from PDB was generated through homology modelling (Muhammed et al., 2019; Muhammed & Aki-Yalcin, 2019). The docking process was validated by redocking the bound ligand. The bound ligand was found to interact with PON1 very well. It interacted with the enzyme via four conventional hydrogen bonds (His115, Asn168, Asn224, and Asp269) and five more other interactions (Ile74, His285, Ile291, and Val346(2)) (Figure 4, Table 1). In a previous crystallographic structure elucidation study, PON1 interacted with the bound ligand via His115, Asn168, and Asp269 amino acids. In the same study, the enzyme interacted to ligands through Ile74, Asn224, and Val346 amino acids (Ben-David et al., 2012). Hence, the docking study results in this study were compatible with the experimental findings. In addition to the strong interaction detected, the previous experimental studies confirmed the docking process.

Esmolol demonstrated strong interaction with the PON1. It interacted with the enzyme with three conventional hydrogen bonds (Ser118, Thr119, and Ala172), four carbon hydrogen bonds (Ile57, Asn227, Asp274, and Pro275), single electrostatic (Glu56), and single hydrophobic interactions (Val273) (Figure 5,

Table 2. IC_{50} and K_i values of deslanoside, digitoxin, esmolol, and adenosine on the inhibition of purified PON1 activity.

Active ingredient	IC_{50} (µM)	R^2	K_i (µM)	R^2	Inhibition type
Deslanoside	96.721 ± 2.82	0.9958	88.54 ± 1.15	0.9184	Non-competitive
Digitoxin	173.329 ± 3.45	0.9966	157.407 ± 4.56	0.9745	Non-competitive
Esmolol	0.131 ± 0.071	0.9905	0.044 ± 0.009	0.9886	Competitive
Adenosine	74.792 ± 1.13	0.9901	65.453 ± 2.07	0.9902	Non-competitive

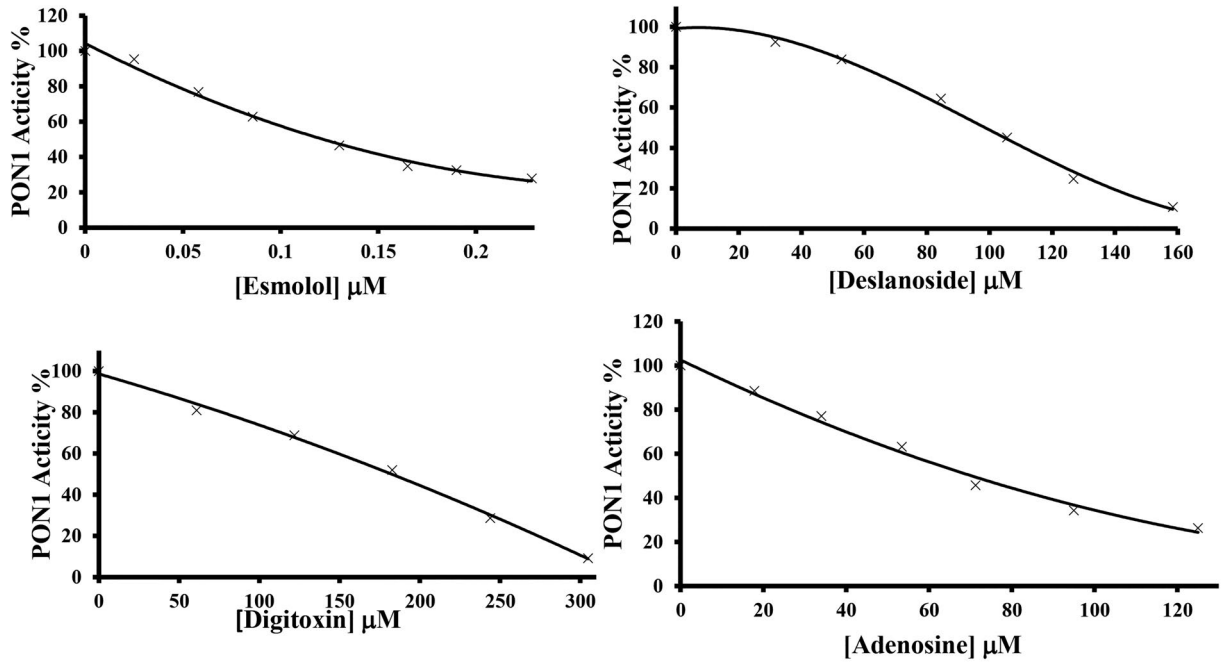

Figure 2. Dose-dependent inhibition curves of esmolol, deslanoside, digitoxin, and adenosine on purified PON1 activity.

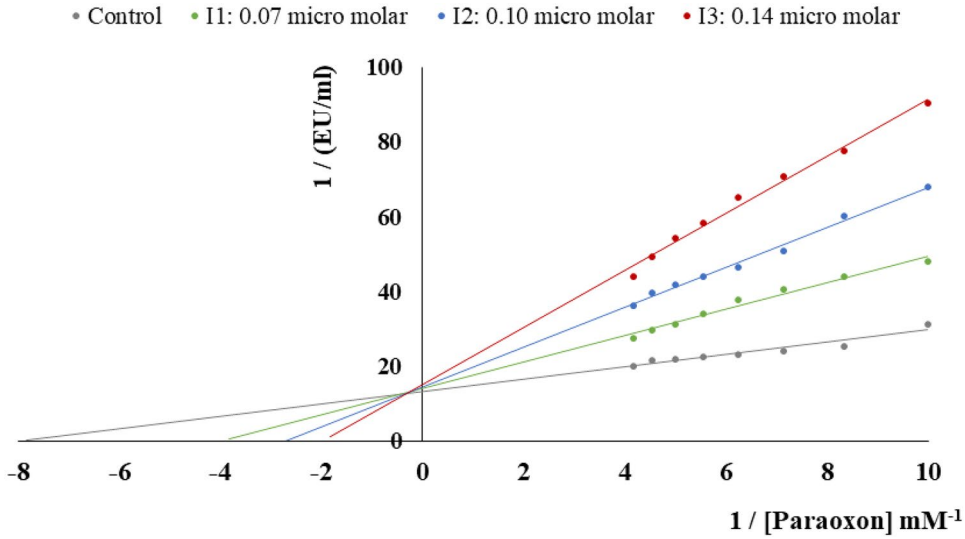

Figure 3. Lineweaver–Burk double reciprocal plot showing the competitive inhibition pattern of esmolol on purified PON1 activity.

Table 3). Most of the hydrogen bonding observed was formed between the hydrogens connected to the electronegative atoms in the esmolol and the respective amino acid residues of PON1 (Figure 5). The strength of the binding for esmolol was similar with that of the bound ligand. The bound ligand had one more conventional bond than esmolol but esmolol had four carbon hydrogen bonds. As hydrogen bonding is crucial in stabilising ligands inside binding region of an enzyme, the stability of the ligands inside the binding region of the PON1 is expected to be similar. As a result, MD simulation was performed to measure the stability level. Though the interaction strength between the bound ligand and esmolol was similar, the binding residues were different. The binding residues of esmolol were different from the binding residues determined through experimental studies. These differences imply a unique binding mode for esmolol.

Molecular dynamics simulation

The molecular docking study revealed that esmolol could bind to PON1 well. Hence, the stability of the PON1-esmolol complex was evaluated through MD

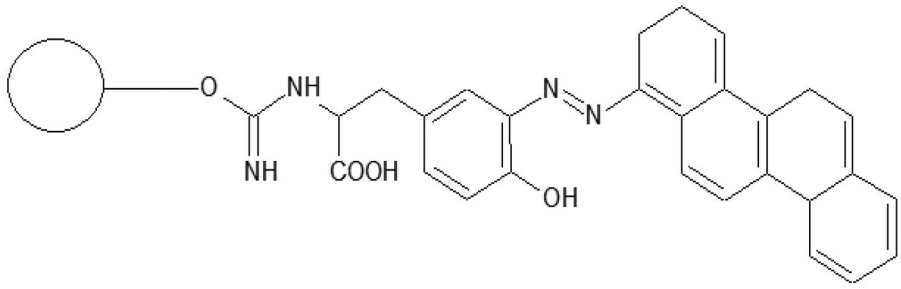

Figure 4. Schematic representation of a Sepharose-4B-L-tyrosine-3-aminophenanthrene hydrophobic gel synthesised using a new method. The L-tyrosine solution prepared in HCl was bound to the CNBr-activated solid support material Sepharose-4B *via* an amide bond through its functional group NH_2. Throughout the docking reaction, the pH was kept constant at 0 °C. The purpose of L-tyrosine in this reaction is to prevent the ligand from forming a cyteric barrier by considering it as an extensor arm. Finally, diazolated 3-aminophenanthrene was docked to the meta position of the L-tyrosine molecule as a ligand. The purification process involved ammonium sulphate precipitation followed by hydrophobic interaction chromatography using Sepharose-4B-L-tyrosin e-6-aminochrysene. Specific activity was calculated as units per milligram of protein.

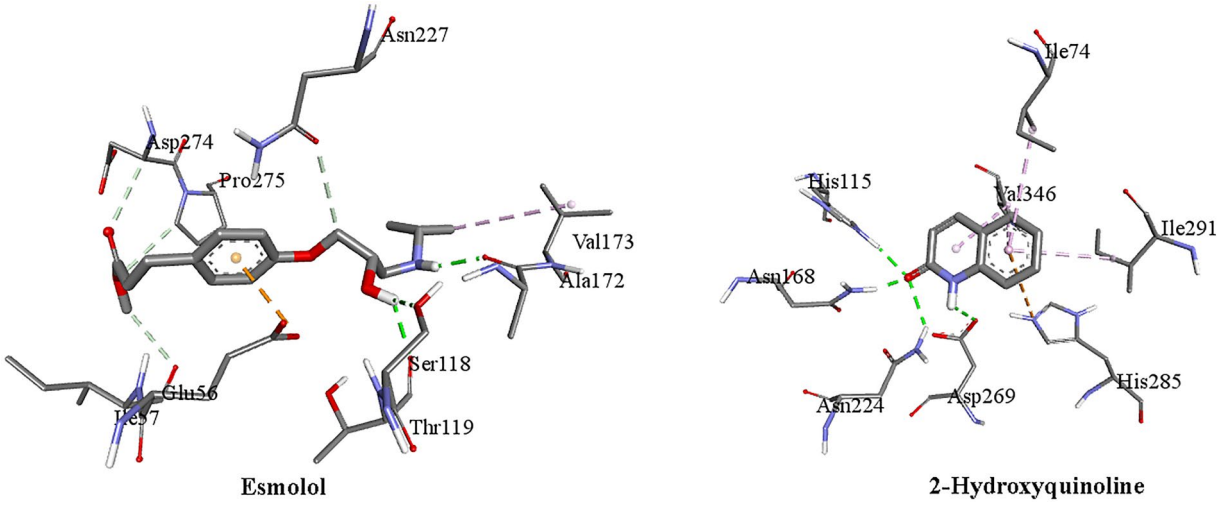

Figure 5. Binding profile of esmolol and 2-hydroxyquinoline with PON1.

simulation. Furthermore, its stability was compared to PON1-bound ligand complex and the apo enzyme. For this purpose, RMSD, RMSF, Rg, and ligand hydrogen bond plots of the complexes and the apo enzyme were drawn (Muhammed et al., 2023). RMSD is used to evaluate deviation of enzyme structures from reference structures in a given simulation period. The relative stability of enzyme structures with or without a ligand is predicted by comparing the RMSD value of enzyme-ligand complexes with each other as well as with the apo enzyme (Işık et al., 2022). In the first 10 ns, the apo enzyme and the PON1-esmolol complex were stabilised. The two had similar RMSD value up to 30 ns. In the 30–95 ns period, the apo enzyme had lower RMSD value than the complex. The overall stability of PON1-esmolol complex was better than the apo enzyme since the apo enzyme had higher level of swinging in the 150 ns period (Figure 6). The PON1-bound ligand complex was stabilised in the first 30 ns. Thereafter, this complex had stable RMSD value. Furthermore, the PON1-esmolol complex had lower RMSD value than PON1-bound ligand complex (Figure 7). The RMSD plot implicated that the PON1-esmolol complex had higher stability than the PON1-bound ligand and the apo enzyme. Therefore, esmolol is expected to remain inside the PON1 binding region throughout the simulation period with better stability.

RMSF plots are used to analyse the perturbation in the amino acid residues during a simulation period (Dong et al., 2018). The RMSF value of esmolol bearing PON1 and the apo enzyme RMSF plots were alike, whereas the 2-hydroxquinoline bearing PON1 had a slender difference with the other two. In the N-terminal 20 amino acid residues, all exhibited significant RMSF fluctuations. In the 65–85 residue region, the PON1-esmolol complex and the apo enzyme had significant fluctuation. In the 100–115 region, the PON1-esmolol complex had higher fluctuation than

Table 3. Binding residues of PON1 in its interaction with esmolol and 2-hydroxyquinoline.

Ligands	Interacting residue	Bonding type	Distance (Å)
2-Hydroxyquinoline	Ile74	Pi-alkyl	4.92
	His115	Conventional hydrogen bond	2.13
	Asn168	Conventional hydrogen bond	1.97
	Asn224	Conventional hydrogen bond	2.49
	Asp269	Conventional hydrogen bond	2.50
	His285	Pi-cation	4.70
	Ile291	Pi-alkyl	4.72
	Val346	Pi-alkyl	4.51
	Val346	Pi-alkyl	4.72
Esmolol	Glu56	Pi-anion	3.65
	Ile57	Carbon hydrogen bond	3.51
	Ser118	Conventional hydrogen bond	2.38
	Thr119	Conventional hydrogen bond	2.27
	Ala172	Conventional hydrogen bond	2.03
	Val173	Alkyl	4.87
	Asn227	Carbon hydrogen bond	3.67
	Asp274	Carbon hydrogen bond	3.71
	Pro275	Carbon hydrogen bond	3.43

the apo enzyme. The PON1-bound ligand complex had higher fluctuations up to 200 amino acid residues (Figure 7). Radius of gyration is used to measure the level of compactness for an enzyme during a simulation period (Maity et al., 2023). After the first 20 ns, the PON1-bound ligand complex had lower Rg value than the rest two with a slender difference. Similarly, the PON1-esmolol complex had lower Rg value than the apo enzyme with a slender difference. A relatively higher difference between them was observed in the 65–100 ns time interval (Figure 6). Hydrogen bonds have a crucial role in stabilising ligands inside the binding region of an enzyme (Ganesan et al., 2017). In general, both complexes had a single bond with intercalated slim two and three hydrogen bonds. In few time intervals, including the first 5 and 23–27 ns interval a sparse three hydrogen bonds were observed for PON1-bound ligand complex. Similarly, in the 7–16 and 130–138 ns period, the PON1-esmolol complex had a sparse two hydrogen bonds. In the molecular docking study, the two complexes were found to interact with more hydrogen bonds than the numbers predicted through MD simulation (Table 3). Hence, there was discrepancy in the hydrogen bond numbers

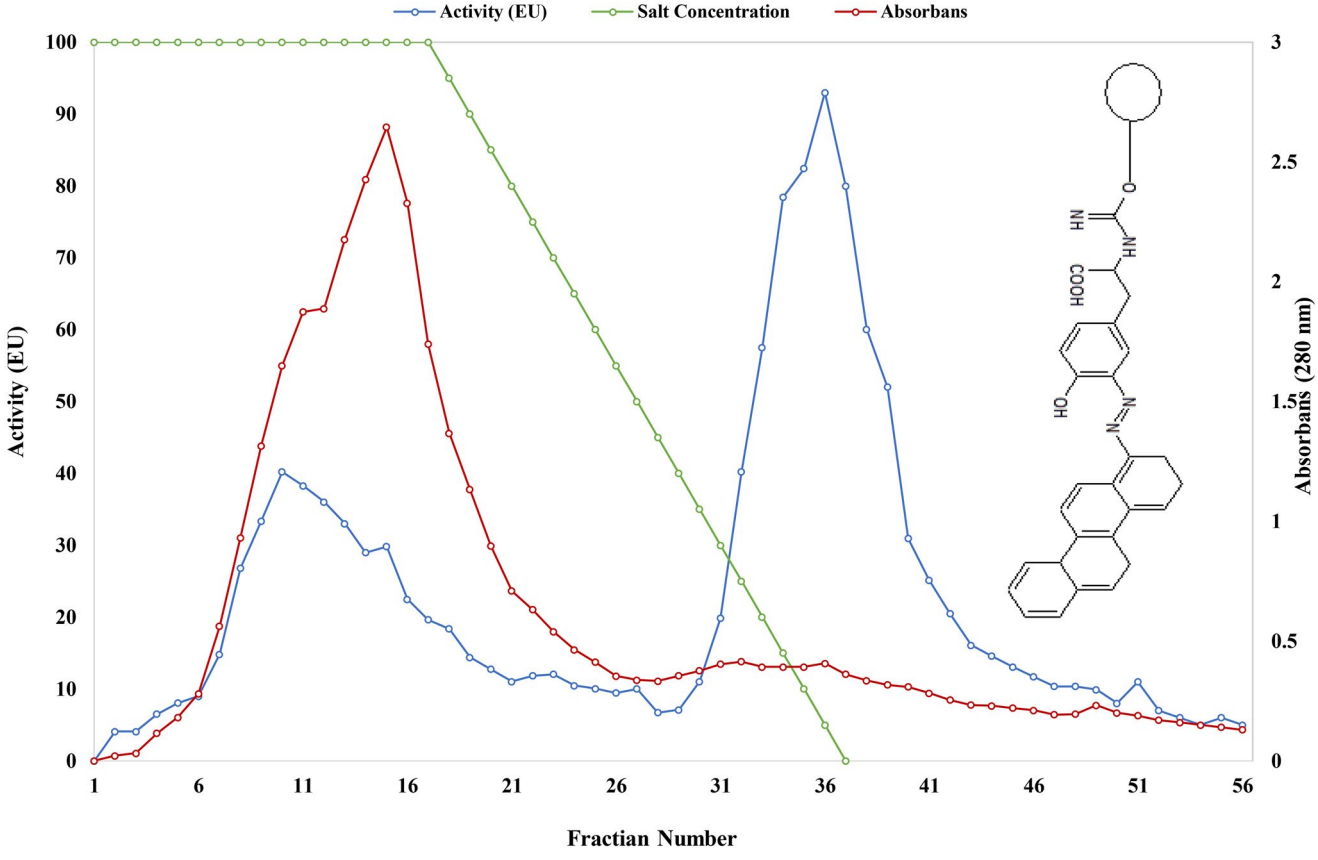

Figure 6. Purification graph of human serum PON1 from newly synthesised sepharose-4B-L-tyrosine-6-aminochrysene hydrophobic interaction chromatographic gel.

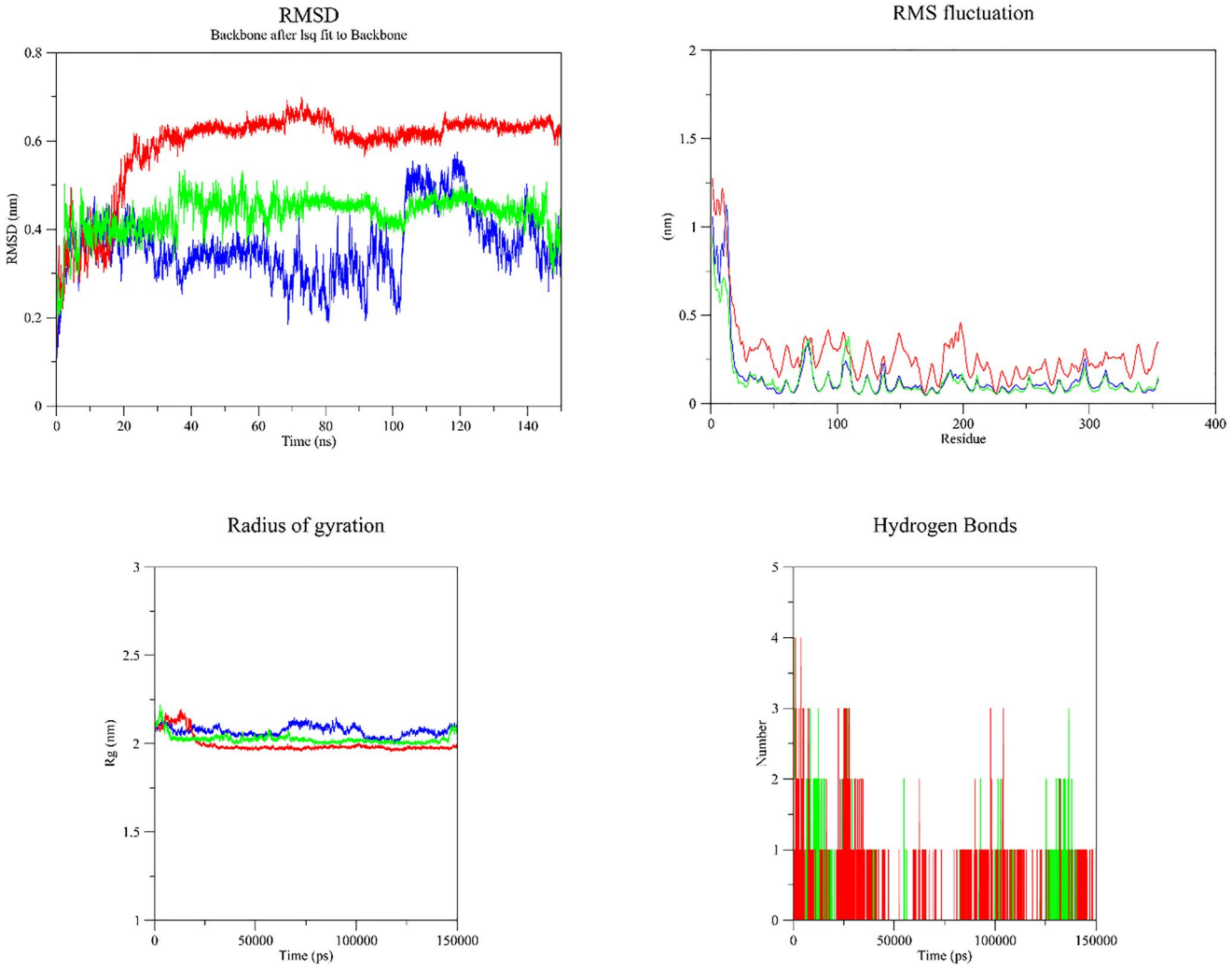

Figure 7. (A) Root mean square deviation (RMSD) profiles of PON1–ligand complexes over 150 ns of molecular dynamics simulation, indicating the structural stability of the complexes. (B) Root mean square fluctuation (RMSF) analysis showing the flexibility of PON1 residues upon drug binding. (C) Radius of gyration (Rg) plots depicting the overall compactness and conformational stability of PON1 throughout the simulation. (D) The number of hydrogen bonds formed between PON1 and the ligands over the simulation time, reflecting the strength and persistence of molecular interactions.

Table 4. Binding energy and the contributing energies for the complexes (in kJ/mol).

Ligands	van der Waals energy	Electrostatic energy	Polar solvation energy	SASA energy	Binding energy
2-Hydroxyquinoline	−160.0 ± 8.2	7.0 ± 4.1	81.3 ± 20.1	−15.4 ± 0.3	−87.2 ± 19.4
Esmolol	−180.1 ± 9.2	−27.4 ± 7.3	54.4 ± 25.3	−27.6 ± 1.2	−180.8 ± 21.8

between the two methods. In short, the MD simulation study revealed that the binding of esmolol to the PON1 was stable.

MMPBSA calculations

MMPBSA is used to calculate the binding energy and contributing energies of target-ligand complexes procured from MD simulation. The MMPBSA binding energy predictions are anticipated to be more accurate than the other energy computation approaches (Ganesan et al., 2017). The MMPBSA computation in this study revealed that the esmolol bearing complex had lower binding energy than that of the 2-hydroxyquinoline (Table 4). This, in turn, implies that esmolol has more affinity towards the PON1. When the binding energy was decomposed into its components, van der Waals energy had the highest contribution to the binding energy in relative to the other components. In relative to their binding energies, the contribution proportion of van der Waals energy in the 2-hydroxyquinoline complex was higher (Table 4). This

implies that hydrophobic interactions might play more important role in stabilising the bound ligand bearing complex than the esmolol bearing ones (Verma et al., 2016). This justifies the higher number of hydrophobic interactions of 2-hydroxyquinoline with PON1 than esmolol in the docking (Table 3).

Conclusion

In this study, the binding mode of esmolol to PON1 was investigated through molecular docking and molecular dynamics (MD) simulation analyses. Molecular docking results revealed that esmolol interacted with PON1 as strongly as the native ligand bound within the enzyme's active site. Furthermore, MD simulations confirmed the stability of esmolol within the binding pocket throughout the simulation period, and MMPBSA calculations indicated a higher binding affinity for esmolol compared to the native ligand.

Paraoxonase 1 (PON1) is a multifunctional enzyme known for its antiatherogenic properties, including the hydrolysis of toxic organophosphates and the prevention of low-density lipoprotein (LDL) oxidation. Low PON1 activity has been associated with an increased risk of cardiovascular diseases, diabetes mellitus, rheumatoid arthritis, hyperthyroidism, cancer, and aging-related conditions. Given its critical physiological functions, therapeutic agents that inhibit or activate PON1 must be thoroughly characterised to avoid unintended clinical consequences.

In addition to investigating drug-enzyme interactions, this study also addressed the longstanding challenge of PON1 purification by developing an alternative chromatographic method using a novel hydrophobic interaction-based ligand, achieving efficient isolation of the enzyme from human serum. Based on these findings, further studies involving *in vivo* experimental models and molecular genetic analyses are warranted to validate the effects of emergency cardiac drugs on PON1 activity and to explore their broader implications for cardiovascular health and drug safety. The expanding knowledge of PON1's biological roles positions it as a promising target for the development of novel therapeutic agents designed to minimise oxidative stress and atherosclerotic risk with fewer adverse effects.

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Author contributions

CRediT: **Başak Gökçe**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing; **Muhammed Tilahun Muhammed**: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; **Melih Yüksel**: Data curation, Investigation; **Nahit Gencer**: Validation, Writing – original draft; **Oktay Arslan**: Writing – review & editing.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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