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In Vitro and In Silico Studies on the Toxic Effects of Antibacterial Drugs as Human Serum Paraoxonase 1 Inhibitor

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The core purpose of the current study was to investigate the interactions of widely used broad-spectrum antibacterial drugs developed in response to the increasing rate of antibiotic-resistant various bacteria and to contribute to the field of drug design. Also, it is to broaden the current knowledge of paraoxonase 1 enzyme (EC: 3.1.8.1; PON1) which is a crucial drug-target enzyme. For this aim, first, we purified PON1 from human serum using rapid chromatographic techniques including, enzyme precipitation, IEX (ion-exchange) chromatography, and SEC (size exclusion chromatography), quickly. Following this, we researched the inhibitory effects of some antibacterial drugs. Finally, molecular docking tests were performed and analyzed in silico data. PON1 was found to be effectively inhibited by tigecycline, linezolid, ciprofloxacin lactate, and ertapenem sodium (K_is in the ranging from 0.018 to 125.540 mM). Drugs showed two different inhibition mechanisms: Linezolid was competitive; others were non-competitive. While Glide GScore of the linezolid for 1 V04 and 3SRE receptors were detected to be −4.442 and −4.915 kcal/mol in the SP mode, monitored as −3.548 and −3.791 kcal/mol in the XP mode, respectively

Introduction

Proper use of drugs, which improve the quality of life of patients, is of great importance all over the world.[1] This situation is more critical for antibacterial medicines which have an essential place in drug consumption in the world. These agents are clinically crucial drugs used in the treatment of diseases caused by pathogenic bacteria.[2] The discovery of antibacterials has been an important milestone for human health, and the use of these drugs in treatment has significantly reduced infectious diseases.[3] The excessive and inappropriate use of these medicines has caused that these drugs are ineffective in treatment nowadays.[4] Today, this situation has become a global threat to public health.[5] Despite the rapidly developing technology and the unconscious use of the medicines, the absence of discovery of novel drugs has caused by many problems to grow day by day.[6]

Tigecycline, a member of the family of glycylcyclines, is a semi-synthetic derivative of antibiotics included in the tetracycline group. It is a first-line drug that has indicated the broad spectrum of activity against a variety of multidrug-resistant (MDR) infections.[7] Linezolid is a drug of belongs to the oxazolidinone class, which is a synthetic class of antibiotics, which shows significant effects in the therapy of MDR gram-positive pathogens. This drug inhibits bacterial development by binding to the 50 S ribosomal subunit during protein synthesis of bacteria.[8] Linezolid, which is called "reserve antibiotic" in the scientific literature, is a drug used as the final alternative in severe infections caused by bacteria that are against resistant to some antibiotics.[9] Ciprofloxacin lactate, a member of the fluoroquinolone class, is an antibiotic displaying broad spectrum activity covering both gram-positive and gram-negative organisms. It deactivates the reproduction of bacteria by inhibiting DNA gyrase and topoisomerase IV enzymes, which play a role in the replication of DNA.[10] Ertapenem is a parenteral carbapenem from the class of β-lactams and is applied as a single drug. This agent occurs superior antibacterial activity against several aerobic and anaerobic organisms.[11]

Atherosclerosis is the underlying reason for more about 50% of deaths in world societies.[12] Atherosclerosis, which is a developing disease in the medium and large coronary arteries,[13] characterized by chronic inflammation, lipid accumulation, extracellular matrix remodeling, and cytotoxic factors[14] is the primary reason for many diseases, such as peripheral vascular diseases, heart attack, and stroke.[15] Oxidative stress plays an essential role in the pathogenesis of this disease. Epidemiological studies have indicated that the most significant components in the blood that contributes to atherogenesis are the plasma lipids.[16] The oxidation of low-density lipoprotein (oxLDL) has been determined to cause some pro-inflammatory cases launching and increasing the atherogenic process.[17]

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In contrast, high-density lipoprotein (HDL) and apolipoprotein A–I antagonize atherosclerosis, prevent oxidative stress and inflammation, and increase cholesterol flow to reduce lesion formation. The uptake and accumulation of oxidatively modified LDL by macrophages initiate a wide variety of biological activities that may lead to the development of atherosclerotic lesions.[18] Therefore, reducing the oxidation of HDL[19] and LDL reduces the risk of cardiovascular events. One of the significant factors which protect serum lipids against oxidation is paraoxonase (paraoxon hydrolase; EC: 3.1.8.1; PON1).[20] PON1, which exhibit anti-inflammatory[21] and antioxidant activities,[22] is an esterase enzyme released by the liver[23] and related to HDL in the blood.[24] In vivo studies, PON1 has been shown to reverse the biological effects of oxidized LDL[25] and to protect LDL against oxidation.[26] It has also been found that PON1 is a therapeutic agent to prevent atherosclerosis and contributes significantly to the antioxidant capacity of HDL.[27] Also, PON1 has been suggested to play an essential role in the hydrolysis of a lactone derived from polyunsaturated fatty acids. This lactone is involved in arachidonic acid metabolism, which is a central regulator of the inflammatory response.[28]

Enzymes are vital molecules for the modulation of critical metabolic processes such as reproduction, signal transduction, and cell death. Many diseases are treated with conventional drugs, which are metabolized by enzymes. Drugs demonstrate their biological influences by interaction with specific enzymes. That is to say, the medicines may modulate enzymes inhibition or induction and contribute to clinically significant drug-enzyme interactions. For example, in inflammatory diseases, all the nonsteroidal anti-inflammatory drugs (NSAIDs) exhibit anti-inflammatory effects by inhibiting the activity of the enzymes of the cyclooxygenase (COX) family. However, such interactions may result in critical adverse medication damages.

Widespread use of drugs is increasing day by day and but sadly, in particular, antibiotics are in the first place. Rational drug use is very important to reduce or prevent prolongation of therapy, superinfections, adverse severe medication reactions, and toxicity of drugs.[29] Moreover, irrational drug use is a potential risk factor for metabolic enzymes. The determining of biological activity of, such enzymes after drug take may evaluate as a pharmacological marker. Our study may provide considerable insight in to arrange doses of the selected drugs that are commonly employed in the treatment of caused diseases by pathogenic bacteria. As stated in the Abstract, in the initial step of the present research, we extended our previous PON1-drug activity relationship studies,[30] and we purified PON1 enzyme from human serum by using simple chromatographic methods. Subsequently, we examined the in vitro effects of some antibacterial drugs, such as tigecycline, linezolid, ciprofloxacin lactate, and ertapenem sodium on PON1 enzyme activity (Figure 1).

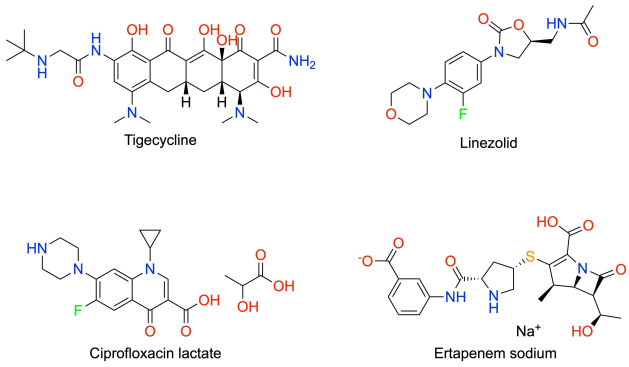

Figure 1. Chemicals structures of tigecycline (PubChem CID: 54686904), linezolid (PubChem CID: 441401), ciprofloxacin lactate (PubChem CID: 149514), and ertapenem sodium (PubChem CID: 23721821).

Results and Discussion

Enzymes are suitable targets for therapeutic practice in pathogenic metabolism. The structure of the enzyme active sites and other allosteric binding sites is naturally suitable for linking drug molecules. The drugs exhibit their biological influences on the active site of the enzyme by competitive, non-competitive, and uncompetitive inhibitors binding or allosteric regulation. While some research has been carried out on PON1, which has been known to be significant by virtue of anti-inflammatory[31] and antioxidant properties,[32] there has still few studies which have investigated the association between PON1 and medication interactions. Therefore, this research purposes of contributing to the academic literature by exploring these interactions.

The PON1 enzyme is available in many tissues, including brain, heart, kidney, liver, lung, small intestine.[33] However, PON1 is synthesized mainly by the liver and bound to HDL and then released into the bloodstream. PON1 is directly responsible for a number of the antiatherogenic properties of HDL.[34] These tasks include to protect lipoproteins and cells against oxidative stress and also to inhibit atherogenesis and lipoprotein peroxidation.[35] This protective effects of PON1 are associated with the enzyme level in the serum.[36] Recent studies found that PON1 levels have low in patients with atherosclerosis, diabetes, and hypercholesterolemia and overexpression of PON1 especially impedes the development of cardiovascular diseases. Also, PON1 has been determined to induce HDL cholesterol efflux from macrophages[37] and to inhibit LDL cholesterol biosynthesis.[38] In other study, it has been shown that PON1 is transported from HDL to the external face of the cell membrane to protect metabolism against oxidative stress-related conditions[39] such as coronary artery disease,[40] diabetes, obesity,[41] rheumatoid arthritis, and neurodegenerative diseases[42] and PON1 activity is inversely related to these diseases.[43] Thus, first of all, we are essential to know which medicates and chemical substances increase PON1 activity and inhibit enzyme activity in order to prevent all the diseases mentioned above related to PON1, especially atherosclerosis.

In this study, it was applied the identical procedure used by our group in previous studies,[44] and PON1 purified from human serum in three simple steps.[45] The purification resulted in with a yield of 20.25%, a specific activity of 3880.83 EU/mg

proteins, and the enzyme was purified approximately 240-fold. This study supports evidence from previous researches.[46] Drug-enzyme interaction researches with metabolic enzymes have importance, and these investigate are increasing day by day in the science world. There is no information about the inhibition mechanism and inhibition type of such antibiotic medications on PON1 enzyme, currently. Thus, in the same study, we focused on the in vitro inhibition role of some antibacterial drugs including tigecycline, linezolid, ciprofloxacin lactate, and ertapenem sodium on PON1 enzyme activity. We identified from the Table 1 that tigecycline showed potent

Table 1. K_i values and inhibition types of antibacterial drugs.

Inhibitor	PubChem CID	K_i (mM)	Inhibition type
Tigecycline	54686904	0.018 ± 0.002	Non-competitive
Linezolid	441401	0.250 ± 0.024	Competitive
Ciprofloxacin lactate	149514	3.492 ± 0.287	Non-competitive
Ertapenem sodium	23721821	125.540 ± 7.170	Non-competitive

The analysis results were exhibited as mean ± standard deviation ($n = 3$ independent experiments).

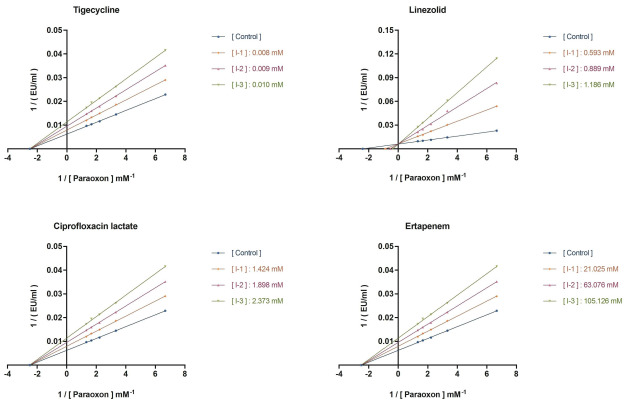

Figure 2. Describing the drug-enzyme interaction may be illustrated in several ways. One of them is Lineweaver-Burk plots, which is defined by the equation. The type of enzyme inhibition (e.g., competitive, non-competitive, and non-competitive inhibitors) and K_i constants may be easily estimated using the Lineweaver-Burk curves. Based on this, the antibacterial drugs (i.e. tigecycline, linezolid, ciprofloxacin lactate, and ertapenem sodium) were analyzed in three different concentrations. Competitive inhibitors (competes with the substrate for the active site) have the same y-intercept as an uninhibited enzyme, but there are different slopes and x-intercepts between the two data sets. Non-competitive inhibitors (binds to both substrate active site and distinct site) plots with the same x-intercept as an uninhibited enzyme but different slopes and y-intercepts.

inhibition profile against PON1. The order of drugs that affect as inhibitor follows: Tigecycline ($C_{29}H_{39}N_5O_8$; K_i: 0.018 ± 0.002 mM) > Linezolid ($C_{16}H_{20}FN_3O_4$; K_i: 0.250 ± 0.024 mM) > Ciprofloxacin lactate ($C_{20}H_{24}FN_3O_6$; K_i: 3.492 ± 0.287 mM) > Ertapenem sodium ($C_{22}H_{24}N_3NaO_7S$; K_i: 125.540 ± 7.170 mM). According to these results, linezolid exhibited competitive inhibition, whereas other drugs showed in non-competitive inhibition (Figure 2).

In addition, in silico molecular modeling studies were performed to find the binding interaction of linezolid to active sites of both 1 V04 and 3SRE receptors (Table 2). The 3D and 2D views of the linezolid with these receptors were schematized in Figures 3–6, respectively. Linezolid composed a hydrophobic cloud with Tyr71, Phe222, Ile291, Phe292, Phe293, Tyr294, Val346, and Phe347 residues of the 1 V04. H-bond was observed between the carbonyl group of the acetamide and Lys192 residue of the 1 V04 in SP mode. Also, in XP mode, the amino group of the ligand formed an h-bond with Asp183. Linezolid showed hydrophobic interactions with Leu69, Tyr71, Val167, Val346, and Phe347 amino acids of 3SRE. Lys192 formed h-bond interactions with the acetamide moiety of the compound in both SP and XP modes of the 3SRE. Molecular docking studies on linezolid showed very similar ligand-receptor interaction between 1 V04 and 3SRE.

Scientists have done various research on PON1-drug interactions. For instance, Ekinci and Beydemir studied the impacts of some antibiotics such as teicoplanin, rifamycin, tobramycin, ceftriaxone sodium, cefuroxime sodium, ceftazidime, ornidazole, and amikacin sulfate were performed on PON1. These agents have exhibited strong inhibition effect on the PON1. IC_{50} values were in the range of 0,077-40,760 mM and K_i constants were 0.090, 0.716, 3.381, 6.427, 6.642, 12.786, 13.501, and 55.437 mM, respectively. In the same study, they also reported that rifamycin and cefuroxime sodium showed competitive inhibition, teicoplanin, ceftazidime, and amikacin sulfate, non-competitive inhibition, and tobramycin, ceftriaxone sodium, and ornidazole, uncompetitive inhibition.[47] In another study, scientists investigated the effects of some antibacterial drugs, including meropenem trihydrate, piperacillin sodium, and cefoperazone sodium on PON1 activity. They found that

Table 2. Binding free energy calculation results for linezolid bound with 1 V04 and 3SRE.

PDB ID	SP Glide GScore	ΔG vdW [a]	ΔG coulomb [b]	Glide energy [c]	Glide model [d]	H-bonds	XP Glide GScore	ΔG vdW [a]	ΔG coulomb [b]	Glide energy [c]	Glide model [d]	H-bonds
1 V04	−4.442	−30.908	−9.517	−40.425	−52.510	Lys192	−3.548	−30.656	−7.274	−37.930	−50.463	Asp183
3SRE	−4.915	−33.474	−6.914	−40.388	−52.505	Lys192	−3.791	−32.911	−6.973	−39.884	−52.846	Lys192

[a] Contribution to the free energy of binding from the van der Waals energy (kcal/mol), [b] Contribution to the free energy of binding from the Coulomb energy (kcal/mol), [c] Energy obtained through Glide module (kcal/mol), [d] Type of energy obtained through Glide module (kcal/mol)

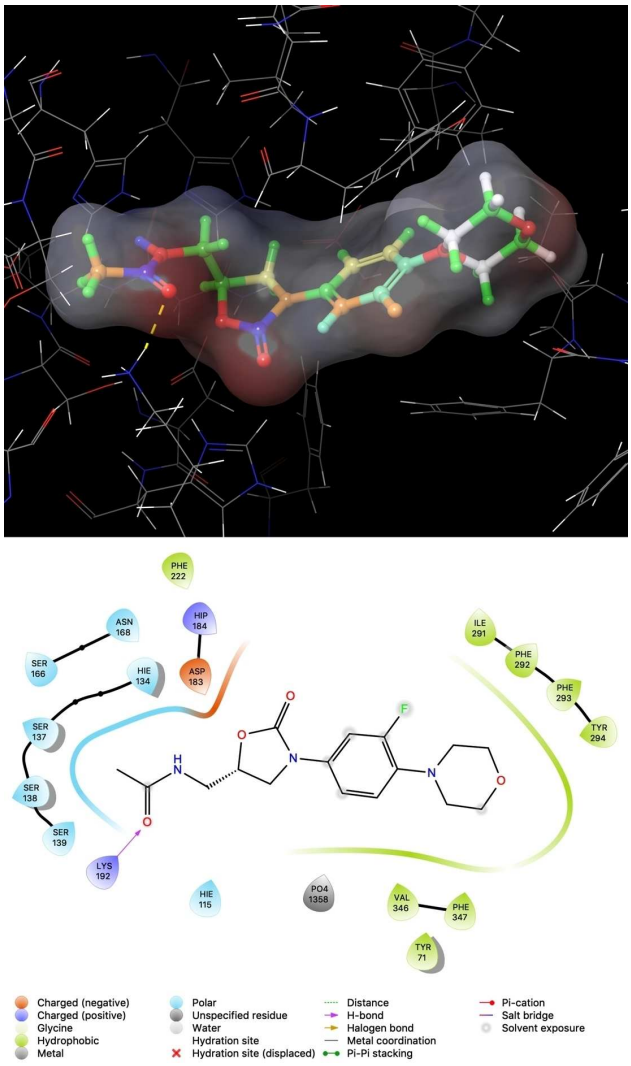

Figure 3. 3D and 2D schematic views of linezolid with 1 V04 in Glide standard precision mode.

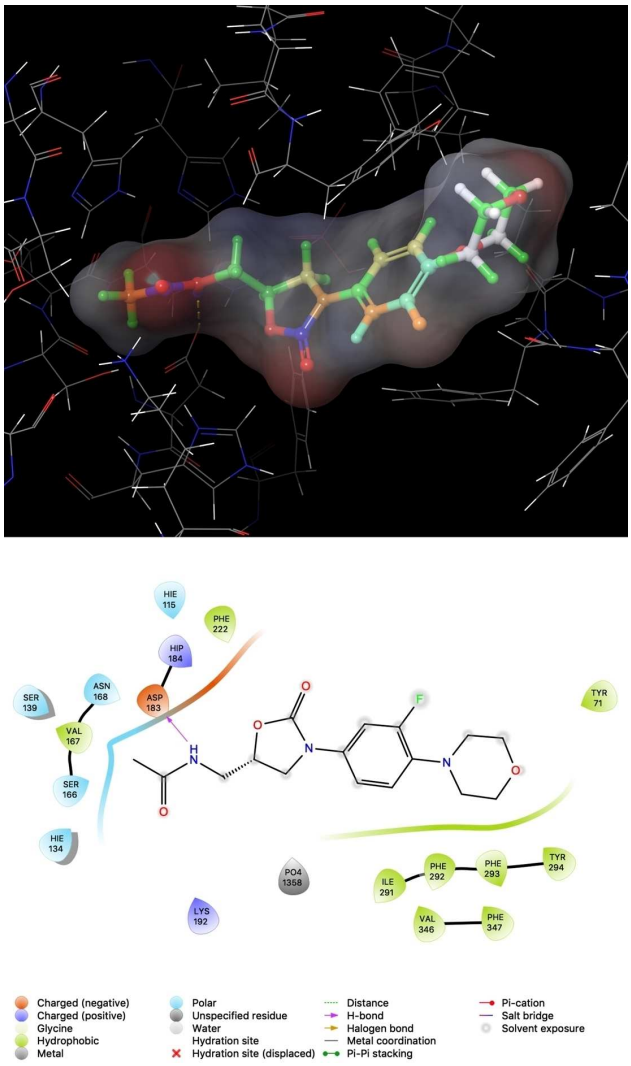

Figure 4. 3D and 2D schematic views of linezolid with 1 V04 in Glide extra-precision mode.

these drugs decreased PON1 activity, and IC_{50} values were 0.481, 23.105, and 25.342 mM, respectively, and K_i constants were in the range of 0.597 ± 0.006-1.414 ± 0.639 mM. Authors reported that meropenem trihydrate showed non-competitive inhibition, other drugs exhibited competitive inhibition.[48]

In our previous a study which set out to investigated PON1-drug interactions, after purifying PON1, we researched that the in vitro effects of various antibacterial drugs such as, moxifloxacin hydrochloride, levofloxacin hemihydrate, cefepime hydrochloride, cefotaxime sodium, and ceftizoxime sodium. IC_{50} values and K_i constants for these drugs were found to be 1.839, 3.959, 21.115, 152.161, and 311.915 mM and 2.641 ± 0.040, 5.525 ± 0.817, 35.092 ± 1.093, 252.762 ± 5.749, and 499.244 ± 10.149 mM, respectively. In their cutting edge paper of 2019, Türkeş and Beydemir analyzed in vitro effects of some antimycotic drugs, including caspofungin acetate, amphotericin B, anidulafungin, and fluconazole on PON1 enzyme activity. They determined that IC_{50} values ranging between 0.037 ± 0.001 to 5.728 ± 0.043 mM and K_i constants were 0.0105 ± 0.0015 mM for caspofungin acetate, 0.3206 ± 0.0196 mM for amphotericin B, 0.1674 ± 0.0233 mM for anidulafungin, and 2.5464 ± 0.1655 mM for fluconazole. These tests revealed that anidulafungin showed mixed inhibition while caspofungin acetate and fluconazole inhibited the PON1 competitively, and amphotericin B showed non-competitive inhibition.[49] We also indicated that moxifloxacin hydrochloride had competitive, whereas the others showed non-competitive inhibition.[30] Also, in our other studies, we selected the drugs, such as diverse nucleoside analogs (gemcitabine hydrochloride, acyclovir, and 5-fluorouracil),[50] some calcium channel blockers (nifedipine, nitrendipine, isradipine, and amlodipine besylate),[51] various antineoplastic medicates (palonosetron hydrochloride, bevacizumab, and cyclophosphamide),[52] several chemotherapeutic agents (vincristine sulfate, epirubicin hydrochloride, and doxorubicin hydrochloride),[53] some gadolinium-based contrast agents (gadoteric acid, gadopentetic acid, gadoxetate disodi-

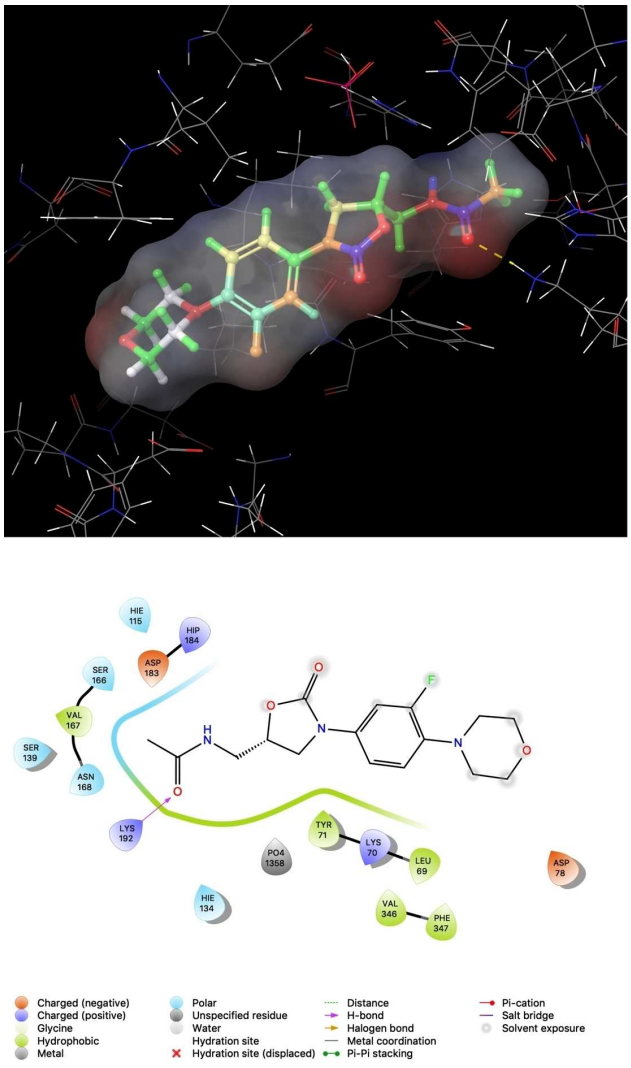

Figure 5. 3D and 2D schematic views of linezolid with 3SRE in Glide standard precision mode.

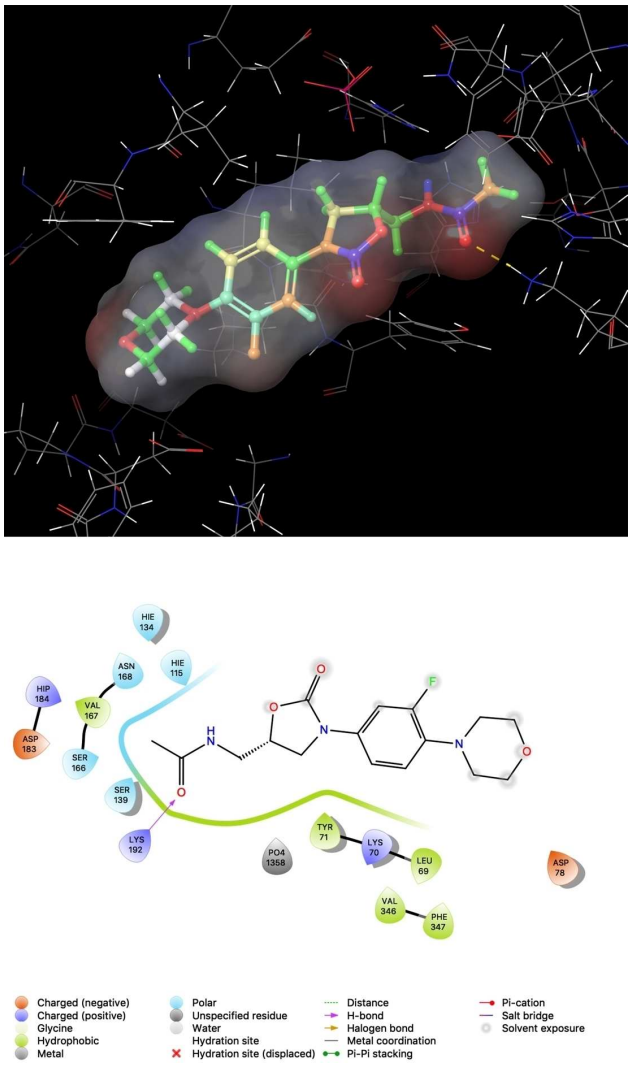

Figure 6. 3D and 2D schematic views of linezolid with 3SRE in Glide extra-precision mode.

um, and gadodiamide),[54] and certain proton-pump inhibitors (pantoprazole, omeprazole, and esomeprazole)[55] for investigation of inhibition effects against human serum PON1 activity. We determined that most of these drugs used in the tests, despite being at therapeutic doses, inhibit PON1 strongly by exhibiting different types of inhibition mechanisms.

Conclusion

Consequently, in our study, it was focalized on the investigation of the in vitro effects of frequently used some antibacterial agents on human serum paraoxonase enzyme activity which has a role in severe cases such as obesity, diabetes, rheumatoid arthritis, neurodegenerative, and cardiovascular diseases. The most striking observation to emerge from the data was that the drug concentrations used in the calculation of the K_i constants in the assays were at the therapeutically doses. A wide variety of risks may occur in patients taking these drugs due to inhibition of PON1. However, these results should be supported by in vivo research. This study will make a significant contribution to this growing drug design field by observing PON1-drug interactions.

Supporting Information Summary

Experimental Section of the current article, the biological and in silico studies procedures and statistical assays are provided in the Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Antibacterial drug · Biological activity · Inhibitors · Molecular docking · Paraoxonase

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