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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, inhibition effects, molecular docking and theoretical studies as Paraoxonase 1 (PON1) inhibitors of novel 1,4-dihydropyridine substituted sulfonamide derivatives

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Abstract

The novel sulfonamide substitute 1,4-dihydropyridine derivatives were synthesized by the method of Hantzsch reaction. They have been characterized by FT-IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. PON1 which is an antioxidant enzyme has important functions in cardiovascular systems. The enzyme has been purified using a two-step method such as ammonium sulfate precipitation and sepharose-4B-l-tyrosine-9-aminophenanthrene hydrophobic interaction chromatography. The results demonstrated that all the synthesized compounds inhibited PON1 enzyme. The best inhibition effect was observed in compound **(1)** for PON1 enzyme (IC_{50}: 8.04 µM, K_i: 5.43 µM). The free radical scavenging for PON1 was discovered as 20.16 mg/mL, while drug score value was reported as 0.13 for compound **(1)**. Furthermore, the lowest binding energy (−1.31 kcal/mol) determined by molecular docking for PON1 enzyme and the lowest LUMO-HOMO gap ($\Delta E = 3.12$ eV) were calculated for compound **(1)**.

Keywords PON1 · Inhibition · Drug score · Molecular docking · 1,4-dihydropyridine

Introduction

Cardiovascular diseases (CVD) are disorders that involve the heart or blood vessels. The leading cardiovascular diseases are coronary heart disease, rheumatic heart disease, cerebrovascular disease, and other conditions [1, 2]. An estimated millions of people die from CVD each year especially a huge proportion of deaths occur in low- and middle-income countries. With this high mortality rate, CVD is the leading cause of death in the world [2, 3]. Build-up of plaques, by the accumulation of lipids, and fibrous elements, inside the artery walls causes atherosclerosis. It is a type of progressive disease named coronary heart disease. Abundance of some plasma lipoproteins has major importance. Because, raised levels of atherogenic lipoproteins are necessary conditions for most forms of the disease [2, 4]. It is well known that high-density lipoprotein (HDL) plays a protective role for the cardiovascular system, and serum HDL levels have been negatively associated with the risk of coronary artery disease (CAD) [2, 5].

The human paraoxonase (PON) gene family consists of three members, PON1, PON2, and PON3. Human serum PON1 is a Ca^{2+}-dependent HDL-associated ester hydrolase enzyme that protects low-density lipoprotein (LDL), and cell membranes from oxidation through hydrolysis of the biologically active lipid peroxides. Therefore, the anti-atherogenic property of HDL is to a great extent conferred by PON1, and its capacity for preventing the formation of oxidized LDL [2, 5, 6].

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1,4-Dihydropyridines (DHPs) are class of nitrogen containing heterocycles and have important pharmacological properties such as antitumor [7], antihypertensive [8], vasodilating [9], antidiabetic [10], and brain-cell-protective activities [11]. It is known that cytochrome P-450 in the living metabolism of these compounds catalyses the redox reactions. These compounds are known as the most important calcium channel blockers. In addition, they are used in the treatment of Alzheimer's disease as an anti-adhesion of blood cells that provide clotting to the normal vascular wall [12]. In addition, the compounds play important roles in medicinal chemistry, for instance, nifedipine, amlodipine, felodipine, and nicardipine, which are the best selling drugs used in the treatment of cardiovascular diseases [13, 14].

The sulfonyl amide structure was first synthesized by Gelmo in 1907 [15]. Their derivatives are collected under the name "Sulfonamides" which are very important molecules for biological systems [16, 17]. Domagk et al. tried to treat hemolytic streptococcal infection by Prontosil, one of a series of azo dyes, in 1935 [18]. Successful chemotherapeutic effect of prontosil treatment has been reported in studies on the sulfonamide group. The compouds have several biological activities, including inhibition of CA, anticancer activity, antibacterial activity, and HIV protease inhibitory activity [19–22]. Many sulfonamide compounds are used clinically, for example, AAZ and brinzolamide as a CA inhibitor for the treatment of glaucoma and seizures, diazoxide for potassium channel activation, chlorothiazide and benzothiadiazine for diuretic activity, benzothiazide for high blood pressure as well as edema, hydroflumethiazide for chronic vascular hypertension, and polythiazide for the treatment of congestive heart failure, hypertension, diabetes insipidus, renal tubular acidosis, edema, and the prevention of kidney stones [23–28].

In this study, novel 1,4-dihydropyridine substituted sulfonamide derivatives were synthesized and characterized. The inhibition effect, drug score, and free radical scavenging values of sulfonamide derivatives on PON1 enzyme were revealed under in vitro conditions. Except that the chemical properties of the synthesized compounds were investigated in computational molecular, bio-based RO5, molecular docking, and frontier molecular orbitals (ΔE, LUMO-HOMO gap) were computed. It is aimed to detect potential new drug active substances for the treatment of cardiovascular diseases by inhibiting the PON1 enzyme with synthesized molecules.

Results and discussion

Synthesis

4-nitro substituted 1,4-dihydropyridine compound was obtained using aldehyde, 1,3-diketone, ammonium acetate and ASA as a solıd acid catalyst by the method of Hantzsch reaction. Short reaction time and no requirement of purification are main important advantages of this developed method for 1,4-dihydropyridines.

Iron in acidic medium was used in the reduction of nitro group because of the high efficiency, and easy purification. The reason for using this method is that the iron is separated by filtration at the end of the reaction and the organic impurities are very low and the products can be synthesized in pure form with high yield. ^{1}H NMR spectrum of the compound showed that when the peaks of aromatic hydrogen, which are under the influence of the NO_2 group, are above 8 ppm, the amino aromatic hydrogen of the product is observed between 7–7.5 ppm, and when examined in the FT-IR spectrum, the nitro peak, which is 1535, and 1315 cm^{-1}, is lost and replaced a new NH_2 peak of 3300 cm^{-1} was observed. At the last step of the synthetic procedure, amine bearing 1,4-dihydropyridine compounds react with the sulfonyl chlorides in basic conditions 6 h at room temperature in DMF. The proposed compounds were shown in Schemes 1–3.

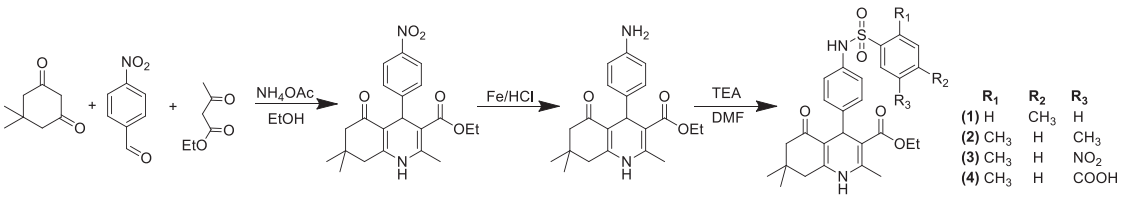
From the FT-IR spectra (given in Supplementary Information Figs. S1–S19) of the prepared compounds, the amine peaks were found to have moved from 3300 cm^{-1} (-NH_2) to the range of 3210–3250 cm^{-1} (NH). The peaks of SO_2 symmetric stretching are seen around 1350 cm^{-1}. In addition, NH_2 chemical shifts were initially observed in the range of 6–6.5 ppm in the proton NMR spectra (given in Supplementary Information Figs. S1–S19) of sulfonamides. After the reaction, NH_2 peaks appearing at around 6 ppm were appeared at around 8.0 ppm. All spectra and elemental analyses support the structure of the synthesized compounds.

PON1 biological activity

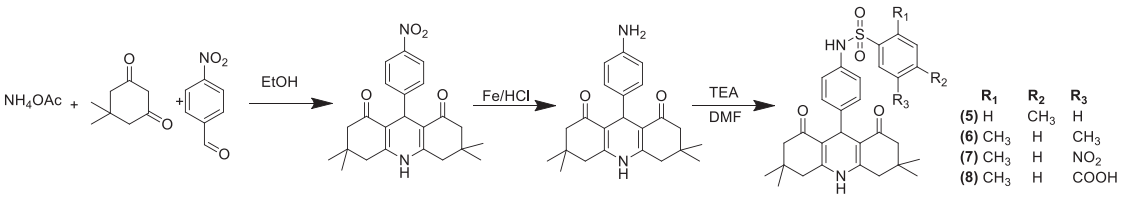
PON1 biological activities of the synthesized compounds were performed under in vitro conditions. The effects of the compounds on PON1 activity were given in Table 1 both IC_{50} and K_i.

IC_{50} values were found for **(1)** to **(12)** in the range of 8.04–36.75 µM. The entire 1,4-dihydropyridine series showed very low inhibition values against PON1. K_i values of the synthesized compounds were calculated between 5.43 to 24.82 µM.

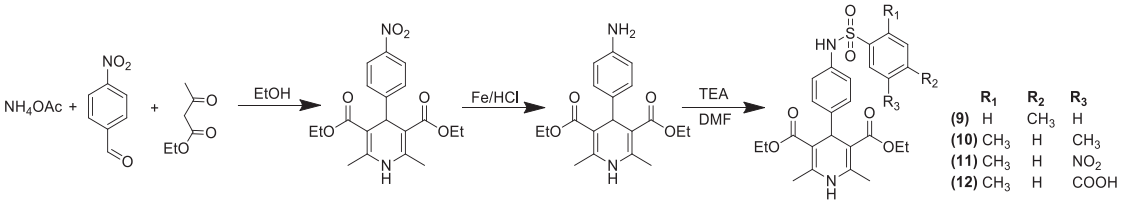
When the data of this study were analysed and the main skeleton was examined, it was observed that the lowest values inhibited the structure of 1,4-dihydropyridine consisting of dimedone and acetoacetate skeleton. Therefore, IC_{50} values were found to be 8.038 µM, 18.580 µM, 20.490 µM and 17.260 µM for molecules of **(1)**, **(2)**, **(3)**, and **(4)**, consisting of dimedone and acetoacetate skeleton, respectively.



Scheme 1 Synthesis of ethyl 2,7,7-trimethyl-5-oxo-4-(4-(phenylsulfonamido)phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives



Scheme 2 Synthesis of N-(4-(3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl)benzene sulfonamide derivatives



Scheme 3 Synthesis of diethyl 2,6-dimethyl-4-(4-(phenylsulfonamido)phenyl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives

Table 1 The effects of structures on PON1 activity; IC_{50}, K_i, and Free Radical Scavenging

Compounds code	IC_{50} Value (μM)	K_i (μM)	R^2	Free Radical Scavenging (mg/mL)
(1)	8.04	5.43	0.94	20.16
(2)	18.58	12.55	0.96	18.32
(3)	20.49	13.84	0.97	26.35
(4)	17.26	11.66	0.95	40.56
(5)	29.19	19.71	0.95	32.18
(6)	27.41	18.51	0.95	15.26
(7)	23.47	15.85	0.95	30.69
(8)	29.06	19.62	0.96	18.62
(9)	21.92	14.80	0.95	28.32
(10)	36.75	24.82	0.97	19.56
(11)	22.95	15.50	0.97	30.25
(12)	31.45	21.24	0.99	30.87
Ascorbic Acid [51]				96.62
2-hydroxyquinoline [29]	1.8			
Naringenin [29]	37.90			

Table 1 analysis showed that the novel synthesized inhibitors (except for **(10)**) were demonstrated excellent inhibition compared with naringenin ($IC_{50} = 37.90$ μM). The **(1)** inhibitor exhibited inhibition very close to the inhibition of 2-hydroxyquinoline ($IC_{50} = 1.8$ μM) which is the best known PON 1 inhibitor in the literature [29]. Additionally, it has been found to show higher inhibition than other sulfonamide derivatives in literature. For instance, **(1)** compound inhibited the PON1 enzyme 23 times better than the 2-amino-5-methyl-1,3-benzenedisulfonamide compound ($IC_{50} = 185$ μM), which was the best inhibition value among the sulfonamide compounds in the study of Alim et al. [30]. Additionally, it was found that the **(1)** molecule inhibited the PON1 enzyme almost three times better than the 4,5-dichlorothiophene-2-sulfonamide compound ($IC_{50} = 24.10$ μM) which is the best inhibitor in an another research published in the literature [31].

Chemoinformatic properties and Lipinski Rule (RO5) evaluation of ligands

The intended compounds were analysed computationally based on bio-molecular chemical properties and RO5. The predicted cheminformatics properties such as polar surface area (PSA), HBD, HBA, molar volume, logP, and drug similarity values of compounds were shown in Table 2. The previous research data confirmed the standard values for molecular weight (MW), and the polar surface area (PSA) are respectively (160 to 550 g/mol), and (<89 Å2) [32]. The sulfonamide compounds (without compound **(9)**) produced

Table 2 Cheminformatics analysis of synthesized compounds

Ligands	Mol.WT (g/mol)	No. HBA	No. HBD	Mol LogP (mg/L)	Mol LogS (mg/L)	PSA(Å^2)	Mol. Vol. (cm^3)	Drug Score
(1)	508.20	5	2	5.03	8.17	85.32	538.63	0.13
(2)	522.22	5	2	5.31	4.85	85.32	560.36	0.20
(3)	553.19	7	2	4.92	9.73	118.71	564.41	−0.39
(4)	552.19	7	3	4.88	22.88	113.73	572.52	0.33
(5)	518.22	4	2	5.35	3.06	78.14	567.02	−0.30
(6)	532.24	4	2	5.63	2.40	78.14	588.71	−0.13
(7)	563.21	6	2	5.24	5.61	111.52	592.75	−0.72
(8)	562.21	6	3	5.20	11.79	106.55	600.87	0.18
(9)	498.18	6	2	4.52	28.39	92.51	510.33	0.16
(10)	512.20	6	2	4.80	13.03	92.51	532.10	0.26
(11)	543.17	8	2	4.41	29.04	125.89	536.06	−0.37
(12)	542.17	8	3	4.37	31.13	120.92	544.17	0.40

Table 3 Docking results of **(1–12)** compounds, and the co-crystallized ligands in the active sites of PON1 receptors (PDB files: 3SRG)

Compounds code	ΔG (kcal/mol)	ΔGligsolvpol (kcal/mol)	Binding Energy (kcal/mol)	Full Fitness (FF, (kcal/mol))	Protein-ligand Site type
(1)	−8.10	−15.89	−1.31	−1756.83	NH_2 SO_2
(2)	−7.68	−16.05	11.55	−1745.84	NH_2 SO_2
(3)	−8.19	−22.96	16.64	−1730.17	NH_2 SO_2
(4)	−8.13	−18.48	5.08	−1748.82	NH_2 SO_2
(5)	−8.29	−17.18	1.01	−1720.62	NH_2 SO_2
(6)	−7.83	−16.87	6.17	−1713.22	NH_2 SO_2
(7)	−8.24	−20.23	6.62	−1705.11	NH_2 SO_2
(8)	−7.58	−20.62	9.44	−1712.97	NH_2 SO_2
(9)	−8.05	−15.22	7.78	−1725.34	NH_2 SO_2
(10)	−24.70	−15.39	22.48	−1718.51	NH_2 SO_2
(11)	−49.19	−19.47	23.98	−1705.56	NH_2 SO_2
(12)	−8.26	−19.55	16.81	−1721.62	NH_2 SO_2

poor results by MW, and PSA (1–12) values were comparable to standard values. RO5 also confirmed the therapeutic potential of all ligands. Hydrogen binding capacity is determined as an important parameter to define the permeability of the drug. Research data has shown that the number of hydrogen bond acceptors ≤10 and the number of hydrogen bond donors ≤5 is low. This completely satisfies the RO5 rule of molecules. Chemo-information analysis confirmed that all the compounds designed have <10 HBA, and <5 HBD. In addition, the MW, and logP values can also be compared to the standard value (<500 g/mol, and <5) [33]. However, there are many examples of a violation of RO5 among current drugs [34]. From the physicochemical, and pharmacokinetic results, it was stated that all new synthesized sulfonamide derivatives (**1–12**) fulfill the requirements of Lipinski's rule, and have appropriate pharmacokinetic properties. The predicted chemoinformatics of the designed ligands were given in Table 3.

Molecular Docking

The docking studies for PON 1 (PDB:3SRG) were carried out by Swiss Dock docking server (http://www.swissdock.ch). Insertion and data acquisition were gathered using the UCSF chimera. Various insertion parameters such as exact fitness (FF, kcal/mol), ΔGligsolvpol (kcal/mol), binding energy (kcal/mol), and Gibbs free energy (ΔG, (kcal/mol)) were examined [35]. When in silico molecular modelling was examined, it was made to explain the binding mode of new 1,4-dihydropyridine substituted sulfonamide derivatives to the active site of the enzyme, based on the data obtained as a result of in vitro PON 1 enzymatic study (given in Supplementary Information Figs. S20–S31). The newly synthesized molecules found Gibbs free energy in the range of −7.58 to −49.19. The approximate binding energy of **(1)** to PON1 enzyme was calculated as −1.31 (kcal/mol). However, the compound **(1)**, which has the lowest binding

Table 4 Interactions observed based on molecular docking results

Ligand	Interaction				
	π-Alkyl	π-Anion	Conventional Hydrogen Bond	Water Hydrogen Bond	Van der Walls
(1)	Pro A:59, Pro A:275, Ile A:228	-	-	Hoh A:384, Hoh A:385	Ile A:121, Hoh A:370, Thr A:119, Phe A:120, Ala A:172, Val A:173, Val A:273, Pro A:230, Val A:276, Hoh A:442
(2)	Ile A:121, Pro A:59, Ile A:228	-	Val A:173	-	Val A171, SerA118, PheA120, ThrA119
(3)	Val A:276, Pro A:275, Phe A:120	-	Pro A:230	Hoh A:370	ILE A:121, Pro A: 219, Hoh A: 392, Ser A:219, Ile A:228, Asn A:227, Glu A:56, Hoh A:362
(4)	Val A:171, Pro A:59, Pro A:275	-	Thr A:219	Hoh A:384, Hoh A:385	Ser A:218, Phe A:120, Ala A:120, Ile A:228, Asn A:227, Hoh A:370, Ile A:121
(5)	Ile A:228, Val A:171, Ile A:121	Glu A:56	Pro A:230	Hoh A:362	Val A:273, Hoh A:384, Asn A:125, Asp A:123, Asp A:122, Ser A:118, Thr A:119, Ala A:172, Hoh A:385, Pro A:275, Leu A:58, Hoh A:385, Phe A:120, Pro A:230
(6)	Pro A:59, Pro A:275	Glu A:56	Thr A:119	Hoh A:464	Ile A:228, Phe A:120, Ala A:172, Ser A:118, Val A:171, Hoh A:369 Asn A:227, Hoh, A384, Val A:273, Hoh A:362, Leu A:58, Ile A:57
(7)	Pro A:59, Pro A:275, Ile A: 121, Ile A:228	-	-	Hoh A:370, Hoh A:369	Hoh A:385, Pro A:230, Ser A:229, Val A:173, Hoh A:392, Hoh A:464, Phe A:120, Asp A:122, Asp A:123, Asn A:125, Hoh A:362, Val A:273, Glu A:56
(8)	Pro A:59, Pro A:275, Ile A:228	Glu A:56	Ala A:172	Hoh A:385, Hoh A:362, Hoh A:384	Pro A:23, Ile A:57, Leu A:58, Thr A:119, Asn A:227, Val A:171, Hoh A:392, Gly A:232, Phe A:120, Ser A:229, Hoh A:370, Ile A:121
(9)	Ile A:121, Pro A:275, Pro A:59, Leu A:58	-	Ala A:172	Hoh A:392, Hoh A:370, Hoh A:384	Val A:273, Hoh A:362, Hoh A:397, Ile A:57, Glu A:56, Hoh A:385, Thr A:119, Pro A:230, Ser A:118, Phe A:120, Ile A228, Ser A:229
(10)	Phe A:120, Pro A:59, Leu A:58	Glu A:56	Ala A:172, Thr A:119, Ser A:118	Hoh A:392, Hoh A:370, Hoh A:369	Ile A:121, Hoh A:362, Hoh A: 384, Gly A:232
(11)	Pro A:59, Pro A:275, Val A:171	-	Glu A:56	Hoh A:385, Hoh A:384	Asp A:123, Val A:276, Pro A:230, Ile A:228, Phe A:120, Ser A:118, Thr A:119, Val A:273, Hoh A:370, Asn A:125, Asp A:122
(12)	Phe A:275, Pro A:59, Leu A:58	-	Ala A:172, Ile A:121	Hoh A:392, Hoh A:370, Hoh A:464	Asn A:227, Ser A:118, Asp A:231, Lys A:250, Pro A:230, GLY A:232, Asp A:122, Phe A:120, Ser A:239, Thr A:119, Hoh A:385, Glu A:56, Ile A:57, Val A:273

energy among the synthesized compounds, can be self-bonded with the PON1 enzyme without extra energy.

Full fitness (FF) was determined between −1705.11 and −1756.83, and the ΔGligsolvpol parameter was shown to be −15.22 to −22.96 (Table 3). All output sets were sorted over two parameters (hydrogen bonding (interactions) and FF). The value with a cluster rank "0" was used as the best FF score. The larger negative value of the FF, the higher the bonding structure of the compounds [36]. In the present study, however, the **(1)** compound was the most effective compound with −1756.83 FF value, and it was consistent with the enzyme inhibition value.

Observed interactions according to molecular docking results are given in Table 4. The best IC_{50} value compound in the sequences, compound **(1)**, displayed a hydrophobic cloud structure with Pro A:59, Pro A:275, Val A:171, and Val A:273. Pro A:59 was formed alkyl interactions with CH_3 and CH_3 at the ester position on 1,4 dihydropyridine structure in the **(1)** molecule. Additionally, Pro A:59 shows π-alkyl interaction with the benzene ring on the 1,4 dihydropyridine. It was revealed that Ile A:228 and the benzene ring of the sulfonamide structure have a pi-alkyl interaction. It was discovered that the sulfonamide structure's sulfur bond oxygen atoms formed the Hoh A:384 and Hoh A:385 water hydrogen bonds. When comparing the by-products of its structure created using two moles of dimedone, the **(7)** molecule offers the best value for inhibition. The Pro A:59 interaction with this compound is a π-anion bond with the aromatic ring on the 1,4-dihydropyridine ring, and a similar interaction was shown with Ile228 on the aromatic ring on the sulfonamide. One of the CH_3's in the dimedone content in the **(7)** compound structure exhibited alkyl interaction with ProA:275, and the CH_3 on the dimedone, which is existing opposite side exhibited the same interaction with Ile A:228. The **(9)** compound, which has a good inhibition value among the 1,4-dihydropyridines compounds synthesized with 2 moles of ethyl acetate as the main skeleton group, was also investigated.

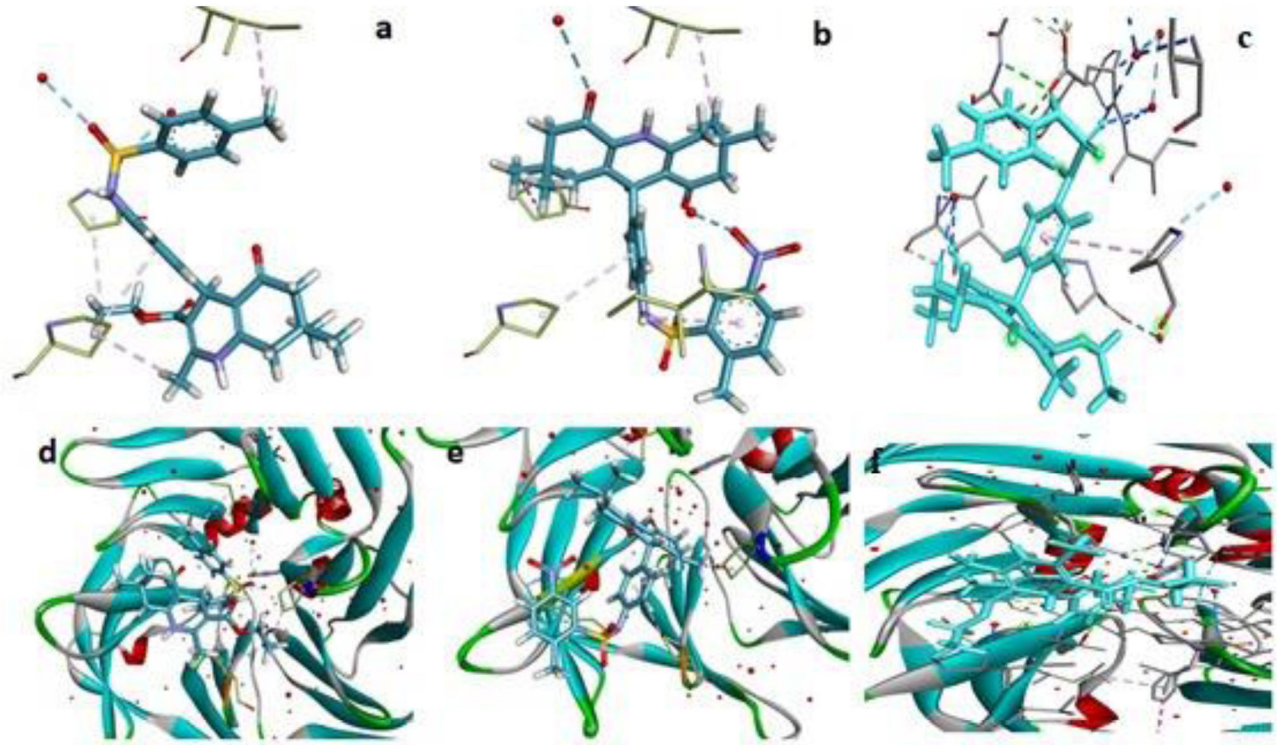


Fig. 1 3D ligand interaction diagrams of docking poses of **(1)** (**a**), **(7)** (**b**), **(9)** (**c**) and 2D ligand interaction diagrams of docking poses of **(1)** (**d**), **(7)** (**e**), **(9)** (**f**) at the binding pocket of paraoxonase (PDB: 3SRG)

Pro A:59, and Pro A:275 structures of **(9)** showed alkyl interaction with the methyl group in the 1,4-dihydropyridines ring. It was observed that there was a water-hydrogen bonding interaction with Hoh A:362 with oxygen in the S = O structure, with C = O in the acetate structure Hoh A:370, and with the methyl hydrogen in the ester structure Hoh A: 384 (Fig. 1).

When the studies in the literature were examined, no detailed study was observed showing the effect of enantiomers on the PON1 enzyme. For this reason, approximate activity values were determined as a racemic mixture. In addition, it has been observed that the calculations made with racemic structures are close to other similar studies.

LUMO-HOMO levels

The energy levels of molecular orbitals, HOMO and LUMO, calculated for the synthesized 1,4-dihydropyridines and sulfonamides derivatives provide information about possible electronic transitions. In addition, HOMO and LUMO provide information about the electrophilic and nucleophilic attraction in the molecule. The LUMO-HOMO gap is very important for chemical reactivity. A low range gap is considered high reactivity (color: green is the positive, purple is the negative value) [37–39]. LUMO-HOMO frontier orbitals and HOMO, LUMO, IP, EA, and ΔE (LUMO-HOMO gap) data of the prepared compounds are shown in Tables 5 and 6, respectively.

While Table 5 was analysed, the molecules have 3.12, 4.99, 7.46, 5.30, 8.12, 5.20, 7.19, 5.54, 7.92, 8.23, 7.25, 7.64 eV LUMO-HOMO gap (ΔE), respectively. Accordingly, the highest reactivity was observed for the **(1)** compound, while the lowest reactivity was observed for the **(5)** compound. In addition, according to the frontier molecular orbital representations in Table 6, it is seen that the molecule will perform an electrophilic attack (positive charge or atoms) on atoms with HOMO orbital, while it will perform a nucleophilic attack (negative charge or electrons) on atoms with LUMO orbital. When Table 6 is examined, possible ionization potential (IP) using negative HOMO energies and possible electron affinity (EA) values using negative LUMO energies were determined.

Structure activity relationship (SAR) study

The SAR study was conducted by examining the in vitro inhibition effect, drug score, free radical scavenging, molecular docking and HOMO-LUMO calculations of 12 newly synthesized and characterized compounds. When the in vitro inhibition effects, and the Molecular Docking data of these compounds were examined, it was determined that the best inhibitor was the **(1)** compound, and the SAR study was conducted for **(1)**. However, in Free Radical

Table 5 LUMO-HOMO frontier orbitals

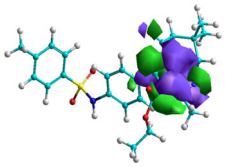
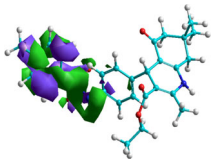
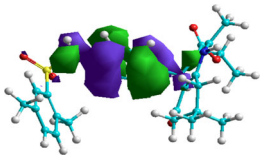
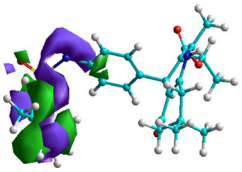
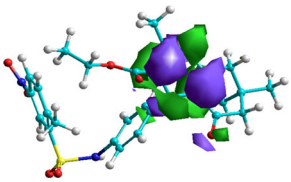
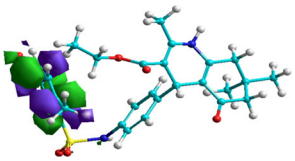
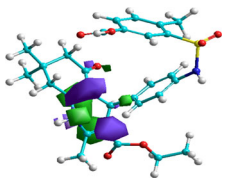
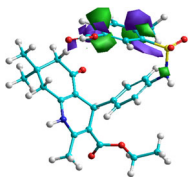
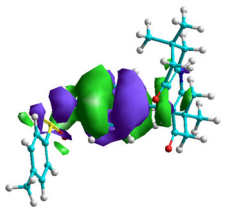
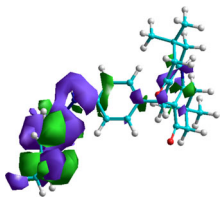
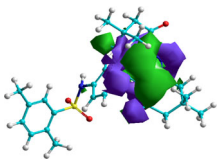
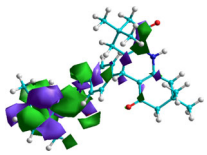
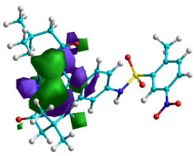
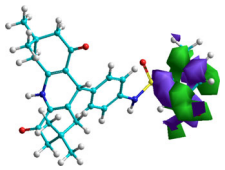
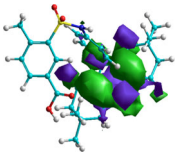
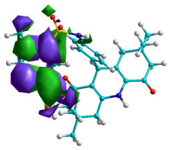
	HOMO eV	LUMO eV
(1)		
(2)		
(3)		
(4)		
(5)		
(6)		
(7)		
(8)		

Table 5 (continued)

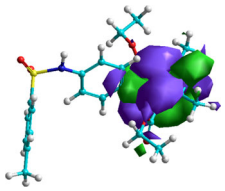
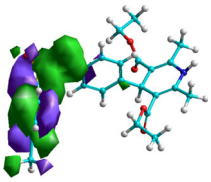
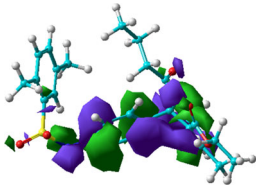
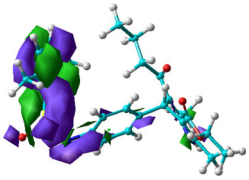
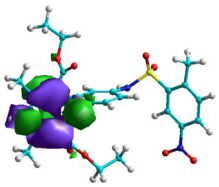
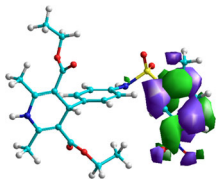
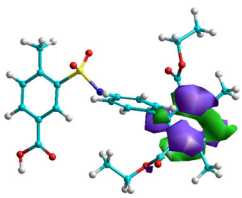
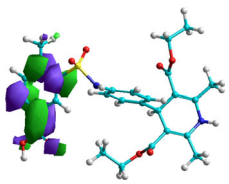
	HOMO eV	LUMO eV
(9)		
(10)		
(11)		
(12)		

Table 6 HOMO, LUMO, IP, EA, and ΔE (LUMO-HOMO gap) data for compounds

Compound	HOMO, eV	LUMO, eV	ΔE, (LUMO-HOMO gap)	Ionization potential (IP), eV	Electron affinity (EA), eV
(1)	−7.50	−4.38	3.12	−7.50	−4.38
(2)	−8.37	−3.37	4.99	−8.37	−3.37
(3)	−8.89	−1.43	7.46	−8.89	−1.43
(4)	−8.71	−3.41	5.30	−8.71	−3.41
(5)	−8.67	−0.55	8.12	−8.67	−0.55
(6)	−8.24	−3.04	5.20	−8.24	−3.04
(7)	−8.67	−1.48	7.19	−8.67	−1.48
(8)	−9.23	−3.69	5.54	−9.23	−3.69
(9)	−8.68	−0.76	7.92	−8.68	−0.76
(10)	−8.86	−0.63	8.23	−8.86	−0.63
(11)	−8.76	−1.51	7.25	−8.76	−1.51
(12)	−8.67	−1.03	7.64	−8.67	−1.03

Scavenging values, it was determined that **(1)** compound was approximately 5 times smaller than the standard material as ascorbic acid. When the drug score values of the synthesized compounds in Table 3 are examined, the fact that all values are greater than **(1)** indicates that the compounds have drug potential [40].

(1) compound was investigated within the scope of molecular docking studies. First of all, the His-His (Histidine-Histidine) amino acid pair, and the calcium ion in the active site of the paraoxonase enzyme, as well as the water molecule are important for the PON1 esterase activity. According to the PON1 esterase mechanism, the ester group on the substrate is cleaved between the water-linked His-His pair in the active site of the enzyme and the Ca^{2+} ion [41]. When the structure of the inhibitors synthesized within the scope of this study is examined (except **(6)**, **(7)** and **(8)**), all compounds contain ester groups. It is thought that these ester groups enter between the His-His pair of the enzyme and the Ca^{2+} ion before the substrate, weakening the esterase activity and causing the inhibition of the enzyme.

All compounds synthesized in this study contain methyl groups. Amino acids bearing methyl groups on the PON 1 enzyme are also quite abundant. As seen in Table 4, the methyl groups of the compounds synthesized with these amino acids (alanine, valine, isoleucine, etc.) make van der Waals (vdW) interactions. In addition, proline, isoleucine, valine, phenylalanine, leucine amino acids, and aromatic compounds of inhibitors form π-alkyl interactions.

Among the synthesized compounds, seven molecules, including **(1)** compound, contain a ketone structure (carbonyl) on the cyclohexenone side group. These carbonyl compounds form unconventional hydrogen bonds with –OH containing amino acids such as serine, tyrosine, etc., directly or through an intervening water molecule.

Finally, although all molecules carry aromatic compounds bound through the sulfonium group, the number and positions of the substituents (-CH_3, -NO_2, -COOH) on the aromatic group are different. In the **(1)** compound, there is only the -CH_3 group in the para position. This allows the amino acids in the active site of the enzyme to form less steric hindrance through water molecules, allowing the sulfonamide structure to hydrogen bonding with the S = O structure (highly electronegative) connected by π-bonds. This is thought to cause a change in the conformation of the active site of the enzyme and its affinity for the substrate.

When the above-mentioned enzyme-inhibitor interactions are examined, it is observed that the **(1)** compound performs all the interactions described. For this reason, **(1)** compound, in which many parameters are effective together, is thought to have the highest inhibition rate.

The HOMO and LUMO values of the synthesized compounds in this study were calculated with the HyperChem program. A compound having a high HOMO energy, and a low LUMO-HOMO gap is a measure of the compound's high reactivity [42, 43]. As seen in Table 6, the highest HOMO energy level belongs to the **(1)** compound (−7.50 eV). In addition, the lowest LUMO-HOMO gap again belongs to **(1)** (3.12 eV). However, as seen in Table 5, the large gap between the HOMO orbitals and the LUMO orbitals increases the reactivity of the compound [43]. Accordingly, it is thought that the **(1)** compound should have the highest reactivity, high interaction with the functional groups in the active site of the PON1 enzyme, and high inhibition effect. HOMO-LUMO gap, HOMO energy level data and in vitro inhibition values support each other and as seen in Table 1, (1) compound shows the best inhibition effect.

Conclusions

In this study, the in vitro effects of 1,4-dihydropyridine-derived sulfonamide structures on the PON1 enzyme, closely associated with cardiovascular diseases, were investigated. Novel twelve sulfonamides (**1–12**) were synthesized in this study. The in vitro inhibition effects, free radical scavenging tests, and drug scores of these obtained sulfonamides on PON1 were investigated. IC_{50}, K_i, free radical scavenging, and drug score values were found to be in the range of 8.04 μM to 36.75 μM, 5.43 to 24.82 μM, 15.26 to 40.56 mg/mL, and −0.72 to 0.40, respectively. It was observed that the lowest binding energy belonged to the **(1)** compound by molecular docking study of twelve sulfonamide derivatives synthesized for inhibition of PON1 enzyme. In addition, when the 2D representations were examined, the π-alkyl interaction of compound **(1)** and the amino acid residues (Pro A:59 and Ile A:228) in the active site of the enzyme showed strong interactions. With these results, strong inhibitory effects were observed for PON1 activity. The HyperChem program with molecular mechanics and semi-empirical PM3 method was used to calculate LUMO-HOMO values, ionization potential, electron affinity, and the most stable conformation of compounds. Compound **(1)** was found as the most reactive compound (ΔE = 3.12 eV). When the PON1 enzyme activity results obtained were examined, it was determined that the highest inhibition was achieved by the compound **(1)** (IC_{50} = 8.04 μM). The fact that the compound **(1)** with the highest reactivity causes high enzyme inhibition, and it is the most suitable compound in molecular docking studies shows that the methods used to support each other. Accordingly, the molecular orbital (LUMO-HOMO gap) data supported the enzyme inhibition data.

The literature shows that many enzymes, and physiological systems are affected differently by sulfonamide derivatives in different ways and at different levels. They have various biological activities such as antibacterial, anticancer, antiobesity, high ceiling

diuretics, glaucoma and Alzheimer's disease treatment. They are widely used for therapeutic and prophylactic purposes in human, and veterinary medicine to combat many dangerous diseases as shown our previous study and others [44, 45]. Our results suggest that the compounds as novel 1,4-dihydropyridine substitute sulfonamide derivatives are likely to be adopted as candidates or pathfinder for the treatment of cardiovascular diseases. Additionally, these reagents should be further evaluated in vivo studies.

Materials and methods/experimental

Materials

FT-IR spectra were measured on a SHIMADZU Prestige-21 (200 VCE) spectrometer with ATR attachment. ^{1}H, and ^{13}C NMR spectra were measured on spectrometers at VARIAN Infinity Plus 300 and 75 Hz, respectively. ^{1}H, and ^{13}C chemical shifts are referenced to the internal deuterated solvent. The elemental analysis was carried out with a Thermo Scientific Flash 2000 instrument. All materials, and equipment were purchased from ISOLAB, Sigma Aldrich, and Alfa Easer Companies. The materials used include ammonium sulfate, Sepharose 4B, L-tyrosine, 9-amino-phenanthrene, paraoxon, and protein assay reagents were obtained from Sigma Chem. Co. All chemicals used were analytical grade.

General procedure for the preparation of 4-nitro-1,4-dihydropyridine derivatives

4-nitrobenzaldehyde (5 mmol), ammonium acetate (5 mmol), dicarbonyl compounds (5 mmol), and alumina sulfuric acid (ASA, 0.6 mmol) as a catalyst was added in 5 mL methanol charged flask and refluxed for overnight. After the reaction was completed, the reaction mixture was cooled to room temperature, poured into 100 ml of ice-cold water, filtered off and dried. The product was crystallized from acetone-hexane and the structures were determined by ^{1}H NMR and ^{13}C NMR spectra [46].

General procedure for the preparation of 4-Amino-1,4-dihydropyridine derivatives

4-Nitro-1,4-dihydropyridine (1 mmol) and iron (6 mmol) in THF/water (7:3) mixture was heated in an oil bath. A few drops of HCl were added to refluxing mixture and refluxed for two more hours. After the reaction was completed, the mixture was filtered through celite while hot and adjusted the pH = 7 then extracted with dichloromethane. The organic phase was washed 2 times with water, dried with sodium sulfate and removed solvent on a rotary evaporator. The product was crystallized from acetone-hexane and the structures were determined by ^{1}H NMR and ^{13}C NMR spectra.

General procedure for the preparation of phenyl benzene sulfonamide derivatives

4-Amino-1,4-dihydropyridine derivatives (1 mmol), sulfonyl chloride derivatives (1 mmol), and a few drops of triethylamine (TEA) were stirred for 6 h at room temperature in DMF. At the end of the reaction, the mixture was cooled to room temperature, poured into 100 mL of ice-cold water, filtered off and dried. The product was crystallized from acetone-hexane (Schemes 1–3). The structures of the prepared compounds were determined by FT-IR, ^{1}H NMR and ^{13}C NMR spectra, elemental analysis.

Ethyl 2,7,7-trimethyl-4-(4-(4-methylphenylsulfonamido) phenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (1)

Yield 87.6%, **m.p**. 97.9 °C, **FTIR (ATR, ν, cm^{-1}):** 3336.15 (NH), 3209.06 (NH), 3089.82 (C-H Aromatic), 2918.30 (C-H Aliphatic), 1701.22 (C = O), 1602.25 (C = O), 1473.62 (C = C), 1342.46 (S = O). **^{1}H NMR (300 MHz, DMSO-d_6, ppm):** 9.78 (NH), 8.83 (NH), 7.60(2H, d, CH), 7.28 (2H, d, CH), 6,98 (2H, d, CH), 6.75 (2H, d, CH), 4.95 (H, s, CH), 4.07 (2H, q, CH_2), 2.42 (2H, dd, CH_2), 2.35 (2H, dd, CH_2), 2.18 (3H, d, CH_3), 1.26 (3H, t, CH_3), 1.20 (3H, s, CH_3), 1.12 (3H, s, CH_3), 0.98 (3H, s, CH_3). **^{13}C NMR (75 MHz, DMSO-d_6, ppm):** 195.25, 169.25, 151.32, 150.12, 137,65, 136.78, 136.56, 135.28, 132.36,130.95, 129.82, 128,96, 128.36, 120.41, 119.57, 112.12, 111.90, 111.86, 102.25, 61.59, 52,30, 41.48, 41.25, 32.85, 27.18, 21.61, 18.86, 14.32. **Elem. Anal**. $C_{28}H_{32}N_2O_5S$, Anal**. Calcd. For**: C:66.12; H:6.34; N:5.51; O:15.73; S:6.30, **Found:** C:66.73; H:6.28; N:5.61; O:15.42; S:5.96.

Ethyl 4-(4-(2,5-dimethylphenylsulfonamido) phenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (2)

Yield 90%, **m.p**. 99.8 °C, **FTIR (ATR, ν, cm^{-1}):** 3290.56 (NH), 3207.62 (NH), 3070.68 (C-H Aromatic), 2960.73 (C-H Aliphatic), 1693.50 (C = O), 1606.70 (C = O), 1485.19 (C = C), 1379.10 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 9.62 (NH), 8.78 (NH), 7,78(H, s, CH), 7.72 (H, d, CH), 7.35 (H, d, CH), 6,92 (2H, d, CH), 6.68 (2H, d, CH), 4.94 (H, s, CH), 4.02 (2H, q, CH_2), 2.39 (2H, dd, CH_2), 2.31 (2H, dd, CH_2), 2.15 (3H, d, CH_3), 1.29 (3H, t, CH_3), 1.24 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.10 (3H, s, CH_3),

0.99 (3H, s, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 195.38, 170.12, 150.31, 150.02, 137,60, 137.28, 136.65, 135.36, 132.36, 129.95, 129.83, 128,75, 128.56, 120.25, 119.87, 112.56, 111.80, 111.66, 103.56, 61.88, 52,67, 42.59, 40.97, 32.56, 28.18, 21.79, 21.54, 18.96, 14.43. **Elem. Anal**. $C_{29}H_{34}N_2O_5S$, Anal**. Calcd. For:** C:66.64; H:6.56; N:5.36; O:15.31; S:6.14, **Found:** C:66.51; H:6.48; N:5.52; O:15.44; S:6.05.

Ethyl 2,7,7-trimethyl-4-(4-(2-methyl-5-nitrophenylsulfonamido) phenyl)-5-oxo-1,4,5,6, 7,8-hexahydroquino line-3-carboxylate (3)

Yield 88.27%, **m.p**. 70,4 °C, **FTIR (ATR, ν, cm^{-1}):** 3329.14 (NH), 3207.62 (NH), 3070.68 (C-H Aromatic), 2954.95 (C-H Aliphatic), 1673.20 (C = O), 1604.77 (C = O), 1477.47 (C = C), 1346.31 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 9.88 (NH), 9.08 (NH), 8.28 (H, s, CH), 7.90 (H, d, CH), 7.47(H, d, CH), 7,13 (2H, d, CH), 6.93 (2H, d, CH), 4.90 (H, s, CH), 4.08 (2H, q, CH_2), 2.43 (2H, dd, CH_2), 2.35 (2H, dd, CH_2), 2.11 (3H, d, CH_3), 1.21 (3H, t, CH_3), 1.16 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.02 (3H, s, CH_3), 0.94 (3H, s, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 195.12, 168.06, 150.76, 150.21, 148.04, 141.98, 140.01, 134.68, 134.47, 129.86, 125.68, 123.58, 119.16, 110.56, 109.98, 103.71, 64.53, 53.68, 41.65, 40.39, 32.78, 27.16, 23.05, 18.95, 13.03. **Elem. Anal**. $C_{28}H_{31}N_3O_7S$, **Anal. Calcd. For:** C: 60.74; H: 5.64; N: 7.59; O: 20.23; S: 5.79, **Found:** C: 60.71; H: 5.68; N: 7.57; O: 20.21; S: 5.83.

3-(N-(4-(3-(ethoxycarbonyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinolin-4-yl) phenyl) sulfamoyl)-4-methylbenzoic acid (4)

Yield 85%, **m.p**. 78,8 °C, **FTIR (ATR, ν, cm^{-1}):** 3207.11 (NH), 3209.06 (NH), 3062.82 (C-H Aromatic), 2958.80 (C-H Aliphatic), 1604.29 (C = O), 1590.25 (C = O), 1481.33 (C = C), 1381.03 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 9.90 (NH), 8.92 (NH), 8.48 (H, s, CH), 7.82 (H, d, CH), 7.17(H, d, CH), 6,95 (2H, d, CH), 6.78 (2H, d, CH), 4.82 (H, s, CH), 4.03 (2H, q, CH_2), 2.41 (2H, dd, CH_2), 2.32 (2H, dd, CH_2), 2.12(3H, d, CH_3), 1.19(3H, t, CH_3), 1.14 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.09 (3H, s, CH_3), 0.92 (3H, s, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 195.10, 170.12, 166.81, 150.12, 147,67, 142.36, 139.88, 133.82, 133.56, 133.24, 130.01, 128.54, 122.71, 119.36, 111.70, 11.54, 102.52, 64.48, 53.63, 41.56, 40.28, 32.82, 27.63 23.04, 18.56, 13.52. **Elem. Anal**. $C_{29}H_{32}N_2O_7S$, **Anal. Calcd. For:** C: 63.03; H: 5.84; N: 5.07; O: 20.27; S: 5.80, **Found:** C: 63.00; H: 5.81; N: 5.06; O: 20.23; S: 5.90.

4-Methyl-N-(4-(3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl) phenyl) benzene sulfonamide (5)

Yield 84%, **m.p**. 122,4 °C, **FTIR (ATR, ν, cm^{-1}):** 3215.34 (NH), 3203.22 (NH), 3052.25 (C-H Aromatic), 2956.87 (C-H Aliphatic), 1660.71 (C = O), 1622.13 (C = O), 1508.33 (C = C), 1359.82 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 7.52 (2H, d, CH), 7.21(2H, d, CH), 6,98 (2H, d, CH), 6.79 (2H, d, CH), 6.92 (NH), 5.78 (NH), 2.39 (4H, dd, CH_2), 2.31 (4H, dd, CH_2), 2.15 (3H, d, CH_3), 1.10 (3H, s, CH_3), 0.99 (3H, s, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 194.88, 154.19, 150.28, 138.94, 137.05, 135.56, 130.05, 127.72, 120.16, 112.05, 111.89, 50.76, 45.18, 32.92, 30.38, 27.64, 21.56. **Elem. Anal**. $C_{30}H_{34}N_2O_4S$, Anal**. Calcd. For:** C: 69.47; H: 6.61; N: 5.40; O: 12.34; S: 6.18, Found**:** C: 69.40; H: 6.58; N: 5.45; O: 12.38; S: 6.19.

2,5-Dimethyl-N-(4-(3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl) phenyl) benzene sulfonamide (6)

Yield 80%, **m.p**. 139,4 °C, **FTIR (ATR, ν, cm^{-1}):** 3230.77 (NH), 3215.36 (NH), 3060.418 (C-H Aromatic), 2956.87 (C-H Aliphatic), 1666.50 (C = O), 1651.07 (C = O), 1510.26 (C = C), 1359.82 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 8.68 (NH), 7.42 (H, s, CH), 7.32 (H, d, CH), 7,21(H, d, CH), 7.18 (2H, d, CH), 6.78 (2H, d, CH), 5.70 (NH), 4.69 (H, s, CH), 2.40 (4H, dd, CH_2), 2.32 (4H, dd, CH_2), 2.10 (3H, d, CH_3), 1.10 (3H, s, CH_3), 0.91 (3H, s, CH_3).**^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 194.88, 150.28, 138.72, 135.46, 133.56, 132.10, 130.29, 127.73, 120.16, 112.05, 111.89, 50.76, 45.18, 32.92, 30.38, 27.64, 21.56, 20.96. **Elem. Anal**. $C_{31}H_{36}N_2O_4S$, **Anal. Calcd. For:** C: 69.90; H: 6.81; N: 5.26; O: 12.01; S: 6.02, Found**:** C: 69.82; H: 6.85; N: 5.30; O: 12.03; S: 6.00.

2-methyl-5-nitro-N-(4-(3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydroacri din-9-yl) phenyl) benzene sulfonamide (7)

Yield 86%, **m.p**. 139.6 °C, **FTIR (ATR, ν, cm^{-1}):** 3280.97 (NH), 3220.35 (NH), 3046.67 (C-H Aromatic), 2956.87 (C-H Aliphatic), 1660.71 (C = O), 1622.13 (C = O), 1523.76 (C = C), 1348.24 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 8.62 (NH), 8.42 (H, s, CH), 8.11 (H, d, CH), 7,31 (H, d, CH), 7.12 (2H, d, CH), 6.82 (2H, d, CH), 5.70 (NH), 4.89(H, s, CH), 2.41 (4H, dd, CH_2), 2.36 (4H, dd, CH_2), 2.11(3H, d, CH_3), 1.14 (3H, s, CH_3), 0.92 (3H, s, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 194.88, 150.28, 146.27, 142.51, 140.86, 130.67, 127.35, 123.00, 120.32, 112.15, 111.75, 50.92,

45.03, 33.21, 30.76, 26.42, 23.61. **Elem. Anal**. $C_{30}H_{33}N_3O_6S$, **Anal. Calcd. For:** C: 63.92; H: 5.90; N: 7.45; O: 17.03; S: 5.69, Found**:** C: 63.89; H: 5.87; N: 7.51; O: 17.01; S: 5.72.

4-methyl-3-(N-(4-(3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)phenyl) sulfamoyl) benzoic acid (8)

Yield 93%, **m.p**. 136.4 °C, **FTIR (ATR, ν, cm^{-1}):** 3250.78(COOH), 3186.40(NH), 3205. 82(NH), 3025.68(C-H Aromatic), 2958.80 (C-H Aliphatic), 1716.65 (C = O), 1668.43 (C = O), 1651.07 (C = O), 1510.26 (C = C) 1361.74 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 8.62 (NH), 8.02 (H, s, CH), 7.89 (H, d, CH), 6,51 (H, d, CH), 7.09 (2H, d, CH), 6.90 (2H, d, CH), 5.75 (NH), 4.82(H, s, CH) 2.41 (4H, dd, CH_2), 2.36 (4H, dd, CH_2), 2.11 (3H, d, CH_3), 1.12 (3H, s, CH_3), 0.93 (3H, s, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 194.88, 170.54, 150.28, 142.63, 137.96, 132.68, 129.06, 128.32, 122.76, 120.16, 112.05, 111.89, 50.76, 45.18, 32.92, 30.38, 27.64, 21.56. **Elem. Anal**. $C_{31}H_{34}N_2O_6S$, **Anal. Calcd. For:** C: 66.17; H: 6.09; N: 4.98; O: 17.06; S: 5.70, **Found:** C: 66.21; H: 6.12; N: 4.90; O: 17.02; S: 5.75.

Diethyl 2,6-dimethyl-4-(4-(4-methyl phenyl sulfonamido) phenyl)-1,4-dihydropyridine-3,5-dicarboxylate (9)

Yield 88%, **m.p**. 130.2 °C, **FTIR (ATR, ν, cm^{-1}):** 3336.85 (NH), 3240.41 (NH), 3016.35 (C-H Aromatic), 2927.94 (C-H Aliphatic), 1697.36 (C = O), 1645.28 (C = O), 1481.33 (C = C) 1345.36 (S = O). **^{1}HNMR (300 MHz, $CDCl_3$, ppm):** 7,69 (2H, d, CH), 7.22 (2H, d, CH), 7,21 (2H, d, CH), 6.91 (2H, d, CH), 6.62 (NH), 5.71 (NH), 4.85 (H, s, CH), 4.05 (2H, q, CH_2), 2.31 (3H, s, CH_3), 2.28(3H, s, CH_3), 1.29 (3H, t, CH_3). **^{13}CNMR (75 MHz, $CDCl_3$, ppm):** 167.96, 150.72, 138.56, 137.34, 135.95, 134.81, 130.05, 128.99, 120.19, 103.64, 68.76, 43.26, 22.19, 15.32. **Elem. Anal**. $C_{26}H_{30}N_2O_6S$, **Anal. Calcd. For:** C: 62.63; H: 6.06; N: 5.62; O: 19.25; S: 6.43, Found**:** C: 62.59; H: 6.10; N: 5.57; O: 19.19; S: 6.45.

Diethyl 4-(4-(2,5-dimethyl phenyl sulfonamido) phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (10)

Yield 87%, **m.p**. 136.2 °C, **FTIR (ATR, ν, cm^{-1}):** 3250.56 (NH), 3212.54 (NH), 3023.35 (C-H Aromatic), 2996.52 (C-H Aliphatic), 1690.21 (C = O), 1635.65 (C = O), 1474.23 (C = C), 1346.98 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 8.24 (NH), 7,92 (H, s, CH), 7.61 (H, d, CH), 7.31 (H, d, CH), 6,94 (2H, d, CH), 6.80 (2H, d, CH), 5.68 (NH) 4.80 (H, s, CH), 4.05 (2H, q, CH_2), 2.32 (3H, s, CH_3), 2.20(3H, s, CH_3), 1.21 (3H, t, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 168.03, 151.24, 138.92, 135.99, 135.95, 134.81, 133.65, 132.91, 129.84, 126.73, 120.19, 104.00, 68.72, 43.93, 22.30, 20.78, 15.64. **Elem. Anal**. $C_{27}H_{32}N_2O_6S$, **Anal. Calcd. For:** C: 63.26; H: 6.29; N: 5.46; O: 18.73; S: 6.26, Found**:** C: 63.31; H: 6.32; N: 5.39; O: 18.69; S: 6.29.

Diethyl 2,6-dimethyl-4-(4-(2-methyl-5-nitro phenyl sulfonamido) phenyl)-1,4-dihydropyridine-3,5-dicarboxylate (11)

Yield 84%, **m.p**. 110.2 °C **FTIR (ATR, ν, cm^{-1}):** 3323.35 (NH), 3225.32 (NH), 3036.63 (C-H Aromatic), 2978.09 (C-H Aliphatic), 1674.21 (C = O), 1651.07 (C = O), 1485.19 (C = C). 1342.63 (S = O), **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 8.84 (NH), 8.68 (NH), 8,12(H, s, CH), 8.02 (H, d, CH), 7.60 (H, d, CH), 6,91 (2H, d, CH), 6.88 (2H, d, CH), 4.88 (H, s, CH), 4.01(2H, q, CH_2), 2.36(3H, s, CH_3), 2.25(3H, s, CH_3), 1.20 (3H, t, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 167.28, 150.73, 146.83, 142.38, 140.62, 135.95, 134.81, 130.97, 127.36, 123.74, 120.37, 103.93, 68.76, 43.78, 22.30, 15.38. **Elem. Anal**. $C_{26}H_{29}N_3O_8S$, **Anal. Calcd. For:** C: 57.45; H: 5.38; N: 7.73; O: 23.55; S: 5.9, **Found:** C: 57.51; H: 5.42; N: 7.68; O: 23.49; S: 5.90.

3-(N-(4-(3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridin-4-yl) phenyl) sulfamoyl) -4-methylbenzoic acid (12)

Yield 91%, **m.p**. 132.6 °C, **FTIR (ATR, ν, cm^{-1}):** 3643.53 (COOH), 3346.50 (NH), 3151.69 (NH), 3032.81 (C-H Aromatic), 2991.59 (C-H Aliphatic), 1689.50 (C = O), 1643.35 (C = O), 1602.35 (C = O), 1477.47 (C = C) 1352.10 (S = O). **^{1}HNMR (300 MHz, DMSO-d_6, ppm):** 8.99 (NH), 8.88 (NH), 8,23(H, s, CH), 8.02 (H, d, CH), 7.60 (H, d, CH), 6,91 (2H, d, CH), 6.88 (2H, d, CH), 4.90 (H, s, CH), 4.05 (2H, q, CH_2), 2.35(3H, s, CH_3), 2.29 (3H, s, CH_3), 1.24 (3H, t, CH_3). **^{13}CNMR (75 MHz, DMSO-d_6, ppm):** 171.65, 167.93, 150.84, 141.53, 138.27, 135.86 134.63, 133.60, 129.75, 127.63, 123.33, 120.05, 103.60, 68.79, 43.31, 22.96, 15.76. **Elem. Anal**. $C_{27}H_{30}N_2O_8S$, **Anal. Calcd. For:** C: 59.77; H: 5.57; N: 5.16; O: 23.59; S: 5.91, **Found:** C: 59.81; H: 5.48; N: 5.21; O: 23.55; S: 5.95.

Purification of PON1

Blood samples were collected from healthy, and voluntary people. PON1 was purified from human serum in two steps. The first step was ammonium sulfate precipitation, and the second one is sepharose-4B-l-tyrosine-9-aminophenanthrene hydrophobic interaction chromatograph, by the procedure of Gencer and Arslan 2009. The procedure was specifically designed for the retained N-terminal hydrophobic signal peptide for PON1 enzyme [47].

PON1 enzyme assay

PON1 activity towards paraoxon substrate was quantified spectrophotometrically through the method defined by Gan et al. [48]. The reaction was followed for 2 min. at 37 °C by monitoring the appearance of p-nitrophenol at 412 nm in a Biotech automated recording spectrophotometer. A molar extinction coefficient (ε) of p-nitrophenol at pH 8.0 in 100 mM Tris–base buffer of 17,100 $M^{-1}cm^{-1}$ was used for the calculation. PON1 activity (1 Ul^{-1}) was defined as 1 µmol of p-nitrophenol formed per minute [47, 49].

In vitro inhibition kinetic studies and Calculation of inhibition constants (K_i)

Different concentrations of novel sulfonamide compounds were added to the enzyme activity for the inhibition studies. PON1 activity with novel sulfonamides was assayed by following the hydration of paraoxon. Activity % values of paraoxonase for six different concentrations of each novel sulfonamide were determined by regression analysis using Microsoft Office Excel. PON1 activity without synthesized compounds was accepted as 100% activity. The inhibitor concentration causing up to 50% enzyme inhibition, known IC_{50} value, was determined from the graphs for each compound [47].

The inhibition constants (K_i) of the newly synthesized 1,4-dihydropyridine substituted sulfonamide derivatives were calculated mathematically using the Cheng & Prusoff equation [50].

Free radical scavenging assay

Radical scavenging activity was determined by modifying the method by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [51]. The assay solution comprises 100 mL of (150 mM) DPPH, 50 µL of increasing concentration of test compounds, and was adjusted to 1000 µL in each. This reaction mixture was incubated at room temperature for 30 min. in a dark environment. Ascorbic acid (Vitamin C) was used as a reference. The measurements were carried out by using UV-Vis spectroscopy at 517 nm. The reaction rates were compared and the percent inhibition due to the presence of tested inhibitors was calculated. Each concentration was analysed in three independent experiments.

Cheminformatics analysis

The ligand molecules (**1–12**) were drawn by the Chemdraw tool and were further minimized by visualizing the UCSF Chimera 1.10.1 software. Different online drug assessment tools, such as Molinspiration (http://www.molinspiration.com/) and Molsoft (http://www.molsoft.com/), have been used to estimate drug resemblance, and biological properties of these designed targeted molecules. The number of rotatable bonds, hydrogen bond acceptors (HBA), and hydrogen bond donors (HBD) were also confirmed by PubChem (https://pubchem.ncbi.nlm.nih.gov/). Moreover, it was analysed using Lipinski's rule of five by Molsoft and Molinspiration tools.

Molecular docking studies

SwissDock online docking platform was used in these docking studies, based on the docking program EADock DSS, which has the following phases in its algorithm. Docking studies were determined for all target gaps, using blind insertion with multiple clamping modes (min. 200) [52, 53].

The crystallographic structure of human serum PON1 was obtained in pdb format from the online RCSB Protein Data Bank (https://www.rcsb.org/) for molecular docking studies. Initially, 2-hydroxyquinoline and water molecules in the crystallographic structure of PON1 were removed from the enzyme with the Discovery studio Client 2021 software. As a second step, the addition of polar hydrogen atoms to the PON1 (PDB ID: 3SRG) enzyme was performed through the same system and the minimization of the added hydrogen atoms was ensured.

The synthesized compounds were drawn by Chemdraw ultra 12.0 software, and saved as mol2. file. Then, both the geometrically optimization and the synthesized molecular series were converted into 3D structures in pdb format and saved via the Avogadro software (https://avogadro.cc/.).

LUMO-HOMO levels

The theoretical HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital), LUMO-HOMO gap (ΔE), ionization potential (IP), and electron affinity (EA) of the synthesized compounds were calculated using the HyperChem Professional 8.0.1 program. For this purpose, a desktop computer with a high core i5 operating system and HyperChem software were used. Two-dimensional structures of the molecules were drawn step by step in the HyperChem program. First, the optimum conformation was determined using the "molecular mechanics method (MM+)" using "single point", and "geometry optimization". Then, the optimum conformation was determined using the "semi-empirical method (PM3)", and "geometry optimization". LUMO-HOMO orbitals, and orbital energies were determined (in vacuo, Polak-Ribiere Algorithm; RMS gradient: 0.1 kcal $Å^{-1}$ mol^{-1} or 100 maximum cycles). The PM3 technique is developed from computational chemistry's Parametric Method number 3, and is included in the semi-empirical method for molecular structure quantum calculations. PM3 is a parameterized Hamiltonian that can recreate a wide range of molecular properties [54].

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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