Research Paper

Benzoxazinones as Human Peroxisome Proliferator Activated Receptor Gamma (PPARγ) Agonists: A Docking Study Using Glide

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The purpose of the present study is to undertake a docking study of some benzoxazinone derivatives on human peroxisome proliferator activated receptor co-crystallized with an alpha-aryloxyphenylacetic acid agonist using Glide 4.5. The QikProp program was used to obtain the absorption, distribution, metabolism and excretion properties of the analogues. The intermolecular hydrogen bonding interaction of the best-fit ligands were found to be associated with Tyr473, Ser289, Hie 449, Hip 323, Ser 342 and Gly 284 amino acid residue at the receptor active site. Among all the observed interaction with similar binding pattern, the presence of methyl carboxypentyl side chain (Lig. No. 21) showed additional interaction with Ser 342 and the affinity was increased by carboxyl oxygen (as hydrogen bond acceptor) with a best Glide score of -14.54 as compared to the co-crystallized aryloxyphenyl acetic acid which achieved a glide score of -12.50.

Key words: Benzoxazinones, docking, glide, PPARγ, QikProp

Peroxisome proliferator activated receptor (PPAR) function as lipid sensors that coordinately regulate the expression of large gene arrays and, thereby, modulate important metabolic events. They are also the targets of drugs that are effective in the treatment of metabolic disorders (type 2 diabetes mellitus and atherosclerosis). To date, three isotypes of PPAR family, PPARγ, PPARα and PPARβ/δ, have been recognized, and found to play an important role in carbohydrate and lipid metabolism[1,2].

PPARα is believed to participate in fatty acid uptake mainly in the liver and heart. PPARβ/δ is involved in fatty acid oxidation in muscle. PPARγ is highly expressed in fat to facilitate glucose and lipid uptake, stimulate glucose oxidation, decrease free fatty acid level and ameliorate insulin resistance. Synthetic ligands for PPARα and γ such as fibric acid and thiazolidinediones have been used in patients with type 2 diabetes and pre-diabetic insulin resistance[3]. They are nuclear fatty acid receptors, which contain a hydrophobic ligand-binding pocket. The thiazolidinediones (TZD) are synthetic ligands of PPARγ. By activating a number of genes in tissues, PPARγ increases glucose and lipid uptake, increases glucose oxidation, decreases free fatty acid concentration, and decreases insulin resistance[4].

TZD comprises a new class of oral antidiabetic agents that selectively enhance and partially mimic certain actions of insulin on carbohydrate and lipid metabolism in type 2 diabetes mellitus (NIDDM) and other conditions of insulin resistance. The first member of this class, ciglitazone and its successors troglitazone, pioglitazone, englitazone, darglitazone and rosiglitazone has been extensively reported. Pioglitazone, darglitazone, and rosiglitazone have progressed in clinical development, while ciglitazone and englitazone due to adverse effects on the liver were discontinued. Troglitazone was introduced in to clinical use but later was discontinued due to hepatotoxicity[5]. Other than TZDs several other potential pharmacophores that binds to the hydrophobic binding pocket of the receptor are well established such as phenylpropionic acid derivatives[6], benzyloxazolidine-2,4-diones[7] and 1,2,4-oxadiazolidine-3,5-diones[8].

The docking studies were done on hPPAR (Human Peroxisome Proliferator Activated receptor)

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Pdb Id: 1Zeo receptor co-crystallized with an alpha-aryloxyphenylacetic acid agonist-(2S)-(4-isopropylphenyl)[(2-methyl-3-oxo-5,7-dipropyl-2,3-dihydro-1,2-benzisoxazol-6-yl)oxy]acetate (CO1). The protein was taken from the Protein Data Bank (www.rcsb.org). The existing non-thiazolidinedione ligand with their activity data reported recently by Rybczynski and coworkers were taken for the present docking study[9]. Few of the best-fit ligands were studied for their ADME properties. To the best of our knowledge, this is the first study to examine the binding interaction of the benzoxazinones ligands.

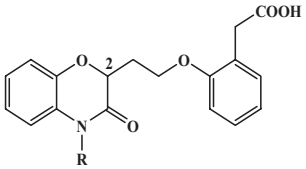
MATERIALS AND METHODS

Ligands were built using Maestro 8.5 build panel and prepared by LigPrep 2.2 version v22208 (Schrödinger, LLC., USA) application that uses Optimized Potentials for Liquid Simulations (OPLS) 2005 force field. The protein was prepared by deleting a chain of the dimer before docking the ligands into the active site of the protein. Further the crystallographically observed water molecules and the active site of the protein was defined for generating the grid. The energy minimized ligands were docked into the prepared grid using Glide (Glide 4.5, Schrödinger, Inc.) on a Linux based (RedHat Linux Enterprise RHEL 5) workstation. The QikProp program (QikProp 3.1, Schrödinger, Inc.) was used to obtain the ADME properties of the analogues. The best-fit ligands were neutralized before being used by QikProp. The neutralizing step is essential, as QikProp is unable to neutralize a structure and no properties will be generated in the normal mode.

Glide docking analysis:

Glide calculations were carried out with Impact version v50208. It performs grid-based ligand docking with energetics and searches for favourable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein. A more negative the glide score indicates better fitting to the receptor active sites[10]. The glide score of 31 ligands were obtained after performing the ligand docking on a Linux based (Red Hat Linux Enterprise RHEL 5) workstation (Table 1). Hydrophobic interaction as depicted by the hydrophobic enclosure reward that indicates the surrounding of the ligand lipophilic atoms or group by the lipophilic protein atoms[11].

TABLE 1: BENZOXAZINONES DERIVATIVES[9] USED FOR THE DOCKING STUDY



Ligand No.	R	Biological activity EC50 (nM)	Glide score	Ligand No.	R	Biological activity EC 50 (nM)	Glide score
1.	$CH_3(CH_2)_4$-	243	-13.80	17.	$OH(CH_2)_6$-	200	-14.20
2.	$CH_3(CH_2)_5$-	100	-13.65	18.	$OH(CH_2)_7$-	149	-13.15
3.	R(2)[b]	100	-12.93	19.	$OHCH(CH_3)(CH_2)_4$-	1000	-13.88
4.	S(2)[b]	1200	-13.11	20.	$CH_3CO(CH_2)_4$-	260	-13.40
5.	$CH_3(CH_2)_6$-	234	-13.20	21.	$CH_3CO(CH_2)_5$-	264	-14.54
6.	$CH_3(CH_2)_7$-	300	-13.03	22.	$CH_3C(NOH)(CH_2)_4$-	10	-12.40
7.	$CH_3(CH_2)_9$-	1000	-14.04	23.	$CH_3C(F)_2(CH_2)_4$-	179	-12.75
8.	*Isoprop*-$(CH_2)_4$-	79	-13.84	24.	R(23)[b]	295	-12.75
9.	*Isobut*-$(CH_2)_4$-	1000	-13.62	25.	S(23)[b]	1000	-13.27
10.	$C_5H_9(CH_2)_3$-	2700	-13.85	26.	$CH_3C(F)_2(CH_2)_5$-	534	-13.87
11.	$C_6H_{11}(CH_2)_2$-	300	-13.35	27.	$F(CH_2)_6$-	117	-13.13
12.	$COOHC(CH_3)_2(CH_2)_4$-	1000	-13.73	28.	$F(CH_2)_7$-	718	-14.28
13.	$CNC(CH_3)_2(CH_2)_4$-	359	-12.95	29.	$CH_3CH_2CH_2S(CH_2)_2$-	380	-13.99
14.	CH_2=$CH(CH_2)_4$-	208	-13.05	30.	$CH_3O(CH_2)_4$	274	-12.63
15.	$OH(CH_2)_5$-	1000	-13.15	31.	R(30)[b]	274	-12.72
16.	$OHC(CH_3)_2(CH_2)_4$-	644	-13.93	CO1	Co-crystallized ligand		-12.50

[b]indicates chirality at 2nd position of benzoxazinone nucleus. A more negative glide score indicates better fitting to the active site of the receptor.

***In silico* ADME Studies on the best-fit ligand:**

One of the main goals in drug discovery is the identification of innovative small molecular scaffolds exhibiting high binding affinity and selectivity for the target together with a reasonable absorption, distribution, metabolism and excretion (ADME) profile, lead and/or drug likeness. Such chemical entities are likely to be able to enter higher phases of the drug development process. This has resulted in a paradigm shift in identifying the drug likeness properties of lead molecules early in the drug discovery process. Thus, *in vitro* approaches are now widely used to investigate the ADME properties of new chemical entities and, more recently, computational (*in silico*) modeling has been investigated as a tool to optimize selection of the most suitable candidates for drug development[12]. The QikProp program was used to obtain the ADME properties of the analogues. It predicts both physically significant descriptors and pharmaceutically relevant properties. The program was processed in normal mode, and predicted the properties for the best-fit molecules (Ligand Nos. 7, 17, 21 and 28), consisting of principal descriptors and physiochemical properties with analysis of the log P (Octanol/Water), % human oral absorption, Lipinski's rule of five violation, CNS activity (Tables 2 and 3). It also evaluates the acceptability of the analogues based on Lipinski's rule of 5 [13] that are essential for rational drug design.

RESULTS AND DISCUSSION

The intermolecular hydrogen bonding interaction of the best-fit ligands were found to be associated with Tyr473, Ser289, Hie449, Hip323, Ser342 and Gly284 amino acid residue at the receptor active site (figs. 1 to 6). Among all the observed interaction with similar binding pattern, the presence of methyl carboxypentyl side chain (Lig. No. 21) showed additional interaction with Ser342 (fig. 3) and the affinity was increased by carboxyl oxygen (as hydrogen bond acceptor) with a best Glide score of -14.54 as compared to the co-crystallized aryloxyphenyl acetic acid which achieved a glide score of -12.50.

The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of PPAR-γ. The best results obtained with docking scores are -14.04, -14.20, -14.54 and -14.28 of ligand 7, 17, 21 and 28 respectively. Based on the glide scores it can be inferred that N-alkyl chain substitution of benzoxazinone nucleus mainly long and branched

TABLE 2: ADME SCREENING BY QIKPROP 3.1 (SCHRÖDINGER, LLC)

Ligand No.	Percentage human oral absorption	Lipinski's rule of five	QPlogPo/w	QPlogS	CNS	#stars	#rtvFG
7.	91.31	1	6.75	-8.17	-2	3	0
17.	82.41	0	4.17	-5.55	-2	0	0
21.	83.62	0	4.52	-6.16	-2	0	1
28.	87.64	1	5.95	-7.25	-2	1	0

TABLE 3: QIKPROP PROPERTIES AND DESCRIPTORS

Descriptor	Description	Recommended range
% Human oral absorption:	It predicts human oral absorption on 0 to 100% scale. The prediction is based on a quantitative multiple linear regression model. This property usually correlates well with human oral-absorption.	>80% is high <25% is poor
Lipinski's rule of five	Lipinski's rules of five are: mol_MW< 500, QPlogPo/w< 5, donorHB≤ 5, accptHB≤ 10. Compounds that satisfy these rules are considered drug like. (The "five" refers to the limits, which are multiples of 5	Lipinski's rule of five
QPlog Po/w:	Predicted octanol/water coefficient	-2.0-6.5
QPlogS	Predicted aqueous solubility, log S. S in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.	-6.5 – 0.5
CNS:	Predictive Central Nervous Activity	Maximum is 4
#stars:	Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. A large number of stars suggest that a molecule is less drug-like than molecules with few stars. The following are some of properties and descriptors are included in the determination of #stars: Molecular weight, dipole, QPlogPw, QPlogPo/w, QlogS, solvent accessible surface area (SASA) etc.	0-5
#rtvFG	This particular descriptor indicates the number of reactive functional groups. The presence of these groups can lead to decomposition, reactivity, or toxicity problems *in vivo*.	0-2

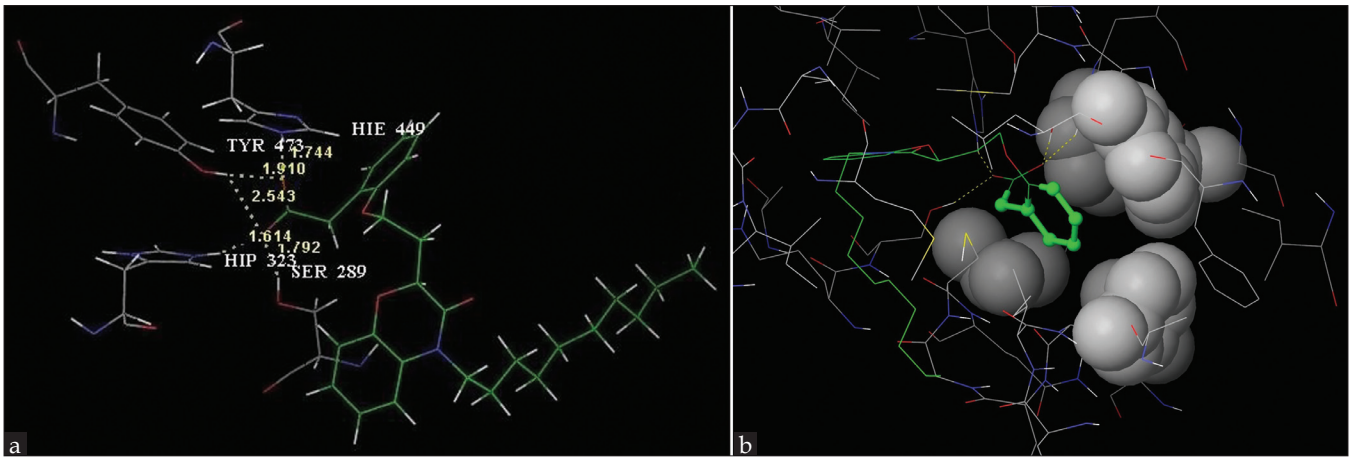


Fig. 1: Interaction of aryloxyphenylacetic acid
(a) Hydrogen bonding interaction of aryloxyphenylacetic acid where the carboxyl groups interact with the Tyr473, Ser 289, Hie449 and Hip 323 residue. (b) Hydrophobic enclosure reward (-0.28) achieved by the hydrophobic group (phenyl) of ligand No.7.

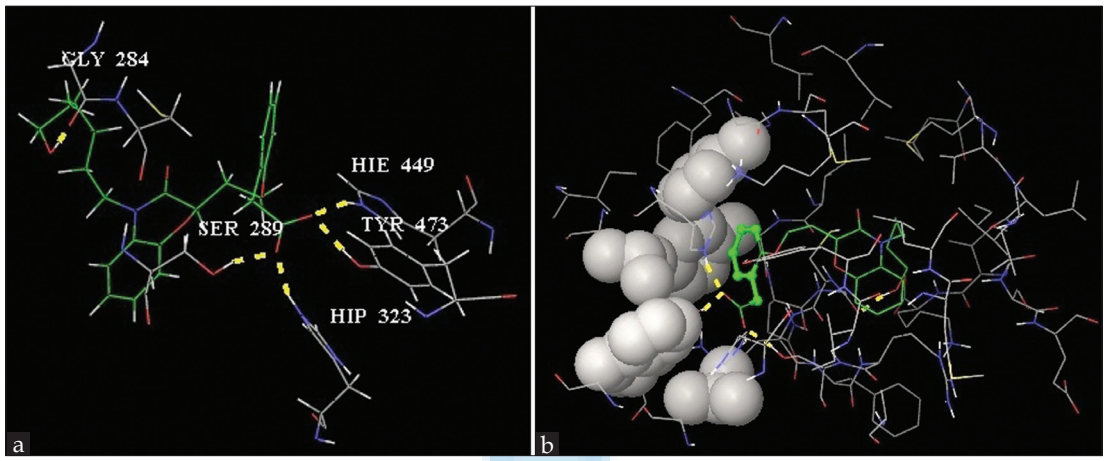


Fig. 2: Interaction of ligand No. 17
(a) Hydrogen bonding interaction and (b) Hydrophobic enclosure reward (-0.15) achieved by the hydrophobic group (phenyl) of ligand No. 17.

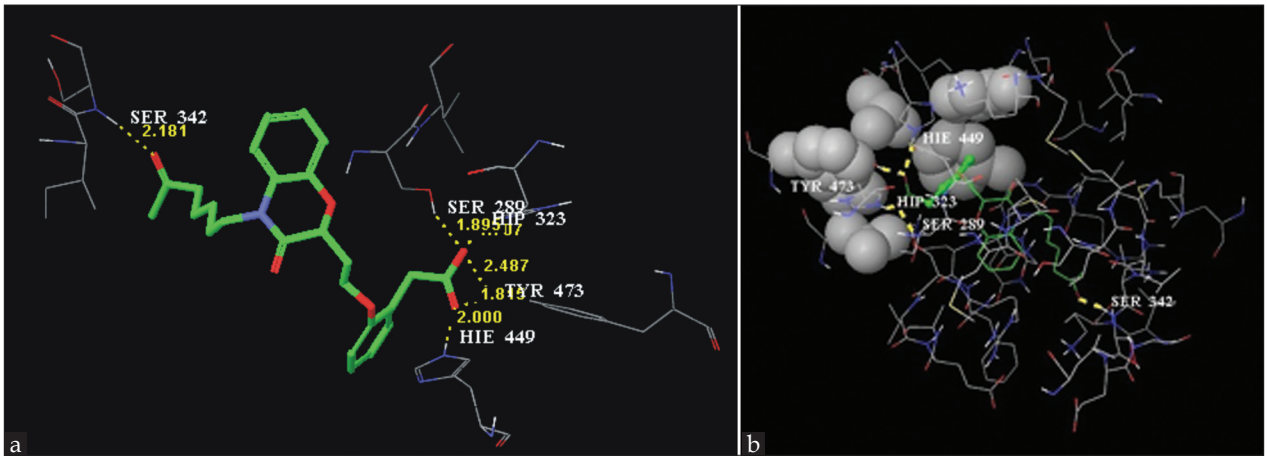


Fig. 3: Interaction of ligand No. 21
(a) Hydrogen bonding interaction and (b) hydrophobic enclosure reward (-0.26) achieved by the hydrophobic group (phenyl) of ligand No. 21.

chain increases the affinity. Further hydroxyl and acyl group substitution of medium length chain gives the best score as the presence of –OH and carbonyl group at the distal side chain assist in hydrogen bond interaction with Gly284 and Ser342 residue, respectively (figs. 2 and 3). In comparison with the co-crystallized ligand (-0.86), most of the ligand received a poor hydrophobic enclosure reward. This

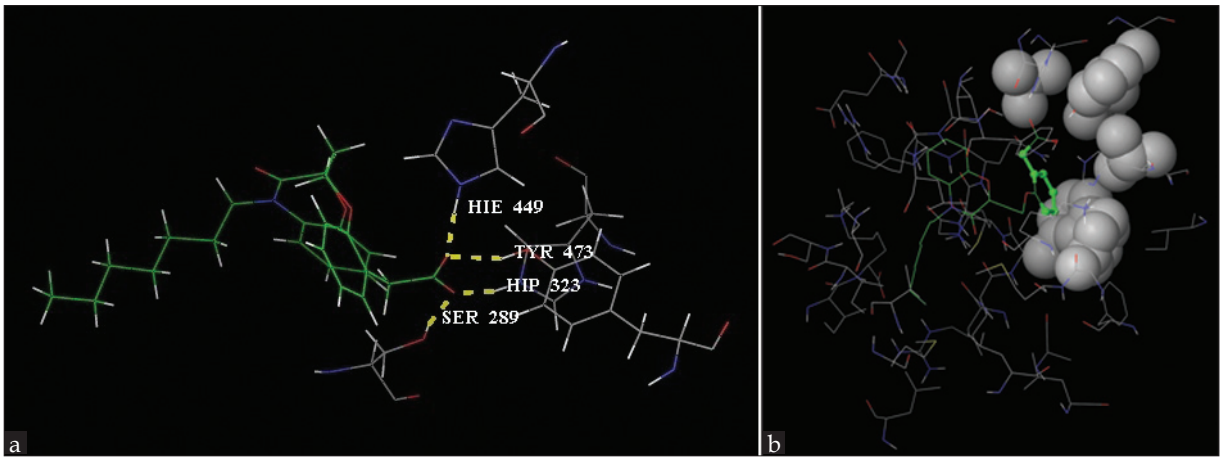


Fig. 4: Interaction of ligand No.28
(a) Hydrogen bonding interaction and (b) hydrophobic enclosure reward (-0.36) achieved by the hydrophobic group (phenyl) of ligand No.28.

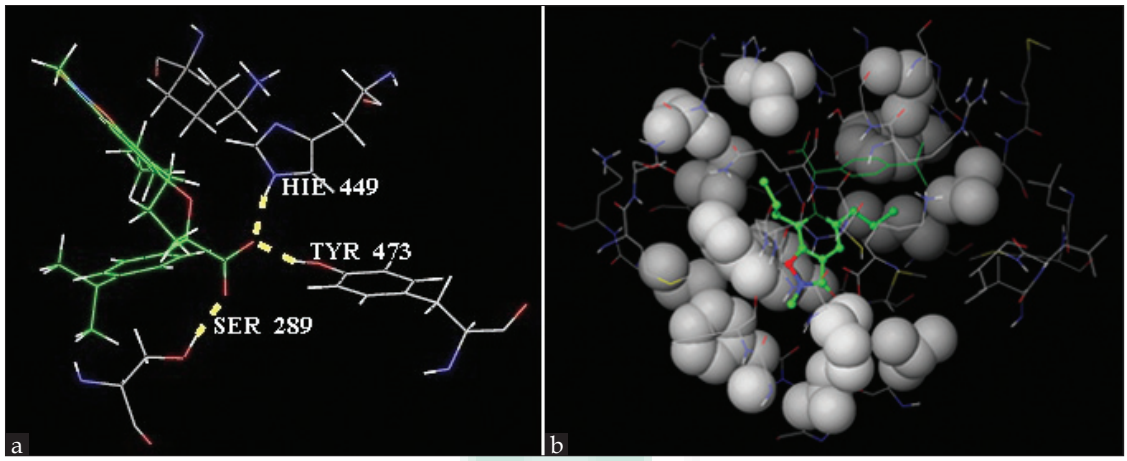


Fig. 5: Interaction of co-crystallized ligand
(a) Hydrogen bonding interaction and (b) hydrophobic enclosure reward (-0.86) achieved by the hydrophobic group (benzisoxazolyl) of co-crystallized ligand (-0.86) occupying the Tyr473, Ser 289 and Hie 449 pocket.

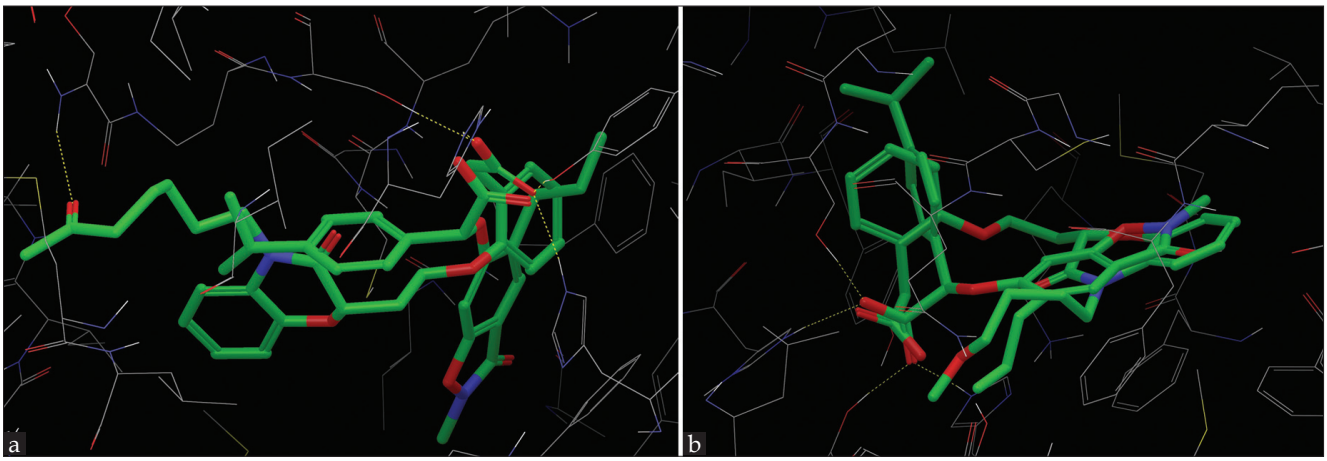


Fig. 6: Graphical picture showing superimposition and binding mode of co-crystallized ligand
Graphical picture showing superimposition and binding mode of co-crystallized ligand (CO1) (a) with ligand 21 (RMSD 1.65Å) and (b) with ligand 31 (RMSD 1.46 Å) docked at the active site of hPPARγ receptor.

may be due to the binding orientation of the molecules as all the derivatives present its phenyl acetic acid moiety towards the active site, which is much less hydrophobic than the benzoxazinone nucleus. Further, the best-fit ligands were subjected to *in silico* ADME screening. Based on the overall analysis it may be

concluded that the series has the potential for the treatment of type 2 diabetes and the benzoxazinone pharmacophore could be used for further development.

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