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# Selectivity profile comparison for certain $\gamma$ -butyrolactone and oxazolidinone-based ligands on a sigma 2 receptor over sigma 1: a molecular docking approach†

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Sigma receptors ( $\sigma_1$ R and  $\sigma_2$ R) are pharmacologically characterized membrane-bound receptors that bind a wide range of chemical compounds. Alzheimer's disease, traumatic brain injury, schizophrenia, and neuropathic pain have all been associated with abnormal  $\sigma_2$  activity. The  $\sigma_2$  receptor has recently been identified as a potential therapeutic target for inhibiting the formation of amyloid plaques. Numerous laboratories are now investigating the potential of  $\sigma_2$  ligands. Small molecule discovery is the focus of current research, with the goal of using target-based action to treat a variety of illnesses and ailments. Functionalized  $\gamma$ -butyrolactone and oxazolidinone-based ligands, in particular, are pharmacologically important scaffolds in drug discovery research and have been thoroughly examined for  $\sigma_2$  receptor efficacy. The purpose of this study was to evaluate the pharmacophoric features of different  $\sigma_2$  receptor ligands using *in silico* techniques. This study used a library of 58 compounds having a  $\gamma$ -butyrolactone and oxazolidinone core. To investigate the binding characteristics of the ligands with the  $\sigma_2$  receptor, a 3D homology model was developed. To understand the binding pattern of the  $\gamma$ -butyrolactone and oxazolidinone based ligands, molecular docking studies were performed on both  $\sigma_1$  and  $\sigma_2$  receptors. Furthermore, MM/GBSA binding energy calculations were used to confirm the binding of ligands on the  $\sigma_2$  over  $\sigma_1$  receptor. These *in silico* findings will aid in the discovery of selective  $\sigma_2$  ligands with good pharmacophoric properties and potency in the future.

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## 1. Introduction

In 1976, W. R. Martin *et al.* published the results of a study on the impact of morphine, ketocyclazocine, and ( $\pm$ )-SKF-100047 in dogs with chronic spinal disease. They found that these three compounds elicited distinctly different responses and hypothesized that each was interacting with a different pharmacological target. They labeled these previously unidentified targets as the  $\mu$ -opioid receptor (morphine type, MOR), the  $\kappa$ -opioid receptor (ketocyclazocine type, KOR), and the  $\sigma$ -opioid receptor (SKF-100047 like).<sup>1</sup> Follow-up studies with the

individual enantiomers of SKF-100047 revealed that each isomer interacted with a different biochemical target. (–)-SKF-100047 interacts with MOR and KOR to produce an opioid type response, but (+)-SKF-100047 produces a non-opioid response through the sigma receptor ( $\sigma$ R).<sup>2</sup> Eventually, it was determined that there are two subtypes of this receptor, which have been designated as sigma-1 ( $\sigma_1$ ) and sigma-2 ( $\sigma_2$ ).<sup>3</sup> An X-ray structure of human  $\sigma_1$  was reported in 2016,<sup>4</sup> but to date there are no known ligands for this receptor.

The true nature of  $\sigma_2$ , however, remained a mystery for nearly 40 years. In 2017, A. C. Kruse *et al.* demonstrated that this receptor is identical to the Transmembrane Protein 97 (TMEM97, also known as MAC30 (Meningioma-associated protein)),<sup>5</sup> and an X-ray structure was published in 2021.<sup>6</sup> Similar to  $\sigma_1$ , there are no known natural functional  $\sigma_2$  ligands. It has been demonstrated that this protein is present in the endoplasmic reticulum (ER) and lysosomes where it binds to cholesterol, but the pharmacological role of  $\sigma_2$  has not been determined.<sup>7</sup> Numerous disease states such as schizophrenia,<sup>8</sup> Alzheimer's disease,<sup>9</sup> neuropathic pain,<sup>10</sup> traumatic brain injury,<sup>11</sup> Niemann-Pick disease,<sup>12</sup> and cancer<sup>13</sup> have been linked to  $\sigma_2$  and this has prompted many research teams to investigate

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the potential therapeutic utility of  $\sigma_2$  ligands. Numerous *in vivo* active  $\sigma_2$  ligands have been identified and some have reached human clinical trials. UKH-1114 (**1**,  $\sigma_2 K_i = 46$  nM) is efficacious in animal models of pain,<sup>14</sup> while Siramesine (**2**,  $\sigma_2 K_i = 0.12$  nM) is efficacious in animal models of depression and anxiety.<sup>15</sup> The radioligand [<sup>18</sup>F]ISO-1 (**3**,  $\sigma_2 K_i = 6.9$  nM) has been studied as a possible PET ligand in the treatment of breast cancer,<sup>16</sup> while CT1812 (**4**,  $\sigma_2 K_i = 8.5$  nM) has been the subject of clinical trials as a potential Alzheimer's disease therapy (Fig. 1).<sup>17</sup>

Recently, we reported our discovery of two novel classes of  $\sigma_2$  ligands, oxazolidin-2-ones (**5**) and functionalized  $\gamma$ -butyrolactones (**6**).<sup>18–20</sup> The synthetic protocol for the synthesis of these two classes of compounds is outlined in the ESI† (Schemes 1 and 2). These compounds demonstrated a range of selectivity for  $\sigma_2$  over  $\sigma_1$ . As part of an effort to develop a better understanding of the structural features that drive selectivity, we have studied generated *in silico* models of both receptors. In addition, we have conducted docking studies and calculated binding energies of the exemplary compounds from our previously disclosed  $\sigma_2$  ligands.

## 2. Materials and methods

### 2.1 Homology modeling

Homology modeling is a technique for creating and predicting an atomic resolution model of a target protein based on the experimentally established 3D structure of a homologous protein called the template protein. The four fundamental stages in homology modeling are (1) locating the template structure sequence, (2) matching the query sequence with the template structure sequence, (3) creating the query's model structure based on the template structure information, and (4) assessing the projected model. As a result, homology modeling may be used to predict the structure of unknown proteins, such as the human sigma 2 receptor.

The model architectures of the human sigma 2 receptor were predicted using a homology modeling approach. Maestro was used for all computational and molecular modeling of the

human sigma 2 receptor (Schrödinger, LLC, 2019-1).<sup>21</sup> The human sigma 2 amino acid sequence, which consists of 176 residues, was retrieved from the Uniprot database (UniProt ID: Q5BJF2).<sup>22</sup> PSI-BLAST was used to scan the nonredundant PDB database for template identification. The X-ray crystalline structure of the *Bos taurus* sigma-2 receptor bound to Z1241145220 (PDB ID: 7M95)<sup>23</sup> exhibited measurable similarities to the query sequence and was therefore utilized to generate the model. Protein Data Bank was used to acquire the coordinates. The alignment of the template and the target was the initial stage. To choose the best alignment, the alignments were ordered by identities, score, positives, expectation value, and gaps, and statistically analyzed.

The Prime Module was used to create the homology models. In the model, the co-crystallized ligand Z1241145220 was retained. Using Prime functionality, loop refinement and numerous loop conformations were produced. Side-chain predictions and all-atom minimizations were used to score these conformations. The model was further optimized and minimized when the model construction calculations were completed.

### 2.2 Molecular docking studies

Schrödinger's Glide docking tool (Schrödinger, LLC, New York, 2019) was utilized to investigate ligand–receptor interactions. Glide is a novel method for finding favourable interactions between proteins and many ligands. As a result, Glide docking allows you to compare the binding mechanism and affinity of different ligands to the protein. In flexible docking, ligand posture refers to the location and orientation of a ligand with regard to the protein, as well as its shape. A number of hierarchical filters are used to analyze the ligand postures obtained by Glide docking. The ChemScore empirical scoring function is used by Glide. This algorithm detects the protein's and ligand's favourable hydrophobic and hydrogen-bonding interactions. Through many evaluation trials, Glide has been regarded as one of the best docking tools presently available.

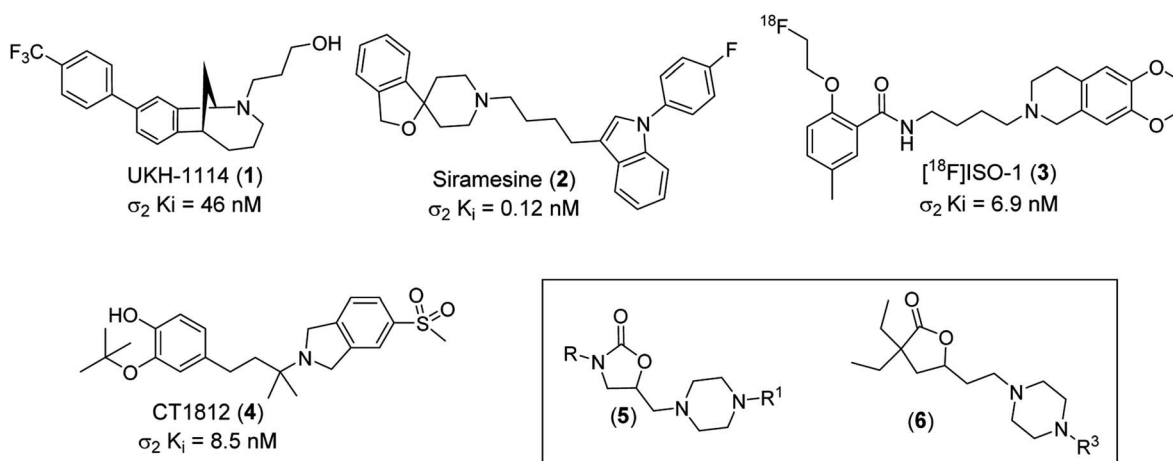


Fig. 1 Structures of UKH-1114 (**1**), Siramesine (**2**), [<sup>18</sup>F]ISO-1 (**3**), CT1812 (**4**), oxazolidinone lead class (**5**) and  $\gamma$ -butyrolactones lead series (**6**).



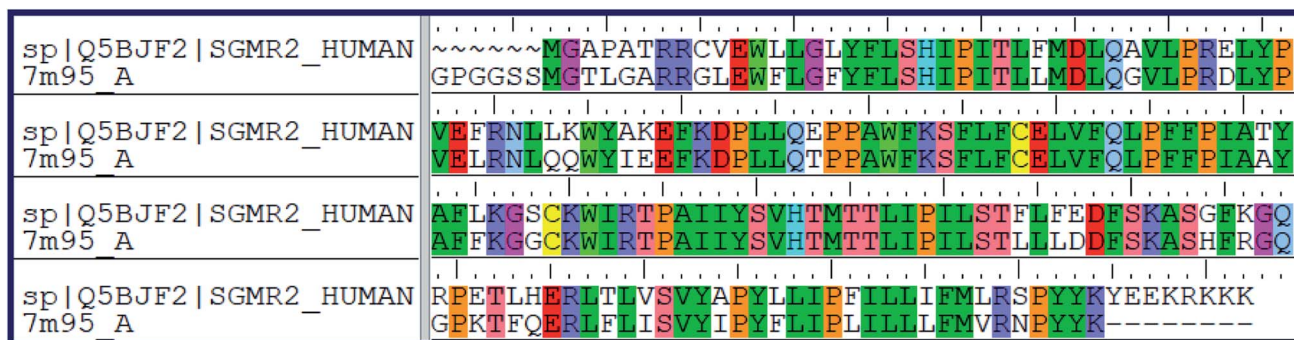


Fig. 2 Sequence alignment of sigma intracellular receptor 2 ( $\sigma_2$ ) from *Homo sapiens* (UniProt ID: Q5BJF2) and bovine sigma-2 receptor from *Bos taurus* (PDB ID: 7M95).

**2.2.1 Ligand preparation.** Glide takes the ligand structure in 3D format as an input file for ligand preparation. Maestro Molecule Builder was used to create the 3D structures of all ligands. The ligand molecule is prepared for Glide docking using the Schrodinger ligand preparation product LigPrep. LigPrep (LigPrep, 2019) is a technique that refines the ligand structure *via* a series of processes. LigPrep was used to remove unnecessary structures, add hydrogens, and optimize and minimize ligand structures. Because of its enhanced parameterization and coverage, the ligand structures were reduced using the OPLS-2005 force field. Furthermore, among the force fields, the OPLS-2005 force field was selected for grid generation due to its metal handling capabilities (LigPrep, 2019). As a consequence, a single low-energy 3D structure was created with the right chiralities. LigPrep is a tool for creating high-quality 3D structures by changing chemical structures like as stereochemistry and protonation state (LigPrep, 2019). As a result, LigPrep was used to establish the protonation states of all the ligands. The protonation states of the ligand may be derived from the structure of the ligand if structural data is available. The structural data for all of the ligands is available, and the protein active site was chosen before molecular docking. As a result, the proper protonation states were assigned to the ligand binding mode.

**2.2.2 Protein preparation.** The RCSB Protein Data Bank was used to get the protein crystal structure of human sigma-1 receptor bound to PD144418 (PDB ID: 5HK1). Using Maestro's Protein Preparation Wizard, the human sigma-1 protein crystal structure and the modelled sigma-2 receptor were refined further. The protein's multimeric complex structure is simplified because a small number of atoms in the complex structure is preferred for computer efficiency. Except for those that have bridging connections between the protein and the ligand, all crystallographic water molecules are eliminated from the receptor molecules' 3D structure.

Customizing the protein, metal ions, and cofactors is simple using the Protein Preparation Wizard. The wizard fills in any missing residues around the protein's active site. The formal charges and ligand bond ordering were changed. Following these stages, the Impact Refinement module was used to perform restrained minimization (Impref, 2019). The revised

ligand/protein/water structures were examined to guarantee the right formal charges, bond ordering, protonation states, and final changes were made to the protein structures. The receptor grid files were created using the prepared protein structures.

**2.2.3 Receptor grid generation.** Several distinct sets of fields were used to depict the shape and features of the grid on the receptor, providing increasingly more precise scoring of the ligand poses. One of the input files for ligand docking is the receptor grid. A prepared structure (an all-atom structure with proper bond ordering and formal charges) is required for receptor grid production, which is obtained during the protein preparation stage. A receptor grid generation panel was used to set up and produce the receptor grid for all of the proteins. The receptor structures were defined using the choices from this panel. Co-crystallized ligands were eliminated during this stage, and the location and size of the active site, which would be indicated by receptor grids, were identified. The active site of the receptor was determined using the ligands in the crystal structures. To properly address metals and a broad variety of atom types described, the force field OPLS 2005 was employed.

**2.2.4 Docking studies.** Glide ligand docking needed previously produced receptor grids and ligand structures built using LigPrep. By choosing XP mode on the Glide ligand docking screen, the docking was completed. The ligand docking panel's flexible docking option with default settings was chosen because it guides the docking process to build conformations internally. In terms of Glide score and docking score, Glide calculated the ligand–receptor binding affinity.

### 2.3 Prime MM/GBSA binding energy calculations

One of the most often used computational approaches for estimating relative binding affinities of target protein–ligand complexes is molecular mechanics with generalised born surface area (MM/GBSA). Through employing the MM/GBSA technology tools available in the Prime module of Schrodinger 2019-1, ligand-binding energies were calculated based on docking complex. The MM/GBSA calculations were performed using the protein–ligand complexes produced from the docking investigations.  $\Delta G_{\text{bind}}$ , the relative binding free energy, was calculated using the following equation:



$$\Delta G_{\text{bind}} = E_{\text{complex}}(\text{minimized}) - [E_{\text{ligand}}(\text{unbound, minimized}) + E_{\text{receptor}}(\text{unbound, minimized})]$$

where  $\Delta G_{\text{bind}}$  is the computed relative free energy that takes into account both ligand and receptor strain energy. The MM/GBSA energy of the minimized complex is  $E_{\text{complex}}(\text{minimized})$ , and  $E_{\text{ligand}}(\text{unbound, minimized})$  is the MM/GBSA energy of the ligand after it has been removed from the complex and allowed to relax. After separating the protein from the ligand, the MM/GBSA energy of the  $E_{\text{receptor}}(\text{unbound, minimized})$  is calculated.

## 3. Results and discussion

### 3.1 Homology modeling and binding site analysis

To the best of our knowledge, the crystal structure for sigma-2 receptor of *Homo sapiens* has not been reported yet. As a result, we used a comparative modeling approach to create a three-dimensional (3D) model for the sigma-2 receptor of *H. sapiens*. The homology model of the *H. sapiens* sigma-2 receptor was created in Schrödinger Suite 2019-1 (Schrödinger, LLC, New York, NY) using Meastro, Prime. The *H. sapiens* sigma intracellular receptor 2 query sequence was obtained from the universal protein resource (Uniprot, Entry Id: Q5BJF2). The template was chosen from the crystallographic structure of the *Bos taurus* sigma-2 receptor (PDB ID: 7M95, 2.41 resolution) with 78.4 percent sequence identity with the target. Prime homology modeling methods were used to create a 3D model of the human sigma-2 receptor. The Ramchandran plot and protein reports were used to analyze the predicted 3D structure. The sequence alignment of the sigma-2 receptor of *H. sapiens* (UniProt ID:Q5BJF2) and *Bos Taurus* (PDB ID: 7M95) is shown in Fig. 2. The homology-modeled structure of the human sigma-2 receptor and its Ramchandran plot are shown in Fig. 3 and 4. The Ramchandran plot of the human sigma-2 receptor structure revealed that >95 percent of residues are favored and allowed  $\phi$ ,  $\psi$  backbone conformational regions.

### 3.2 Molecular docking

All of the compounds were subjected to molecular docking studies in order to discover significant binding mechanisms responsible for their action on the sigma 1 and 2 receptors. Molecular docking was used to confirm the binding pose and conformation of these analogues. All of the compounds were docked into the active site of a homology-modeled sigma 2 receptor as well as the crystal structure of the sigma 1 receptor. The results of molecular docking, hydrogen bonding, and arene-arene interactions of compounds with sigma 1 and 2 receptors are shown in Tables 1 and S1.†

The initial set of compounds (**LACT1**–**LACT41**) comprised of a series of  $\gamma$ -butyrolactone analogues with a heterocyclic primary core and varied electron donating, electron withdrawing, hydrophobic, H-bond acceptor, and donor groups. When compared to the 1-diethyl lactone derivative (**LACT1**) of the  $\gamma$ -butyrolactone family, the 1,1-dimethyl lactone derivative (**LACT2**) demonstrates a restricted number of interactions with the active site of sigma 2 receptor. The active site residues

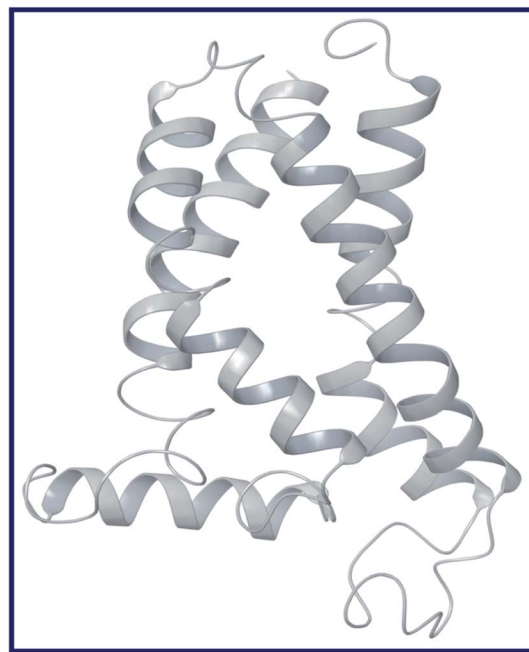


Fig. 3 Homology modelled 3D structure of human sigma intracellular receptor 2 ( $\sigma_2$ ).

(Asp29, Glu73) of  $\sigma_2$  R formed two hydrogen bonds with **LACT1**. The protonated N-atom of phenyl piperazine serves as an H-bond donor, forming an H-bond with Asp29 ( $d = 2.57 \text{ \AA}$ ). Butyrolactone's carbonyl oxygen atom functions as an H-bond acceptor, forming an H-bond with Glu73 ( $d = 2.49 \text{ \AA}$ ). Furthermore, piperazine's N-atom forms a Pi-cation interaction with Tyr147. **LACT1** has also exhibited a variety of hydrophobic interactions with active site residues. The phenyl ring of **LACT1** forming a Pi-Pi (arene-arene) contact with Tyr50. Furthermore, **LACT1** formed only one Pi-Pi interaction with the  $\sigma_1$  R residue Tyr103 and made no H-bond interactions with it. There are fewer interactions with both  $\sigma_1$  and  $\sigma_2$  R for the 1,1-dimethyl lactone derivative (**LACT2**).

**LACT3** with carbonyl group on its ethyl linker chain has lower docking scores towards both  $\sigma_1$  &  $\sigma_2$  R. **LACT4** with piperazin-2-one ring has average docking scores with fewer interactions on  $\sigma_1$  &  $\sigma_2$  R. **LACT5** with piperadine ring has shown good docking scores with  $\sigma_1$  &  $\sigma_2$  R. The N-atom of piperadine scaffold has made two strong Pi-cation interactions with the active site residues Tyr147 ( $d = 5.59 \text{ \AA}$ ) and Tyr150 ( $d = 4.55 \text{ \AA}$ ) of  $\sigma_2$  R. Interestingly, the N-atom of piperadine also made a salt bridge with Asp29 ( $d = 3.07 \text{ \AA}$ ) of  $\sigma_2$  R. Further, the phenyl ring of **LACT5** established two Pi-Pi interactions with the active site residues Tyr103, Tyr206 of  $\sigma_1$  R. In case of **LACT6**, the protonated nitrogen atom was observed at cyclohexyl connected N-atom of the piperazine ring and this N-atom interact with Asp29 *via* salt-bridge. In addition, the protonated N-atom of the piperazine ring has made a Pi-cation interaction Tyr150 of  $\sigma_2$  R. Further, the protonated N-atom of the piperazine ring of **LACT6** has established an H-bond and Pi-cation interaction with Glu172 and Phe107 of  $\sigma_1$  R, respectively. Compounds with electron withdrawing substituent's like CN



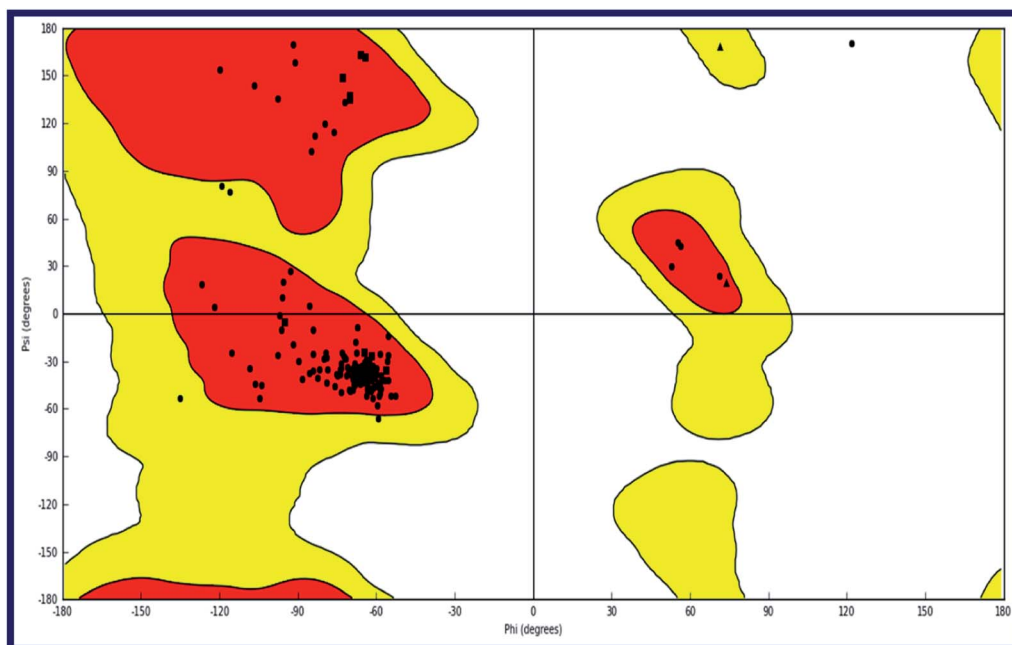


Fig. 4 Ramachandran plot for the modeled sigma intracellular receptor 2 ( $\sigma_2$ ) of *Homo sapiens*. The plot is organized as follows: glycine, proline and all other residues are plotted as triangles, squares, and circles respectively. The red, yellow and white regions represent the favoured, allowed and the disallowed regions respectively.

Table 1 GLIDE docking score for certain  $\gamma$ -butyrolactone and oxazolidinone-based ligands at the active sites of sigma intracellular receptor 1 and 2 ( $\sigma_1$  and  $\sigma_2$ )<sup>a</sup>

S. no	Ligand ID	Receptor name	Ki (nM)	Docking score	Interactions		
					H- bonds	Pi-Pi stacking	Hydrophobic
1	LACT11	$\sigma_1$	10 000	-5.705	—	Tyr103	Val84, Trp89, Met93, Leu95, Ala98, Tyr103, Leu105, Phe107, Tyr120, Ile124, Phe133, Val162, Trp164, Met170, Ile178, Leu182, Ala185, Tyr206
		$\sigma_2$	14	-6.850	—	Tyr50	Ile24, Met28, Trp49, Tyr50, Leu59, Phe66, Phe69, Leu70, Cys72, Leu111, Ile114, Val146, Tyr147, Tyr150
2	LACT21	$\sigma_1$	1168	-5.681	—	—	Val84, Ala86, Trp89, Met93, Leu95, Tyr103, Leu105, Phe107, Tyr120, Ile124, Phe133, Val162, Trp164, Met170, Ile178, Leu182, Phe184, Ala185, Tyr206
		$\sigma_2$	44	-7.550	Asp29	—	Ile24, Met28, Trp49, Tyr50, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Val146, Tyr147, Tyr150
3	LACT22	$\sigma_1$	195	-4.693	—	—	Val84, Trp89, Met93, Leu95, Ala98, Tyr103, Leu105, Phe107, Tyr120, Ile124, Phe133, Val152, Val162, Trp164, Met170, Ile178, Leu182, Phe184, Ala185, Leu186, Tyr206
		$\sigma_2$	5.9	-7.891	Asp29	—	Ile24, Met28, Trp49, Tyr50, Phe54, Leu59, Phe66, Leu70, Leu111, Ile114, Val146, Tyr147, Pro149, Tyr150
4	LACT26	$\sigma_1$	10 000	-4.983	Glu172	—	Val84, Trp89, Met93, Leu95, Ala98, Tyr103, Leu105, Tyr120, Ile124, Phe133,



Table 1 (Contd.)

S. no	Ligand ID	Receptor name	Ki (nM)	Docking score	Interactions		
					H- bonds	Pi-Pi stacking	Hydrophobic
5	LACT29	$\sigma_2$	142	-7.914	Asp29, Glu73	—	Val162, Trp164, Ile178, Leu182, Phe184, Ala185, Tyr206
		$\sigma_1$	2167	-6.486	Glu172	Tyr103, Tyr206	Ile24, Met28, Trp49, Tyr50, Phe54, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Val146, Tyr147, Tyr150
6	LACT35	$\sigma_2$	32	-7.915	Asp29	—	Val84, Trp89, Met93, Leu95, Ala98, Tyr103, Leu105, Tyr120, Ile124, Phe133, Val152, Val162, Trp164, Ile178, Leu182, Phe184, Ala185, Tyr206
		$\sigma_1$	125	-7.416	—	His154	Ile24, Met28, Trp49, Tyr50, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Val146, Tyr147, Tyr150
7	LACT37	$\sigma_1$	59	-7.642	—	Tyr103	Val84, Trp89, Met93, Leu95, Tyr103, Leu105, Phe107, Tyr120, Ile124, Phe133, Val162, Trp164, Met170, Ile178, Leu182, Phe184, Ala185, Tyr206
		$\sigma_2$	6.1	-10.62	Asp29	—	Ile24, Met28, Tyr50, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Val146, Tyr147, Tyr150
8	LACT41	$\sigma_1$	10 000	-7.582	Glu172	—	Val84, Trp89, Met93, Leu95, Ala98, Tyr103, Leu105, Phe107, Tyr120, Ile124, Phe133, Val162, Trp164, Ile178, Leu182, Phe184, Ala185, Tyr206
		$\sigma_2$	2.8	-9.880	Asp29	—	Ile24, Met28, Trp49, Tyr50, Leu59, Phe66, Leu70, Leu111, Ile114, Val146, Tyr147, Tyr150
9	OXAZ2	$\sigma_1$	10 000	-5.445	—	Tyr103	Val84, Trp89, Met93, Leu95, Ala98, Tyr103, Leu105, Phe107, Tyr120, Ile124, Phe133, Val152, Val162, Trp164, Ile178, Leu182, Phe184, Ala185, Leu186, Tyr206
		$\sigma_2$	119	-7.113	Asp29	Tyr50	Ile24, Met28, Trp49, Tyr50, Leu59, Phe66, Leu70, Leu111, Ile114, Val146, Tyr147, Pro149, Tyr150
10	OXAZ3	$\sigma_1$	10 000	ND	—	—	—
		$\sigma_2$	465	-7.708	Asp29, Glu73	Phe54	Ile24, Met28, Trp49, Tyr50, Phe54, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Val146, Tyr147, Pro149, Tyr150
11	OXAZ4	$\sigma_1$	10 000	ND	—	—	—
		$\sigma_2$	206	-7.627	Asp29	Phe54	Ile24, Met28, Trp49, Tyr50, Phe54, Leu59, Phe66, Leu70, Phe81, Tyr103, Leu111, Ile114, Val146, Tyr147, Pro149, Tyr150
12	OXAZ5	$\sigma_1$	10 000	-4.569	—	Tyr103, Tyr206	Val84, Trp89, Met93, Leu95, Ala98, Tyr103, Leu105, Tyr120, Ile124, Phe133, Val152, Val162, Trp164, Ile178, Leu182, Phe184, Ala185, Leu186, Tyr206
		$\sigma_2$	530	-6.371	—	—	Ile24, Met28, Leu46, Trp49, Tyr50, Phe54, Leu59, Phe66, Phe69, Leu70, Phe81, Tyr103, Leu111, Ile114, Val146, Tyr147, Tyr150
13	OXAZ6	$\sigma_1$	10 000	ND	—	—	—
		$\sigma_2$	192	-7.609	Asp29	—	Ile24, Met28, Trp49, Tyr50, Phe54, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Val146, Tyr147, Pro149, Tyr150



Table 1 (Contd.)

S. no	Ligand ID	Receptor name	Ki (nM)	Docking score	Interactions		
					H- bonds	Pi-Pi stacking	Hydrophobic
14	<b>OXAZ7</b>	$\sigma_1$	10 000	ND	—	—	—
		$\sigma_2$	91	-8.164	Asp29	—	Ile24, Met28, Leu46, Trp49, Tyr50, Phe54, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Vall46, Tyr147, Tyr150
15	<b>OXAZ8</b>	$\sigma_1$	2847	-1.104	—	Trp121	Tyr120, Trp121, Ala183, Phe184, Ala187, Phe191
		$\sigma_2$	36	-10.15	—	—	Ile24, Met28, Leu46, Trp49, Tyr50, Phe54, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Vall46, Tyr147, Tyr150
16	<b>OXAZ13</b>	$\sigma_1$	10 000	ND	—	—	—
		$\sigma_2$	49	-8.832	Asp29	Tyr150	Ile24, Met28, Trp49, Tyr50, Phe54, Leu59, Phe66, Phe69, Leu70, Leu111, Ile114, Vall46, Tyr147, Pro149, Tyr150

<sup>a</sup> ND# not docked at the active site.

(**LACT7**),  $\text{CF}_3$  (**LACT8**), and Cl (**LACT9**) in the 4-position of the phenyl ring has shown good docking scores with both the  $\sigma_1$  &  $\sigma_2$  R. The protonated N-atom of the piperazine ring of **LACT7**, **LACT8** and **LACT9** has shown a salt-bridge and Pi-cation interaction with Asp29 and Tyr147 of  $\sigma_2$  R, respectively. Additionally, an H-bond interaction was observed in between **LACT9**

and Val146 of  $\sigma_2$  R. Further, a salt-bridge and a Pi-cation interaction were observed for both **LACT7**, **LACT8** with Glu172 and Phe107 of  $\sigma_1$  R, respectively. A Pi-Pi interaction was observed for 4-trifluoromethyl substituted phenyl ring of **LACT8**, **LACT9** with Tyr103 and Leu182 of  $\sigma_1$  R, respectively.

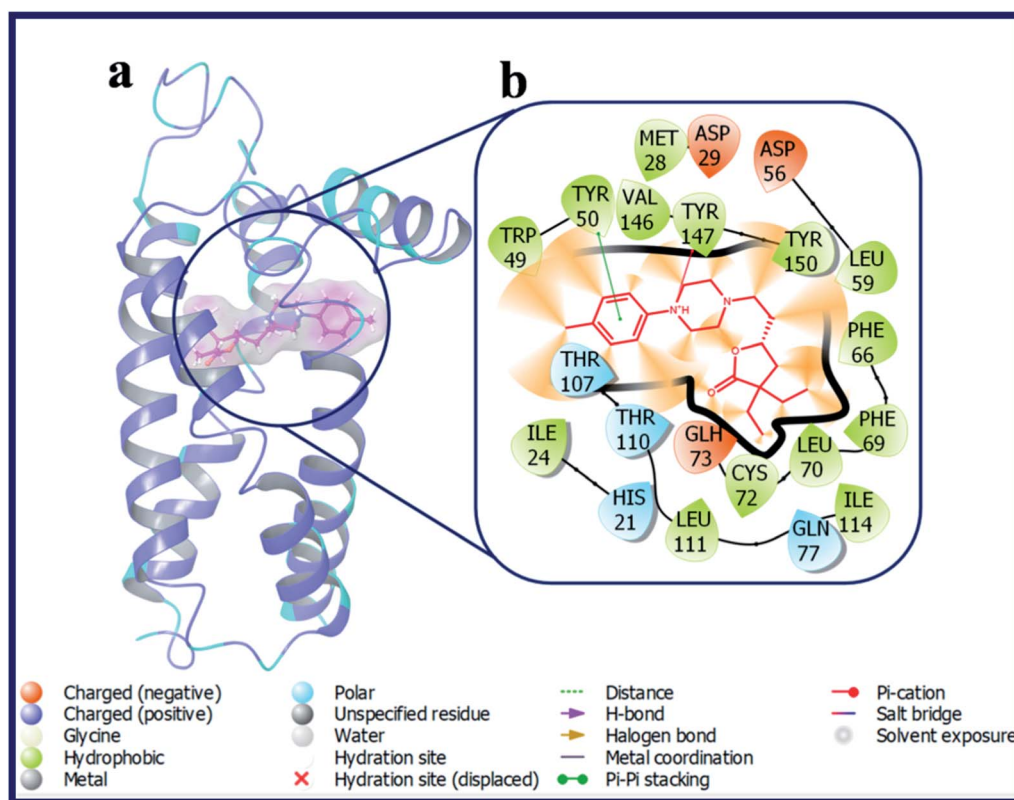


Fig. 5 (a) Docking pose of compound **LACT11** (purple colour stick) and (b) its ligand–protein interactions in the active site of modeled sigma intracellular receptor 2.



Compounds with electron donating substituent's like OMe (**LACT10**) and Me (**LACT11**) in the 4-position of the phenyl ring has shown good docking scores on  $\sigma_2$  R over  $\sigma_1$  R. **LACT10** established two hydrogen bonds with the active site residues (Asp29, Glu73) of  $\sigma_2$  R. The protonated N-atom of phenyl piperazine has made an H-bond interaction with Asp29 and the carbonyl oxygen atom of butyrolactone shown H-bond interaction with Glu73. Further, the N-atom of piperazine is established a Pi-cation interaction with Tyr147. With respect to the  $\sigma_1$  R, compound **LACT10** has formed an H-bond interaction with Glu172 and Pi-Pi interaction with His154. The detailed binding pose and protein-ligand interactions of **LACT11** with  $\sigma_2$  R was depicted in Fig. 5. 4-Me substituted phenyl ring of **LACT11** established a Pi-Pi interaction with Tyr50 and N-atom of phenyl piperazine has made a Pi-cation interaction Tyr147. Further, several hydrophobic interactions were observed for **LACT11** and the active site residues of  $\sigma_2$  R, which stabilizes the lodging of **LACT11** in the active pocket.

Compounds **LACT12**–**LACT14** include electron withdrawing substituent's at 3-position of the phenyl ring based derivatives endowed with an improved docking scores with  $\sigma_2$  R if compared with the  $\sigma_1$  R. The carbonyl oxygen atom of butyrolactone of **LACT12** has shown an H-bond interaction with Glu73. Further, the protonated N-atom of phenyl piperazine of

both **LACT12** and **LACT13** has made a salt-bridge and a Pi-cation interaction with Asp29 and Tyr147 of  $\sigma_2$  R, respectively. **LACT14** established two hydrogen bonds with the active site residues Asp29 ( $d = 2.29$  Å), Glu73 ( $d = 2.3$  Å) and a Pi-cation interaction with Tyr147 of  $\sigma_2$  R. Further, compound **LACT15** and **LACT16** with electron donating substituent's at 3-position of the phenyl ring showed almost similar docking scores on both  $\sigma_1$  &  $\sigma_2$  R.

Interestingly, compound **LACT17**–**LACT19** with electron withdrawing substituent's at 2-position of the phenyl ring showed good docking scores on  $\sigma_2$  R if compared with the  $\sigma_1$  R. **LACT17**, **LACT18** and **LACT19** has shared a common H-bond interaction, as well as Pi-cation interaction with the active site residues Asp29 and Tyr147 of  $\sigma_2$  R, respectively. Additionally, compounds with electron donating substituent's at 2-position of the phenyl ring (**LACT20** and **LACT21**) has also shown good docking scores on  $\sigma_2$  R over  $\sigma_1$  R. Mounting the steric bulk in the 2-position of the phenyl ring by placing an isopropyl group (**LACT22**) improved  $\sigma_2$  R selectivity. Fig. 6 displays the docking pose of **LACT22** and its ligand-protein interactions in the active site of  $\sigma_2$  R. **LACT22** with 2,4-di-Me substitution on phenyl ring displayed high docking score for both  $\sigma_1$  &  $\sigma_2$  R. **LACT24** with 2-pyridine ring showed poor docking score at  $\sigma_2$  R. In this analogue, the 2-pyridine ring was involved in the Pi-Pi stacking

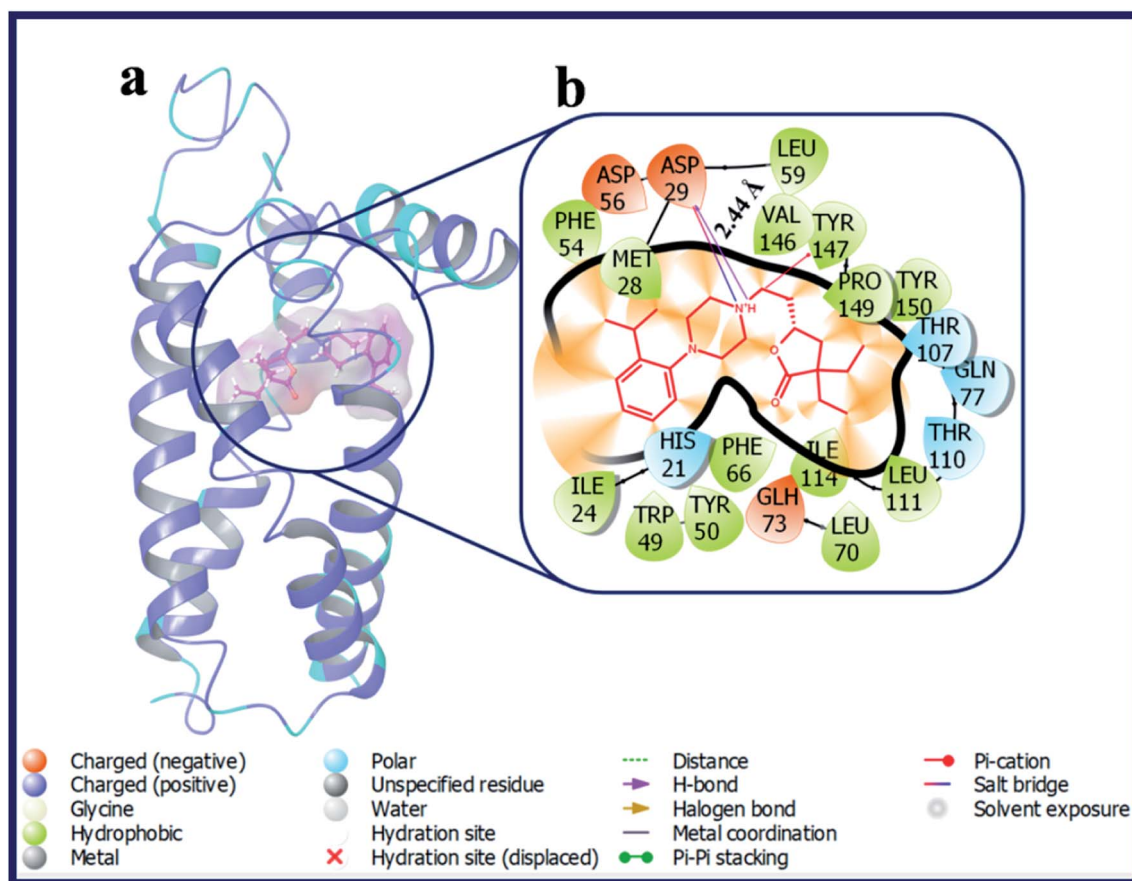


Fig. 6 (a) Docking pose of compound **LACT22** (purple colour stick) and (b) its ligand-protein interactions in the active site of modeled sigma intracellular receptor 2.



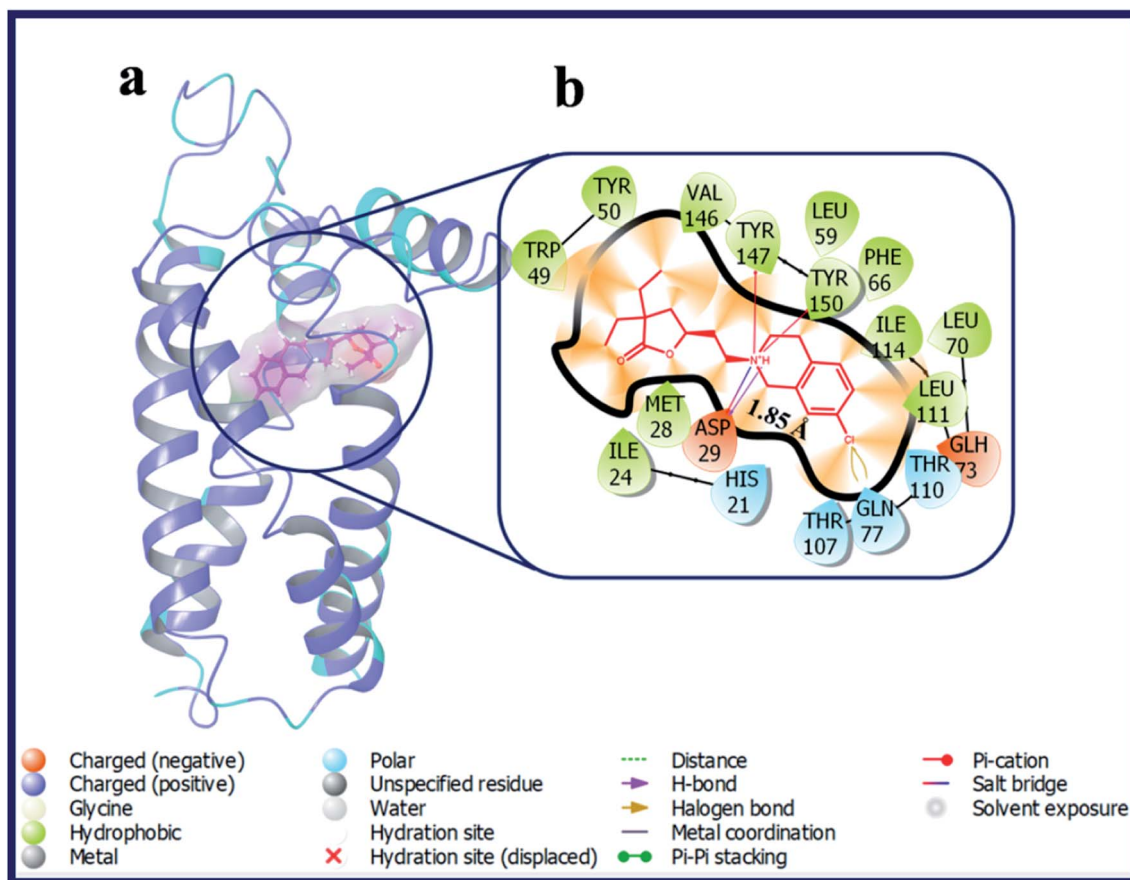


Fig. 7 (a) Docking pose of compound LACT37 (purple colour stick) and (b) its ligand–protein interactions in the active site of modeled sigma intracellular receptor 2.

interaction with Tyr50 of  $\sigma_2$  R. With respect to the  $\sigma_1$  R, compound LACT24 has formed an H-bond interaction with Glu172 ( $d = 2.55$  Å). In case of 3-pyridine ring containing analogue (LACT25) and 4-pyridine ring containing analogue (LACT26), we observed a common H-bond interactions (Asp29, Glu73) and Pi–cation interaction (Tyr147) with  $\sigma_2$  R. In addition, the 3-pyridine ring of LACT25 also involved in the Pi–Pi stacking interaction with Tyr50 of  $\sigma_2$  R. Further, LACT25 did not show any H-bond, Pi–Pi, salt-bridge and Pi–cation interactions with  $\sigma_1$  R. LACT26 exhibited an H-bond between protonated N-atom of phenyl piperazine and Glu172, and Pi–cation interaction between N-atom of 4-pyridine ring and His154 of  $\sigma_1$  R.

LACT27 with linker chain length of “3C” displayed good docking score on  $\sigma_2$  R over  $\sigma_1$  R. The protonated N-atom of phenyl piperazine ring of LACT27 established an H-bond, salt-bridge, Pi–cation interactions with Val146 ( $d = 2.56$  Å), Asp29 ( $d = 4.68$  Å), and Tyr147 ( $d = 4.51$  Å) of  $\sigma_2$  R, respectively. Compound LACT28 include linker chain length of “4C” also shown greater docking score on  $\sigma_2$  R over  $\sigma_1$  R. LACT28 established an H-bond with the active site residue Asp29 ( $d = 2.34$  Å) and a Pi–cation interaction with Tyr147 ( $d = 5.06$  Å) of  $\sigma_2$  R. 1-Naphthyl piperazine containing  $\gamma$ -butyrolactone analogue (LACT29) displayed good docking score on  $\sigma_2$  R. LACT29 made an H-bond with the active site residue Asp29 ( $d = 2.13$  Å) and

a Pi–cation interaction with Tyr147 ( $d = 4.98$  Å) of  $\sigma_2$  R. With respect to the  $\sigma_1$  R, the protonated N-atom of piperazine ring of LACT29 has formed an H-bond interaction with Glu172 ( $d = 2.33$  Å) and the 1-naphthyl part made two Pi–Pi interactions with the active site residues Tyr103 and Tyr206 of  $\sigma_1$  R. The 4-pyrimidine ring containing  $\gamma$ -butyrolactone analogue (LACT30) displayed poor docking scores on both  $\sigma_1$  and  $\sigma_2$  R.

Captivatingly, compounds with prospective piperazine bioisosteres like homopiperazine analogue (LACT31), 2,6-diazaspiro[3.3]heptane analogue (LACT32), and hexahydropyrrolo [3,4-*c*]pyrrole analogue (LACT33) shown excellent docking scores on  $\sigma_2$  R over  $\sigma_1$  R. LACT31, LACT32 and LACT33 has shared a common H-bond interaction, as well as Pi–cation interaction with the active site residues Asp29 and Tyr147 of  $\sigma_2$  R, respectively. Further, LACT32 and LACT33 have shown additional Pi–cation interaction with Tyr150 of  $\sigma_2$  R. Replacing of phenyl ring in LACT33 with 4-pyridine led to increase in number interactions with the active site residues of  $\sigma_2$  R. The N-atom of 4-pyridine of LACT34 established H-bond interaction with Gln77 ( $d = 2.61$  Å), and also 4-pyridine formed a Pi–Pi interaction with His21 of  $\sigma_2$  R. Further, LACT34 has made H-bond interaction, as well as Pi–cation interaction with the active site residues Asp29 ( $d = 2.03$  Å) and Tyr147 of  $\sigma_2$  R, respectively.



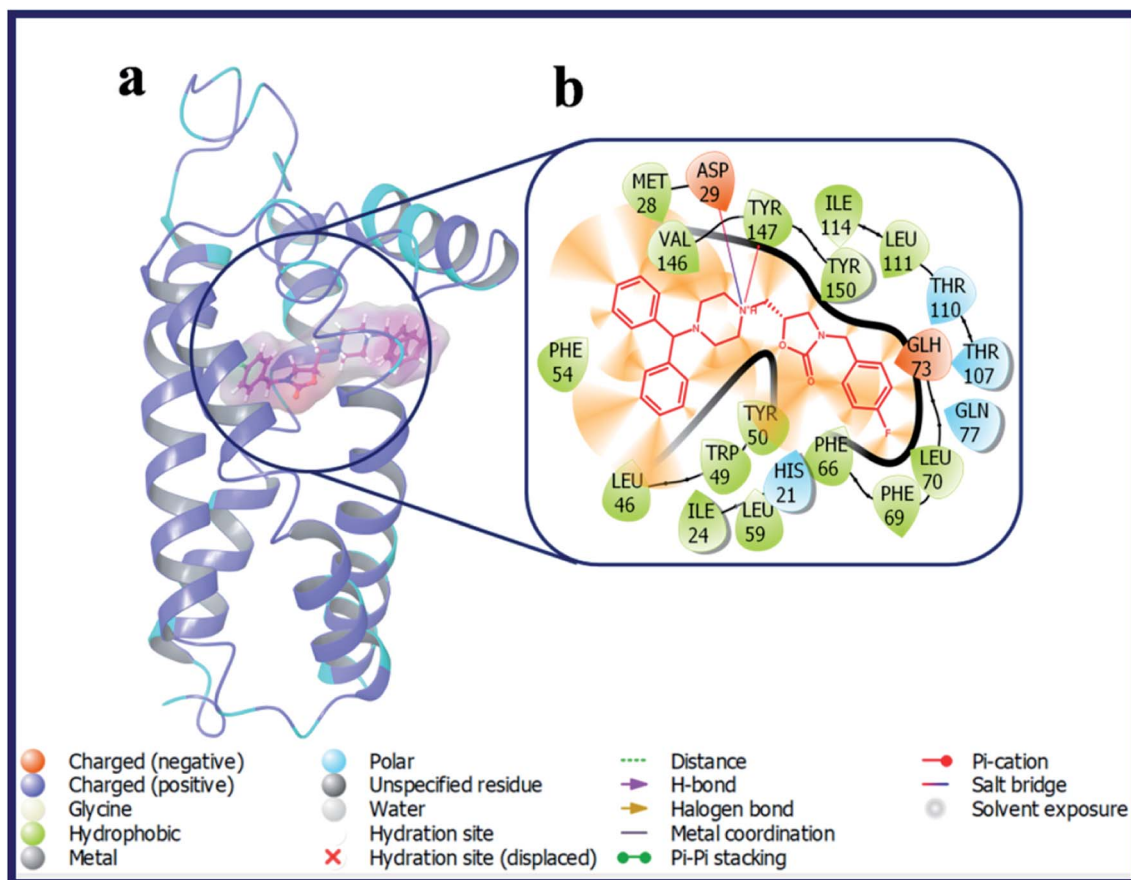


Fig. 8 (a) Docking pose of compound OXAZ8 (purple colour stick) and (b) its ligand–protein interactions in the active site of modeled sigma intracellular receptor 2.

According to our calculations, fascinatingly, compound **LACT35–LACT38** with tetrahydroisoquinoline scaffold have shown admirable docking scores with the  $\sigma_2$  R. Compound **LACT35–LACT38** has shared a common H-bond interaction, as well as two Pi–cation interactions with the active site residues Asp29 and Tyr147 & Tyr150 of  $\sigma_2$  R, respectively. The protonated N-atom of tetrahydroisoquinoline involved in both H-bond and Pi–cation interactions. In addition, the carbonyl oxygen atom of butyrolactone of **LACT35** is acting as H-bond acceptor and made an H-bond interaction with Gln77 ( $d = 3.38$  Å). Fig. 7 illustrates the binding pose of compound **LACT37** and its ligand–protein interactions in the active site of  $\sigma_2$  R. Similarly, compound **LACT39–LACT41** with pyridine type nitrogen atom in the tetrahydroisoquinoline ring also exhibited the good docking scores and interactions against both  $\sigma_1$  and  $\sigma_2$  R, but their actual biological  $K_i$  values are not at all potent towards the both  $\sigma_1$  and  $\sigma_2$  R. The *in silico* docking scores for these compounds is probably due to the close structure resemblance with that of simple tetrahydroisoquinoline scaffold containing analogues (**LACT35–LACT38**).

The second set of compounds (**OXAZ1–OXAZ17**) consisted of a series of oxazolidinone-based derivatives with a heterocyclic primary core and various electron donating, electron withdrawing, hydrophobic, H-bond acceptor, and donor groups.

Within this series, compounds have various substituent's capped at amide group of oxazolidin-2-one ring. The unsubstituted oxazolidin-2-one ring containing compound **OXAZ1** displayed very poor docking scores towards both  $\sigma_1$  and  $\sigma_2$  R. Compounds with 3 to 6 membered cycloalkane group attachments, *i.e.* cyclopropyl (**OXAZ2**), cyclobutyl (**OXAZ3**), cyclopentyl (**OXAZ4**) and cyclohexyl (**OXAZ5**) have shown good to moderate docking scores against  $\sigma_2$  R. Compound **OXAZ2** established one hydrogen bond with the active site residue (Asp29) of  $\sigma_2$  R. The protonated N-atom of phenyl piperazine is acting as H-bond donor and made an H-bond interaction with Asp29 ( $d = 1.80$  Å). Further, the N-atom of piperazine is established two Pi–cation interactions with Tyr147 and Tyr150. Additionally, one of the phenyl groups of benzhydryl part established a Pi–Pi interaction with Tyr50. With respect to the  $\sigma_1$  R, the protonated N-atom of piperazine ring of **OXAZ2** has formed a salt-bridge with Glu172 and one of the phenyl groups of benzhydryl part established a Pi–Pi interaction with Tyr103. **OXAZ3** and **OXAZ4** have shared a common H-bond interaction, as well as Pi–cation interaction and Pi–Pi interaction with the active site residues Asp29, Tyr147 and Phe54 of  $\sigma_2$  R, respectively. Further, **OXAZ3** have shown additional H-bond interaction with Glu73 of  $\sigma_2$  R. On the contrary, compound **OXAZ3** and **OXAZ4** was not docked at the active site of  $\sigma_1$  R. Compound **OXAZ5** exhibited a Pi–





Table 2 The average  $\Delta G_{\text{Bind}}$  binding free energy (kcal mol<sup>-1</sup>) results from MM-GBSA calculations

S. no.	Ligand name	Receptor name	<sup>a</sup> $\Delta G_{\text{Bind}}$	<sup>b</sup> $\Delta G_{\text{Bind}}$ Coulomb	<sup>c</sup> $\Delta G_{\text{Bind}}$ covalent	<sup>d</sup> $\Delta G_{\text{Bind}}$ H-bond	<sup>e</sup> $\Delta G_{\text{Bind}}$ lipo	<sup>f</sup> $\Delta G_{\text{Bind}}$ solv GB	<sup>g</sup> $\Delta G_{\text{Bind}}$ packing	<sup>h</sup> $\Delta G_{\text{Bind}}$ vdW
1	<b>LACT11</b>	$\sigma_1$	-72.692	-36.141	5.125	-0.110	-64.419	51.599	-0.516	-28.230
		$\sigma_2$	-84.578	-12.003	3.137	-0.042	-65.936	18.129	-0.182	-27.682
2	<b>LACT21</b>	$\sigma_1$	-70.828	-42.736	15.959	-0.490	-64.396	51.490	-0.803	-29.853
		$\sigma_2$	-100.593	-18.207	1.251	-0.751	-61.938	27.402	-0.546	-47.804
3	<b>LACT22</b>	$\sigma_1$	-82.630	-50.328	12.790	-0.742	-75.122	59.170	-0.875	-27.523
		$\sigma_2$	-93.976	-8.363	2.000	-0.564	-63.601	22.203	-0.137	-45.515
4	<b>LACT26</b>	$\sigma_1$	-62.239	2.854	7.838	-0.012	-57.765	22.889	-0.996	-37.048
		$\sigma_2$	-107.986	1.463	7.707	-0.751	-61.362	-4.223	-0.446	-50.375
5	<b>LACT29</b>	$\sigma_1$	-60.303	-1.503	16.626	-0.042	-70.189	34.138	-0.958	-38.376
		$\sigma_2$	-107.352	-12.577	2.049	-0.886	-66.412	20.676	-0.822	-49.380
6	<b>LACT35</b>	$\sigma_1$	-67.810	-2.586	6.614	-0.012	-57.729	26.364	-0.485	-39.977
		$\sigma_2$	-95.993	-15.515	5.009	-0.914	-61.545	23.716	-0.093	-46.650
7	<b>LACT37</b>	$\sigma_1$	-84.106	-4.010	6.139	-0.011	-67.887	24.824	-0.371	-42.789
		$\sigma_2$	-90.984	-22.915	9.645	-1.181	-57.791	28.408	-0.001	-47.149
8	<b>LACT41</b>	$\sigma_1$	-65.730	-0.511	5.299	-0.039	-54.189	26.266	-1.028	-41.529
		$\sigma_2$	-95.398	-14.991	5.238	-0.901	-59.999	21.977	-0.093	-46.629
9	<b>OXAZ2</b>	$\sigma_1$	-2.382	2.861	27.555	-0.037	-72.857	29.786	-3.059	13.370
		$\sigma_2$	-99.275	-3.132	8.131	-0.925	-62.137	9.443	-1.060	-49.596
10	<b>OXAZ3</b>	$\sigma_1$	—	—	—	—	—	—	—	—
		$\sigma_2$	-114.210	-12.314	3.431	-1.004	-65.374	12.458	-1.244	-50.162
11	<b>OXAZ4</b>	$\sigma_1$	—	—	—	—	—	—	—	—
		$\sigma_2$	-111.426	-12.105	2.296	-0.810	-63.686	17.158	-1.379	-52.898
12	<b>OXAZ5</b>	$\sigma_1$	-2.018	4.574	32.115	-0.004	-81.300	32.921	-2.689	12.365
		$\sigma_2$	-120.283	-14.860	9.246	-0.038	-68.995	15.282	-0.186	-60.732
13	<b>OXAZ6</b>	$\sigma_1$	—	—	—	—	—	—	—	—
		$\sigma_2$	-97.551	-5.157	9.508	-0.711	-57.962	11.774	-0.987	-54.016
14	<b>OXAZ7</b>	$\sigma_1$	—	—	—	—	—	—	—	—
		$\sigma_2$	-103.179	-3.886	5.441	-0.478	-61.168	11.805	-0.022	-54.871
15	<b>OXAZ8</b>	$\sigma_1$	-45.625	-4.248	3.120	-0.224	-26.243	16.691	-0.444	-34.277
		$\sigma_2$	-103.320	-10.931	10.362	-0.415	-63.127	15.993	-0.220	-54.983
16	<b>OXAZ13</b>	$\sigma_1$	—	—	—	—	—	—	—	—
		$\sigma_2$	-104.800	-11.373	5.611	-0.774	-63.712	18.488	-1.227	-51.812
17	<sup>i</sup> <b>PD144418</b>	$\sigma_1$	-92.772	-52.669	4.382	-0.784	-52.904	56.883	-0.219	-47.460
18	<sup>j</sup> <b>Z1241145220</b>	$\sigma_2$	-92.982	-23.867	4.044	-1.290	-51.544	29.613	-0.276	-49.662

<sup>a</sup> Free energy of binding. <sup>b</sup> Coulomb energy. <sup>c</sup> Covalent energy (internal energy). <sup>d</sup> Hydrogen bonding correction. <sup>e</sup> Lipophilic energy. <sup>f</sup> Electrostatic solvation energy. <sup>g</sup> Pi-pi packing correction. <sup>h</sup> van der Waals energy. <sup>i</sup> PD144418 = 3-(4-methylphenyl)-5-(1-propyl-3,6-dihydro-2H-pyridin-5-yl)-1,2-oxazole. <sup>j</sup> Z1241145220 = 3-[1-(3-phenylpropyl)-1,2,3,6-tetrahydropyridin-4-yl]-1H-pyrrolo[2,3-*b*]pyridine.

2.34 Å) and a Pi-cation interaction with Tyr147 of  $\sigma_2$  R. Further, compound **OXAZ15**, **OXAZ16** and **OXAZ17** showed very poor binding score with the active site of  $\sigma_1$  R.

### 3.3 Prime MM/GBSA binding energy calculations

To further authenticate the binding affinity of docked  $\gamma$ -butyrolactone and oxazolidinone-based ligands at the active sites of the sigma 1 and sigma 2 receptors, binding free energy was calculated using the molecular mechanics generalised born surface area (MM-GBSA) approach available in the Prime module. Table 2 summarizes the binding energies of certain  $\gamma$ -butyrolactone and oxazolidinone-based ligands based on biological  $K_i$  values and the  $\sigma_2/\sigma_1$  ratio.

The estimated binding free energy of docked poses of molecules with  $\sigma_1$  receptor ranged from  $-2.018$  to  $-84.106$  kcal mol<sup>-1</sup>, whereas the BFG score for the  $\sigma_2$  receptor ranged from  $-84.578$  to  $-114.210$  kcal mol<sup>-1</sup>. The study shows that produced chemicals bind to the  $\sigma_2$  receptor at a higher rate

than those that bind to the  $\sigma_1$  receptor. The docking complexes for the chemicals **OXAZ3**, **OXAZ4**, **OXAZ6**, **OXAZ7**, and **OXAZ13** were not identified. So, their binding energies were assumed to be 0 kcal mol<sup>-1</sup>. The compound **OXAZ3** with the  $\sigma_2$  receptor has the greatest BFG score of around  $-114.210$  kcal mol<sup>-1</sup>. The van der Waal energy ( $G_{\text{vdW}} = -50.162$  kcal mol<sup>-1</sup>) and lipophilic energy ( $G_{\text{lipo}} = -65.374$  kcal mol<sup>-1</sup>) terms are the major contributors to **OXAZ3** binding activity in the active pocket of the  $\sigma_2$  receptor, while coulombic energy ( $G_{\text{cou}} = -12.314$  kcal mol<sup>-1</sup>) moderately favours ligand binding, according to this study. Furthermore, the ligand's binding activity inside the active site of the  $\sigma_2$  receptor is deemed unfavourable due to covalent energy and electrostatic solvation energy terms. Nonetheless, when compared to the  $\sigma_1$  receptor, the compounds had lower BFG, van der Waal energy, lipophilic energy, and coulombic energy. The results show that the produced compounds had a higher affinity for the  $\sigma_2$  receptor than the  $\sigma_1$  receptor.



## 4. Conclusion

In this work, essential components responsible for the binding process between the  $\sigma_2$  receptor and its ligands were identified utilizing molecular docking and MM/GBSA binding energy calculations. Molecular docking study provides a useful prediction of structural features for certain series of compounds belonging to  $\gamma$ -butyrolactone and oxazolidinones to bind at the active sites of  $\sigma_1$  and  $\sigma_2$  receptor. A 3D homology model for the  $\sigma_2$  receptor was built to perform the modeling studies. The Ramchandran plot examination was conducted to assess the correctness of modeled  $\sigma_2$  receptor. The putative binding site on the modeled  $\sigma_2$  receptor was identified using SiteMap analysis. In this present work, a total of 58 compounds have been used. The molecules were docked into both the active site of a homology-modeled  $\sigma_2$  receptor and the crystal structure of the  $\sigma_1$  receptor. The structural features required for ligand binding to  $\sigma_1$  and  $\sigma_2$  receptors were studied in 2D and 3D. With the  $\sigma_2$  receptor, the ligands showed higher BFG, van der Waal energy, lipophilic energy, and coulombic energy. The top-ranked molecules, according to the above study, had much more interactions and binding energy for the  $\sigma_2$  receptor than the  $\sigma_1$  receptor. Thus, the present study provides an understanding of the specific pharmacophoric features and interactions of ligands that are responsible for  $\sigma_2$  selectivity over  $\sigma_1$  receptor. Future research on these molecules might lead to the discovery of novel and potential selective  $\sigma_2$  ligands.

## Conflicts of interest

The authors declare no conflicts of interest.

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