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Volume 9(19)

# Sequence analysis, structure and binding site prediction of Sigma 1 receptor protein by *in silico* method

**Narayanasamy Lokeswaran Latha[1], Garimella Gyananath[1]* & Pudukulathan Kader Zubaidha[2]**

[1]School of Life Sciences, S. R. T. M. University, Nanded - 431606, India; [2]Chemical Sciences, S. R. T. M. University, Nanded-431606, India; Garimella Gyananath - Email: gyananath52@gmail.com; Fax: 91-2462-29245; *Corresponding author

Received October 25, 2013; Accepted November 03, 2013; Published December 06, 2013

**Abstract:**
Sigma 1 Receptor is a subtype of opioid receptor that participates in membrane remodeling and cellular differentiation in the nervous system. Sigma1 Receptor protein with amino acid length ranging from 229 is widely distributed in the liver and moderately in the intestine, kidney, white pulp of the spleen, adrenal gland, brain, placenta and the lung. In this study, the three dimensional structure for sigma 1 receptor protein has been developed by *in- silico* analysis based on evolutionary trace analysis of 37 sigma proteins from different sources. The present work focus on identification of functionally important residues and its interaction with antipsychotic drugs reported in literature.

**Key words:** Sigma receptor, Evolutionary trace, Binding sites.

**Background:**
Sigma 1 receptor is a subtype of opioid receptor that participates in membrane remodeling and cellular differentiation in the nervous system. Sigma receptors are subdivided in to 2 subtypes, sigma – 1 and sigma -2. The sigma -1 receptor is a 25K Da protein possessing one putative transmembrane domain and an endoplasmic reticulum retention signal. The cloned sigma-1 receptor reveals no homology to any known mammalian protein **[1].** The amino acid sequence of sigma I receptors exhibits more than 90% identity in different mammalian species including guinea pig, human, rat, and mouse **[2].** The cloned cDNA, when functionally expressed in mammalian cells, enhances the binding of sigma – 1 receptor ligands. Sigma-1 receptors contain 3 hydrophobic domains namely at the N- and the C termini, and at the center of the protein.

Evolutionary Trace (ET-method) is a method in which protein sequences of a particular protein family is partitioned in to different groups, which originate from the common node in the phylogenic tree and it also involves evolutionary time cut-off. Detailed analysis on the evolutionary conservation information extracted from multiple sequence alignment is an important tool used for prediction of functional properties as well as prediction of ligands binding sites; protein interface surfaces etc, and the detection of conserved residues would be useful in identifying the functionally important residue, even in the absence of structural information. According to the ET method each residue is reported as either conserved or class specific or variable based on the conservation properties **[3].** Parthiban *et al* has reported the use of evolutionary trace in identifying conserved and class specific residues close to the putative binding site in nocitinic acetylcholine receptors **[4].** LIU Yang Don *et al* **[5]** has reported the use of evolutionary trace analysis in identifying 11 trace residues in superoxide dismutase of extremophile Thermoplasma acidophilum, of which three residues (Asn39, Gly105 and Glu162) were scattered over the structure and the rest of the residues were identified near the Fe binding site.

 

In this work, we made an attempt to explore the information about functionally important residues of sigma -1 receptor through evolutionary conservation method and to predict the three dimensional structure of Sigma 1 protein and its interaction with antipsychotic drugs.

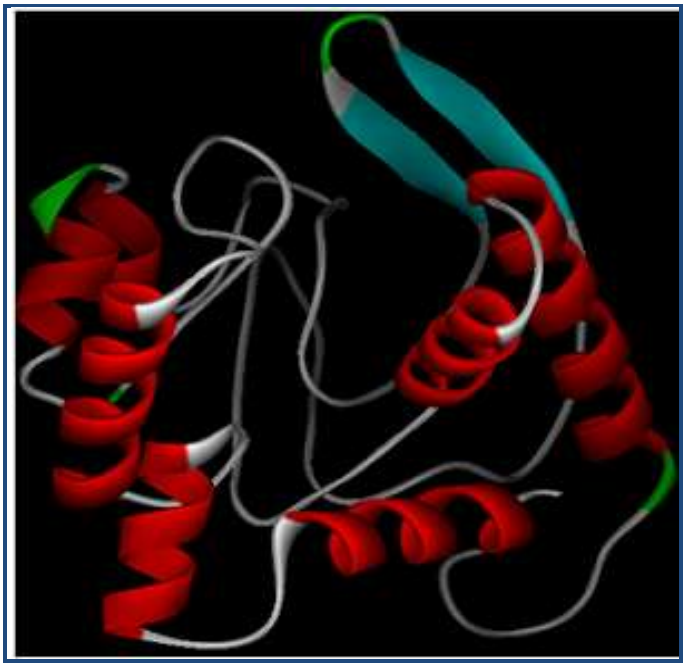

**Figure 1:** Predicted three dimensional structure of sigma 1 protein.

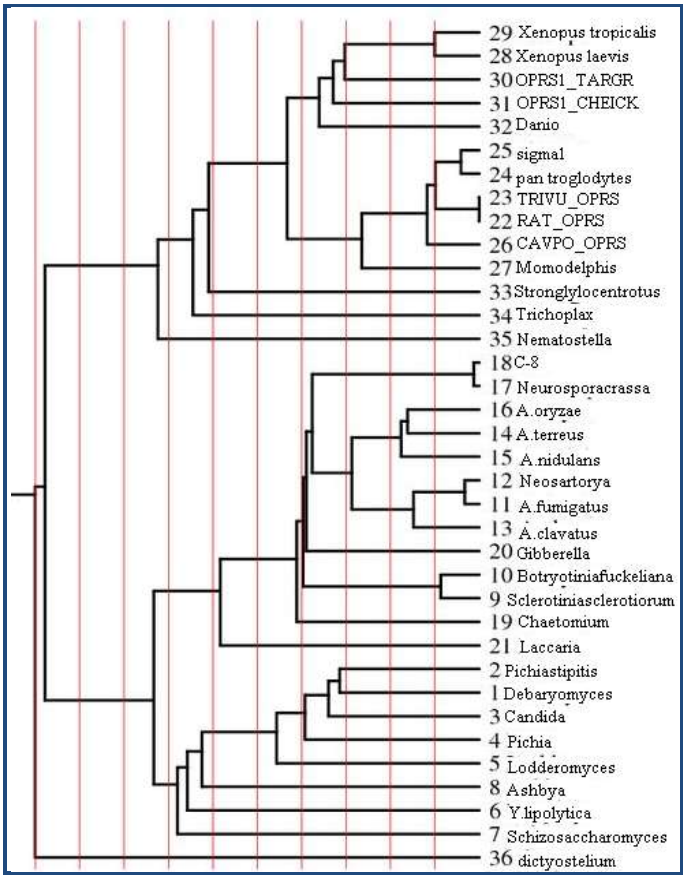


**Figure 2:** Vertical lines in Dendrogram A to J show different partition identity cutoffs (PICs) each PIC represents an individual group. A represents the most conserved 10th trace. As PIC increase A to J partition comprises decrease group from 10 to 1.

**Methodology:**

***Sequence analysis and ab initio structure prediction of Sigma 1 protein***

The Sigma 1 protein sequence of *Homo sapiens* was retrieved from NCBI (NC_). Domain and pattern were analysed using PFAM **[6]** and PROSITE **[7]** database respectively. The secondary structure of the protein was predicted using different JPRED **[8],** SOPM **[9]**, SOPMA **[10]** and GOR4 **[11]** servers. The three dimensional structure of sigma 1 protein was predicted using I-TASSER server (http: //zhanglab.ccmb.med.umich.edu /I-TASSER/) **[12].** 3D models are built based on multiple-threading alignments by LOMETS and iterative TASSER assembly simulations; function insights are then derived by matching the predicted models with protein function databases.

***Evolutionary trace analysis***

A total of 74 non-redundant protein sequences of sigma-1 receptor from various organisms were retrieved from Swiss prot database. Of the 74 sequences, only 36 sequences have been selected for multiple sequence alignment and showed in **Table 2 (see supplementary material).** The selection criteria were based on the (a) sequences having functionally similar domains, (b) sequences with >25% sequence identity, (c) sequences with full length sigma 1 proteins were selected. We performed multiple sequence alignment for 36 Sigma 1 receptor sequences using CLUSTALW. ET analysis for Sigma 1 Receptor sequences were carried out using ET server **[13].**

**Results & Discussion:**

The 223 amino acids long sigma receptor protein of *Homosapiens* has an ERG2_Sigma1R. No pattern/signature could be identified from prosite database. No homologous protein with solved structure could be identified from PDB database using BLAST tool. Similarly, threading servers such as PHYRE **[14]** and 3DPSSM **[15]** also didn't identify any significant templates that could be used for developing three dimensional models for sigma 1 receptor protein.

Hence, *ab initio* method was opted for predicting the structure of sigma 1 receptor protein. The protein sequence was submitted to I-TASSER server and five models were predicted. Of the five models, the best model was selected based on TM-score. The C-score and TM-score are -4.12 and 0.28±0.09 respectively. The predicted model was identified to have seven helices and four strands **(Figure 1).** Based on statistics, if a template/model has a TM-score around or below 0.17, it means the prediction is nothing more than a random selection from PDB library. Hence the predicted model is taken as a significant model.

Based on the evolutionary trace analysis, the phylogenetic tree **(Figure 2)** was split into 10 evenly distributed partitions, namely P01-P10 in order of evolutionary time cut-off (ETC). Analysis of the mapped traces for partitions P01-P10 revealed clusters of conserved residues occurring on the surface of the protein. Eleven residues were identified to be well conserved in the selected 37 sequences and predicted to be functionally important residues **(Figure 3).**

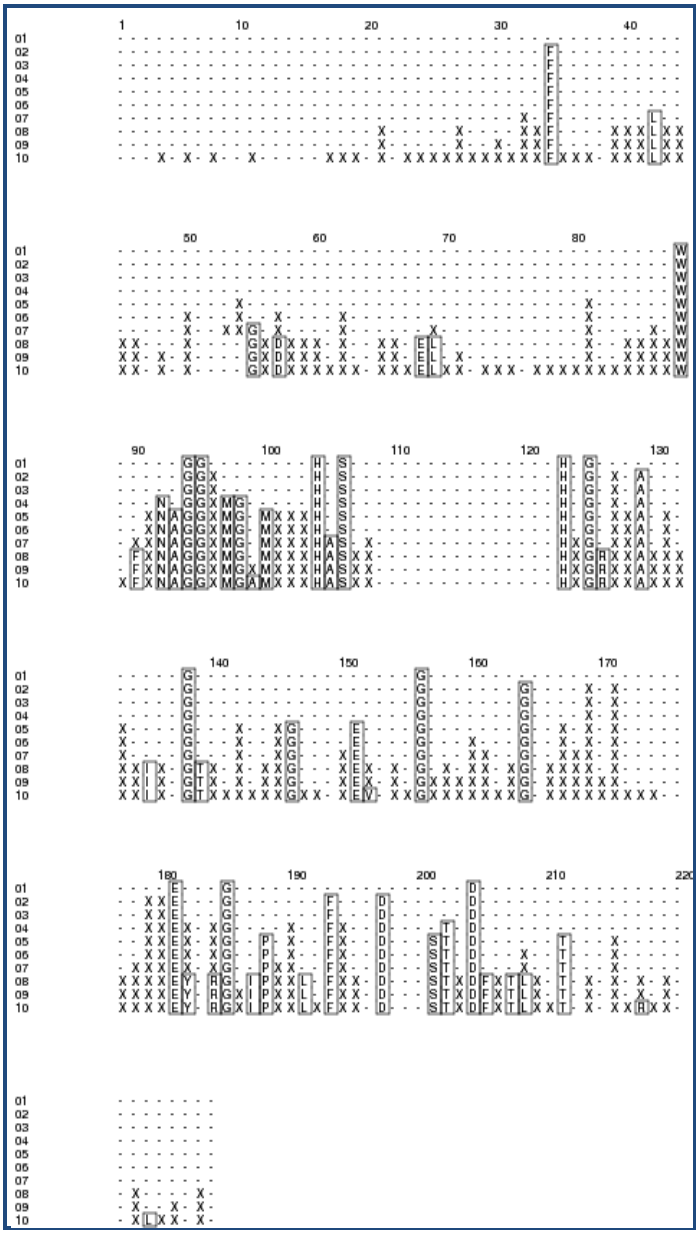

**Figure 3:** Evolutionary Trace shows conserved consensus pattern.

***Active site prediction of Sigma protein and docking analysis***

The predicted sigma protein model was submitted to Q-Site server for prediction of the active site. The first predicted active site of the ten different predictions was selected based on the volume of the site. We then docked the antipsychotic compound (tBOC) into the active site of our target to understand the binding affinity of the compound. Based on the docking results, tBOC binds into the cavity of sigma 1 protein with GOLD score 40.04. The binding of tBOC to sigma 1 protein will be validated using experimental methods. We further docked our compound (tBOC) along with 23 available drug molecules available in PDB database in complex to protein tyrosine phosphatase 1B of *Homo sapiens* which is an important target for cancer, diabetic and obesity to understand the affinity level of our compound to that of the available molecules. Based on our results **Table 1 (see supplementary material)**, tBOC was ranked sixth among the 23 drug molecules docked to protein tyrosine phosphatase 1B. This result predicts that tBOC has binding affinity with the protein tyrosine phosphatase 1B and can be probably an alternative drug for obesity, diabetics and cancer which has to be further confirmed through wet lab experiments. Further, docking studies were carried out for all these above mentioned 24 drug molecules to sigma 1 protein of human using GOLD software. Based on our results, 2FJ was identified to have a better affinity to sigma 1 protein (GOLD score: 76.0) when compared to tBOC (40.04) **(Figure 4).**

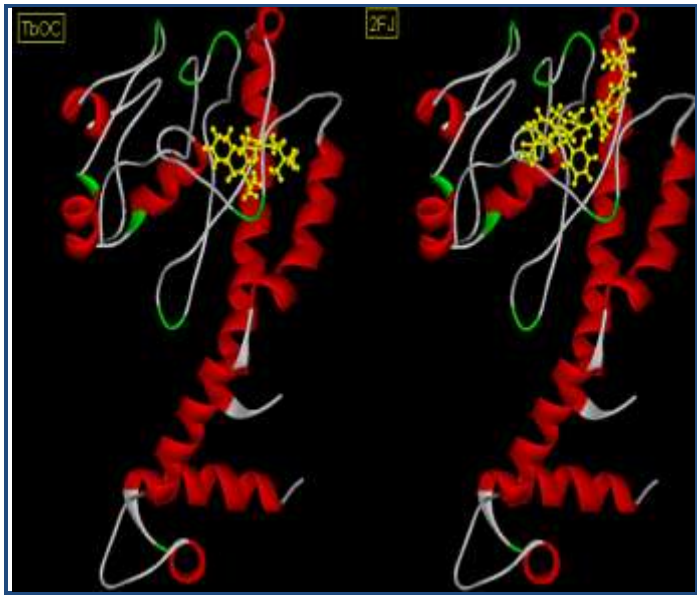


**Figure 4:** Docked conformation of tBOC and 2FG in the active sites of sigma 1 protein of *Homo sapiens.*

**Conclusion:**

Sigma 1 Receptor protein is a potential target for studying basic mechanisms of behavioral studies were shown to be involved in higher ordered brain functions including memory and drug dependence. Since no experimental structures are available for sigma1 receptor protein, our study was first to predict the three dimensional structure of sigma 1 protein. Further we compared the binding of our compound TbOC to sigma 1 protein which proves a potential antipsychotic drug. The predicted functionally important residues and the 3D structure will be helpful for understanding the function of sigma1 receptor protein and for structure based drug designing studies.

**References:**

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**[3]** Nagarajan S & Marimuthu P, *Bioinformation*. 2006 **1**: 180 [PMID: 17597884]
**[4]** Parthiban M *et al. J Mol Model*. 2010 **16**: 1651 [PMID: 20204665]
**[5]** Liu Yuan Dong *et al. Progress in Modern Biomedicine*. 2008 **8:** 6
**[6]** Finn RD *et al. Nucleic Acids Res*. 2010 **38**: D211 [PMID: 19920124]
**[7]** Hulo N *et al. Nucleic Acids Res*. 2006 **34**: D227 [PMID: 16381852]
**[8]** Cuff JA *et al. Bioinformatics*. 1998 **14**: 892 [PMID: 9927721]
**[9]** Geourjon C & Deléage G, *Protein Eng*. 1994 **7**: 157 [ PMID: 8170919]

**[10]** Geourjon C & Deléage G, *Comput Appl Biosci*. 1995 **6**: 681 [PMID: 8808585]
**[11]** Garnier J *et al. Methods Enzymol*. 1996 **266**: 540 [PMID: 8743705]
**[12]** Yang Y, *BMC Bioinformatics*. 2008 **9**: 40 [PMID: 18215316]
**[13]** Innis CA *et al. Protein Eng*. 2000 **13**: 839 [PMID: 11239083]
**[14]** Kelley LA & Sternberg MJE, *Nature Protocols*. 2009 **4**: 363 [PMID: 19247286]
**[15]** Kelley LA *et al. J Mol Biol*. 2000 **299**: 499 [PMID: 10860755]

**Edited by P Kangueane**
**Citation**: **Latha *et al.*** Bioinformation 9(19): 944-951 (2013)

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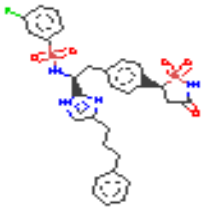
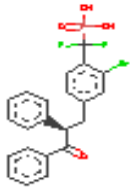
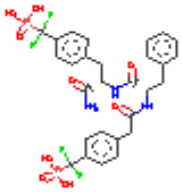
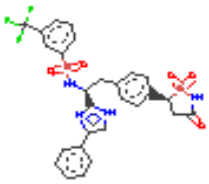
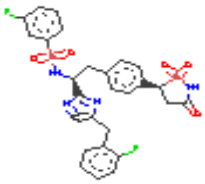
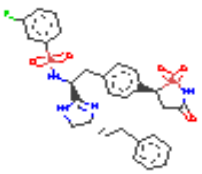
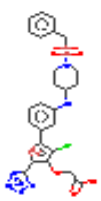
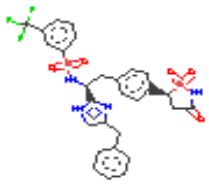
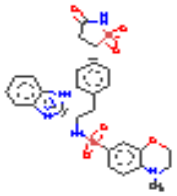
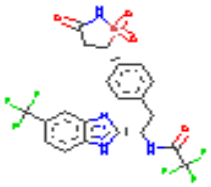
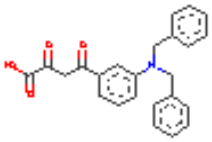
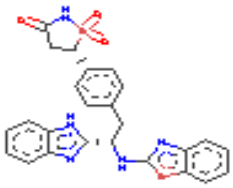
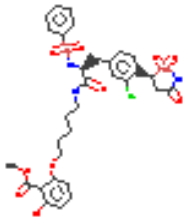
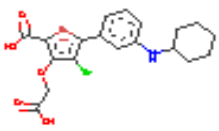
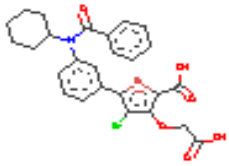
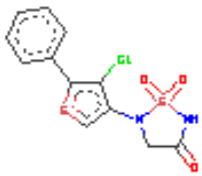
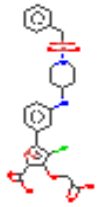
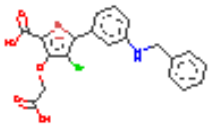
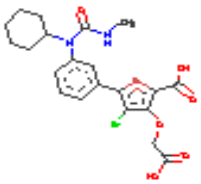
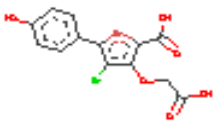
## Supplementary material:

**Table 1:** List of drug molecules bound with tyrosine phosphatase 1B and their GOLD score.

| S.No | PDB Code | Drug | Structure | Molecule Name | Formula | Gold Score |
|---|---|---|---|---|---|---|
| 1 | 2VEY | IZ5 |  | N-{(1S)-2-{4-[(5S)-1,1-dioxido-3-oxoisothiazolidin- 5-yl]phenyl}-1-[4-(3 -phenylpropyl)-1H-imidazol- 2 yl]ethyl}-3-fluorobenzenesulfonamide | C29 H29 F N4 O5 S2 | 69.07 |
| 2 | 3CWE | 825 |  | [{2-bromo-4-[(2R)-3-oxo-2,3-diphenylpropyl]phenyl} (difluoro)methyl]phosphonic acid | C22 H18 Br F2 O4 P | 68.99 |
| 3 | 2CNE | DFJ |  | N-({4-[DIFLUORO(PHOSPHONO)METHYL]PHENYL}ACETYL) - L-PHENYLALANYL-4-[DIFLUORO (PHOSPHONO)METHYL]-L-PHENYLALANINAMIDE | C28 H29 F4 N3 O9 P2 | 68.95 |
| 4 | 2VEU | IZ1 |  | N-[(1S)-2-{4-[(5S)-1,1-dioxido-3-oxoisothiazolidin- 5-yl]phenyl}-1-(4-phenyl-1H -imidazol-2-yl)ethyl]- 3-(trifluoromethyl) benzenesulfonamide | C27 H23 F3 N4 O5 S2 | 65.02 |
| 5 | 2VEW | IZ3 |  | 3-fluoro-N-[(1S)-1-[4-[(2-fluorophenyl)methyl]imidazol- 2-yl]-2-[4-[(5S)-1,1,3 -trioxo-1,2-thiazolidin- 5-yl]phenyl]ethyl] benzenesulfonamide | C27 H22 F2 N4 O5 S2 | 64.34 |
| 6 | 2VEX | IZ4 |  | N-{(1S)-2-{4-[(5S)-1,1-dioxido-3-oxoisothiazolidin- 5-yl]phenyl}-1-[(4R)-4-(2 -phenylethyl)-4,5- dihydro-1H-imidazol-2-yl]ethyl}-3-fluorobenzenesulfonamide | C28 H29 F N4 O5 S2 | 60.63 |
| 7 | 2NT7 | 902 |  | {[5-(3-{[1-(BENZYLSULFONYL)PIPERIDIN-4-YL]AMINO}PHENYL)-4-BROMO-2-(2H-TETRAZOL -5-YL)-3-THIENYL]OXY}ACETIC ACID | C25 H25 Br N6 O5 S2 | 59.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 2VEV | IZ2 |  | N-[(1S)-1-(4-benzyl-1H-imidazol-2-yl)-2-{4- [(5S)-1,1-dioxido-3 -oxoisothiazolidin-5-yl]phenyl}ethyl]- 3-(trifluoromethyl)benzenesulfonamide | C28 H25 F3 N4 O5 S2 | 59.34 |
| 9 | 2CNH | IZB |  | N-[(1S)-1-(1H-BENZIMIDAZOL-2-YL)-2-{4-[(5S)- 1,1-DIOXIDO-3-OXOISOTHIAZOLIDIN-5 -YL]PHENYL}ETHYL]- 4-METHYL-3,4-DIHYDRO-2H-1,4-BENZOXAZINE-7-SULFONAMIDE | C27 H27 N5 O6 S2 | 59.02 |
| 10 | 2CNG | IZE |  | N-{(1S)-2-{4-[(5R)-1,1-DIOXIDO-3-OXOISOTHIAZOLIDIN-5-YL]PHENYL}-1-[5-(TRIFLUOROMETHYL)-1H-BENZIMIDAZOL- 2-YL]ETHYL}-2,2,2-TRIFLUOROACETAMIDE | C21 H16 F6 N4 O4 S | 58.37 |
| 11 | 3EB1 | LZQ |  | 4-[3-(dibenzylamino)phenyl]-2,4-dioxobutanoic acid | C24 H21 N O4 | 56.84 |
| 12 | 2CNF | F32 |  | (5S)-5-{4-[(2S)-2-(1H-BENZIMIDAZOL-2-YL)- 2-(1,3-BENZOTHIAZOL-2 -YLAMINO)ETHYL]PHENYL}ISOTHIAZOLIDN-3-ONE1,1-DIOXDE | C25 H21 N5 O3 S2 | 56.66 |
| 13 | 2CNI | IZF |  | METHYL 2-{[5-({3-CHLORO-4-[(5S)-1,1-DIOXIDO- 3-OXOISOTHIAZOLIDIN-5-YL]-N-(PHENYLSULFONYL)- L-PHENYLALANYL}AMINO)PENTYL]OXY}-6-HYDROXYBENZOATE | C31 H34 Cl N3 O10 S2 | 55.04 |
| 14 | 2QBS | 24 |  | 4-BROMO-3-(CARBOXYMETHOXY)-5-[3-(CYCLOHEXYLAMINO) PHENYL]THIOPHENE- 2 -CARBOXYLIC ACID | C19 H20 Br N O5 S | 48.79 |

| | | | | | |
|---|---|---|---|---|---|
| 15 | 2ZN7 | 410 |  | 4-bromo-3-(carboxymethoxy)-5-{3-[cyclohexyl (phenylcarbonyl) amino]phenyl}thiophene- 2-carboxylic acid | C26 H24 Br N O6 S | 48.45 |
| 16 | 2NTA | 521 |  | 5-(4-CHLORO-5-PHENYL-3-THIENYL)-1,2,5-THIADIAZOLIDIN- 3-ONE 1,1-DIOXIDE | C12 H9 Cl N2 O3 S2 | 47.22 |
| 17 | 2QBP | 527 |  | 5-(3-{[1-(BENZYLSULFONYL)PIPERIDIN-4-YL]AMINO}PHENYL)- 4-BROMO-3-(CARBOXYMETHOXY)THIOPHENE-2-CARBOXYLIC ACID | C25 H25 Br N2 O7 S2 | 47.21 |
| 18 | 2QBR | 910 |  | 5-[3-(BENZYLAMINO)PHENYL]-4-BROMO-3-(CARBOXYMETHOXY) THIOPHENE- 2-CARBOXYLIC ACID | C20 H16 Br N O5 S | 46.17 |
| 19 | 2ZMM | 35B |  | 4-bromo-3-(carboxymethoxy)-5-{3-[cyclohexyl (methylcarbamoyl)amino]phenyl}thiophene- 2-carboxylic acid | C21 H23 Br N2 O6 S | 46.01 |
| 20 | 2H4G | 694 |  | 4-BROMO-3-(CARBOXYMETHOXY)-5-(4-HYDROXYPHENYL) THIOPHENE- 2-CARBOXYLIC ACID | C13 H9 Br O6 S | 37.48 |
| 21 | tBoc | | | Tertiary Butyl Oxycarbony | | 37.42 |

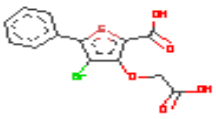
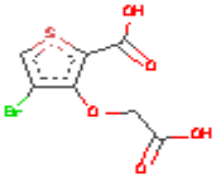
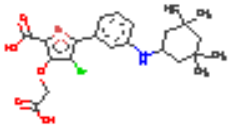
| | | | | | |
|---|---|---|---|---|---|
| 22 | 2H4K | 509 |  | 4-BROMO-3-(CARBOXYMETHOXY)-5-PHENYLTHIOPHENE-2-CARBOXYLIC ACID | C13 H9 Br O5 S | 34.27 |
| 23 | 2HPI | 512 |  | 4-BROMO-3-(CARBOXYMETHOXY)THIOPHENE-2-CARBOXYLIC ACID | C7 H5 Br O5 S | 28.73 |
| 24 | 2QBQ | 4B3 |  | 4-BROMO-3-(CARBOXYMETHOXY)-5-{3-[(3,3,5,5 – TETRAMETHYLCYCLOHEXYL)AMINO]PHENYL}THIOPHENE-2-CARBOXYLIC ACID | C23 H28 Br N O5 S | 20.25 |

**Table 2:** List of selected Sigma 1 receptor sequences with its database accession number and e value which are involved in multiple sequence alignment

| Original S.NO | PDB code NO | Organism | Accession Number | Domain | E value |
|---|---|---|---|---|---|
| 1 | 81882051 | OPRS 1_ RAT | SP/Q9ROC9.1 | ERG2-Σ1r | 1.30E-137 |
| 2 | 75040821 | OPRS 1 _TRIVU | SP/Q5PXE2.1 | ERG2-Σ1r | 1.30E-137 |
| 4 | 81862796 | OPRS 1CAVPO | SP/Q60492.1 | ERG2-Σ1r | 1.20E-125 |
| 6 | 114624248 | pan troglodytes | XP-001163744.1 | ERG2-Σ1r | 2.10E-111 |
| 10 | 126335022 | Momodelphis domistica | XP-001378743.1 | ERG2-Σ1r | 9.00E-114 |
| 17 | 148235761 | Xenopuslaevis | NP-001087013.1 | ERG2-Σ1r | 2.40E-94 |
| 18 | 82083438 | OPRS1_TARGR Rec | SP/Q645J3.1 | ERG2-Σ1r | 4.00E-101 |
| 19 | 56118624 | Xenopus tropicalis | NP-001008207.1 | ERG2-Σ1r | 6.80E-95 |
| 20 | 41055792 | Danio rerio | NP-957271.1 | ERG2-Σ1r | 2.00E-98 |
| 21 | 82082452 | OPRS1 CHEICK | SPQ5ZL84.1 | ERG2-Σ1r | 2.80E-94 |
| 23 | 72015009 | Stronglylocentrotus purpuratus | XP-7838051 | ERG2-Σ1r | 3.10E-91 |
| 24 | 190583472 | Trichoplax adhaerens | gb/EDV23543.1 | ERG2-Σ1r | 8.70E-79 |
| 25 | 156398915 | Nematostella vectensis | XP-001638433.1 | ERG2-Σ1r | 3.10E-64 |
| 29 | 70990744 | Aspergillus fumigatus Af293 | XP-750221.1 | ERG2-Σ1r | 1.80E-131 |
| 30 | 119496837 | Neosartorya fischeri NRRL 181 | XP-001265192.1 | ERG2-Σ1r | 3.10E-131 |
| 31 | 121702847 | Aspergillus clavatus NRRL 1 | XP-001269688.1 | ERG2-Σ1r | 1.10E-132 |
| 33 | 66826757 | dictyostelium discoideum AX4 | XP-646733.1 | ERG2-Σ1r | 1.00E-33 |
| 34 | 169771107 | Aspergillusoryzae RIB40 | XP-001820023.1 | ERG2-Σ1r | 3.30E-132 |
| 36 | 115390859 | Aspergillusterreus NIH2624 | XP-001212934.1 | ERG2-Σ1r | 3.70E-130 |
| 38 | 46125875 | Gibberella zeae PH-1 | XP-387491.1 | ERG2-Σ1r | 5.50E-134 |
| 40 | 67516339 | Aspergillus nidulans FGSC a4 | XP658055.1 | ERG2-Σ1r | 3.70E-133 |
| 42 | 156053141 | Sclerotiniasclerotiorum 1980 | XP-001592497.1 | ERG2-Σ1r | 1.50E-116 |
| 44 | 149239506 | Lodderomyces elongisporus NRRL YB-4239 | XP-001525629.1 | ERG2-Σ1r | 2.20E-101 |
| 45 | 50420709 | Debaryomyces hansenii CBS767 | XP-458891.1 | ERG2-Σ1r | 3.40E-110 |
| 46 | 164426517 | Neurosporacrassa OR74A | XP-961312.2 | ERG2-Σ1r | 9.30E-158 |
| 47 | 1575320 | C-8 sterol isomerase | GB/AABO9470.1 | ERG2-Σ1r | 3.70E-156 |
| 49 | 154318271 | Botryotiniafuckeliana B05.10 | XP-001558454.1 | ERG2-Σ1r | 4.10E-118 |
| 50 | 170106151 | Laccaria bicolor S238N-H82 | XP-001884287.1 | ERG2-Σ1r | 2.40E-108 |
| 53 | 146413541 | Pichia guilliermondii ATCC 6260 | XP-001482741.1 | ERG2-Σ1r | 1.00E-113 |
| 54 | 68473770 | Candida albicans SC5314 | XP-718988.1 | ERG2-Σ1r | 2.10E-106 |
| 56 | 126137844 | Pichiastipitis CBS 6054 | XP-001385445.1 | ERG2-Σ1r | 2.10E-112 |
| 58 | 116197971 | Chaetomium globosum CBS 148.51 | XP-001224797.1 | ERG2-Σ1r | 3.30E-127 |
| 61 | 50547073 | Yarrowia lipolytica | XP-504668.1 | ERG2-Σ1r | 4.50E-87 |
| 62 | 50554519 | Yarrowialipolytica | XP-501006.1 | ERG2-Σ1r | 2.10E-91 |
| 65 | 19114236 | Schizosaccharomyces pombe 972h-1 | NP-593324.1 | ERG2-Σ1r | 1.20E-153 |
| 70 | 45198340 | Ashbya gossypii ATCC 10895 | NP-985369.1 | ERG2-Σ1r | 9.20E-126 |
| 74 | 5032117 | Sigma 1 | NP-005857 | ERG2-Σ1r | 6.50E-130 |