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Special Issue: Computational drug designing and molecular docking analyses

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In silico elucidation of potent drug targets of the melanocyte-stimulating hormone receptor 1 protein

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Abstract

Most of the genes, including melanocortin 1 receptor (MC1R) involved in melanogenesis. The black to brown eumelanin and the yellow to red phaeomelanin are the two kinds of pigment recognized in human skin. The primary cause of the dermal reaction to ultraviolet radiation (UVR) is cutaneous pigmentation, subsequently of the risk of developing skin cancer. Melanin pigmentation protects the skin against the harmful effects of ultraviolet radiation. The death rate of melanoma depends upon the type of skin cancer. Different computational approaches were utilized for 3D modeling and protein-protein docking analyses of MC1R leads to virtual screening. Comparative modeling and threading techniques were employed to predict the 3D structure of MC1R and 95.79% of quality factor was calculated. STRING database was utilized and 1Y7K was observed as interacting partner of MC1R. Protein-protein docking was performed, and potential interacting residues were observed. Virtual screening was performed against FDA library from ZINC database and molecular docking was performed by AutoDock Vina. The compound ZINC131 showed least binding energy of -10.3 kcal/mol. The suggested molecule may be used for further analyses in the drug discovery processes. In conclusion, the findings of current work may be helpful for novel therapeutic targets against MC1R.

Introduction

The melanocortin receptor system has five receptors named as MC1R, MC2R, MC3R, MC4R, MC5R related to G-proteins and involved in the biological functions for multicellular animals and human beings [1]. Melanocyte-stimulating hormone (MSH) influenced through melanocyte receptors from melanoma. Such melanoma cells encode melanocyte-stimulating hormone receptor (MSHR), and coding DNA emulation also isolated from melanoma cells. The protein of 317 amino acids with transmembrane structural features of G-protein coupled receptors (GPCR) encoded from cloned coding DNA [2]. The melanin pigmentation can protect the skin from damaging possessions of ultraviolet radiation (UVR). In human skin, there are mainly two types of melanin, as red phaeomelanin and black eumelanin. Eumelanin has the potential to make free radicals against UVR. Hence, eumelanin is photo protective. The living beings with red hair having a prevalence of phaeomelanin in skin and hair and may abridged the aptitude to produce eumelanin. In mammals, MSH regulates a relative amount of phaeomelanin and eumelanin. MSH receptors increase the production of eumelanin [3, 4].

UVR may cause severe and prolonged effects on the skin of human beings. The result of ultraviolet (UV) radiation persuaded oxidative stress and defense through paracrine factors on the skin of living beings. The adaption in melanocytes due to heat stress influenced was reliant on melanin level and apoptosis reduction. The survival mechanism adopted by the role of δ-MSH in cell survival and melanocytes under ultraviolet oxidative stress [5]. About 65% of all melanomas are preventive measures from the protection of the sun. UVR is responsible for the appearance of all melanoma [6]. The primary gene present in humans is Melanocyte-Stimulating Hormone Receptor 1 (MC1R), where polymorphic coding regions show the foremost phenotypic variant factors in pigmentation. Though, distinct MC1R alleles influence the color of skin and hair [7]. MC1R member of GPCR may articulate in melanoma cells and MSH receptor. The extent and nature of major phenotypic variation factors may control by MSH receptor [8]. The phenotypic changes occur due to genes, mainly during clear Mendelian inheritance and the presence of no transitional phenotypes [9]. Some species including mouse, drosophila [10] and color carp [11] show pigmentation with the protagonist of mRNA or specific genes.

Furthermore, this gene plays an essential role in pigmentation leads to decisively phenotype and phenotype of beings affect due to gene interfaces [9], [10], [11], [12]. Due to pigmentation of MC1R, its variations may cause skin cancer [13]. MC1R has 80 variants. Out of these variants, some variants have partial loss of receptor signaling ability and enabled to produce cyclic adenosine monophosphate (cAMP) as the wild type receptor give active response to alpha-MSH stimulation. The red color phenotype belongs to the assessable change of melanin synthesis from eumelanin to phaeomelanin [14], [15]. The wide distribution of receptor is involved in several biological functions including pigmentation, antipyretic and anti-inflammatory actions [16].

The other variations in MC1R may increase the risk of evolving melanoma in the deficiency of UVR related to skin damage. Cancers are associated with mutations in the genes relate to melanoma risk including BRAF and CDKN2A. The activity of the receptor is mediated by GPCR which activate adenylate cyclase.

The primary purpose of this work was computational analyses, 3D structural prediction of MSHR, Protein-protein interaction studies and molecular socking analyses [17, 18]. For the completion of these objectives, sequence analysis, comparative modeling approach, comparative molecular docking approaches were utilized for analyses [19]. The result of trailed approaches confirmed that these strategies were accomplished of identifying the correspondent effective molecule.

Materials and Methods

The MC1R has the accession number Q01726 in Uniprot Knowledge Base. In present work, computational approaches including sequence analyses, 3D structure prediction, comparative protein-protein docking analyses and molecular docking analyses were performed.

ProtParam [20] and ProtScale [21] were used for the compositional properties analyses of the amino acid sequences.

The amino acid sequence of MC1R isoform was retrieved from Uniprot KB (https://www.uniprot.org/uniprot/Q01726) [22], and subjected to BLASTp against Protein Data Bank (PDB) [23] for the selection of suitable template identification. Homology modeling was done for 3D structure prediction of MC1R employed by using the automated protein modeling program MODELLER 9.20 [24]. Numerous other tools including SWISS MODEL [25], I-TASSER [26], PSIPred [27], robetta

[28], Raptor-X [29], M4T [30], Phyre2 [31], SPARKS-X [32], IntFOLD [33], esyPred3D [34], HHpred [35], MOD-WEB [36], 3D-JigSaw [37] and CPHmodels [38] were utilized for 3D structure prediction. UCSF Chimera 1.13 [39] and PyMol [40] visualizing tools were used for the visualization of protein 3D structures. UCSF Chimera 1.13 was used for the minimization of predicted protein structure. For structure evaluation, MolProbity [41] online server was used. For the determination, the accuracy of protein sequence residues and protein structure quality, some other evaluation tools were used including Rampage [42], [43], Anolea [44], Errat [45], ProCheck [46] and Verify 3D [47]. Errat [48] evaluation tool was used for the structure prediction of the quality factor and Anolea was used to validate the Z-score value of the predicted structures.

STITCH [49] (Search Tool for InTeracting CHemical) [50] and STRING (Search Tool for the Retrieval of Interacting Genes/Protein) [51] database were used for the analyses of the functional interacting partner of MC1R [52].

Comparative molecular docking analyses were performed AutoDock Vina for library screening of FDA library of ZINC database for blind docking analyses. Through comparative molecular docking, the least energy of zinc compound was observed. The molecular structure of the protein-ligand docking was visualized by UCSF Chimera 1.13.

Results and Discussion

Skin cancer is termed as Melanoma that reveals tumor heterogeneity features [53] and develops different research approaches as immunotherapy [53] [54] for the treatment and detection of skin cancer and melanoma risk [54]. The study of structural bioinformatics and oncology is the field of exploring information and efficiently providing research to understand better about melanoma. Research is providing better and possible resources for the treatment of skin cancer. Not only just reduction of melanoma risk or predictions of skin cancerous proteins are beneficial from the structural bioinformatics. The mechanism of MSHR protein role by using computational methods in neurological diseases and disorders [55] [56]. Vaccines also used for targeting melanoma tumor cells, as a form of active immunotherapy [54, 57].

Sequence Analyses

Many observations from the literature and examination of the biological database revealed information about the sequence MC1R. The UCSC genome browser was employed to examine the exact location of MC1R in human (**Fig. 1**).

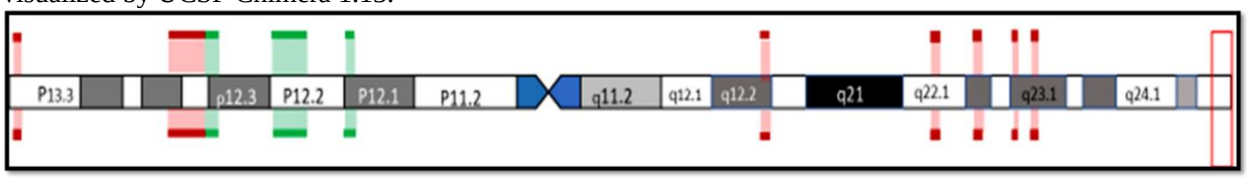

Fig. 1: Location of MC1R on chromosome 16.

UCSC genome browser BLAT, Ensemble [58] and NCBI BLAST tools were used for the alignment of MC1R against the mouse and rat through Clustal Omega (**Fig. 2**).

COILS and ProtParam were employed to analyze the protein composition and ProtScale tools were used to calculate the composition of amino acids. It was observed that the molecular weight of the protein was 34705.51 based on the average isotope masses of amino acids [59] and the common isotope masses of amino acid and the common isotope mass of one water molecule. Theoretical pI which depends upon the side chain plays an important role in determining the pH of the protein. The half-life of the protein was observed 30 hours in vitro. The number of atoms having negative and positive charges along with the total number of atoms and aliphatic index were also calculated (**Fig. 3**).

The neural network-based protein disorder was predicted in MC1R region and Pondr tool [60] was used to identify the natural disordered regions (**Fig. 4**).

Structure prediction

The 3D structure prediction of MC1R by using comparative modeling and threading approaches [61]. The NMR and X-ray crystallographic structure of MC1R was not reported. The sequence was submitted to BLASTp to retrieve the suitable

```
sp|Q9NPB9|ACKR4_HUMAN       MALEQNQSTDYYYEENEMNGTYDYSQYELIC----IKEDVREFAKVFLPVFLTIVFVIG
                                                                                55
sp|Q60614|AA2BR_MOUSE       ----------------------MQLETQDALYVALELVIAALAVAGNVLV-----CAAVG
                                                                                33
sp|Q9QZN9|CNR2_RAT          MA--GC--RE-LELTNGSNGGLEFNPMKEYM-----ILSDAQQIAVAVL-----CTLMG
                                                                                44
                                                                                 :*
sp|Q9NPB9|ACKR4_HUMAN       LA----GNSMVVAIYAYYKKQRTKTDVYILNLAVADLLL--LFTLPFWA-----VNAVH
                                                                               103
sp|Q60614|AA2BR_MOUSE       ASSAL----------------QTPTNYFLVSLATADVAVGLFAIPFAITISLGFCTDFH
                                                                                76
sp|Q9QZN9|CNR2_RAT          LLSALENVAVLYLILSSQRLRRKPSYLFIGSLAGADFLASVIFACNFV-----IFHVFH
                                                                                98
                                                                                 .*
sp|Q9NPB9|ACKR4_HUMAN       GWVLGKIMCKITSALYTLNFVSGMQFLACISIDRYVAVTKVPSQSGVG-KPCWIICFCV
                                                                               161
sp|Q60614|AA2BR_MOUSE       GCLFLACFV-L-----VLTQSSIFSLLAVAVDRYLAIRVPLRYKGLVTGTRARGIIAVL
                                                                               129
sp|Q9QZN9|CNR2_RAT          GVDSRNIFLLKIGSVTMTFTASVGSLLLTAVDRYLCLCYPPTYKALVTRGRALVALGVM
                                                                               157
                                                                                 :
sp|Q9NPB9|ACKR4_HUMAN       WMAAILLSIPQLVFYTVNDNA-------------RCIPI---FPRYLGTSMKALIQM
                                                                               202
sp|Q60614|AA2BR_MOUSE       WVLAFGIGLTPFLGWNSKDSATSNCTELGDGIANKSCCPVTCLF-ENVVPMSYMVYF-N
                                                                               186
sp|Q9QZN9|CNR2_RAT          WVLSALISYLPLMGWTC--------------------CPSPCSELFPLIPNDYLLGW-L
                                                                               195
                                                                                 :
sp|Q9NPB9|ACKR4_HUMAN       LEICIGFVVPFLIMGVCY---------FITARTLMKMPNIK------ISR--PLKVLLT
                                                                               244
sp|Q60614|AA2BR_MOUSE       FFGCV--LPPLLIMLVIYIKIFMVACKQLQRMELMDH-----SRTTLQREIHAAKSLAM
                                                                               238
sp|Q9QZN9|CNR2_RAT          LFIAI--LFSG---IIYTYGYVLWKAHQHVASLAEHQDRQVPGIARMRLDVRLAKTLGL
                                                                               249
                                                                                 * *
sp|Q9NPB9|ACKR4_HUMAN       VVIVFIVTQLPYNIVKFCRAIDIIYSLITSCNMSKRMDIAIQVTESIALFHSCLNPILYV
                                                                               304
sp|Q60614|AA2BR_MOUSE       IVGIFALCWLPVHAIN------CITLFHPALAKDKPKWVMNVAILLSHANSVVNPIVYA
                                                                               291
sp|Q9QZN9|CNR2_RAT          VMAVLLICWFPALALM-------GHSLVTTLSD-KVKEAFAFCSMLCLVNSMINPIIYA
                                                                               300
                                                                                 :*:***:*.
sp|Q9NPB9|ACKR4_HUMAN       FMGASFKNYVMKVAKKYGSWRRQRQSVEEFPFDSEGPTEPT-----------------
                                                                               345
sp|Q60614|AA2BR_MOUSE       YRNRDFRYSFHKIISRYVLCQAETKG----GSGQAGAQSTLSLGL--------------
                                                                               332
sp|Q9QZN9|CNR2_RAT          LRSGEIRSAAQHCLTGWKKYL----Q---GLGSEGKEEAPKSSVTETEAEVKTTTGPGS
                                                                               352
                                                                                 .
sp|Q9NPB9|ACKR4_HUMAN       --STFSI-          350
sp|Q60614|AA2BR_MOUSE       --------          332
sp|Q9QZN9|CNR2_RAT          RTPGCSNC          360
```

Fig. 2: Multiple sequence alignment through Clustal Omega against the sequences of mouse and rat showed conserved residues having *.

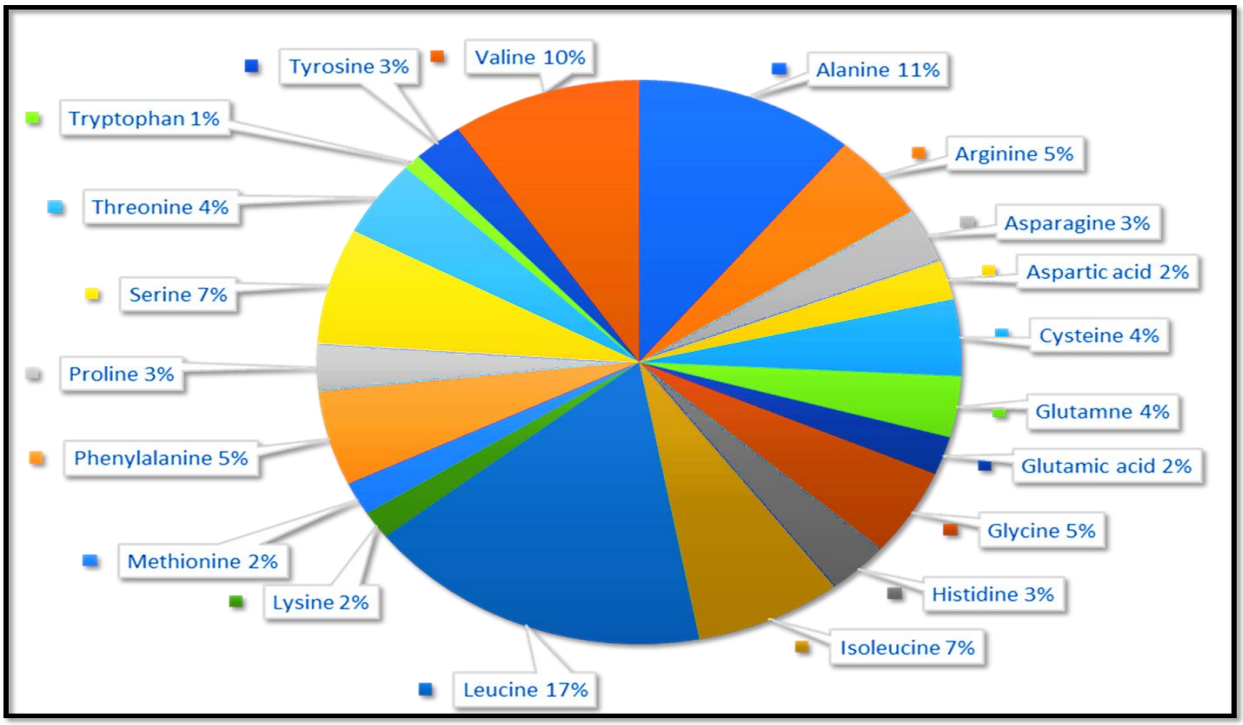

Fig. 3: Pie chart represents the composition of amino acid of MC1R and calculated percentage values.

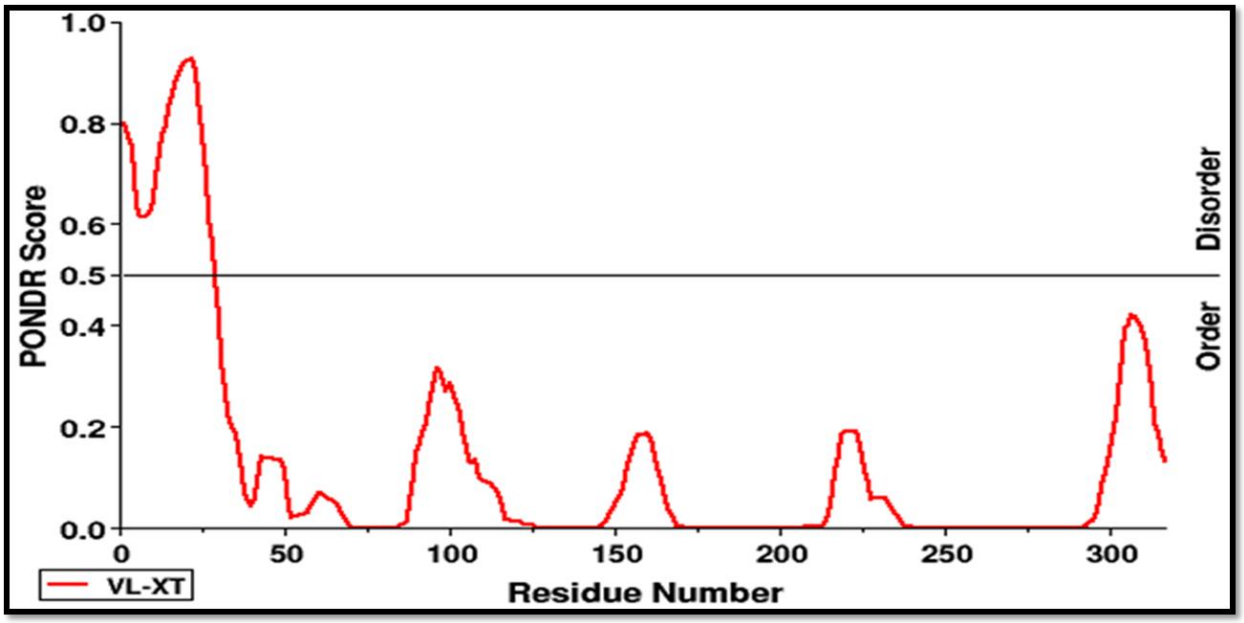

Fig. 4: Disorder Residue from PONDR tool of Protein.

templates against PDB. The top ranked five aligned suitable templates having maximum identity, query coverage, E values and total scores were selected for homology modeling. The selected templates were utilized to generate the 3D structure of MC1R. The overall query coverage and similarity for the used templates and MC1R protein showed 70% from end to end and was considered reliable for the homology modeling approach.

Several 3D models were predicted for MC1R by utilizing various tools through in silico approaches (threading and comparative modeling) to satisfy the sequence.

All the generated models were evaluated based on the quality factor, favored region, allowed region, and outliers. By using homology modeling and threading approaches, the graph was generated comparatively for all the predicted models and the reliable structure was retrieved from the generated graph (**Fig. 5**) for further analyses.

ERRAT evaluation tool was utilized and the observed overall quality factor of the selected structure of MC1R was 95.79%. For the evaluation of predicted models, the Ramachandran plot was utilized which reveals the φ and ψ distributed and 99.3% residues were observed in favored region and allowed region and only 1 residue was observed in outlier region. For the improvement of stereochemistry, the structural minimization on the predicted structure was applied and considered the model for the most optimal purpose. By using UCSF Chimera 1.13, the most optimal structure was minimized on 1000 steepest and 1000 conjugates gradients run after the critical examination at evaluation parameters (**Fig. 6**).

Protein-Protein Interactions

The protein-protein docking analyses were performed and determined by using GrammX online server [62]. The interacting residues of the complex were visualized through UCSF Chimera 1.13 (**Fig. 7**) (**Table 1**).

Comparative Molecular Docking

Different binding energies and complexes were revealed by comparative molecular docking analyses. The best complex was determined by analyzing the least binding energy and selected for the interactional studies. The compound ZINC00131 showed least binding energy of -10.4 kcal/mol (**Table 2**). The 3D structure analyses of comparative molecular docking was visualized by UCSF Chimera 1.13 (**Fig. 8**) [66]. The 2D structure for the compound was drawn by ChemDrawUltra 8.0 [65] (**Fig. 9**).

Proteins are involved in cellular processes and are the molecule of life. The functionality of proteins depends on its structure. The field of bioinformatics covered various disciplines including structural bioinformatics, computing, mathematics, artificial intelligence, computational chemistry and biostatistics approaches to facilitate the discovery of

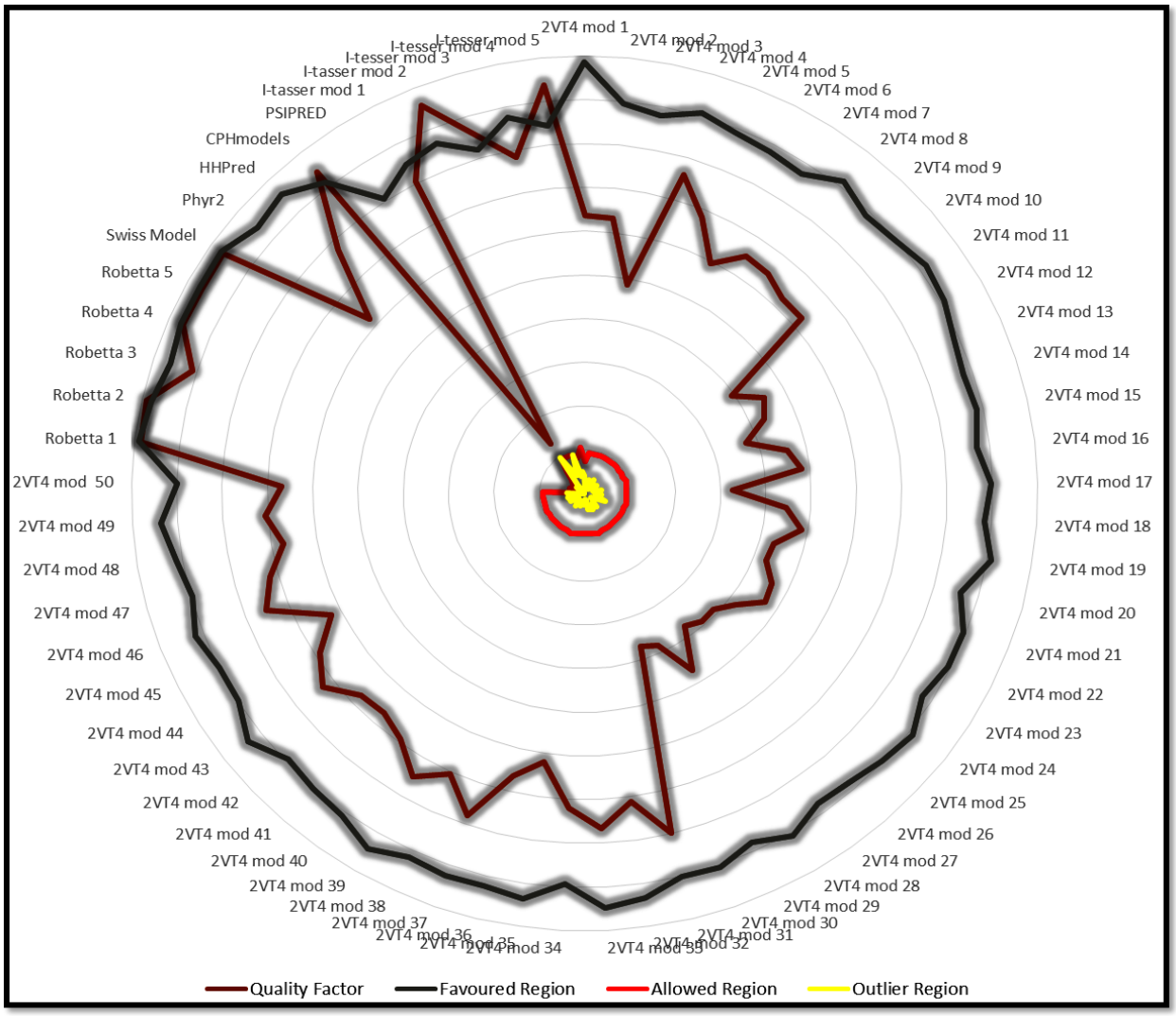

Fig. 5: Graph of quality factor, favored region, allowed region and outliers.

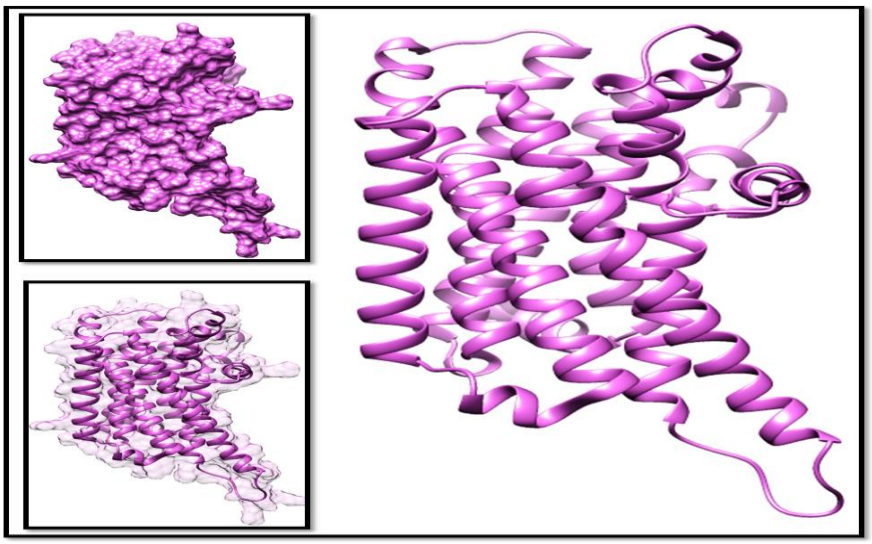

Fig. 6: 3D predicted structure of MC1R.

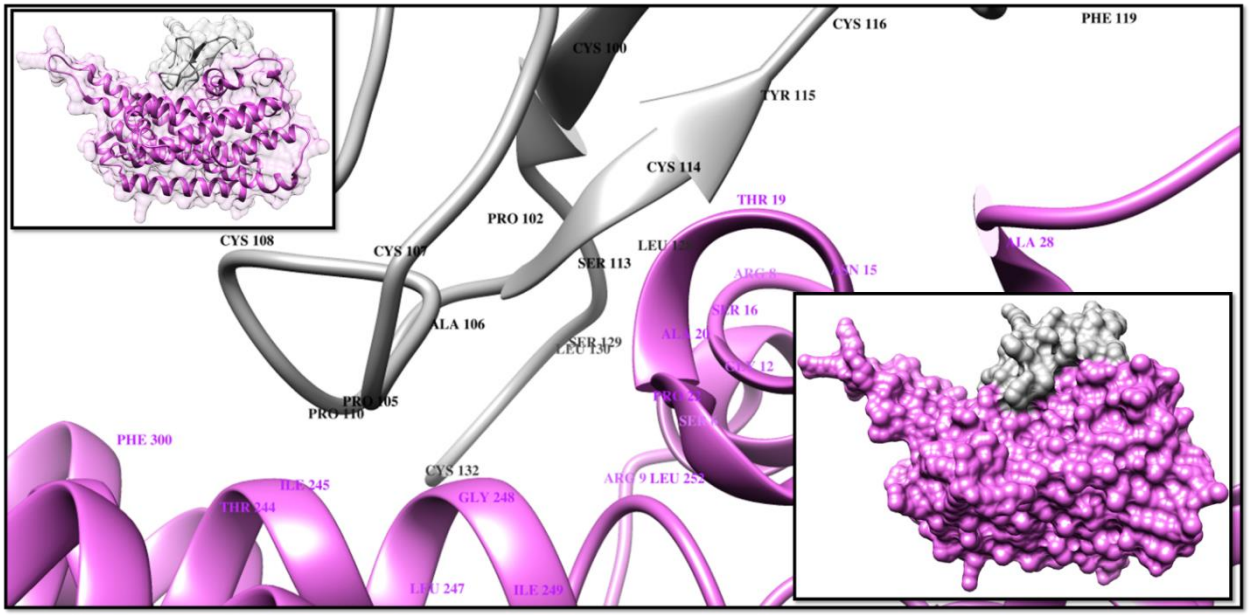

Fig. 7: Protein-protein docking analyses.

Table 1: Residues of protein-protein docking as receptor protein and ligand protein.

Targeting Protein Name	Targeting Protein residues	Interacting Protein Name	Interacting Protein residues
Melanocyte-Stimulating Hormone Receptor 1	PHE 300, THR 244, ILE 245, GLY 248, LEU 247, ILE 249, ARG 9, LEU 252, SER 6, PRO 22, GLY 12, ALA 20, SER 16, ARG 8, ASN 15, ALA 28,	1Y7K	CYS 108, PRO 110, PRO 105, CYS 107, ALA 106, CYS 132, PRO 102, LEU 130, SER 129, SER 113, LEU 128, CYS 114, CYS 100, TYR 115, CY

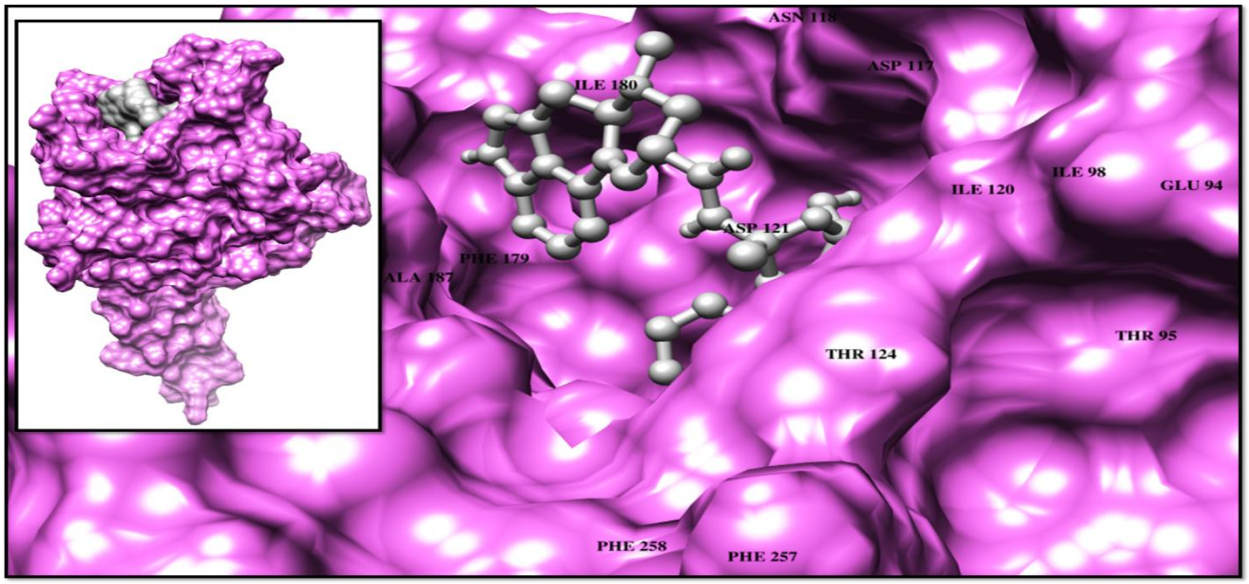

Fig. 8: Molecular docking analyses of ZINC131 compound having least binding energy.

Table 2: Top four least binding energy compounds from molecular docking analyses.

Compound	Binding Affinity kcal/mol	RMSD/ upper binding	RMSD/ Lower binding
ZINC00131	-10.0	0	0
ZINC00087	-9.7	0	0
ZINC00059	-9.5	0	0
ZINC00092	-9.4	0	0

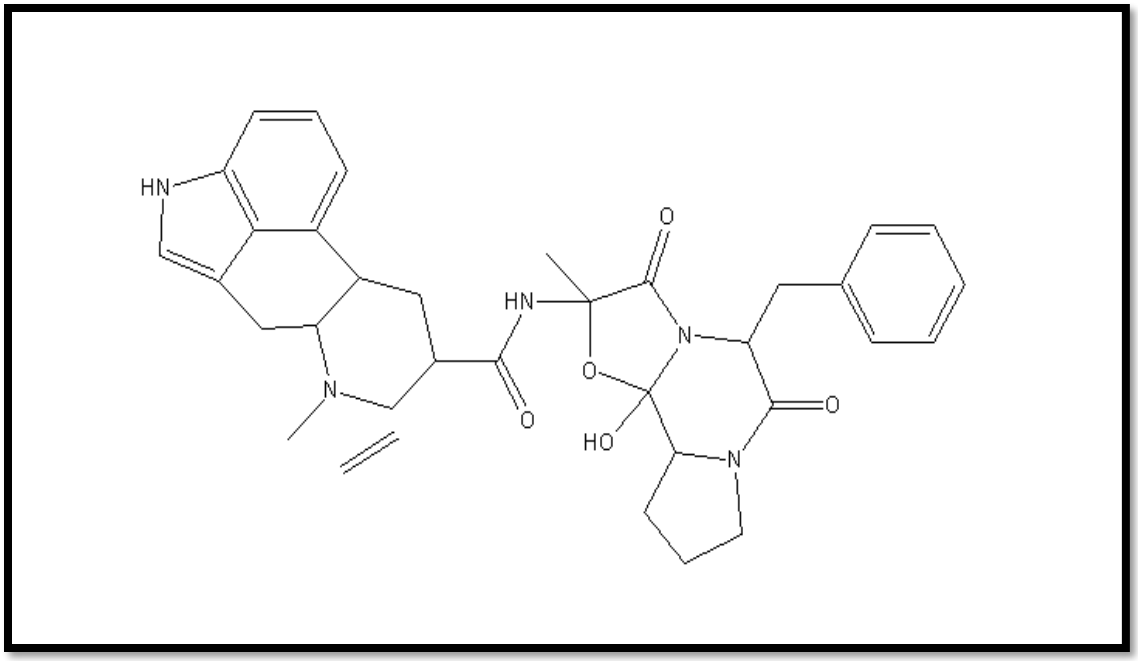

Fig. 9: 2D structure of ZINC131 compound.

new biological ideas [67]. Over the last ten years, the structural bioinformatics has undergone many improvements. Biological data increases with the help of computational recourses and methodology to develop the size and resolution of study as well as created complex questions to research [68, 69]. The approaches of computational analyses lower the time phases and very useful to the researcher in the field of research [70]. By using *in silico* methods and computational approaches predict the protein structure of MC1R.

Conclusion

Various computational approaches were applied for the structure prediction of MC1R helped to develop novel drug targets. Protein-protein docking analyses were performed and interactional residues were reported. Comparative molecular docking was utilized and ZINC131 compounds showed least binding energy.

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Conflicts of Interest

The authors declare no conflict of Interest.

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