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Regulation of Type-2 Diabetes through IP_3R and MTrs along with SERCA and CRAC Channels by Agomelatine, Luzindol, 2-APB and Thapsigargin: A Computational Exploration

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Abstract

Type-2 diabetes speedily disseminates from corner to corner in the humanity. Activation of insulin secretion mainly depends on melatonin receptors, IP_3 receptor as well as sarcoplasmic endoplasmic reticulum Ca^{2+}ATPase channel. Agomelatine and IP_3 have a very important role in the increased Ca^{2+} level in the cytoplasm followed by the secretion of insulin; both are the activator of MTrs and IP_3 receptors respectively. Luzindol and 2-APB are the inhibitor of MTrs and IP3 receptor, mediated by the inhibition of the PLC pathway caused reduction of IP_3 which is a very important element for the insulin secretion. Keyhitch in type-2 diabetes is failing the quantity of insulin in the pancreas by exhaustion of Ca^{2+} in cytoplasm. IP_3 will be activated by the existence of agomelatine consequential in elevation of the Ca^{2+} level in the cytoplasm follow by lack of Ca^{2+} levels in the endoplasmic reticulum. Collectively, our computational studies suggest that MTrs and IP_3R are the narrative and hopeful targets to be used against type-2 diabetes.

Keywords: Agomealtine; Luzindol; Melatonin receptor; IP_3R; SERCA; CRAC

Introduction

Ca^{2+} is the greatest vital second messengers essential for cell [1]. There are lots of routes or contrivance in the cell can adjust the signalling of the calcium. These routes can prompt and obstruct the cellular Ca^{2+} signalling. If some disruptionarises in these routes they will begin some many types of diseases [2]. Like Type 1- child or juvenile or (IDDM) diabetes mellitus, Type 2- diabetes (NIDDM) or Adult -onset diabetes or insulin resistance diabetes mellitus, Type 3- diabetes mellitus (Brain insulin diabetes), Gestational diabetes mellitus (GDM or Early pregnancy diabetes). Ca^{2+} activating agonist was added to the its receptors on plasma membrane consequences the rise the number of calcium ion in the cytoplasm which persist in the absence of extracellular concentration of calcium ion, after that will be secreted from intracellular store. The regulation of calcium depends on some receptors like melatonin receptors, IP_3 receptor, CRAC and SERCA these are responsible to play major roles. Melatonin receptors regulate several other second messengers: cyclic guanosine 3', 5'-monophosphate [3], diacylglycerol (DAG) [4], arachidonic acid, inositol-1, 4, 5-trisphosphate (IP_3) [5-11] and [Ca^{2+}]. In secretion of Insulin the major pathway is through the inhibition of adenylcyclase and guanylcyclase by inhibiting the formation of the cAMP and cGTP from ATP and GTP by MT_1 and MT_2 receptors respectively. Used agomelatine, because it is an analogue of melatonin and it has good binding affinity to MT_1 and MT_2 receptors. IP_3 is the hydrophilic compound but basically it is derived from a lipid moiety, it can bind to IP_3R present on ER and caused the rising of Ca^{2+} level. Calcium release activated channel (CRAC) is specialized plasma membrane Ca^{2+} ion channels. When Ca^{2+} are depleted in the endoplasmic reticulum (a major store of Ca^{2+}) of pancreatic beta cells, the CRAC channel is activated to slowly replenish the level of calcium in the endoplasmic reticulum. The Sarco- and Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) are an intracellular membrane bound enzyme that utilizes the free energy of ATP to transport Ca^{2+} against a concentration gradient. The physiological role of SERCA is to sequester cytosolic Ca^{2+} into membrane-bound intracellular compartments. The stored Ca^{2+} is in turn released as a general messenger for cellular signaling [12] use thapsigargin drug, it is an antagonist of SERCA. It will mainly use for the maintaining the concentration of calcium in the cytoplasm. It causes inhibition of the incorporation of calcium ion into ER.In the present study, we explore the possible pathway through (KEGG pathway database) by the help of interactions: MTrs with agomelatine, luzindol, IP_3R with IP_3, SERCA with thapsigargin and ORAI1 with STIM1 in CRAC channel. We also investigate the possible combination of drugs, which one is suitable for the control of Ca^{2+} in cytoplasm to increase the insulin secretion, therefore we investigate the best pathway for the control of type-2 diabetes.

Material and Methods

Materials

SWISS-MODEL was used for the preparation of MTrs, Pubchem of NCBI database and chemspider were used for the finding of drug SDF file, Pyrx was used for the conversion of SDF file to PDB file, Swiss PDB viewer was used for the energy minimization of drugs as well as receptors, Arguslab was used for the stabilization of drugs, RCSB database was used for the finding of receptors PDB file, DoGSitesScorer: Active site prediction and analysis server was used for the determination of active site in the receptor with respect to its drug, Hex5.1 was used for the docking of drug with receptors, VEGA was

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used for the checking of stability of receptor by procheck software.The KEGG pathway database was used for the analysis of drug effect.

Methods

Homology modelling of melatonin receptor and finding the PDB structure of other receptors and ligands: Melatonin receptor was modelled by the fasta format of MTr amino acid sequence, with the help of SWISS- MODEL online server [13-15], and because there is no 3D structure of MTr is available in the online database like RCSB and NCBI.The structure of IP_3R, SERCA and CRAC was found in the RCSB pdb data bank. In which download the PDB file of receptors and prepare for the further studies by the pyrx software, to remove unwanted hetero atom, which can create problems at the time of docking.Download the pdb structure of ligands and optimization the structure with the help of argus lab software.

Energy minimization and structure stabilization of receptors: Energy minimization was needed to perform every docking studies, in which used Swiss PDB viewer to minimize the energy of receptors or protein structure.

Active site prediction: In the prediction of active site we were used DoGSiteScorer: Active Site Prediction and Analysis Server, it is an automated pocket detection and analysis tool which can be used for the protein druggability assessment. Prediction with DogSiteScorer are based on designing size, shape and chemical features of automatically expected pockets, merged into a support vector machine for druggability estimation. Input the 4-letter pdb code of the structure to be treated, or upload a pdb file. After clicking Investigate and analyze pockets [16-18].

Molecular docking of ligands to its receptors: In molecular docking we were used Hex5.1 software for the binding sites of desired ligands [19]. Correlative blind searching was set to reveal information on shape, electrostatics. Molecular mechanical refinementswere set as a post docking process. After modeling final MTr was used in the docking studies with the agomelatine (an agonist of MTr) and luzindol (antagonist of MTr). MTr belong to the super family of G-protein-coupled receptors (GPCR). They are involved in the modulation of a large spectrum of physiological functions, including regulation of circadian and seasonalrhythms [4]. MTr regulate several other second messengers: cyclic guanosine 3', 5'-monophosphate [3], diacylglycerol (DAG) [4], arachidonic acid, inositol-1, 4, 5-trisphosphate (IP_3) [5-11] and [Ca^{2+}]. In secretion of Insulin the major pathway are through the inhibition of adenylcyclase and guanylcyclase by inhibiting the formation of the cAMP and from ATP by MTr. Use agomelatine, because it is an analogue of melatonin and it has good binding affinity to MTr. There are some of intracellular messenger those are transfer the signal from melatonin receptor to inside the cell PLC, PIP_2, etc. Hydrolysis of PIP_2 catalyzed by PLC results water-soluble IP_3 will be formed [20]. The calcium (Ca^{2+}) level increase in cytoplasm, and they cause the changing the potential between inside and outside of the membrane. Ca^{2+} from store in mitochondrial by increasing the micromolar concentrations of IP_3 in pancreatic acinar cells [21].

In the CRAC receptor protein ORAI1 is a structural component ORAI1 interacts with the STIM1 protein. STIM1 is a transmembrane protein of the endoplasmic reticulum (ER). STIM1 can sense the concentration of Ca^{2+} inside the ER. When the concentration of Ca^{2+} inside the ER becomes low, STIM1 proteins aggregate and interact with ORAI1 located in the cell surface membrane [22]. When concentration of Ca^{2+} inside the ER approaches the upper set point, another protein, SARAF (TMEM66) associates with STIM1 to inactivate the store-operated calcium channel (SOCE) [23].

SERCA (Sarco Endoplasmic Reticulum ATPase) present in the plasma membrane of the Sarco endoplasmic reticulum. SERCAis an intracellular membrane bound enzyme that utilizes the free energy of ATP to transport Ca^{2+} against a concentration gradient from cytoplasm to the endoplasmic reticulum. Thapsigargin is non-competitive inhibitor of SERCA, it has raised cytosolic (intracellular) calcium concentration by blocking the ability of the cell to pump calcium into the sarcoplasmic and endoplasmic reticulum which causes these stores to become down. Store-depletion can secondarily activate plasma membrane calcium channels, permitting an entry of calcium into the cytosol.

Results

Modeling of MTr and pdb strcture of other receptors

Homology modeling is a appropriate process to predict 3D protein structure using templates in PDB. The SWISS-MODEL Workspace is a web-based integrated service committed to protein structure homology modelling. It contribute and lead the user in building protein homology models at different levels of complexity (Figure 1).

In Figure 1, a model for melatonin receptor (MTr) has been generated in automated mode. Upper panel: the green line represents the target sequence (364 residues). Blue lines indicate for which segments of the target models have been generated, in this case for residues 1 to 348. Middle panel: information on the template structure (1u19, chain B) and quality (sequence identity, E-value) of the target–template sequence alignment shown in the lower panel docking [15]. In section A describe the quaternary information and model information of template in which template id was 1u19, single chain in dimeric form and model residue range was 13to 330, sequence identity was 19.325 and Qmean Z-score was -9.382 (Figure 2).

Figure 2 shows the QMEAN4 scores with the help of x-ray crystallography techniques produced structure. In Figure 2, A shows Z-score of the individual components of QMEAN, the average Q-MEAN of high resolution structure is 0. Q-MEAN score estimating the quality of protein strucutre models is avital value in protein structure prediction. Often one ends up in having a set of alternative models (e.g. from different modeling servers or based on alternative template structures and alignments) from which the best candidate shall be selected. Or a singe model has been built from which the absolute quality needs to be predicted in order to have an idea about its suitability for subsequent experiments. The QMEAN score provides access to two scoring functions for the quality estimation of protein structure models which allow to rank a set of models and to identify potentially unreliable region within these.

The QMEAN4-score is a composite score consisting of a linear combination of 4 statistical potential terms (estimated model reliability between 0-1). The pseudo-energies of the contributing terms are given in Table 1 together with their Z-scores with respect to scores obtained for high-resolution experimental structures of similar size solved by X-ray crystallography.

Table 1 represents the parameters of the selected template for MTr receptors, in which there are four mode of energy defined the qmean4 score of Z-score were used regarding the selection of the appropriate model for molecular docking of melatonin receptor. In parameters model residue range, sequence identity and q- mean Z- score were play crucial role for the stability and quality of the receptors or protein structures (Figure 3 and 4).

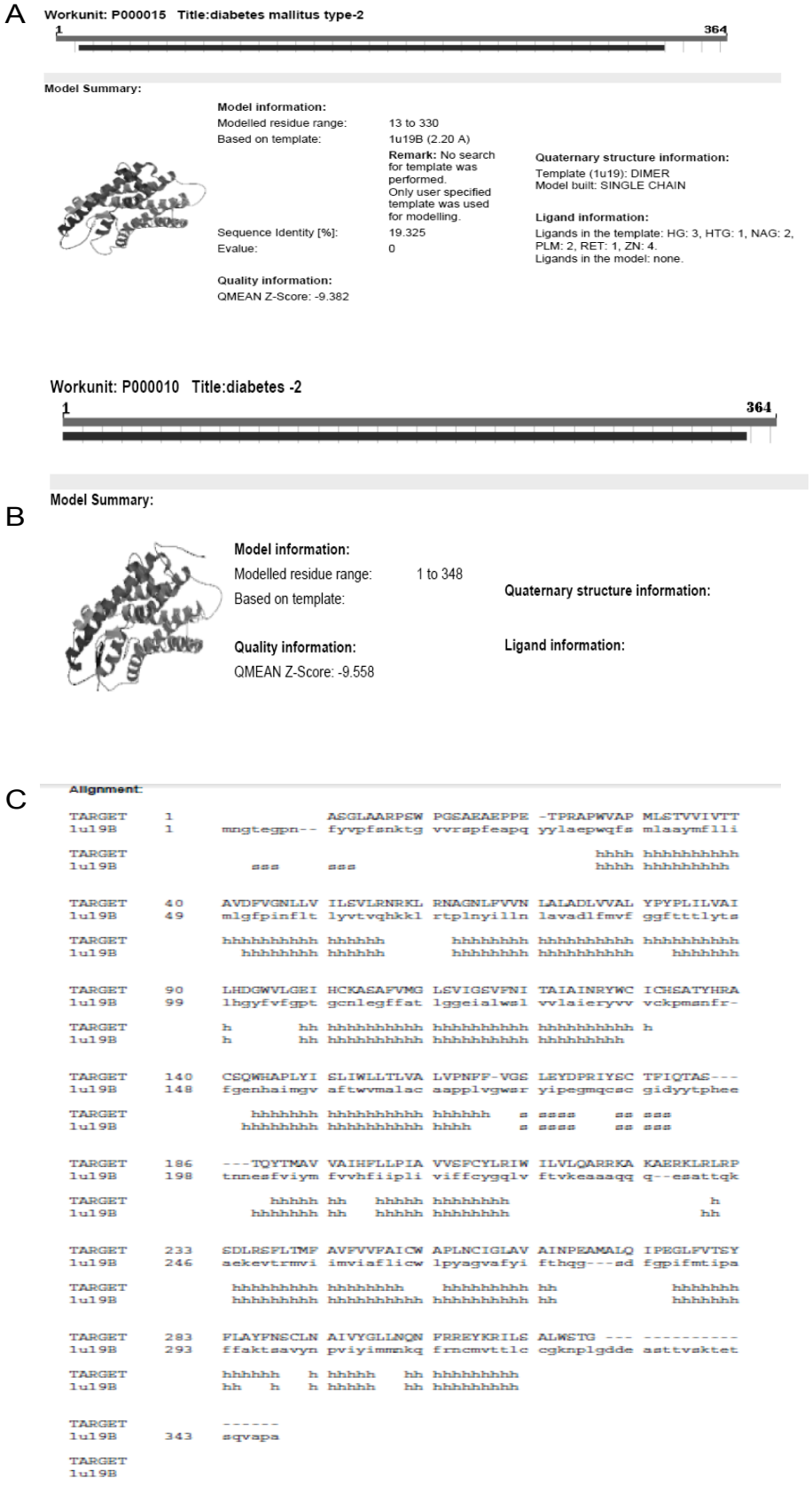


Figure 1: Typical view of a SWISS-MODEL workspace result in A,B and C [15].

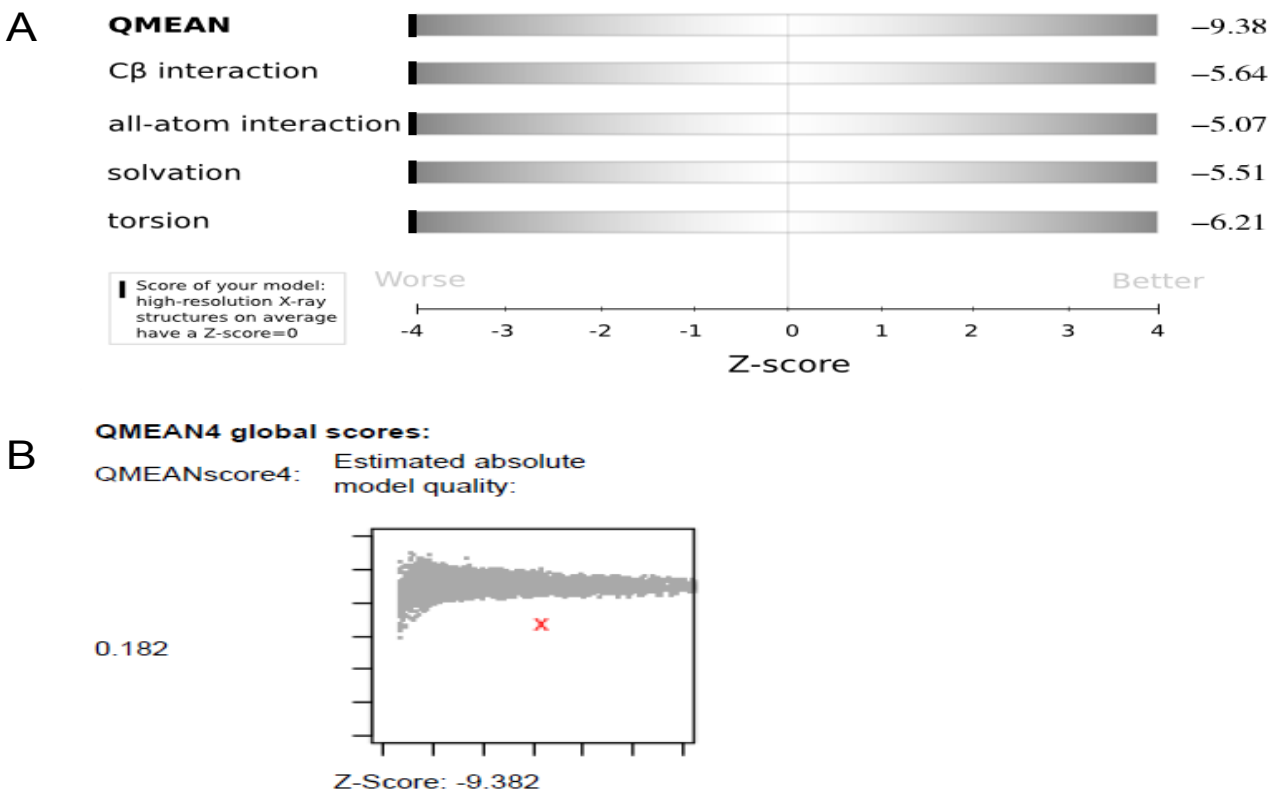


Figure 2: A- QMEAN4 global scores and B- Score components.

Scoring function term	Raw score	Z-score
C_beta interaction energy	218.73	-6.55
All-atom pairwise energy	3843.10	-5.54
Solvation energy	20.80	-5.51
Torsion angle energy	39.42	-6.27
QMEAN4 score	0.188	-9.56

Table 1 : Parameters of the selected template for MTr modeling [24].

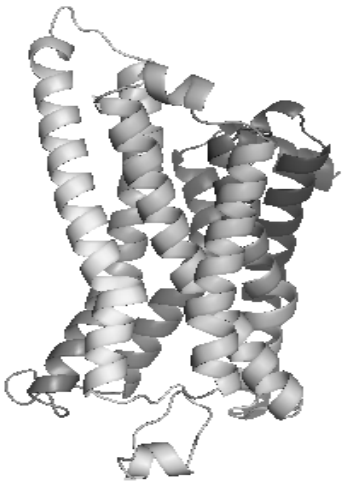


Figure 3: 3D structure of MTr, by SWISS MODEL online server [13-15].

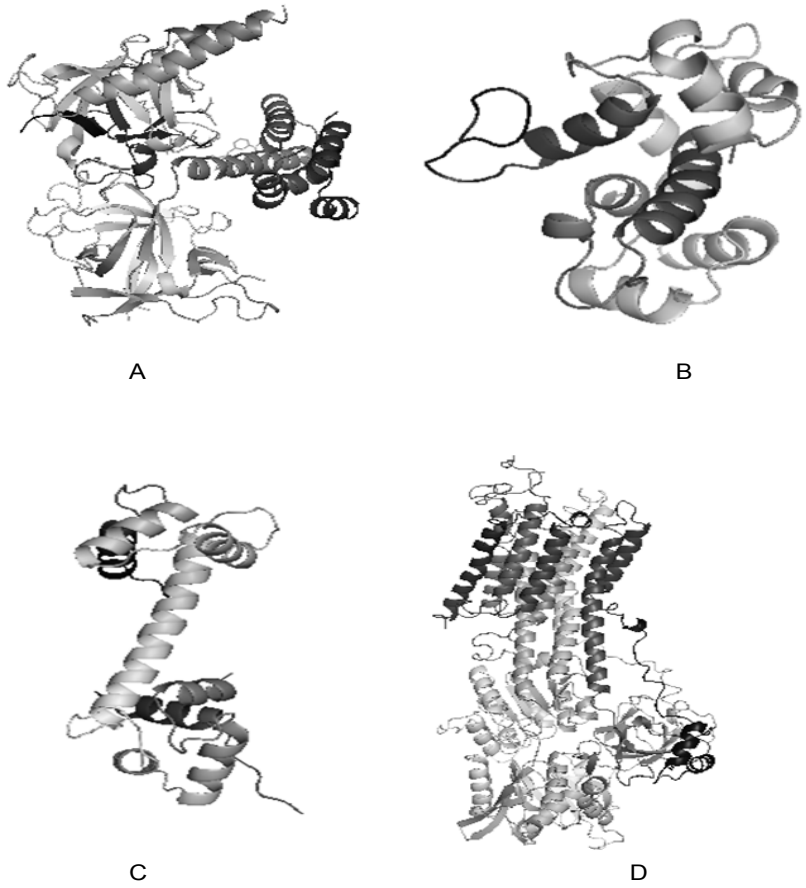


Figure 4: 3D structure of receptors A-IP3R, B-STIM1 of CRAC, C-ORAI1 of CRAC and D-SERCA.

Receptors	Bonds (KJ/mol)	Angles (KJ/mol)	Torsion (KJ/mol)	Improper (KJ/mol)	Non-bonded (KJ/mol)	Electrostatic (KJ/mol)	Total (KJ/mol)
MTr	1008.284	3276.276	5789.394	821.414	-31405.40	-29656.97	-50167.004
IP3R	296.87	1625.375	2846.691	421.600	-15791.08	-15357.51	-25958.039
STIM-1	121.809	533.975	598.140	184.468	-5331.84	-4784.28	-8677.726
ORAI-1	94.163	639.810	747.475	256.970	-4529.61	-3564.45	-6355.641
SERCA	963.082	3648.042	4836.976	1030.017	-26717.09	-23666.48	-39905.453

Table 2: Energy Minimization of the receptors on the basis of six parameters [13,15,25-27].

Energy minimization and stabilization of receptors

In the energy minimization we have used swiss pdb viewer software. It can repair distorted geometries by moving atoms to release internal constraints [13,15,25-27] (Table 2).

Stability has defined by the VEGA ZZ 3.0.1 software with the help of PROCHEK, in which the RamaPlot plug-in allows to calculate the Phi and Psi protein angles and to show their values in a two dimensional plot (Figure 5 and Table 3).

Active site prediction

Based on the 3D coordinates of a protein, its possible active sites on the protein surface are calculated with DoGSite. DoGSite is a grid-based function prediction method which uses a Difference of Gaussian filter to detect potential pockets on the protein surface and splits them into subpockets. Consequently, global things, relating the size, shape and chemical geographies of the predicted pockets are calculated (Figure 6).

Molecular docking of ligands to its receptors

Docking is a method which predicts the desired orientation of one molecule to a second whenbound to each other to form a stable complex [30]. Understanding of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. In molecular docking we was used HEX 5.1 softwareHex, is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein molecules. Hex can also calculate protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes.

Molecular docking between Agomelatine, luzindole and MTr: Agomelatine is an analogue of the melatonin hormone and act as same as melatonin in which stimulate the MTr and caused the increasing the amount of Ca^{2+} through IP_3. MTr agonists and antagonist are analogues of melatonin that bind to and activate or inhibit the MTr

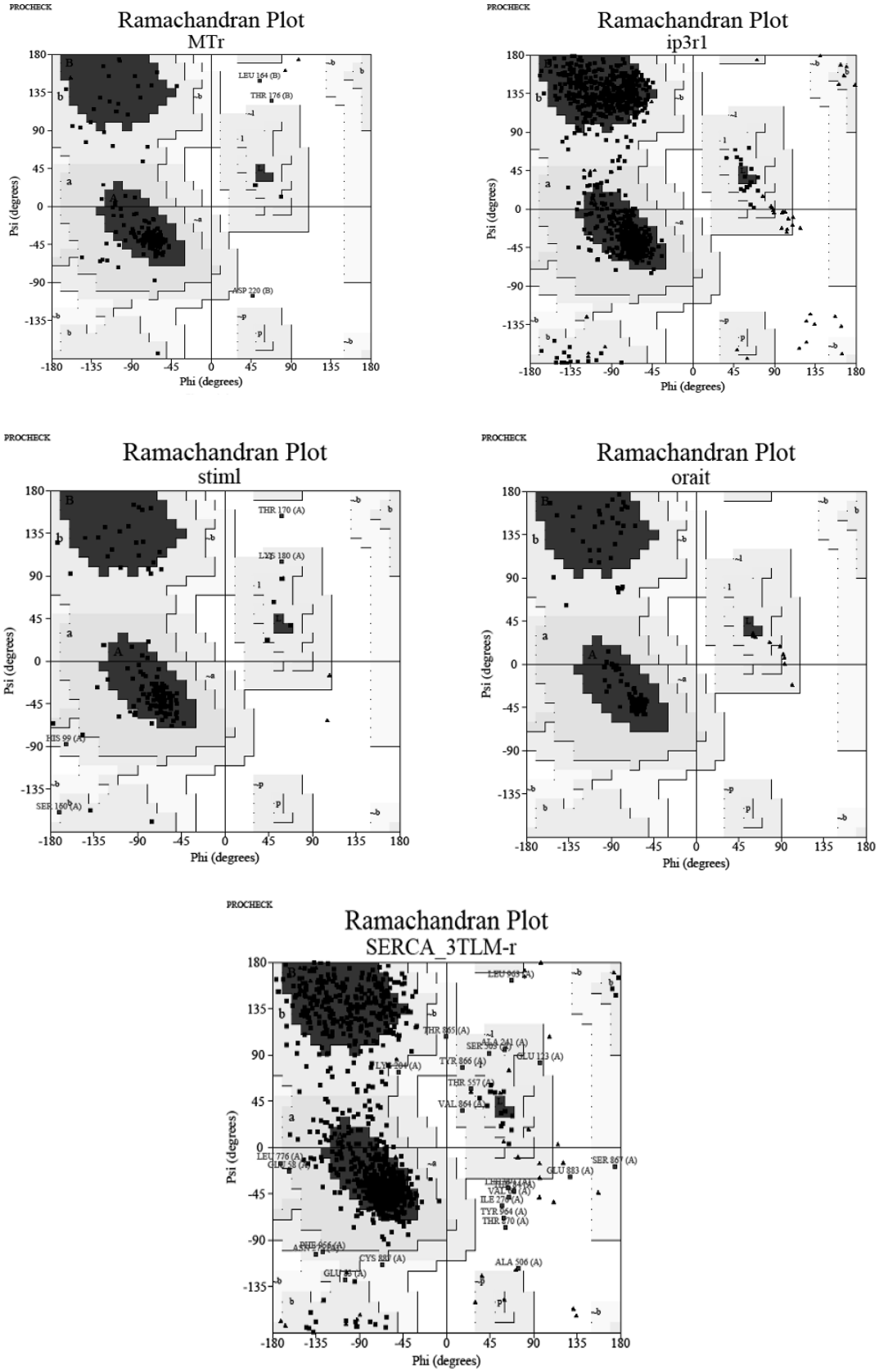


Figure 5: Ramachandran plots of MTr, IP3R, STIM1, ORAI1 of CRAC and SERCA receptors [28,29].

Ramachandran Plot Statistics	MTr		IP3R		STIM-1		ORAI-1		SERCA	
Residues in most favored regions [A,B,L]	240	91.3%	765	89.7%	111	81.6%	142	95.9%	687	78.9%
Residues in additional allowed regions [a,b,l,p]	20	7.6%	88	10.3%	21	15.4%	6	4.1%	160	18.4%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%	0	0.0%	3	2.2%	0	0.0%	15	1.7%
Residues in disallowed regions	3	1.1%	0	0.0%	1	0.7%	0	0.0%	9	1.0%
Number of non-glycine and non-proline residues	263	100%	853	100%	136	100%	148	100%	871	100%
Number of end-residues (excl. Gly and Pro)	2		30		9		4		4	
Number of glycine residues (shown as triangles)	10		54		8		11		68	
Number of proline residues	12		39		3		2		44	
Total number of residues	287		976		156		165		987	

Table 3: Ramachandran Plot statistics.

[31]. Agonists of the MTr have a number of therapeutic applications including treatment of sleep disorders and depression but now used for the treatment of diabetes type-2. The discovery and development of MTr agonists was motivated by the need for more potent analogues than melatonin, like agomelatine with better pharmacokinetics and longer half-life [31].

After ligand site prediction of MTr with respect to Agomelatine and luzindole was showing similar ligand site in which active pocket no (Figure 7).

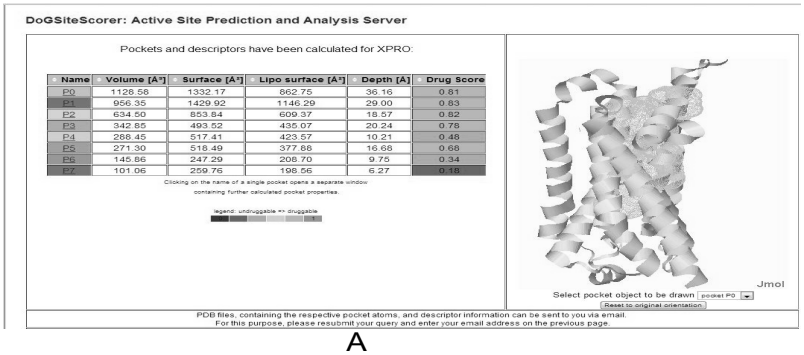
In Figure 8 agomelatine was bound to its appropriate site in MTr, in which agomelatine an analogue of the melatonin which is a potent agonist of MTr. After binding it was activated the MTr caused regulates the function of the cell through intracellular second messengers. For example, in many tissues, melatonin has been found to decrease intracellular concentration of cAMP [32,33]. Predicted interactions of MTr protein with agomelatine, luzindole, are shown in Figure 8. Also, related docking binding energies (kJ/mol) presented in Table 4.

In Figures 8 and 9 shows the binding site of the agomeltine and luzindole in the MTr, in which agomelatine binds to its suitable ligand site with minimum energy and caused stable binding to stimulate MTr and luzindole acts as inhibitor of the MTr. The stimulatory effect of melatonin on IP_3 release seems to be transduced by the membrane-bound melatonin MTr, in which according to KEGG pathway database, only the MT1 MTr is being expressed [34-36]. However, the type of MT_1 receptor coupled G-protein and the subunit, stimulating PLC, can only be hypothesized, as the signaling ability of a given receptor depends on the type of cell expressing the receptor [11]. MTrs (MT_1, MT_2 and MT_3) *in vitro* exhibit differential abilities to stimulate PLC via Gq-proteins [37,38], and the MT1 subtype has been seen to couple with Gq-proteins in an agonist-dependent and guanine nucleotide-sensitive manner in cells [39]. However, in cells expressing the human MT_1 receptor, melatonin markedly elevates the IP3-liberating effect of PGF2a, presumably via the Gbc-subunit [40]. Receptor-activated G-proteins stimulate the PLC-mediated breakdown of phosphatidylinositol 4, 5-bisphosphate to the second messengers DAG and IP3. The latter mobilizes intracellular Ca^{2+} stores by binding to IP_3Rs that are ligand-activated Ca^{2+} channels and are regulated by Ca^{2+} itself [41]. This process is most likely to happen in pancreatic beta cells after stimulation with agomelatine, as our computational analysis indicate that $[Ca^{2+}]$ increases even in a Ca^{2+} free medium. In addition, a successive Ca^{2+} influx from extracellular sources (capacitative Ca^{2+} entry) via so-called store operated Ca^{2+} channels and a release of Ca^{2+} triggered by Ca^{2+} itself (Ca^{2+} induced Ca^{2+} release) are possible processes as well [41,42]. Nevertheless, present data strongly indicate that Ca^{2+} release

from intracellular sources, like the endoplasmic reticulum, is an important mechanism in reaction to melatonin analogue agomelatine in pancreatic beta cells. Elevation of $[Ca^{2+}]$ i is a key regulator of stimulus secretion coupling. The main secretory product of the pancreatic b-cell is insulin. i.e. Melatonin and its analogue agomelatine stimulated IP3 release have only a minor impact on direct stimulation of insulin secretion. The inhibitory effect of luzindole was inhibited the formation of PLC and has participated to reduce the amount of insulin.

Molecular docking between 2-APB, IP_3 and IP_3R: IP_3R agonists (IP_3) in light white colour in the Figure 8 binds to IP_3 Receptor and antagonist (2-APB) in dark greycolour in the Figure 9 binds to IP_3R, both play an important role to activate or inhibit the endoplasmic membranous receptor IP_3R, this is the Ca^{2+} channel associated receptor, help to increase the amount of Ca^{2+} in the cytoplasm and decreased in the lumen of endoplasmic reticulum. 2-APB is a membrane permeable modulator of intracellular inositol triphosphate (IP3)-induced calcium release. Studied the regulation of Ca^{2+} influx and out flux for the secretion of insulin. After the formation of IP_3 from PIP2 with the help of PLC,it has bound to IP_3R and active to its associated Ca^{2+} channel and caused Ca^{2+} influx in the cytoplasm according to KEGG pathway database, in which if the IP_3 has bound to previously bounded IP3R with 2-APB drug, it has not activated IP_3R and inhibit them. Predicted docking was authenticated along with the binding energy of the docking in Table 4. 2-APB and IP_3 both were binding to different ligand site. The binding site of the 2-APB in the IP_3R is near to binding site of the IP_3 in the IP_3R. The combination of IP_3 and 2-APB was used for the selection of most accurate pathway for the increasing the amount of Ca^{2+} in the cytoplasm, this will be beneficial for the selection of the perfect pathway for the secretion of insulin [43].

After Molecular docking between IP_3R and IP_3, 2-APB Binding energy obtained was observed to be -171 KJ/Mol and -184 KJ/Mol respectively. Here IP_3R was used as a receptor, IP_3 and 2-APB was used as a ligand. From this value of energy it could be interpreted that the efficient binding between IP_3R and IP_3, 2-APB has resulted in the successful activation and inhibition of the IP_3R and its associated channel, which caused activation the entry of Ca^{2+} into the cytoplasm from the lumen of endoplasmic reticulum in the pancreatic beta cell by the action of IP_3 and inhibition of the entry of Ca^{2+} into the cytoplasm by 2-APB. This combination of activator and inhibitor were used for the checking the possible pathway led to increase in the level of Ca^{2+} in the cytoplasm and caused the release of insulin from insulin vesicles.



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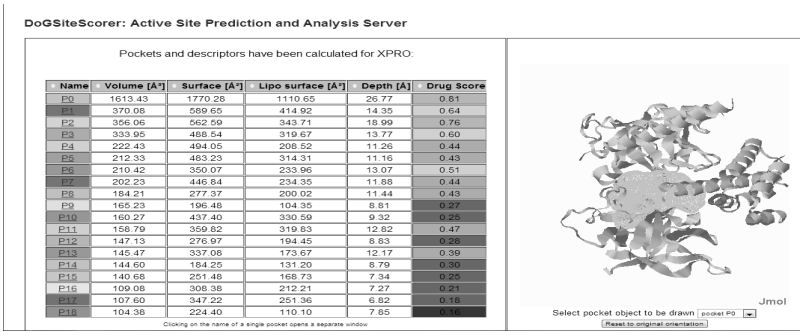

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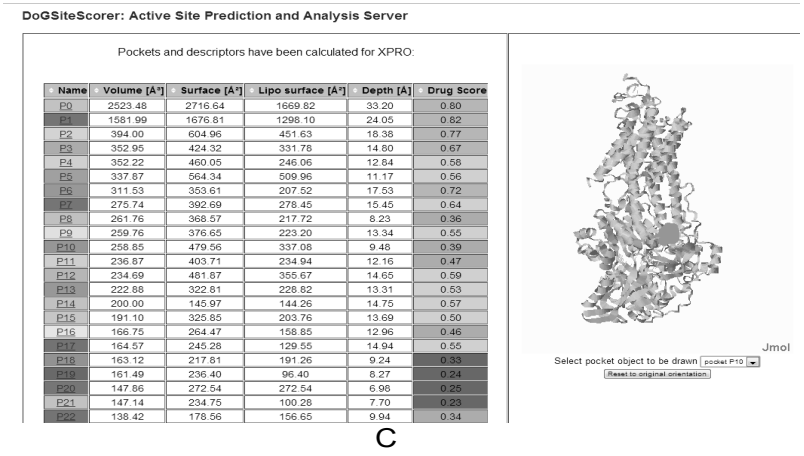

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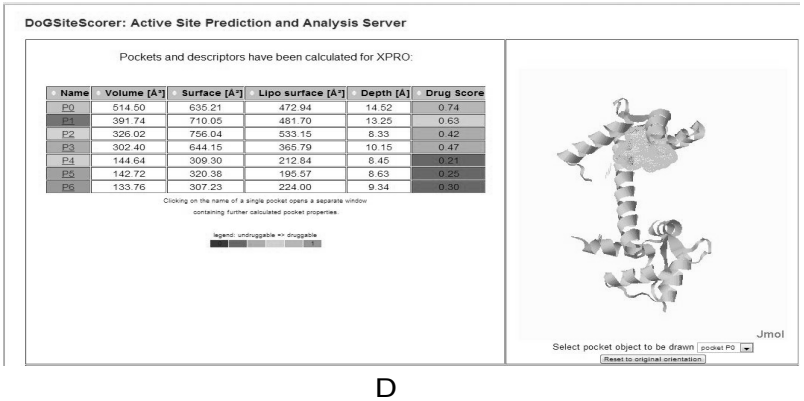


D

Figure 6: Active site prediction of receptors against with its suitable ligands, A- active site prediction of MTr with respect to Agomelatine drug and luzindol, B- active site prediction of IP3R with respect to 2-APB drug and IP_3, C-active site prediction of SERCA with respect to thapsigargin drug, D- active site prediction of ORAI1 domain of CRAC with respect to STIM1 protein [17].

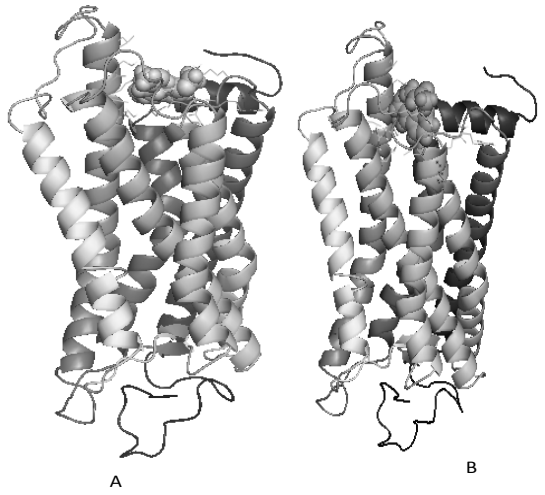


Figure 7: Interaction of MTr with Agomelatine (A) and luzindole (B) [19].

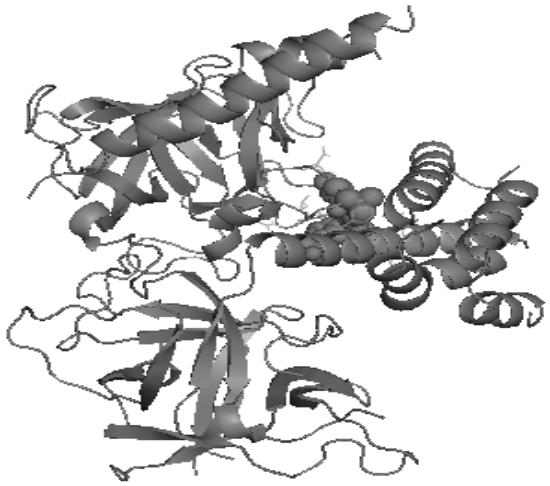


Figure 8: Interaction of IP_3R with 2-APB [19].

Molecular docking model	Docking binding energy (KJ/mol)
MTr with agomelatine (agonist)	-220
MTr with luzindole (antagonist)	-256
IP_3R with 2-APB	-184
IP_3R with IP_3	-171
SERCA with thapsigargin (inhibitor)	-285
ORAI-1 of CRAC with STIM-1	-594

Table 4: Binding energy after the molecular docking.

Molecular docking between thapsigargin and SERCA: SERCA resides in the sarcoplasmic reticulum (SR) within cells. It is a Ca^{2+} ATPase that transfers Ca^{2+} from the cytosol of the cell into the lumen of the SR at the expense of ATP hydrolysis during insulin secretion. The role of ATP-dependent calcium uptake into intracellular storage sections is a vital feature of hormonally prompted calcium signalling. Thapsigargin, a non-phorboid tumour developer, gradually is being used to operate calcium stores because it induces a hormone-like rise of cytosolic calcium. It has been recommended that thapsigargin acts through inhibition of the endoplasmic reticulum calcium pump [44].

In Figure 10 thapsigargin binds to its suitable binding site which were present in the SERCA. After binding of thapsigargin they changed the conformation of SERCA and caused the closed the Ca^{2+} channels. The interaction of thapsigargin with the SERCA isoforms is quick, stoichiometry, and fundamentally irretrievable. These properties determine that thapsigargin interacts with a recognition site found solitary in all members of the endoplasmic and sarcoplasmic reticulum calcium pump family. Binding of thapsigargin is very important to SERCA in figure 12 because it is necessary to stimulate the closing of Ca^{2+} on the endolplasmic reticulum to cause the prohibition of the entry of the Ca^{2+} from cytoplasm to inside the lumen of endoplasmic reticulum (Figures 11 and 12).

Binding energy obtained from Molecular docking between SERCA

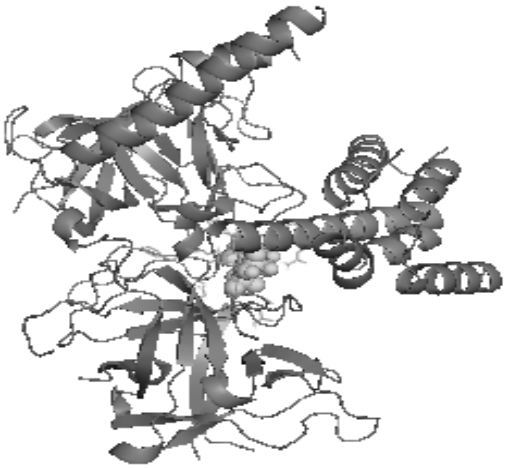


Figure 9: Interaction of IP3R with IP_3 [19].

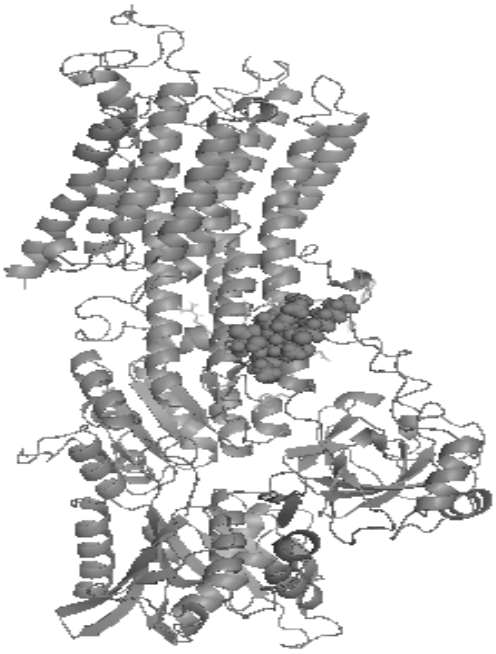
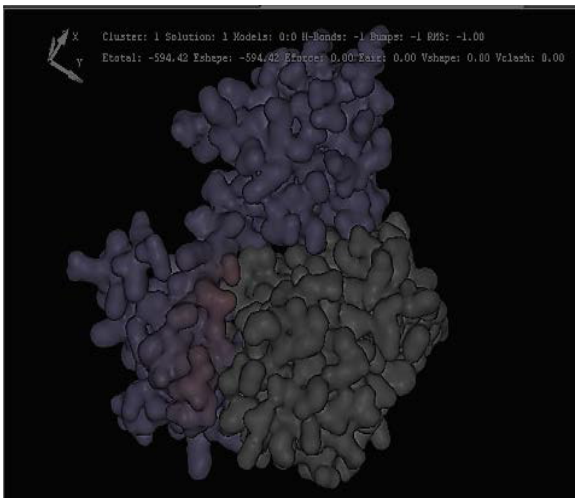


Figure 10: Interaction of SERCA with thapsigargin [19].



A

Figure 11: Interaction of CRAC domain ORAI-1(blue color) with STIM-1(grey color) [19].

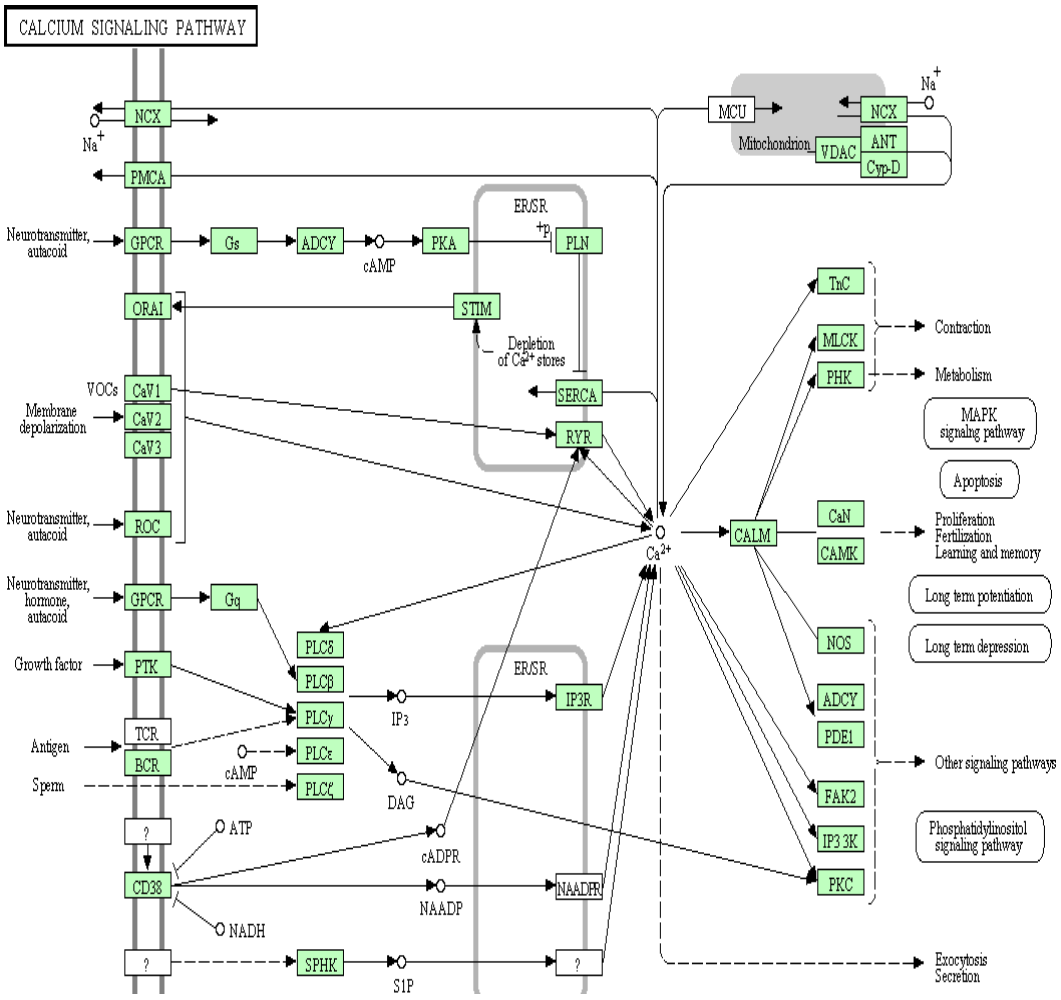


Figure 12: Ca^{2+} signalling KEGG database pathway shows the regulation of Ca2+ by GPCR (MTr), IP3R, SERCA and ORAI-1 with STIM-1 [46-54].

and thapsigargin was observed -285 KJ/mol. Here SERCA was used as a receptor and thapsigargin was used as a ligand. From this value of energy it could be interpreted that the efficient binding between SERCA and thapsigargin has resulted in the successful inhibition of the SERCA which caused inhibited the entry of Ca^{2+} into the lumen of endoplasmic reticulum in the pancreatic beta cell. This finally led to increase in the level of Ca^{2+} in the cytoplasm and caused the release of insulin from insulin vesicles (Table 4).

Molecular docking between binding domain of CRAC (ORAI-1) and STIM-1: Store-operated Ca^{2+} channels activated by the depletion of Ca^{2+} from the endoplasmic reticulum (ER) are a major Ca^{2+} entry pathway in non-excitable cells and are essential for T cell activation and adaptive immunity. Following store depletion, the ER Ca^{2+} sensor STIM1 and the CRAC channel protein Orai1 redistribute to ER-plasma membrane (PM) junctions.

The plasma membrane protein "Orai" (ORAI1 and ORAI2 in humans) forms the core of CRAC channel. The protein ORAI1 is a structural component of the CRAC calcium channel. ORAI1 interacts with the STIM1 protein. STIM1 is a trans-membrane protein of the endoplasmic reticulum (ER). STIM1 can sense the concentration of Ca^{2+} inside the endoplasmic reticulum. When the concentration of Ca^{2+} inside the ER becomes low, STIM1 proteins aggregate and interact with ORAI1 located in the cell surface membrane [22] (Figure 11).

To identify the CRAC-activating domain of STIM1, series of soluble cytosolic STIM1 fragments for their ability to activate an NFAT-dependent luciferase reporter gene (*NFAT-luc*) which is important to binding to ORAI-1 of the CRAC. Severalfragments of STIM-1 are available in the cytoplasm but STIM1342–448 (D5) cluster or fragment are important to bind with ORAI-1 and it was sufficient to activate *NFAT-luc*, D5 also known as CAD (CRAC activating domain). Because cytosolic CAD is a potent activator of ORAI-1 channels and is not associated with the ER [45].

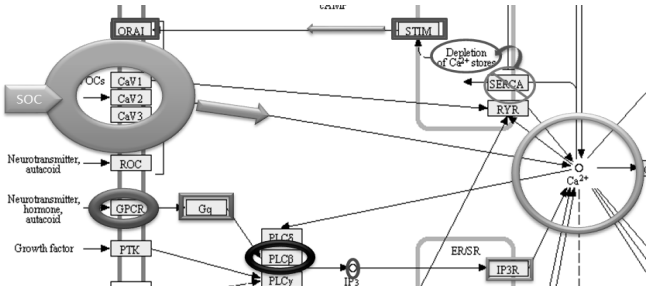


Figure 13: Possible pathway for the increasing the amount of Ca^{2+} in the cytoplasm and insulin secretion from the pancreatic beta cell by KEGG pathway database [46-54].

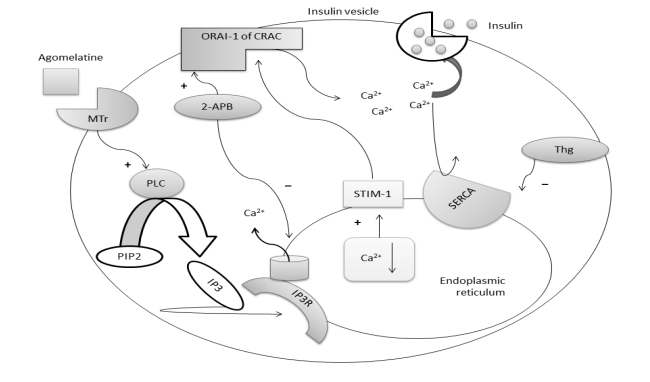


Figure 14: Shematic diagram of action of drug in pathway for insulin secretion.

Binding energy obtained from Molecular docking of ORAI-1 of CRAC and STIM-1 was observed -594 KJ/mol (Table 4), from this observed value it could be interpreted that the efficient binding between ORAI-1 and STIM-1has resulted in the activation of the CRAC associated store operated Ca-channel which further resulted in the influx of the Ca^{2+} into the cytoplasm from outside of the cell membrane. This finally led to increase in the level of Ca^{2+} in the cytoplasm and hence secretion of insulin from insulin vesicles.

In Figure 12 shows that the Ca^{2+} signalling cascade from the KEGG pathway database on the pancreatic beta cell, in which representation of the whole controlling unit for Ca^{2+} signalling and regulate the homeostasis of the Ca^{2+} in the cytoplasm as well as in the lumen of endoplasmic reticulum. In present study focused main pathway for Ca^{2+} signallingrepresented in Figure 13 in which highlighted region in red colour GPCR like MTr activated with the help of agomelatine to form PLC through a Gq unit of GPCR and these PLC cause formation of IP_3. IP_3 was bound to IP_3R on the surface of the endoplasmic reticulum and open its association Ca channel to secrete Ca^{2+} into the cytoplasm. By the help of thapsigargin drug SERCA was inhibited and caused the depletion Ca^{2+} inside the ER and sense STIM-1 to bind with ORAI-1 domain of the CRAC to open the store operated Ca channel in orange colour and increase the amount of Ca^{2+} inside the cytoplasm, finally the main purpose of the present study was to increase the amount of insulin in the cytoplasm, to stimulate the secretion of more amount of insulin from insulin vesicle of the pancreatic beta cell to control the diabetes type-2 (Figure 14).

Discussion

From the primary structure analysis it was concluded that MTr receptor is a surface protein which is hydrophilic in nature. Its secondary structure analysis confirmed that it contains more alpha helices and no beta sheets. Its 3D structure was formed with the help of Swiss model online server through homology modelling and 3D structure of IP_3R, SERCA and ORAI-1 of CRAC and STIM-1 was obtained in the form of PDB id and viewed in a Swiss PDB viewer. The PDB structure of ligand Agomleatine, luzindole, IP_3, 2-APB and thapsigargin was obtained by chemspider and PubChem online database. Various confirmatory tools were used for validation of the results. The geometry and energy of the ligands were optimized in Arguslab. Using Dog site scorer online server for the active pocket in the receptors. The candidate drug agomelatine were docked to the target receptor MTr. The drug agomelatine showed the best docking result with docking Energy of -220Kj/Mol. As the Table 4 was successful in HEX5.1 it can be concluded that agomelatine is a potential activator of MTr.

In molecular docking studies Agomelatine was bound to MTr, activated it and stimulated to form PLC required for the conversion of IP_3 from PIP2 and also formed DAG. IP_3 was binding to IP_3R in the surface of the endoplasmic reticulum and can be stimulated its associated Ca^{2+} channel and caused the influx of Ca^{2+} in the cytoplasm. 2-APB was bound to IP_3R and inhibit them, luzindole also act as an inhibitor of MTr and caused the inhibition of MTr. Thapsigargin was act as an inhibitor of SERCA and caused the efflux of Ca^{2+} from cytoplasm to ER lumen, so it providesfavourable environments to increase the

amount of Ca^{2+} in cytoplasm. In CRAC channel the ORAI-1 domain bind to the STIM-1 domain of ER and sense the Ca^{2+} depletion inside the ER and they helped to stimulate the SOC channel to influx the Ca^{2+} from outside of the cytoplasm and increased the amount of Ca^{2+} in cytosol. The present study investigated the possible pathway to increase the amount of Ca^{2+} in the cytoplasm to stimulate the insulin vesicle to secretion of insulin.

The main problem in diabetes type-2 disease is that it is not a curable disease; it can only controlled with the help of some drugs or combinational drug therapy like an intramuscular injection of insulin and oral administration of insulin along with the drug. Now days big problem is that lots of disease are occurring in every one person from the six persons like diabetes type-2, depression, insomnia, epilepsy, etc. The most dangerous disease is diabetes type-2 and depression it caused the environment to develop another disease. So in present study have to focused to treat or control more than one disease on the single therapy of the drugs, in which used agomelatine to control both diabetes type-2 as well as depressive disorder because of the agomleatine is the MTr agonist to stimulate the elevate the level of Ca^{2+} in the cytoplasm of the pancreatic beta cell and cause the formation of Ca^{2+} complex in the neuronal cell to cause the anti-depressive effect. So the agomelatine is the key drug for the diabetes as well as depressive disorder, by work as an antidiabetic and antidepressive drug.

Diabetes type-2 research in the past few years has impressively advanced our understanding about molecular mechanisms and physiological function of the insulin secretion. Studies have established roles of several receptors involved in the regulation of the pathway which controls secretion of insulin through an increasing Ca^{2+} level in the cytoplasm of pancreatic beta cells. Several important pathways have been identified which may play vital role in the regulation of insulin secretion. However, the signalling cascade meets on MTr, and IP_3R receptor which initiates the signalling of IP_3 and Ca^{2+} responsible for the secretion of insulin. Crystal structures of ORAI-1domain of CRAC which is present on the cell membrane and STIM-1 forming a complex provides valuable insights about various residues involved in intermolecular contacts between the two proteins. From the literature, binding of STIM-1 motif containing proteins have been identified to interact with ORAI-1 and stimulate the store operated Ca channel and caused influx of Ca^{2+} in the cytoplasm.

However, Regulation of Ca^{2+} by SERCA receptor thapsigargin has evolved and caused the inhibition of Ca^{2+} into the ER from the cytoplasm. 2-APB can inhibit the IP_3R which is present on the ER's membrane and caused the prohibition of binding of IP_3 to IP_3R, hence proved IP_3 binds to IP_3R and stimulate the IP_3 associated Ca channel to secrete Ca^{2+} from ER. Detailed *in silico* analysis was performed and binding of agomelatin, luzindole, 2-APB, thapsigargin drugs and IP_3, STIM-1 was tested on MTr, IP_3R, SERCA and ORAI-1 of CRAC. The possible involvement of the agomelatine in the signalling cascade of melatonin has gained growing interest in the last years. There is obviously no uniform pathway, and a number of authors suggest different mechanisms. Stimulatory and inhibitory effects of agomelatine and luzindole respectively on IP_3 release seem to be dependent on cell type, subtype of the melatonin receptor, availability of Gq-proteins and downstream signalling cascade proteins. agomelatine or its physiological counterpart melatonin is well-characterized stimulants that mediate physiological functions of the pancreatic system. It is a well-established fact that melatonin and its analogue stimulates insulin secretion in pancreatic b-cells by activation of muscarinic acetylcholine receptors, PLC-b, IP_3 release and, ultimately, the elevation of Ca^{2+} in the cytoplasm. Thapsigargin show indirectly positive effect in the increase of Ca^{2+}, through inhibition of SERCA receptor in the ER. In the present study, both agomelatine and thapsigargin showed stimulatory effects on Ca^{2+} release of pancreatic cells.

Results from molecular docking of MTr, IP_3R, CRAC and SERCA domain using 4 ligands and 2 natural active domains suggest that Mtr-agomelatine showed the lowest binding energy in KJ/Mol. This suggests that MTr-agomleatine possesses the highest binding affinity and lowest dissociation. It means that MTr-agomleatine will cause to the formation of PLC which can participate in the production of IP_3 from PIP2. However, apart from this complex, other ligands also gave lower binding energy such as IP_3R-2-APB and IP_3 which could also be considered as additional ligands which could possess the potential to stimulate and inhibit the influx of Ca^{2+} from the ER to the cytoplasm. SERCA-thapsigargin also shows the lowest binding energy in KJ/Mol, suggests that inhibition of entry of Ca^{2+} into ER from Cytoplasm. ORAI-1 of CRAC–STIM-1 lowest binding energy in KJ/mole suggests that activation of SOC to entry of Ca^{2+} into the cytoplasm; 2-APB also has a small role in the activation of SOC according to concentration of it.

The present study of the combinations of drugs was performed for the first time in pancreatic beta cell for the secretion of insulin. From the study, the regulation of insulin secretion depends on the some main receptors. Finally the ligand molecules which could have great potential to activate the pathway to increase Ca^{2+} in the cytoplasm were tested insilico using tools. Results confirmed that if agomelatine, thapsigargin and 2-APB were used in combination therapy they could increase the secretion amount of insulin in Diabetic type-2 patient and also useful for the depressive disorder because of the antidepressive activity of the agomelatine.

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