Transcriptome signatures reveal candidate key genes in the peripheral blood mononuclear cells of patients with coronary artery disease and prediction of small drug molecules

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Research Article

Abstract

Coronary artery disease (CAD) is one of the most common disorders in the cardiovascular system. This study aims to explore potential signaling pathways and important biomarkers that drive CAD development. The CAD GEO Dataset GSE113079 was featured to screen differentially expressed genes (DEGs). The pathway and Gene Ontology (GO) enrichment analysis of DEGs were analyzed using the ToppGene. We screened hub and target genes from protein-protein interaction (PPI) networks, target gene - miRNA regulatory network and target gene - TF regulatory network, and Cytoscape software. Validations of hub genes were performed to evaluate their potential prognostic and diagnostic value for CAD. A final, molecular docking study was performed. 1,036 DEGs were captured according to screening criteria (525upregulated genes and 511downregulated genes). Pathway and Gene Ontology (GO) enrichment analysis of DEGs revealed that these up and down regulated genes are mainly enriched in thyronamine and iodothyronamine metabolism, cytokine-cytokine receptor interaction, nervous system process, cell cycle and nuclear membrane. Hub genes were validated to find out potential prognostic biomarkers, diagnostic biomarkers and novel therapeutic target for CAD. A small drug molecule was predicted. In summary, our findings discovered pivotal gene expression signatures and signaling pathways in the progression of CAD. CAPN13, ACTBL2, ERBB3, GATA4, GNB4, NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 might contribute to the progression of CAD, which could have potential as biomarkers or therapeutic targets for CAD.

Introduction

Coronary artery disease (CAD) remains the one of leading healthy issues worldwide and 23.3 million people will die of CAD by 2030 [1]. The risk factors for CAD mainly smoking, high blood pressure, high blood cholesterol levels, diabetes, overweight or obesity, physical inactivity, high stress and unhealthy diet [2]. At present, surgery has been applied to improve survival of CAD patients [3]. However, the molecular pathogenesis of CAD advancement is still largely unknown.

As an inventive and high-throughput investigation facilitate the concurrent analysis of expression changes in thousands of genes in CAD samples and contributes to diagnosis, prognosis and new drug discovery [4]. In current years, there have been huge research on the molecular pathogenesis of CAD occurrence and progression by finding and analyzing differentially expressed genes (DEGs) with microarray technologies. Genes such as human paraoxonase/arylesterase (HUMPONA) [5], apolipoprotein E (apo E) [6], MMP-2, MMP-3, MMP-9 and MMP-12 [7], endothelial nitric oxide synthase (eNOS) [8] and angiotensin II type 1 (AT1) receptor [9] were associated with CAD progression. Signaling pathway such TLR4 signaling pathway [10], mTOR signaling pathway [11], CXCR4 signaling pathway [12], eNOS-activating pathways [13] and Akt pathway [14] were involved in development of CAD. Presently, a combination of gene expression profiling and bioinformatics analysis allows us to comprehensively detect mRNA expression changes in CAD and subsequently identify hub genes, target genes and pathways that exist in the protein - protein interaction network (PPI), target gene - miRNA regulatory network and target gene - TF regulatory network of differentially expressed genes.

In the present study, gene expression dataset GSE113079 was downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) [15], which is a public functional genomics data. DEGs were diagnosed by the comparison of CAD and normal tissue based on R software. Pathway and gene ontology (GO) enrichment analysis, PPI network and module analysis, target gene - miRNA regulatory network and target gene - TF regulatory network analysis. Hub genes were validated. Finally, small drug molecules was predicted.

Materials And Methods

Microarray data and data preprocessing

Raw expression profile data from the Agilent microarray file GSE113079 [16] were downloaded from the public NCBIGEO database and executed on the GPL20115 platform. GSE113079 contains 93 CAD patients and 48 healthy controls. The raw expression files of microarray dataset was pre-processed according to the loess and quantile method [17] and probe identification numbers were converted to gene symbols using as a reference the Agilent-067406 Human CBC lncRNA + mRNA microarray V4.0 (Probe name version). When multiple probes compare to the same gene, the probe with the high p value from the downstream differential analysis was picked to resolve the differential gene expression value.

Identification of DEGs

The linear models for microarray data Limma package in Bioconductor [18] was used to identify DEGs by comparing the expression values between peripheral blood mononuclear cells from CAD patients and peripheral blood mononuclear cells from healthy control. The corresponding P value of the gene symbols after t test were used, and adjusted P < 0.05 and |logFC| > 0.97 for up regulated genes, and |logFC| < -0.963 for down regulated genes were used as the selection criteria.

Pathway enrichment analysesof DEGs

BIOCYC (https://biocyc.org/) [19], Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) [20], Pathway Interaction Database (PID, http://pid.nci.nih.gov/) [21], Reactome (https://reactome.org/PathwayBrowser/) [22], Molecular signatures database (MSigDB, http://software.broadinstitute.org/gsea/msigdb/) [23], GenMAPP (http://www.genmapp.org/) [24], Pathway Ontology (https://bioportal.bioontology.org/ontologies/PW) [25], PantherDB (http://www.pantherdb.org/) [26] and Small Molecule Pathway Database (SMPDB) (http://smpdb.ca/) [27] are a databases resource for understanding high-level functions and biological systems from large-scale molecular datasets generated by high-throughput experimental technologies. The ToppGene (ToppFun) (https://toppgene.cchmc.org/enrichment.jsp) [28] in online tool was used to perform the pathway enrichment analyses of the DEGs. P < 0.05 was considered statistically significant.

Gene ontology (GO) enrichment analysis of DEGs

The GO (http://www.geneontology.org/) [29] is a represented terminology of terms defines gene products according to the biological process (BP), molecular function (MF), and cellular component (CC). We used ToppGene (ToppFun) (https://toppgene.cchmc.org/enrichment.jsp) [28], a web-accessible program that integrates functional genomic annotations, to view the GO enrichment of DEGs; a p value <0.05 was considered statistically significant.

PPI network construction and module analysis

STRING (https://string-db.org/) [30] is a protein-protein interaction (PPI) network analysis online tool. The current version of the STRING PPI database is 11.0, which screen more than 5,090 species and 24.6 million proteins and holds the upload of genome level data sets. To resolve which proteins encoded by the DEGs acts a dominant role in CAD, the DEGs were applied to STRING v.11.0 with medium confidence scores of 0.4. To find the hub genes, we visualized the PPI network using Cytoscape v.3.7.2 software (http://www.cytoscape.org/) [31] and analyzed the topological properties of these nodes using the Network Analyzer tool. Then we selected the nodes with high degrees centrality [32], high betweenness centrality [33], high stress centrality [34], high closeness centrality [35] and low clustering coefficient [36] values as hub genes. The PEWCC1 [37] built in Cytoscape is an automated method that was used to evaluate highly interconnected modules as molecular complexes or clusters. The analysis parameters were establish by default. The pathway and GO enrichment analysis was executed for DEGs, from which four significant modules of genes were diagnosed with p < 0.05 set as the threshold.

Construction of target gene - miRNA regulatory network

The NetworkAnalyst (https://www.networkanalyst.ca/) [38] online platform was used combine the results of mRNA (DEGs) with known miRNAs of human to construct the target gene - miRNA network and to predict target genes with differential expression miRNAs. In addition, we predicted the target genes for miRNAs using two online software: DIANA-TarBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) [39] and miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/php/download.php) [40]. All 3 procedural predicted genes were selected as targets for DEGs to construct differentially expressed miRNA. Target genes were arranged into the miRNA regulatory network separately to access each miRNA regulatory network which were visualized using Cytoscape (http://www.cytoscape.org/) [31]. DEGs (up and down regulated) interaction with more number of miRNAs consider as target genes.

Construction of target gene - TF regulatory network

Transcription factor gene data of the NetworkAnalyst (https://www.networkanalyst.ca/) [38] was used to identify the transcription factor regulatory networks linked with the target genes. The NetworkAnalyst describes transcription factor (TF) to genes from the perspective of ChEA database (http://amp.pharm.mssm.edu/lib/chea.jsp) [41] database resource. The NetworkAnalyst illustrate a more extensive transcription factor regulation network. Target genes were arranged into the TF regulatory network separately to access each transcription factor regulatory network which were visualized using

Cytoscape (http://www.cytoscape.org/) [31]. DEGs (up and down regulated) interaction with more number of TFs consider as target genes.

Validations of hub genes

The human protein atlas database (HPA) (www.proteinatlas.org) [42] was used to analyze protein expression of hub genes in peripheral blood mononuclear cells in bone marrow. A receiver operating characteristic (ROC) curve was produce using the pROC package of the R software [43], and the area under the curve (AUC) was determined using generalized linear model (GLM) in machine learning algorithms to assess the predictive accuracy of hub genes.

Molecular docking studies

Surflex-Docking docking studies were conducted on active components by using SYBYL-X 2.0,perpetual software module. The molecules were sketched using ChemDraw software and were saved into sdf. format using Open Babel free software by importing. The genes of over expressed genes of ACTBL2 (Actin beta-like 2), CAPN13 (Calpain 13), ERBB3 (Erythroblasticleukemia viral oncogene homolog 3), GATA4 (GATA binding protein 4), GNB4 (Guanine nucleotide binding protein beta polypeptide 4) and their X-RAY crystallographic structure and co-crystallized PDB code 2FF3, 2I7A, 3LMG, 3DFV and 6UQ3 respectively were selected for docking and were extracted from Protein Data Bank [44-48]. Optimization of the designed molecules was done by transforming the 3D concord structure, applying TRIPOS force field and applying GasteigerHuckel (GH) charges, In addition, MMFF94s and MMFF94 algorithm processes have been used for energy minimization. Protein processing was performed after introduction of the protein. The co-crystallized ligand and all the water molecule were ejected from the crystal structure; added more of hydrogen and refined the side chain. To minimize structure complexity, the TRIPOS force field was used and the interaction efficiency of the compounds with the receptor was represented by the Surflex-Dock score in kcal/mol units. The best position was inserted into the molecular area between the protein and the ligand. Using Discovery Studio Visualizer, the simulation of ligand interaction with receptors is accomplished.

Results

Data preprocessing and identification of DEGs

The gene expression profile with accession numbers GSE113079 was downloaded from GEO database. The results of before and after normalizing the microarray gene expression are shown in Fig. 1A and Fig. 1B. DEGs between peripheral blood mononuclear cells from CAD patients and peripheral blood mononuclear cells from healthy control were screened using limma package in R bioconductor (P-value <0.05, |logFC| > 0.97 for up regulated genes, and |logFC| < - 0.963 for down regulated genes). In this study, 1,036 total DEGs (525 up regulated genes and 511 down regulated genes, respectively) in GSE113079 was screened. The total number of DEGs collected for each subject in the differential gene expression

analysis is given in Table 1. A volcano diagram was constructed for the DEGs and is shown in Fig. 2. The DEGs (up and down regulated genes) are presented by a cluster heatmap in Fig. 3A and Fig. 3B.

Pathway enrichment analysesof DEGs

Pathway enrichment analyses were performed using ToppGene, analyzing the pathway classification of DEGs (up and down regulated genes). Pathways of up regulated were mainly enriched in thyronamine and iodothyronamine metabolism, trehalose degradation, cytokine-cytokine receptor interaction, Olfactory transduction, FOXA1 transcription factor network, lissencephaly gene (LIS1) in neuronal migration and development, signaling by GPCR, GPCR downstream signaling, C21 seroid hormone metabolism, androgen and estrogen metabolism, ensemble of genes encoding extracellular matrix and extracellular matrix-associated proteins, ensemble of genes encoding ECM-associated proteins including ECM-affilaited proteins, ECM regulators and secreted factors, cortocotropin releasing factor receptor signalingpathway, 5HT4 type receptor mediated signaling pathway, corticotropin-releasing hormone signaling, G protein signaling via galphaq family, ornithine transcarbamylase deficiency (OTC deficiency and intracellular signaling through PGD2 receptor and prostaglandin D2 according to the BIOCYC, KEGG, PID, Reactome, MSigDB, GenMAPP, Pathway Ontology, PantherDB and SMPDB pathway analysis results (Table 2), whereas pathways of down regulated were mainly enriched in inosine-5'-phosphate biosynthesis, sulfate activation for sulfonation, antigen processing and presentation, graft-versus-host disease, Fc-epsilon receptor I signaling in mast cells, TGF-beta receptor signaling, signaling by interleukins, generic transcription pathway, glycosaminoglycan degradation, sterol biosynthesis, ras-independent pathway in NK cell-mediated cytotoxicity, hypoxia and p53 in the cardiovascular system, FAS signaling pathway, angiogenesis, pathway of folate cycle/metabolism, vascular endothelial growth factor signaling, sarcosinemia and purine metabolism according to the BIOCYC, KEGG, PID, Reactome, MSigDB, GenMAPP, Pathway Ontology, PantherDB and SMPDB pathway analysis results (Table 3).

Gene ontology (GO) enrichment analysis of DEGs

The Gene Ontology (GO) enrichment analyses were conducted using online tool ToppGene. GO terms of the up regulated and down regulated genes s were listed in Table 4 and Table 5, respectively. Gene Ontology (GO) enrichment analysis showed that the up regulated genes were mainly associated with nervous system process, G protein-coupled receptor signaling pathway, intrinsic component of plasma membrane, extracellular matrix, transmembranesignaling receptor activity and receptor regulator activity. The down regulated genes were mainly associated with cell cycle, regulation of immune system process, nuclear membrane, nuclear chromatin, DNA-binding transcription factor activity, RNA polymerase II-specific and signaling receptor binding.

PPI network construction and module analysis

The PPI network of the up and down regulated genes was analyzed by using online STRING database. A total of 3715 nodes with 6518 edges were reflected in PPI network of up regulated genes is shown in Fig 4A. CAPN13, EGFR, ACTBL2, ACTL8, ERBB3, PRMT5, GATA4, RHOV, CHD5, MAGEL2, THNSL2, SLC38A8,

THPO and SPTSSB were the hub genes with high node degree distribution, betweenness centrality, stress centrality, closeness centrality and low clustering coefficient in the network are listed in Table 6. A total of 5135 nodes with 10628 edges were reflected in PPI network of down regulated genes is shown in Fig 4B. FYN, PAK2, CUL3, RPS6, NOTCH2, PDE4D, SPATA21, MYBL1, SMURF1, PDGFRB, DLG3, ADHFE1, NMB, SLC25A36, MLLT1 and RNF2 were the hub genes with high node degree distribution, betweenness centrality, stress centrality, closeness centrality and low clustring coefficient in the network are listed in Table 6.

Four significant modules were selected for each up and down regulated genes using the PEWCC1E plug-in. The top four modules for up regulated genes were selected (module 13, 105 nodes and 235 edges; module 20, 77 nodes and 97 edges; module 21, 73 nodes and 81 edges; module 34, 53 nodes and 58 edges) are shown in Fig. 5A. The results revealed that hub genes (ACTG2, GATA4, EGFR, TP73, ACTBL2, FOXJ1, BMP7 and CDK5R2) in these significant modules were mostly enriched in the muscle contraction, notch-mediated HES/HEY network, cytokine-cytokine receptor interaction, E2F transcription factor network, actin cytoskeleton, epithelial cell differentiation, biological adhesion and neuron projection. Similarly, top four modules for down regulated genes were selected (module 1, 92 nodes and 186 edges; module 2, 56 nodes and 187 edges; module 5, 49 nodes and 144 edges; module 11, 29 nodes and 57 edges) are shown in Fig. 5B. The results revealed that hub genes (RPS6, PAK2, PODN, LMNA, EIF1AX, RPS27, HSPA8, FYN and LMNB1) in these significant modules were mostly enriched in the mTOR signaling pathway, Fc-epsilon receptor I signaling in mast cells, ensemble of genes encoding extracellular matrix and extracellular matrix-associated proteins, caspase cascade in apoptosis, postsynapse, cell cycle, regulation of immune system process and positive regulation of signal transduction.

Construction of target gene - miRNA regulatory network

NetworkAnalyst was applied to screen the miRNAs of the up and down regulated genes. The miRNAs predicted by at least two databases (among the following databases: DIANA-TarBase and miRTarBase) were diagnosed as the miRNAs of the target genes. Then, Cytoscape software was used to draw the target gene - miRNA regulatory network. The target gene - miRNA regulatory network for up regulated genes included 1867 nodes and 3735 edges (Fig. 6A). As shown in Table 7, TRIM72 regulates 123 miRNAs (ex,hsa-mir-4537), TET3 regulates 105 miRNAs (ex,hsa-mir-3148), NFIB regulates 89 miRNAs (ex,hsa-mir-4517), SLC19A3 regulates 80 miRNAs (ex,hsa-mir-4500) and SMOC1 regulates 123 miRNAs (ex,hsa-mir-6133) were considered as target gene. We performed pathway and GO enrichment analysis of these predicted target genes, which mainly enriched in muscle contraction, FOXA1 transcription factor network, intrinsic component of plasma membrane and biological adhesion. The target gene - miRNA regulatory network for down regulated genes included 2529 nodes and 10243 edges (Fig. 6B). As shown in Table 7, PPP1R15B regulates 168 miRNAs (ex, hsa-mir-7150), WEE1 regulates 167 miRNAs (ex,hsa-mir-3926), RPRD2 regulates 152 miRNAs (ex,hsa-mir-4452), LCOR regulates 146 miRNAs (ex,hsa-mir-4310) and SAR1A regulates 145 miRNAs (ex,hsa-mir-5698) were considered as target gene. We performed pathway and GO enrichment analysis of these predicted target genes, which mainly enriched in regulation

of hydrolase activity, cell cycle, gene expression, nuclear chromatin and protein processing in endoplasmic reticulum.

Construction of target gene - TF regulatory network

NetworkAnalyst was applied to screen the TFs of the up and down regulated genes. The TFs predicted by database (ChEA database) was diagnosed as the TFs of the target genes. Then, Cytoscape software was used to draw the target gene - TF regulatory network. The target gene - TF regulatory network for up regulated genes included 539 nodes and 5790 edges (Fig. 7A). As shown in Table 8, ACTL8 regulates 145 TFs (ex, EGR1), LHFPL3 regulates 132 TFs (ex,SOX2), CXCL12 regulates 119 TFs (ex,SUZ12), GLI2 regulates 117 TFs (ex, AR) and C7 regulates 114 TFs (ex, TP53) were considered as target gene. We performed pathway and GO enrichment analysis of these predicted target genes, which mainly enriched in epithelial cell differentiation, cytokine-cytokine receptor interaction, pathways in cancer and innate immune system. The target gene - TF regulatory network for down regulated genes included 608 nodes and 10262 edges (Fig. 7B). As shown in Table 8, PRIM2 regulates 218 TFs (ex, SOX2), regulates 211 TFs (ex, MYC), GMDS regulates 210 TFs (ex, SPI1), C5ORF58 regulates 190 TFs (ex, RUNX1) and C10orf88 regulates 180 TFs (ex, FLI1) were considered as target gene. We performed pathway and GO enrichment analysis of these predicted target genes, which mainly enriched in metabolic pathways, gene expression, asparagine N-linked glycosylation and cell cycle.

Validations of hub genes

The ten hub genes (up and down regulated) were selected for further validation of their potential prognostic value. Upon comparing the expression of hub genes in the human protein atlas database (Fig. 8), it showed that CAPN13, ACTBL2, ERBB3, GATA4 and GNB4 were highly expressed in peripheral blood mononuclear cells of bone marrow, while NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 were less expressed in peripheral blood mononuclear cells of bone marrow ROC analysis was performed from the 10 hub genes from GSE113079. The ROC curves of these ten hub genes all indicated favorable prognostic values for CAD. In addition, the area under ROC curve (AUC) of CAPN13, ACTBL2, ERBB3, GATA4, GNB4, NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 were 0.855 (p = 2.406664e-08), 0.923 (p = 5.413565e-10), 0.829 (p =2.857413e-08), 0.903 (p = 4.513268e-09), 0.918 (p = 6.358925e-09), 0.891 (p = 4.367291e-09), 0.927 (p = 1.344048e-10), 0.911 (p = 5.076899e-10), 0.892 (3.057148e-08) and 0.904 (p = 1.902203e-08), respectively (Fig. 9).

Molecular docking studies

The prevailing work aimsto discover the significant interactions responsible for complex stability with the receptor of the binding sites by docking studies. The docking studies was executed using Sybyl X 2.1 software on designed molecules which includes derivatives of dihydropyridine heterocyclic nucleus found in amlodipine a beta blocker normally used in in coronary artery disease. Beta-blockers suppress the heart's sympathetic activation, decreasing heart rate and contractility that lower the need for myocardial oxygen and thereby prevent or alleviate angina in CAD patients. Since beta-blockers suppress the heart

rate during exercise, the initiation of angina or the ischemic threshold is postponed or stopped during exercise. In the treatment of exertional angina, all forms of beta-blockers tend to be equally successful. Based on the structure of amlodipine containing dihydropyridine heterocyclic nucleus the molecules containing dihydropyridine are designed to identify for docking studies in the present research. A total of 34 common dihydropyridine derivatives were developed and amlodipine was used as a standard for docking studies on over-expressed proteins, and the structures are shown in Fig.10, respectively. The one protein from each over expressed genes in coronary artery diseases such as ACTBL2 (Actin beta-like 2), CAPN13 (Calpain 13), ERBB3 (Erythroblasticleukemia viral oncogene homolog 3), GATA4 (GATA binding protein 4), GNB4 (Guanine nucleotide binding protein beta polypeptide 4)and their X-RAY crystallographic structure and co-crystallized PDB code 2FF3, 2I7A, 3LMG, 3DFV and 6UQ3 respectively were constructed for docking. To identify the potential molecule and its binding affinity to proteins, the docking was carried out on built molecules. It is said that the molecule with C-score greater than 5 are active and few molecules with particular proteins obtained greater than 8 respectively. Docking experiments were carried out on a total of 34 designed molecules, few obtained an outstanding C-score greater than 8 and few molecules obtained an optimum binding score of 4-4.9 then obtained less binding sore of 2.0-3.0 respectively. The molecule IM1, IM4, IM7, IM9, IM10,IM11, IM12, IM13, IM14, IM15, IM16, TZ19, TZ21, TZ25, TZ26, TZ28, TZ29 and IM1, IM9, IM10, IM11, IM13, TZ26 and IM12 with protein of PDB code 2FF3 and 3LMG and 3DFV respectively obtained excellent binding score of more than 7.Good binding score of 5 to 6.99 obtained from the molecules areIM2, IM3, IM5, IM6, IM8, IM17, TZ18, TZ20, TZ22, TZ23, TZ24, TZ27, TZ30, TZ31, TZ32, TZ33, TZ34 and IM7, IM11, IM12, TZ27 and IM2, IM3, IM4, IM5, IM6, IM7, IM8, IM12, IM14, IM16, IM17, TZ18, TZ19, TZ20, TZ21, TZ22, TZ23, TZ24, TZ25, TZ28, TZ29, TZ30, TZ31, TZ32, TZ33 and IM1, IM2, IM3, IM4, IM6, IM7, IM8, IM9, IM10, IM11, IM13, IM14, IM16, TZ18, TZ20, TZ23, TZ24, TZ26, TZ27, TZ28, TZ29 and IM7, IM8, IM10, IM11, IM12, IM13, IM13, IM16, TZ23, TZ25, TZ26 with PDB protein of 2FF3, 2I7A, 3LMG, 3DFV and 6UQ3 respectively. Molecules with optimum binding score are IM1, IM2, IM3, IM4, IM5, IM6, IM8, IM9, IM10, IM13, IM14, IM15, IM16, IM17, TZ18, TZ19, TZ20, TZ21, TZ22, TZ23, TZ24, TZ25, TZ26, TZ28, TZ29, TZ30, TZ31, TZ32, TZ33, TZ34 and TZ27, TZ34 and IM5, IM15, IM17, TZ19, TZ21, TZ22, TZ25, TZ30, TZ31, TZ32, TZ33, TZ34 and IM1, IM2, IM3, IM4, IM5, IM6, IM9, IM14, IM15, IM17, TZ18, TZ19, TZ20, TZ21, TZ22, TZ24, TZ27, TZ28, TZ29, TZ30, TZ31, TZ32, TZ33, TZ34with PDB code of 2I7A, 3LMG, 3DFV and 6UQ3 and the molecule IM7 obtained highest binding score of 9.00 greater than the standard amlodipine with PDB 2FF3 respectively the values are depicted in Table 9. The standard amlodipine obtained good binding score with 3LMG, 2FF3 and 6UQ3, and obtained optimum binding score with PDB 2I7A and 3DFV respectively. The Fig. 11 and Fig. 12 depicts 3D hydrogen bonding interactions of lignd with Protein, with aminoacids and other bonding interactions with amino acids and Fig. 13 depicts the 2D interactions with amino acids and their distance with protein code 2FF3 of molecule IM7 are depicted by 3D and 2D respectively.

Discussion

Currently, genetic and genomics related researches progress rapidly and provide new viewpoint to illuminate the molecular pathogenesis of CAD. And bioinformatics analysis also has show and devotes to

search for candidate biomarkers to provide more precise screening, prompt diagnosis, sophisticated prognostic and new therapeutic targets for CAD based on massive genetic and genomics data [49]. In the present study, 1,036 DEGs were identified in the CAD group compared with normal control samples, including 525 up regulated genes and 511 down regulated gene. Genes such as PTGDS (prostaglandin D2 synthase) [50] and PDE4D [51] were responsible for development of stroke. Oncostatin M receptor (OSMR) was liable for progression of atherosclerosis [52]. Genes such as SLC19A3 [53] and RCN2 [54] were liable for progression of diabetes, but these genes may be responsible for advancement of CAD. Genes such as KLKB1 [55], PRMT5 [56], F2R [57] and IL18RAP [58] were liable for progression of CAD. AKAP5 was associated with progression of hypertension [59], but this gene may be identified with progression of CAD.

Some of the DEGs enriched in pathways from different pathway databases. DIO2 was linked with development of hypertension [60], but this gene may be responsible for progression CAD. Genes such as CCR2 [61], CCL19 [62], CX3CL1 [63], CXCL12 [64], IL20 [65], epidermal growth factor receptor (EGFR) [66], ERBB3 [67], adrenomedullin (ADM) [68], SCUBE1 [69], LMAN1L [70] and EGFL7 [71] were responsible for pathogenesis of CAD. Genes such as CXCL6 [72], BMP7 [73], RXFP2 [74], BRS3 [75], FFAR3 [76], neuropeptide B (NPB) [77], SPON2 [78], FCN3 [79], REG3A [80] and ornithine carbamoyltransferase (OTC) [81] were culpable for pathogenesis of diabetes, but these genes might be involved in development of CAD. Genes such as COL18A1 [82], cortistatin (CORT) [83], guanine nucleotide binding protein (G protein) [84] and MUC2 [85] were involved in development of obesity, but these genes might be associated with pathogenesis of CAD. Genes such as ADRA1A [86], corticotropin releasing hormone (CRH) [87], CRHR1 [88], GRIN1 [89], HSD3B1 [90] and nerve growth factor (beta polypeptide) (NGF) [91] were answerable for progression of hypertension, but these genes might be linked with development of CAD. ADAMTS2 was associated with progression of myocardial infarction [92], but this gene might be liable for progression of CAD. CFC1 was responsible for development of congenital cardiac disease [93], but this gene might be associated with progression of CAD. Genes such as HSPA8 [94], HIF1A [95], CCL4 [96], CCL20 [97], IL1B [98], NCAM1 [99], IL18R1 [100], CXCL1 [101], CXCL2 [102], oncostatin M (OSM) [103], CD80 [104], IL27 [105] and lamin A/C (LMNA) [106] were liable for progression of CAD. Genes such as KIR2DL2 [107], KIR3DL1 [108], KLRC3 [109], KLRD1 [110], PIK3R1 [111] and PAK2 [112] were involved in the progression of diabetes, but these genes might be linked with progression of CAD. MAP2K4 was liable for progression of ischemic stroke [113]. Genes such as S1PR1 [114] and CUL3 [115] were involved in progression of hypertension, but these genes may be associated with development of CAD. RAR-related orphan receptor A (RORA) was linked with development of obesity [116], but these genes might be involved in pathogenesis of CAD.

Some of the DEGs enriched in GO terms. Genes such as noggin (NOG) [117], very low density lipoprotein receptor (VLDLR) [118] and AQP10 [119] were responsible for progression of obesity, but these genes might be involved in development of CAD. Genes such as TRPM5 [120], crystallin, alpha A (CRYAA) [121], PAX6 [122], SORBS1 [123], SLC38A1 [124], complement component 7 (C7) [125] and PAX8 [126] were linked with advancement of diabetes, but these genes might be associated with pathogenesis of CAD. Genes such as KCNJ11 [127], PKD2L1 [128], CSMD1 [129], SLC6A2 [130] and ATP2B3 [131] were liable

for advancement of hypertension, but these genes might be involved in progression CAD. ASGR1 was linked with advancement of CAD [132]. Genes such as CDKN1C [133], NR4A1 [134] and ZNF627 [135] were responsible for progression of myocardial infarction, but these genes might be associated with development of CAD. Genes such as PPP1R15A [136], protein kinase C, theta (PRKCQ) [137], LPIN1 [138], NOTCH2 [139], Shwachman-Bodian-Diamond syndrome (SBDS) [140], SOX13 [141] and FOXP4 [142] were culpable for advancement of diabetes, but these genes might be linked with progression of CAD. Genes such as ABCB1 [143], CAMK2N1 [144], HES1 [145], TNFAIP3 [146], proliferating cell nuclear antigen (PCNA) [147], IKZF2 [148], ZNF208 [149], NRF1 [150], EGR3 [151] and SMAD7 [152] were important for progression of CAD. Filamin A (FLNA) was involved in development of hypertension [153], but this gene might be responsible for progression of CAD. Genes such as PHLDA1 [154], PLK2 [155], IER3 [156] and thymopoietin (TMPO) [157] were identified with development of ischemic cardiomyopathy, but these genes might be involved in progression of CAD. TOR1AIP1 was associated with heart failure [158]. Enriched genes such as CERS6 [159], KLF3 [160] and NUCKS1 [161] were responsible for advancement of obesity, but these genes might be involved in progression of CAD. cAMP responsive element modulator (CREM) was linked with progression of cardiac arrhythmia [162], but this gene might be important for development CAD.

In the PPI network, hub genes with a high node degree distribution, betweenness centrality, stress centrality, closeness centrality and low clustring coefficient were selected. GATA4 was important for progression of CAD [163]. Genes such as MAGEL2 [164], ADHFE1 [165] and neuromedin B (NMB) [166] were associated with development of obesity, but these genes might be liable for progression of CAD. SMURF1 was liable for advancement of hypertension [167], but this might be involved in pathogenesis of CAD. In addition, modules were extracted from PPI network, which involved 17 up regulated genes and 20 down regulated genes. TBX2 was involved in the progression of hypertension [168], but this gene might be associated with development of CAD. Genes such as podocan (PODN) [169] and PAS domain containing serine/threonine kinase (PASK) [170] were liable for progression of diabetes, but these genes might be linked with progression of CAD.

In the target gene - miRNA regulatory network, 5 up regulated genes and 5 down regulated genes with a high node degree was chosen as target gene. TRIM72 was associates with development of cardiac fibrosis [171], but this gene might be liable for development of CAD. TET3 was responsible for progression of CAD [172]. PPP1R15B was important for progression of diabetes [173], but this gene might be involved in advancement of CAD. CAPN13, ACTBL2, ACTL8, ras homolog gene family, member V (RHOV), CHD5, THNSL2, SLC38A8, serine palmitoyltransferase, small subunit B (SPTSSB), SPATA21, DLG3, SLC25A36, ACTG2, ACTL6B and RAS, EF-hand domain containing (RASEF), LHX9, FOXJ1, TP73, CDK5R2, EIF1AX, HNRNPA0, RPS27, LGR6, granzyme B (GZMB), RPRD2 and SAR1A are the novel biomarkers for CAD.

In the target gene - TF regulatory network, 5 up regulated genes and 5 down regulated genes with a high node degree was chosen as target gene. GLI2 was linked with progression of obesity [174], but this gene might be responsible for advancement of CAD. Our study found that LHFPL3 is up regulated in CAD and

has potential as a novel diagnostic and prognostic biomarker, similarly, our study found that EXOSC10, GDP-mannose 4,6-dehydratase (GMDS), C5ORF58 and C10orf88 are down regulated in CAD and has potential as a novel diagnostic and prognostic biomarker, and therapeutic target.

In conclusion, 1,036 DEGs (525 up rand 511 down regulated gene) were screened out from the GSE113079 dataset, which might contain hub genes contributing to the pathogenesis of CAD. Through our bioinformatics analysis, hub genes including CAPN13, ACTBL2, ERBB3, GATA4, GNB4, NOTCH2, EXOSC10, RNF2, PSMA1 and PRKAA1 might contribute to the progression of CAD, which could serve as novel diagnostic and prognostic biomarkers and therapeutic targets for CAD.

Declarations

Acknowledgement

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

No informed consent because this study does not contain human or animals participants.

Availability of data and materials

The datasets supporting the conclusions of this article are available in the GEO (Gene Expression Omnibus) (https://www.ncbi.nlm.nih.gov/geo/) repository. [(GSE113079) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113079)]

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Author Contributions

V. K. - Methodology and validation

B. V. - Writing original draft, and review and editing

C. V. - Software and investigation

I. K. - Supervision and resources

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Tables

Due to technical limitations, Tables 1-9 are only available as a download in the Supplemental Files section.
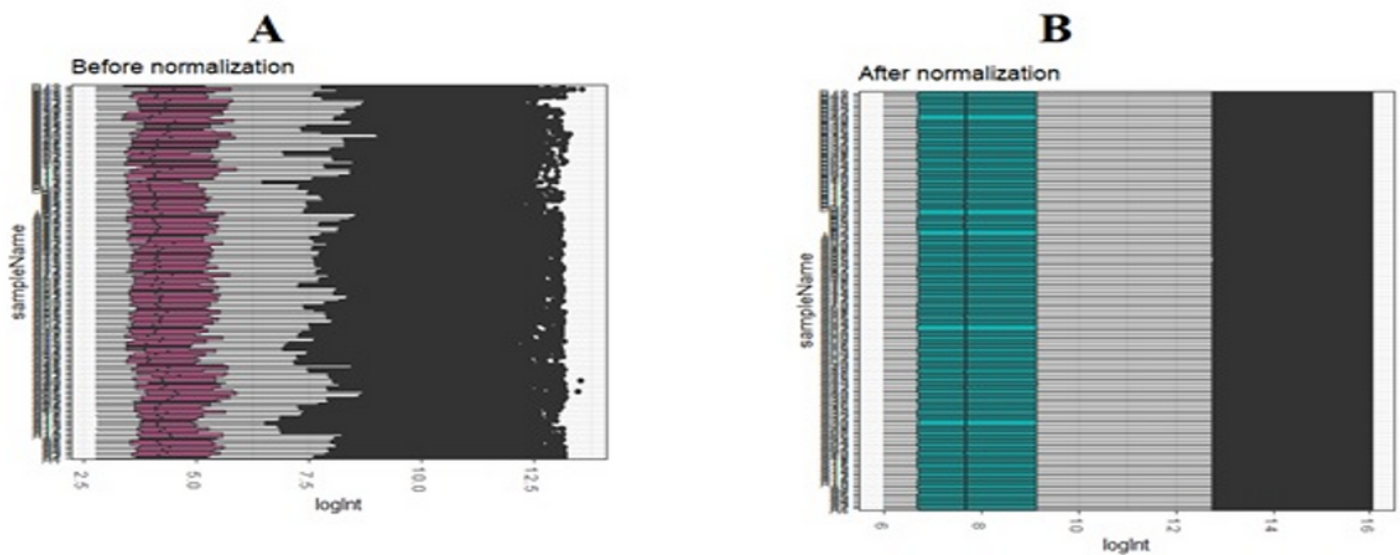
Figures

Figure 1

Box plots of the gene expression data before (A) and after normalization (B). Vertical axis represents the sample symbol and the Horizontal axis represents the gene expression values. The black line in the box plot represents the median value of gene expression. (A1-A48 = healthy controls; B1-B93 = CAD patients)
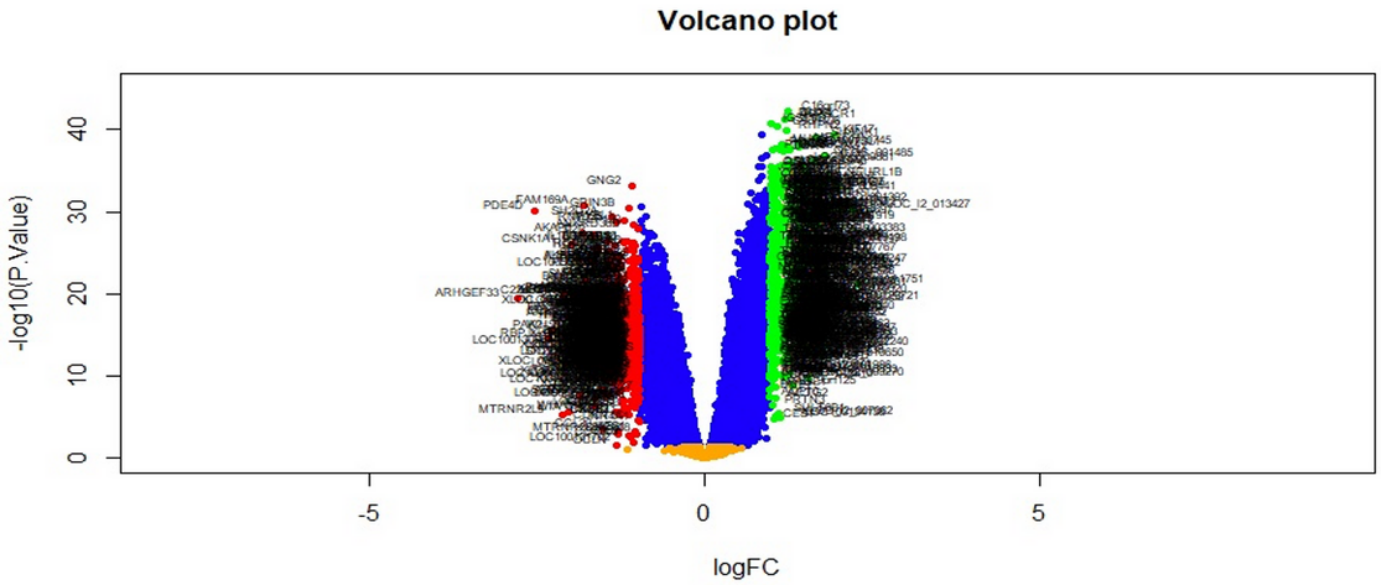
Figure 2

Volcano plot of differentially expressed genes. Genes with a significant change of more than two-fold were selected.
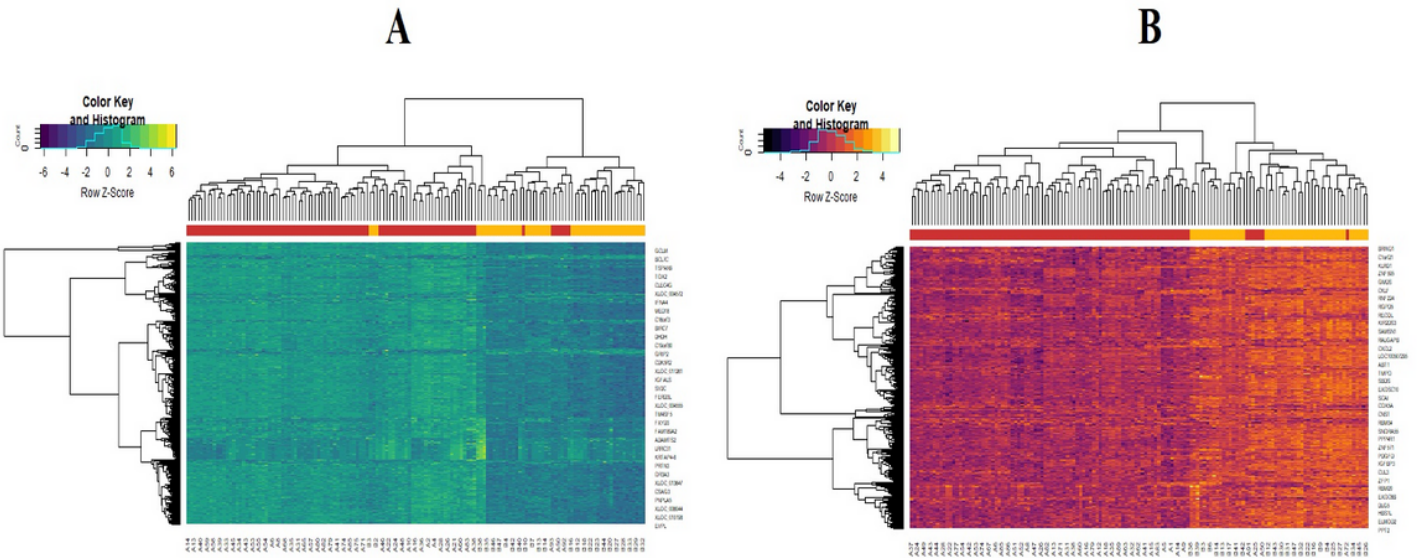
Figure 3

Heat map of (A) up regulated differentially expressed genes (B) down regulated differentially expressed genes. Legend on the top left indicate log fold change of genes. (A1-A48 = healthy controls; B1-B93 = CAD patients)
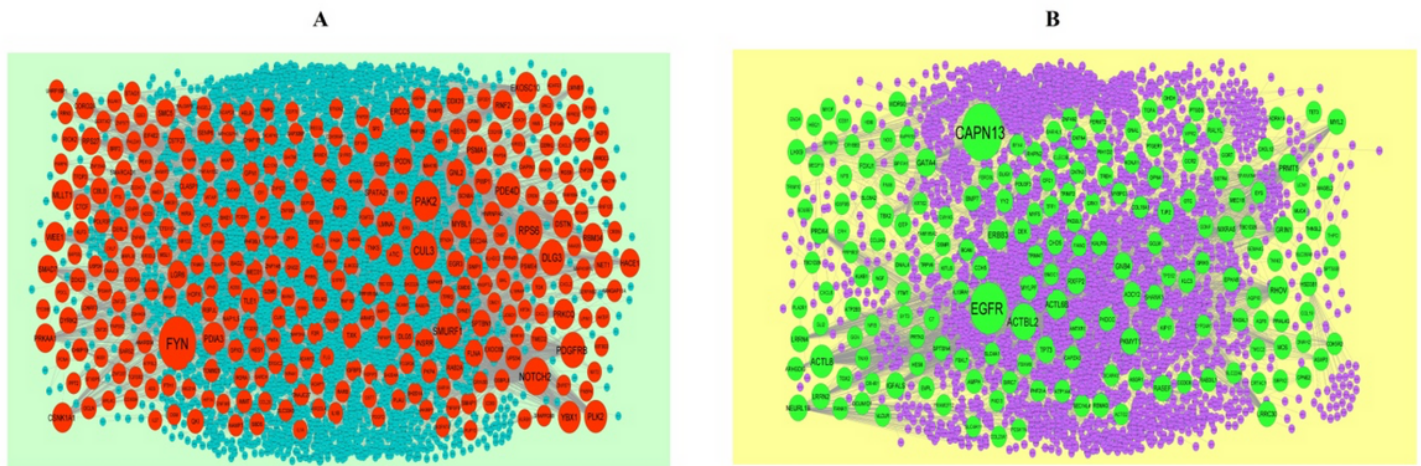
Figure 4

Protein–protein interaction network of (A) up regulated differentially expressed genes (B) down regulated differentially expressed genes. Green nodes denotes up regulated genes and red nodes denotes down regulated genes.
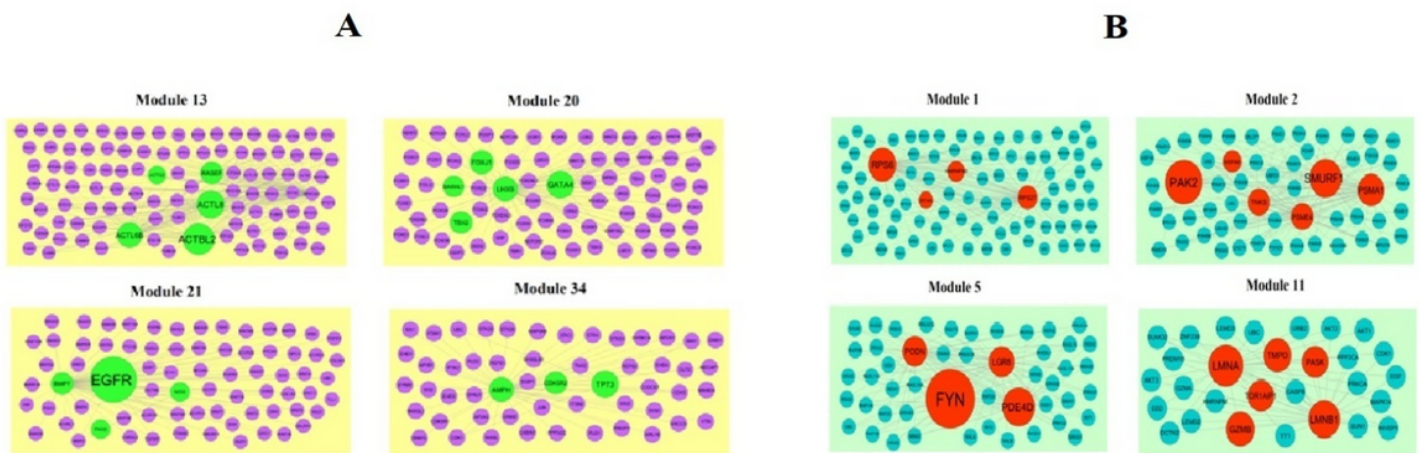
Figure 5

Modules in PPI network. (A) Green nodes denote the up regulated genes (B) Red nodes denote the down regulated genes
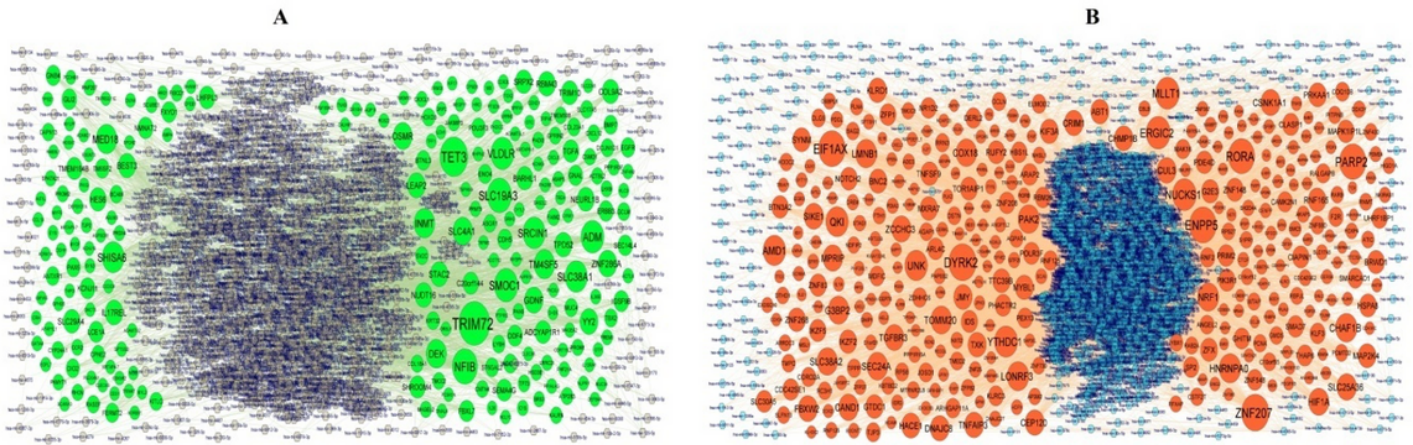
Figure 6

(A) The network of up regulated genes and their related miRNAs. The green circles nodes are the up regulated DEGs and gray diamond nodes are the miRNAs (B) The network of down regulated genes and their related miRNAs. The red circle nodes are the down regulated DEGs and blue diamond nodes are the miRNAs
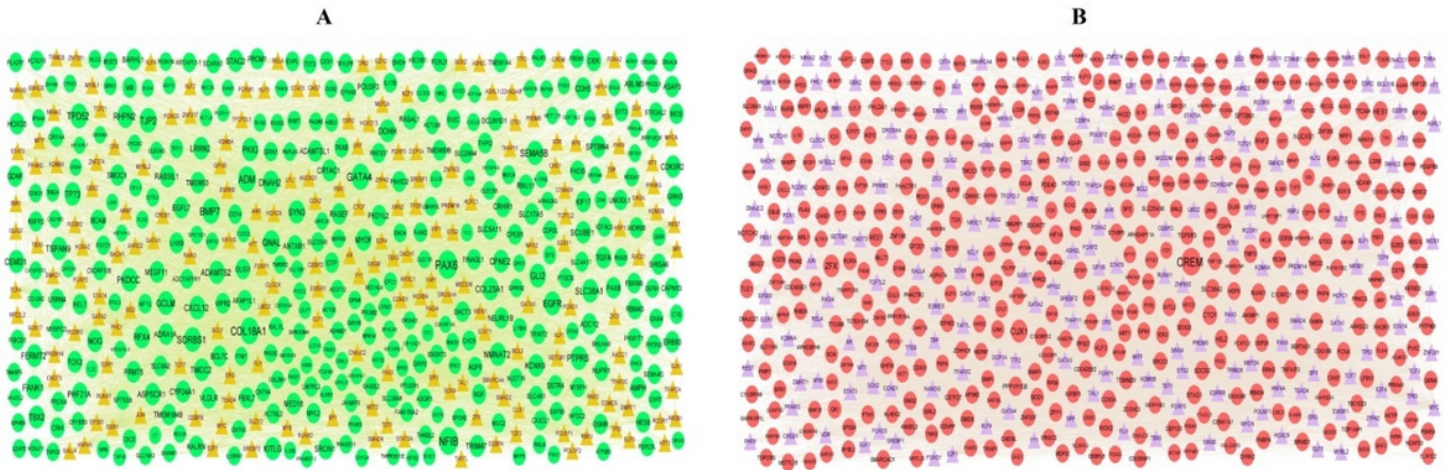
Figure 7

(A) The network of up regulated genes and their related TFs. (Yellow triangle - TFs and green circles-target up regulated genes) (B) The network of down regulated genes and their related TFs. (Purple triangle - TFs and red circles - target down regulated genes)
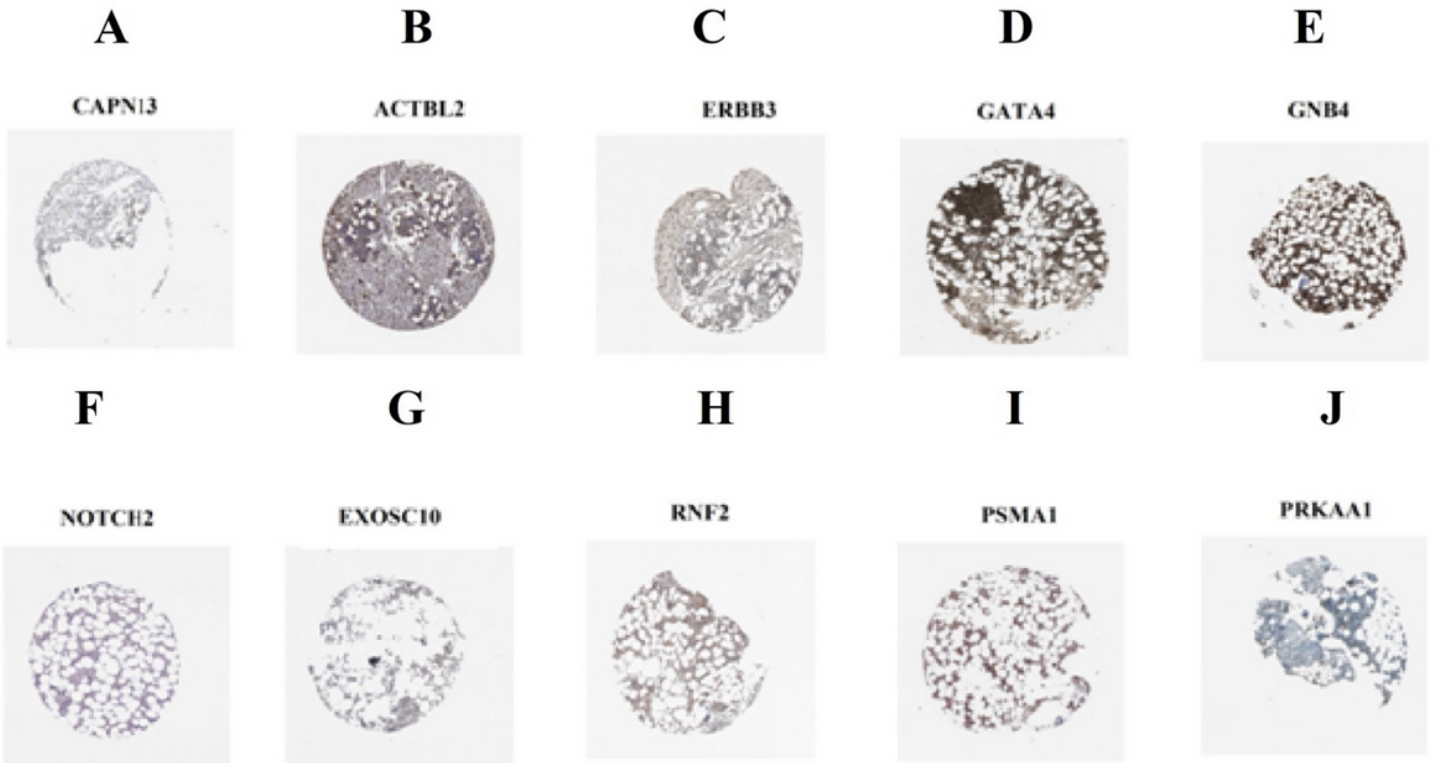
Figure 8

Immune histochemical analyses of hub genes were produced using the human protein atlas (HPA) online platform. A) CAPN13 B) ACTBL2 C) ERBB3 D) GATA4 E) GNB4 F) NOTCH2 G) EXOSC10 H) RNF2 I) PSMA1 J) PRKAA1
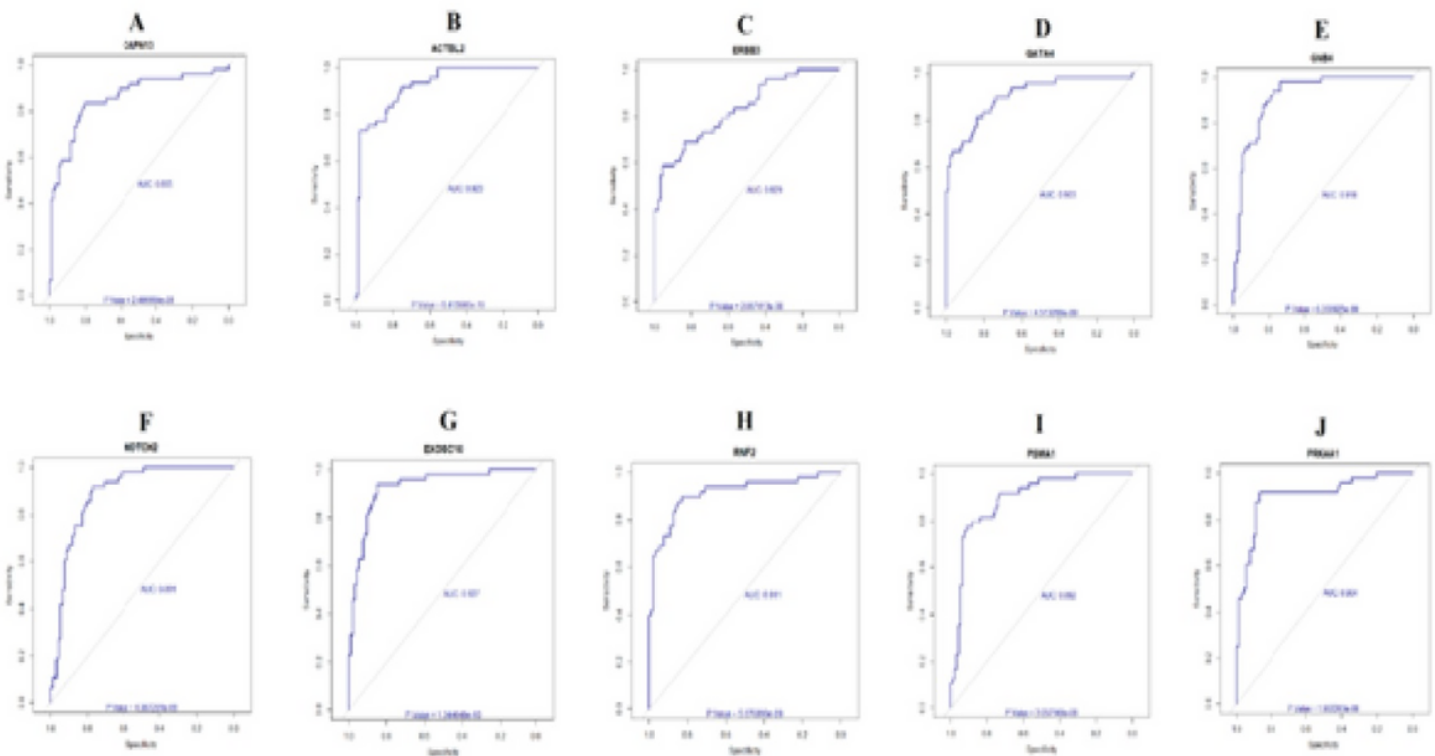
Figure 9

ROC curve validated the sensitivity, specificity of hub genes as a predictive biomarker for CAD prognosis. A) CAPN13 B) ACTBL2 C) ERBB3 D) GATA4 E) GNB4 F) NOTCH2 G) EXOSC10 H) RNF2 I) PSMA1 J) PRKAA1
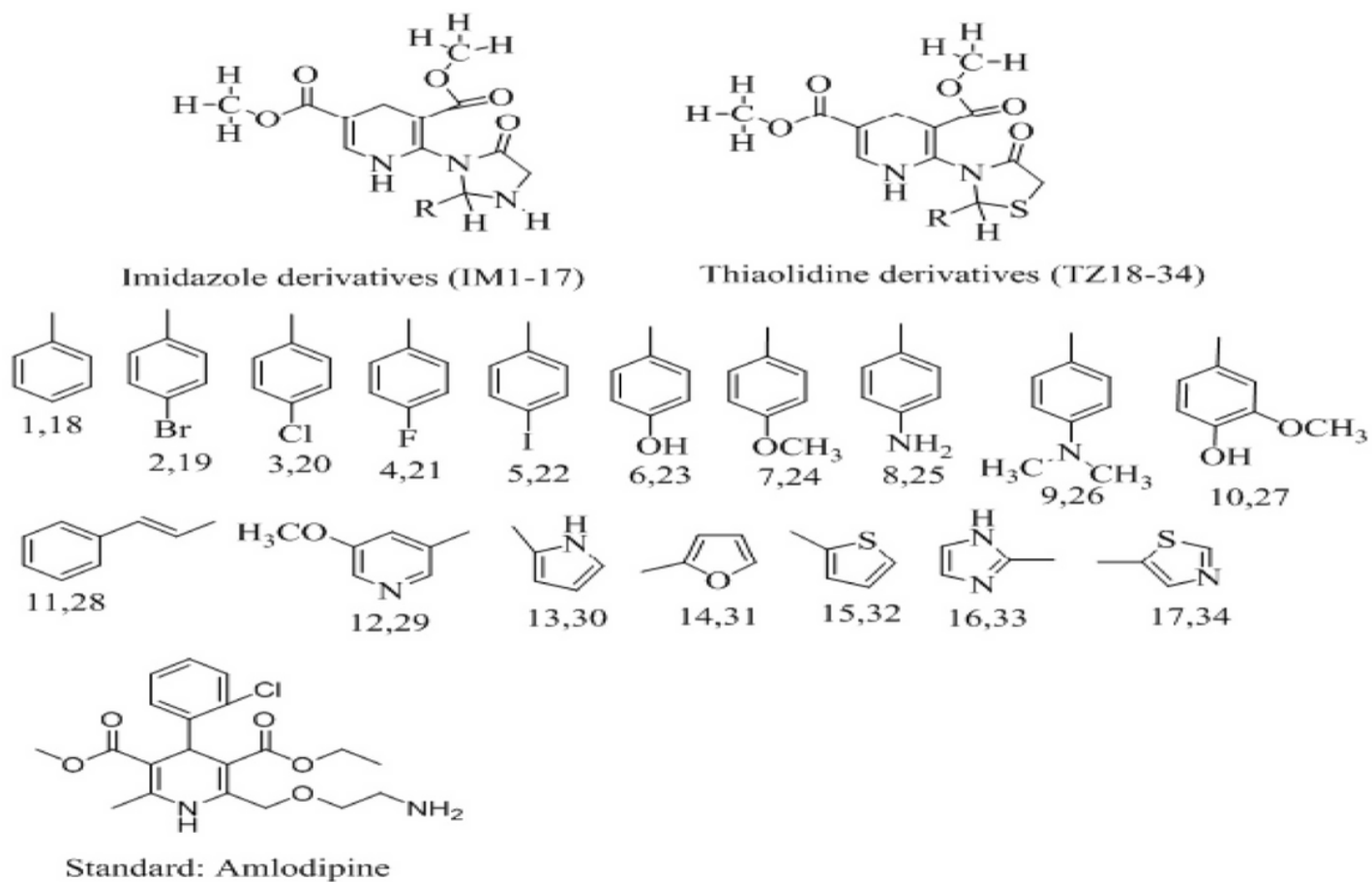

Imidazole derivatives (IM1-17)   Thiaolidine derivatives (TZ18-34)

1,18   Br 2,19   Cl 3,20   F 4,21   I 5,22   OH 6,23   OCH₃ 7,24   NH₂ 8,25   H₃C-N-CH₃ 9,26   OCH₃ OH 10,27

11,28   H₃CO 12,29   13,30   14,31   15,32   16,33   17,34

Standard: Amlodipine

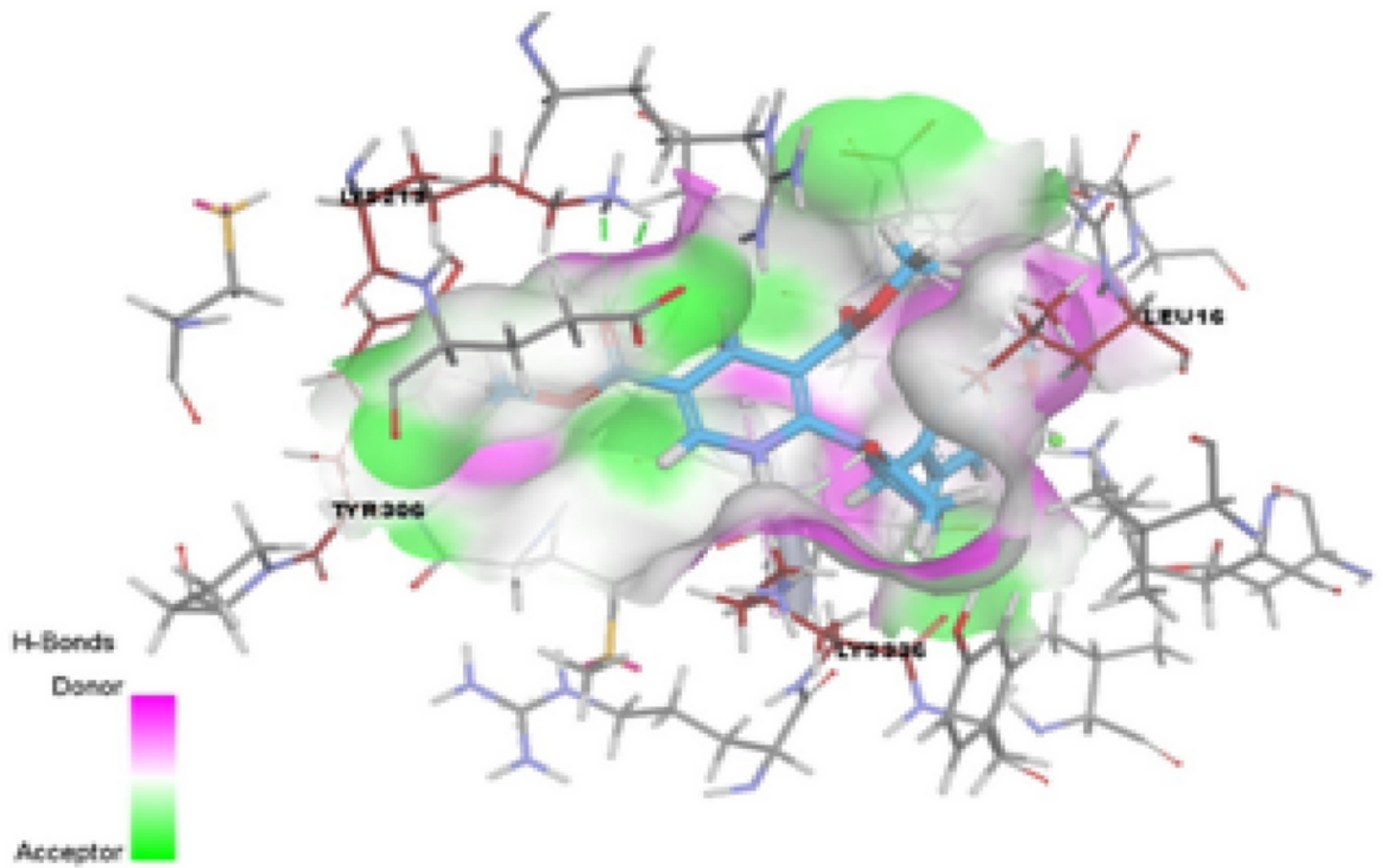# Figure 10

Structures of Designed Molecules

Figure 11

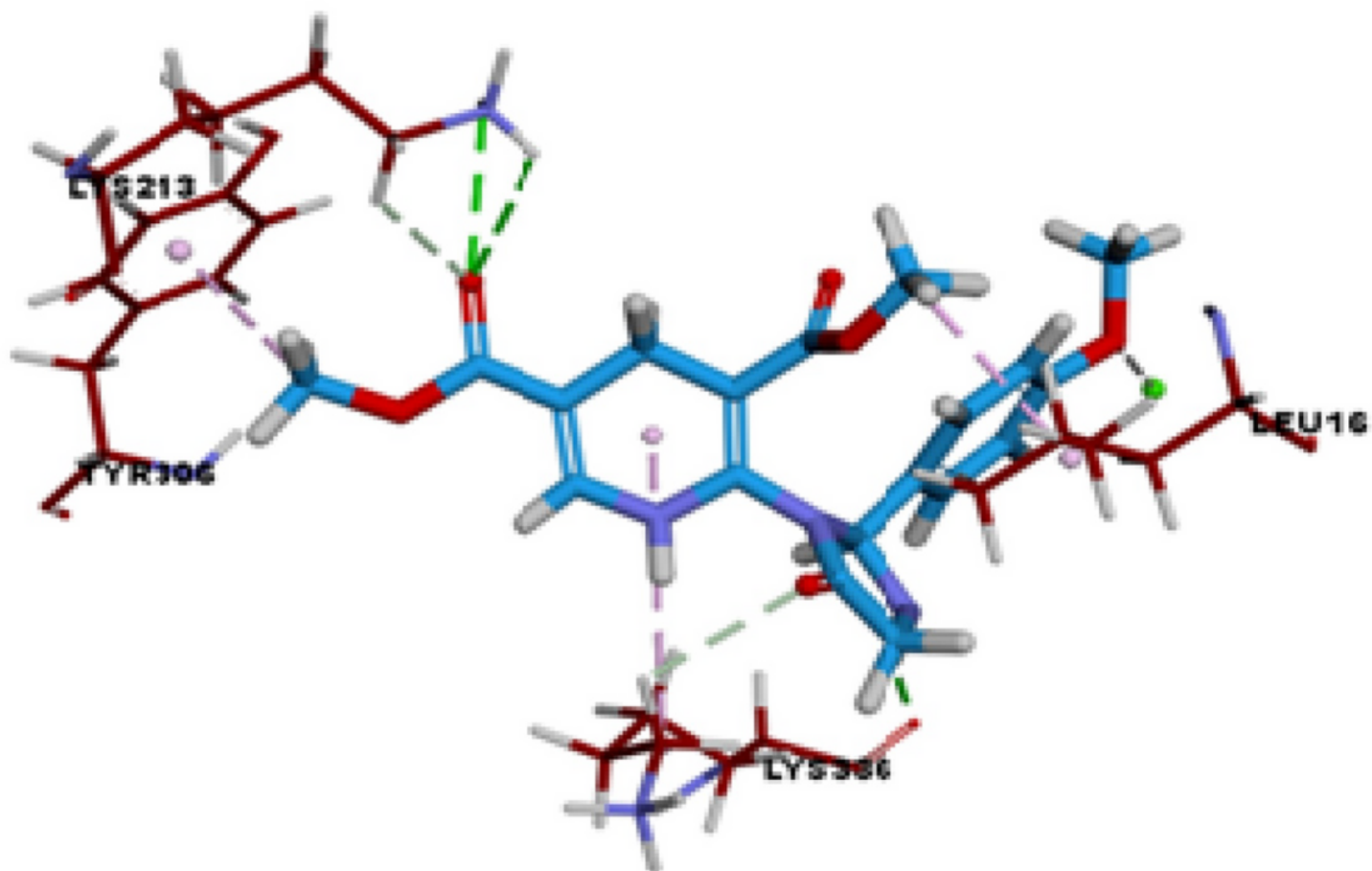Hydrogen bonding Interactions of Ligand with Protein

Figure 12
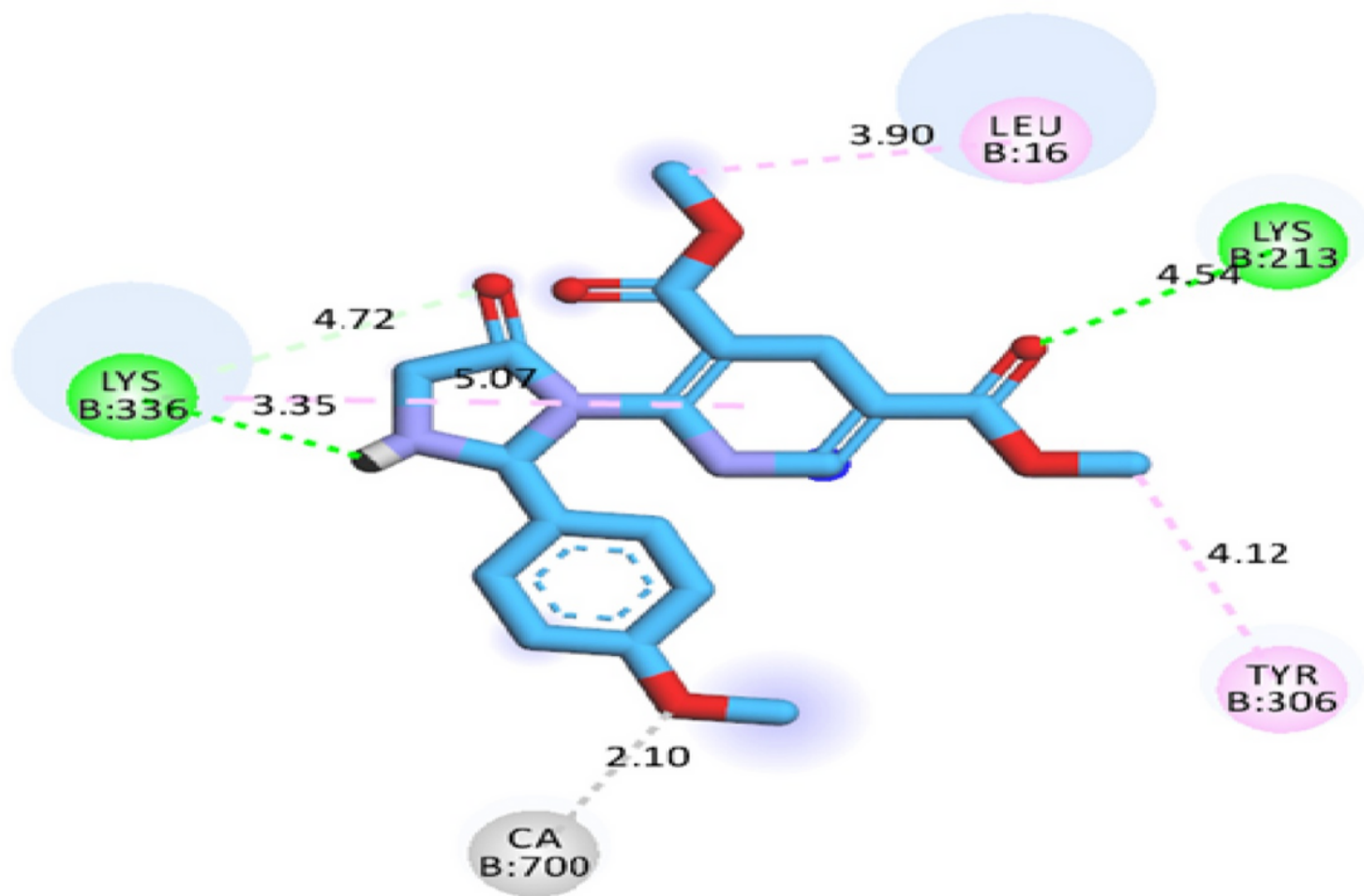
3D Representation of Molecule with Amino acids

Figure 13

2D Binding of Molecule HES with 5M5R

Supplementary Files

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- Tables.docx