

# Investigation of Candidate Genes and Mechanisms Underlying Obesity Associated Type 2 Diabetes Mellitus Using Bioinformatics Analysis and Screening of Small Drug Molecules

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## Research Article

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# Abstract

## Background

Obesity associated type 2 diabetes mellitus is a metabolic disorder ; however, the etiology of obesity associated type 2 diabetes mellitus remains largely unknown. There is an urgent need to further broaden the understanding of the development mechanism of obesity associated type 2 diabetes mellitus.

## Methods

To screen the differentially expressed genes (DEGs) that may play essential roles in obesity associated type 2 diabetes mellitus, the public expression profiling by high throughput sequencing data (GSE143319) were downloaded and screened for DEGs. Then, Gene Ontology (GO) function analysis and REACTOME pathway analysis were performed. To screen hub and target genes, the protein–protein interaction network, miRNA-target genes regulatory network and TF-target gene regulatory network were constructed. The Receiver operating characteristic (ROC) curve analysis and RT-PCR analysis of hub genes in obesity associated type 2 diabetes mellitus were also analyzed. Final molecular docking studies performed for screening small drug molecules.

## Results

There were 409 up regulated and 411 down regulated genes detected, and the biological processes of the GO analysis were enriched in regulation of ion transmembrane transport, intrinsic component of plasma membrane, transferase activity, transferring phosphorus-containing groups, cell adhesion, integral component of plasma membrane and signaling receptor binding, whereas, the REACTOME pathway analysis was enriched in integration of energy metabolism and extracellular matrix organization. The hub genes CEBPD, TP73, ESR2, TAB1, MAP3K5, FN1, UBD, RUNX1, PIK3R2 and TNF, which might play a essential role in obesity associated type 2 diabetes mellitus was further screened.

## Conclusions

The present study could deepen the understanding of the molecular mechanism of obesity associated type 2 diabetes mellitus, which could be useful in developing clinical treatments of obesity associated type 2 diabetes mellitus.

## Introduction

Obesity associated type 2 diabetes mellitus are a core challenge for metabolic disorder research around the globe [1]. Type 2 diabetes mellitus is characterized by insulin deficiency due to pancreatic  $\beta$ -cell inactivation and insulin resistance [2]. Genetic factors, hyperinsulinemia, atherogenic dyslipidemia, glucose intolerance, hypertension, prothrombic state, hyperuricemia, and polycystic ovary syndrome are the risk factors linked with progression of type 2 diabetes mellitus [3]. Obesity associated type 2 diabetes mellitus constitutes another main type of common and chronic disease that affects the vital organs such

as heart [4], brain [5], kidney [6] and eye [7]. Do not fully resolve the etiology and advancement of obesity associated type 2 diabetes mellitus. Therefore, the potential molecular mechanisms add to the pathogenesis of type 2 diabetes mellitus remain to be precisely exhibit to find potential target genes for the avoidance and treatment of obesity associated type 2 diabetes mellitus.

Developing evidence has shown that genetic predisposition plays a key role in the advancement of obesity associated type 2 diabetes mellitus [8]. Recently, several genes and pathways have been found to participate in the occurrence and advancement of obesity associated type 2 diabetes mellitus [9], including FGF21 [10], pro-opiomelanocortin (POMC) [11], PI3K/AKT pathway [12] and JAK/STAT pathway [13]. However, the current knowledge is insufficient to explain how these crucial genes are associated with advancement of obesity associated type 2 diabetes mellitus. Therefore, there is a great need to find new biomarkers and to advance novel techniques to enlighten the mechanism controlling obesity associated type 2 diabetes mellitus.

Bioinformatics analysis of expression profiling by high throughput sequencing has shown great promise to discover potential key genes and signaling pathways with roles in metabolic disorder [14], to identify new biomarkers and biological processes implicated in obesity associated type 2 diabetes mellitus. In this investigation, using bioinformatics analysis, we aimed to investigate expression profiling by high throughput sequencing data from dataset to determine differentially expressed genes (DEGs) and significant pathways in obesity associated type 2 diabetes mellitus. After searching the Gene Expression Omnibus (GEO) database [15], we identified dataset GSE143319 with RNA sequencing data of T2DM. Subsequently, we performed Gene Ontology (GO) enrichment analysis of the signaling pathways involved, and a protein-protein interaction (PPI) network, miRNA-target genes regulatory network, TF-target gene regulatory network constructed and analyzed, and validation of hub genes were developed, all of which will improve our understanding of the pathogenesis of obesity associated type 2 diabetes mellitus. Final molecular docking studies performed for screening small drug molecules.

## Materials And Methods

### RNA sequencing data

The expression profiling by high throughput sequencing data for GSE143319 deposited by Ding et al [16] into the GEO database were obtained on the GPL20301 platform (Illumina HiSeq 4000 (Homo sapiens)). The expression profiling by high throughput sequencing is provided for 30 samples, including 15 samples of a metabolically healthy obese and 15 samples of a metabolically unhealthy obese.

### Identification of DEGs

The limma [17] in R bioconductor package was utilized to screen differentially expressed genes (DEGs) between metabolically healthy obese and metabolically unhealthy obese. These DEGs were identified as important genes that may play an important role in the development of T2DM with obesity. The cutoff

criterion were  $|\log \text{fold change (FC)}| > 0.2587$  for up regulated genes, were  $|\log \text{fold change (FC)}| < -0.2825$  for down regulated genes and  $P < 0.05$ .

### **Gene ontology and pathway enrichment analyses**

ToppGene (ToppFun) (<https://toppgene.cchmc.org/enrichment.jsp>) [18] which is a useful online platform database that integrates biologic data and provides a comprehensive set of functional annotation information of genes as well as proteins for users to analyze the functions or signaling pathways. GO (<http://geneontology.org/>) [19] enrichment analysis (biologic processes [BPs], cellular components [CCs], and molecular functions [MFs]) is a strong bioinformatics tool to analyze and annotate genes. The REACTOME (<https://reactome.org/>) [20] is a pathway database resource for understanding high-level gene functions and linking genomic information from large-scale molecular data sets. To analyze the function of the diagnosed DEGs, biologic analyses were performed using GO enrichment and REACTOME pathway analysis via ToppGene online database.

### **PPI network construction and module analysis**

IMEX interactome (<https://www.imexconsortium.org/>) [21] online database was using to predicted the PPI network information. Analyzing the interactions and functions between DEGs may provide information about the mechanisms of generation and development of disease (PPI score $>0.4$ ). Cytoscape (version 3.8.2) ([www.cytoscape.org](http://www.cytoscape.org)) is a bioinformatics platform for constructing and visualizing molecular interaction networks [22]. Therefore, the node degree [23], betweenness centrality [24], stress centrality [25], closeness centrality [26] were statistically analyzed in networks using Network Analyzer to obtain the significant nodes or hub genes in the PPI network. Network Analyzer, a Java plugin for Cytoscape, is capable of predicting key nodes in a given network by several topological algorithms. The plug-in Molecular Complex Detection (MCODE) of Cytoscape was applied to detect densely connected regions in PPI networks. The PPI networks were constructed using Cytoscape and the most significant module in the PPI networks was selected using PEWCC1 (<http://apps.cytoscape.org/apps/PEWCC1>) [27]. The criteria for selection were set as follows: Max depth = 100, degree cut-off = 2, Node score cut-off = 0.2, PEWCC1 scores  $>5$ , and K-score = 2.

### **Target gene – miRNA genes regulatory network construction and analysis**

T2DM with obesity relating miRNAs and experimentally validated target genes were extracted from miRNet database (<https://www.mirnet.ca/>) [28]. T2DM with obesity relating miRNA-target gene pairs were identified through comparing the DEGs with the downloaded miRNA-target pairs. Then the target genes - miRNA regulatory network was constructed using Cytoscape software.

### **Target gene – TF network regulatory construction and analysis**

T2DM with obesity relating TFs and experimentally validated target genes were extracted from TFs database NetworkAnalyst database (<https://www.networkanalyst.ca/>) [29]. T2DM with obesity relating

TF-target gene pairs were identified through comparing the DEGs with the downloaded TF-target pairs. Then the target genes -TF regulatory network was constructed using Cytoscape software.

### **Receiver operating characteristic (ROC) curve analysis**

The ROC curve was used to calculate classifiers in bioinformatics applications. To further assess the predictive accuracy of the DEGs, ROC analysis was performed to discriminate metabolically healthy obese from metabolically unhealthy obese. ROC curves for hub genes were generated using pROC in R [30] based on the obtained DEGs and their expression profiling by high throughput sequencing data from dataset. The area under the ROC curve (AUC) was evaluated and used to compare the diagnostic value of hub genes.

### **Validation of the expression levels of candidate genes by RT-PCR**

Quantitative RT-PCR was conducted to validate the expressions of these hub genes in obesity with T2DM. Total RNAs were extracted from Primary Subcutaneous Pre-adipocytes; Normal, Human (ATCC® PCS-210-010™) and 3T3-L1 cells (ATCC® CL-173) using TRI Reagent® (Sigma, USA) according to instruction, followed by reverse transcription with Reverse transcription cDNA kit (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA amplification through 7 Flex real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The expressions of these hub genes were normalized to against beta-actin expression. The data were calculated by the  $2^{-\Delta\Delta Ct}$  method [31]. Primers used in the current investigation were listed in Table 1.

### **Molecular docking studies**

The Surflex-Docking docking studies for the designed molecules were performed using module SYBYL-X 2.0 perpetual software. Using ChemDraw Tools, the molecules were sketched and imported and saved into sdf. Format using open free software from Babel. The co-crystallised protein structures of CEBPD, TP73, ESR2, TAB1 and MAP3K5 of its PDB code 3L4W, 2XWC, 1U3Q, 5NZZ & 2CLQ was extracted from Protein Data Bank 1262 [32-36]. Together with the TRIPOS force field, GasteigerHuckel (GH) charges were added to all designed derivatives for the structure optimization process. Furthermore, energy minimization was carried out using MMFF94s and MMFF94 algorithm process. The processing of protein was accomplished after the incorporation of protein. The co-crystallized ligand and all water molecules were expelled from the crystal structure; more hydrogen was added and the side chain was optimized. TRIPOS force field was used to minimize complexity of structure. The interaction efficiency of the compounds with the receptor was expressed in kcal / mol units by the Surflex-Dock score. The best spot between the protein and the ligand was inserted into the molecular region. The visualisation of ligand interaction with receptor is done by using discovery studio visualizer.

## **Results**

### **Identification of DEGs**

As presented in the cluster heat map of Fig. 1, total 820 DEGs, comprising 409 up regulated and 411 down regulated genes, were identified between metabolically healthy obese samples and metabolically unhealthy obese samples. DEGs expressions were illustrated by volcano plot (Fig.2), and the top up regulated and down regulated DEGs are listed in Table 2.

### **Gene ontology and pathway enrichment analyses**

DEGs were divided into up regulated genes and down regulated genes. GO categories and REACTOME pathway enrichment analysis were conducted for these genes. Results of GO categories were presented by functional groups, which were group BP, CC, and MF and are listed in Table 3. In group BP, up regulated genes were significantly enriched in regulation of ion transmembrane transport and oxoacid metabolic process, while the down regulated genes were mainly enriched in cell adhesion and response to endogenous stimulus. For group CC, up regulated genes mainly enriched in intrinsic component of plasma membrane and mitochondrion, and down regulated genes mainly enriched in integral component of plasma membrane and supra molecular fiber. In addition, GO results of group MF showed that up regulated genes mainly enriched in transferase activity, transferring phosphorus-containing groups and transporter activity and down regulated genes mainly enriched in signaling receptor binding and molecular transducer activity. Several significant enriched pathways were acquired through REACTOME pathway analysis (Table 4). The enriched pathways for up regulated genes included integration of energy metabolism and neuronal system. Meanwhile, down regulated genes strikingly enriched in extracellular matrix organization and GPCR ligand binding.

### **PPI network construction and module analysis**

PPI network complex consisted of 3648 nodes and 6305 edges, wherein node and edge represented gene and interaction between 2 genes (Fig. 3A). Moreover, CEBPD, TP73, ESR2, TAB1, MAP3K5, FN1, UBD, RUNX1, PIK3R2 and TNF were identified as central genes and are listed in Table 5. In addition, module analysis was conducted to detect the highly connected regions of PPI network, and two significant modules were obtained (Fig.3B and Fig.3C). Further GO and pathway enrichment analysis revealed that genes in these modules were mostly implicated in regulation of ion transmembrane transport, oxoacid metabolic process, intrinsic component of plasma membrane, extracellular matrix organization and supra molecular fiber.

### **Target gene – miRNA regulatory network construction and analysis**

The target genes - miRNA regulatory network was constructed, including 1982 miRNAs and 245 target genes. As shown in the integrated target genes - miRNA regulatory network (Fig. 4), FASN targeted 147 miRNAs (ex, hsa-mir-4314), SREBF1 targeted 81 miRNAs (ex, hsa-mir-5688), CKB targeted 72 miRNAs (ex, hsa-mir-583), CACNA1A targeted 69 miRNAs (ex, hsa-mir-632), ESR2 targeted 61 miRNAs (ex, hsa-mir-3176), MAP1B targeted 249 miRNAs (ex, hsa-mir-1299), RUNX1 targeted 125 miRNAs (ex, hsa-mir-4530), PRNP targeted 106 miRNAs (ex, hsa-mir-4477a), FN1 targeted 105 miRNAs (ex, hsa-mir-606) and DAB2 targeted 75 miRNAs (ex, hsa-mir-1343-3p6) and are listed in Table 6.

## Target gene-TF s regulatory network construction and analysis

The target genes -TF regulatory network was constructed, including 333 TFs and 204 target genes. As shown in the integrated target genes -TF regulatory network (Fig. 5), SREBF1 targeted 94 TFs (ex, ATF4), FASN targeted 71 TFs (ex, CUX1), SLC9A3R1 targeted 63 TFs (ex, MBD2), CKB targeted 50 TFs (ex, IRF4), TGM2 targeted 50 TFs (ex, SIN3A), PIK3R2 targeted 73 TFs (ex, ZNF143), FLNC targeted 53 TFs (ex, SMARCE1), RUNX1 targeted 53 TFs (ex, ZBTB7A), FN1 targeted 45 TFs (ex, CREB1) and TRIM63 targeted 22 TFs (ex, RELA) and are listed in Table 6.

## Receiver operating characteristic (ROC) curve analysis

The ROC curve was used to assess the predictive accuracy of hub genes. AUC was determined and used to prefer the most appropriate cut-off gene expression levels. ROC curves and AUC values are presented in Fig. 6. All AUC values exceeded 0.72, while the up regulated genes CEBPD, TP73, ESR2, TAB1 and MAP3K5, and down regulated genes FN1, UBD, RUNX1, PIK3R2 and TNF had AUC values > 0.75.

## Validation of the expression levels of candidate genes by RT-PCR

To further verify the expression level of hub genes in obese samples, RT-qPCR was performed to calculate the mRNA levels of the ten hub genes identified in the present study (CEBPD, TP73, ESR2, TAB1, MAP3K5, FN1, UBD, RUNX1, PIK3R2 and TNF) in obese samples. As illustrated in Fig. 7, the expression of CEBPD, TP73, ESR2, TAB1, MAP3K5 were significantly up regulated in obese samples compared with normal control tissues, while FN1, UBD, RUNX1, PIK3R2 and TNF were significantly down regulated in obese samples compared with normal control tissues. The present RT-PCR results were in line with the prior bioinformatics analysis, suggesting that these essential genes might be associated to the molecular mechanism underlying obesity associated type 2 diabetes mellitus.

## Molecular docking studies

In the current research, the docking simulation was conducted to recognize the active site conformation and major interactions responsible for complex stability with the binding sites receptor. Drug design software Sybyl X 2.1 was used to perform docking experiments on novel molecules containing thiazolidinedioneheterocyclic ring. Molecules containing the heterocyclic ring of thiazolidinedione are constructed based on the pioglitazone structure and are most widely used alone or in conjunction with other anti-diabetic drugs. Obesity associated type 2 diabetes mellitus is a chronic disorder that prevents insulin from being used by the body the way it should. It's said that people with obesity associated type 2 diabetes mellitus have insulin resistance, oral hypoglycaemic agents are used either alone or in combination of two or more drugs. Pioglitazone (Glitazones) are commonly used either alone or in combination in obesity associated type 2 diabetes mellitus. The one protein in each over expressed genes in obesity associated type 2 diabetes mellitus are selected for docking studies. The X-RAY crystallographic structure of one protein from each over-expressed genes of CEBPD (CCAAT enhancer binding protein delta), TP73 (tumour protein P73), ESR2 (Estrogen receptor 2), TAB1 (TGF-beta activated

kinase 1 MAP3K7 binding protein 1) and MAP3K5 (Mitogen activated protein kinase kinase 5) and their co-crystallized PDB code of 4LY9, 2XWC, 2IOG, 5NZZ and 5UP3 respectively were selected for docking. The examination of the designed molecules were performed to recognize the potential molecule. The foremost of the designed molecules obtained C-score greater than 6 and are said to be active. A total of 24 designed molecules few molecules have excellent good binding energy (C-score) greater than 8 respectively. Few of the designed molecules obtained good binding scores such as molecule TZP20, TZPS8, TZP22, TZPS10 (Fig.8) obtained binding core of 12.212, 11.489, 11.013 and 10.851 with 5UP3 and molecule TZP22, TZPS8, TZPS10 obtained binding score of 9.482, 9.329 and 9.252 with 2XWC and molecule TZP20, TZPS10 obtained binding score 7.359 and 6.848 with 5NZZ and molecule TZP22, TZP21, TZPS9 obtained binding score 11.053, 10.716 and 10.669 with 2IOG respectively. The molecule TZP23, TZPS5, TZPS2 obtained bind score 4.336 to 4.319 with 5NZZ and molecule TZPS10 of binding core 4.633 with 2IOG respectively. The binding score of the predicted molecules are compared with that of the standard Pioglitazone obtained bind score of 10.1314, 9.834, 9.8244, 9.8284 and 7.4321 with 2IOG, 2XWC, 4LY9, 5UP3 and 5NZZ, the values are depicted in Table 7. The molecule TZP22 obtained good binding score with all proteins and hydrogen bonding and other bonding interactions with amino acids with protein code 2IOG are depicted by 3D (Fig Fig.9) and 2D figures (Fig.10).

## Discussion

Obesity associated type 2 diabetes mellitus is the most common aggressive metabolic disorder [37]. However, the most key challenge in treating obesity associated type 2 diabetes mellitus is the presence of complexity [38]. Although previous investigations have reported various potential molecular markers linked with the advancement of obesity associated type 2 diabetes mellitus, the potential molecular mechanism underlying its pathogenesis has not been generally studied [39]. In the present investigation, a total of 820 DEGs were identified, containing 409 up regulated genes and 411 down regulated genes. SULT1C2 [40] and UBD (ubiquitin D) [41] were responsible for progression of kidney diseases, but these genes might be liable for advancement of obesity associated T2DM. HLA-DQA1 was associated with progression of T2DM [42]. SPX (spexin hormone) [43] and APOB (apolipoprotein B) [44] are a critical proteins plays an important role in obesity associated type 2 diabetes mellitus.

The GO and pathway enrichment analysis of DEG are closely related to obesity associated type 2 diabetes mellitus genes and advancement. KCNE5 [45], SHANK3 [46], CASQ2 [47], EDNRA (endothelin receptor type A) [48], EPHB4 [49], ALPK3 [50], WNT11 [51], IRAK2 [52], FBN1 [53], SFRP2 [54], CLCA2 [55], NEXN (nexilin F-actin binding protein) [56], PALLD (palladin, cytoskeletal associated protein) [57], DAB2 [58], NRP2 [59], THBS2 [60], CSF1R [61], KCNA2 [62], CACNA1C [63], F2R [64], UCHL1 [65], CCL18 [66], ITGB1BP2 [67] and FMOD (fibromodulin) [68] were reportedly involved in cardio vascular diseases, but these genes might be key for progression of obesity associated type 2 diabetes mellitus. Hu et al. [69], Liu et al. [70], Eltokhi et al. [71], Cai et al. [72], Pfeiffer et al. [73], Lin et al. [74], Royer-Zemmour et al. [75], Pastor et al. [76], Goodspeed et al. [77], Zhang et al. [78], Rogers et al. [79], Su et al. [80] and Foale et al. [81] reported that NRXN1, CRHR1, SHANK2, PSEN2, CKB (creatine kinase B), CD200R1, SRPX2, PTPRZ1, SLC6A1, GABRB2, KCNA1, ASAH1 and LINGO1 were linked with progression of neuropsychiatric



disorders, but these genes might be involved in advancement of obesity associated type 2 diabetes mellitus. Reports indicate that SPHK2 [82], NPC1L1 [83], CNTFR (ciliary neurotrophic factor receptor) [84], SLC2A4 [85], EDA (ectodysplasin A) [86], TGM2 [87], GCK (glucokinase) [88], FASN (fatty acid synthase) [89], FAP (fibroblast activation protein alpha) [90], PRNP (prion protein) [91], LYVE1 [92], SERPINE1 [93], TNF (tumor necrosis factor) [94], FASLG (Fas ligand) [95], HGF (hepatocyte growth factor) [96], FNDC5 [97], LBP (lipopolysaccharide binding protein) [98] and LOX (lysyl oxidase) [99] were found in obesity associated T2DM. Hirai et al [100], Vuori et al [101], Porta et al [102], Nomoto et al [103] and Blindbæk et al [104] demonstrates that VAMP2, CACNB2, SLC19A3, PFKFB3 and MFAP4 are essential for progression of type 1 diabetes, but these genes might be key for advancement of obesity associated type 2 diabetes mellitus. CACNA1A [105], ALK (ALK receptor tyrosine kinase) [106], SLC4A4 [107], STOX1 [108], COL3A1 [109], VNN1 [110], SLC4A7 [111], BDKRB2 [112], DRD1 [113] and LPAR1 [114] have reported significantly linked with hypertension, but these genes might be crucial for progression of obesity associated type 2 diabetes mellitus. KCNE2 [115], DLL1 [116], ACVR1C [117], RGS3 [118], MLXIPL (MLX interacting protein like) [119], PAG1 [120], SLC2A10 [121] and GRB14 [122] play important role in type 2 diabetes mellitus progression. A recent investigation has indicated that GPIHBP1 [123], FGFRL1 [124], DAPK2 [125], MAP3K5 [126], ANKK1 [127], GK (glycerol kinase) [128], SPHK1 [129], GNG3 [130], FSTL3 [131], SLIT2 [132], CCDC80 [133], RND3 [134], PTGER4 [135], RUNX1 [136], ADAM12 [137], OLR1 [138], THBS1 [139], CD28 [140], TRPV4 [141], ATRN (attractin) [142], MRC1 [143], SEMA3C [144], HTR2B [145], NOX4 [146], TACR1 [147], BAMBI [148], PDGFD (platelet derived growth factor D) [149], APLN (apelin) [150], MFAP5 [151] and LUM (lumican) [152] are associated with a development of obesity. A previous investigation found that DDR1 [153], TAB1 [154], NEK8 [155], SERPINE2 [156], FCGR2B [157], ANGPT2 [158], FN1 [159], SOCS5 [158], SMOC2 [160], CD2 [161] and SCN9A [162] expression were associated with a kidney diseases, but these genes might be responsible for advancement of obesity associated type 2 diabetes mellitus.

In addition, an investigation reported that hub genes serve an essential role in maintaining the entire PPI network and its modules are indispensable. Investigation has demonstrated that CEBPD (CCAAT enhancer binding protein delta) is one of the most important genes involved in obesity [163]. An investigation by Domingues-Montanari et al. [164] demonstrated that ESR2 is key for progression of cardio vascular disease, but this gene might be responsible for progression of obesity associated type 2 diabetes mellitus. TP73, PIK3R2, SLC9A3R1, KRT5, KRT14 and TFAP2C are novel biomarkers for pathogenesis of obesity associated type 2 diabetes mellitus.

The miRNA-target gene regulatory network and TF-target gene regulatory network highlighted in the current investigation provides new theoretical guidance for further exploring the mechanism of obesity associated type 2 diabetes mellitus and provides a new perspective for understanding the underlying biological processes of obesity associated type 2 diabetes mellitus, and miRNA and TF targeted therapy. Eberlé et al [165], Cheng et al [166], Cavallari et al [167], Qi et al [168] and Yan et al [169] indicated that SREBF1, MBD2, IRF4, CREB1 and RELA (Nuclear factor- $\kappa$ B) were responsible for advancement of obesity associated type 2 diabetes mellitus. Matsha et al [170] and Ding et al [171] demonstrated that hsa-mir-1299 and hsa-mir-4530 were liable for progression of type 2 diabetes mellitus. Hall et al [172] and

Salazar-Mendiguchía et al [173] reported that FLNC (filamin C) and TRIM63 were involved in progression of cardio vascular disease, but these genes might be essential for development of obesity associated type 2 diabetes mellitus. Xiao et al [174], Stratigopoulos et al [175] and Zhou et al [176] noted that ATF4, CUX1 and ZBTB7A were responsible for advancement of obesity. MAP1B, hsa-mir-4314, hsa-mir-5688, hsa-mir-583, hsa-mir-632, hsa-mir-3176, hsa-mir-4477a, hsa-mir-606, hsa-mir-1343-3p6, SIN3A, ZNF143 and SMARCE1 are the novel biomarkers for pathogenesis of obesity associated type 2 diabetes mellitus.

In conclusion, with the integrated bioinformatics analysis for expression profiling by high throughput sequencing in obesity associated type 2 diabetes mellitus, Ten hub genes associated with the pathogenesis and prognosis of obesity associated type 2 diabetes, including CEBPD, TP73, ESR2, TAB1, MAP3K5, FN1, UBD, RUNX1, PIK3R2 and TNF. These hub genes were all unregulated in obesity associated type 2 diabetes mellitus and first five (CEBPD, TP73, ESR2, TAB1 and MAP3K5) of them might be linked with targeted therapy. These hub genes may be regarded as new diagnostic and prognostic biomarkers for obesity associated type 2 diabetes mellitus. However, further in-depth investigation (in vivo and in vitro experiment) is necessary to elucidate the biological function of these genes in obesity associated type 2 diabetes mellitus.

## **Declarations**

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### **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. [(GSE143319) (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE143319>)]

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Author Contributions

P. G - Methodology and validation

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

A. T - Formal analysis and validation

C. V - Software and investigation

I. K - Supervision and resources

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## Tables

Due to technical limitations, table 1-7 is only available as a download in the Supplemental Files section.

## Figures

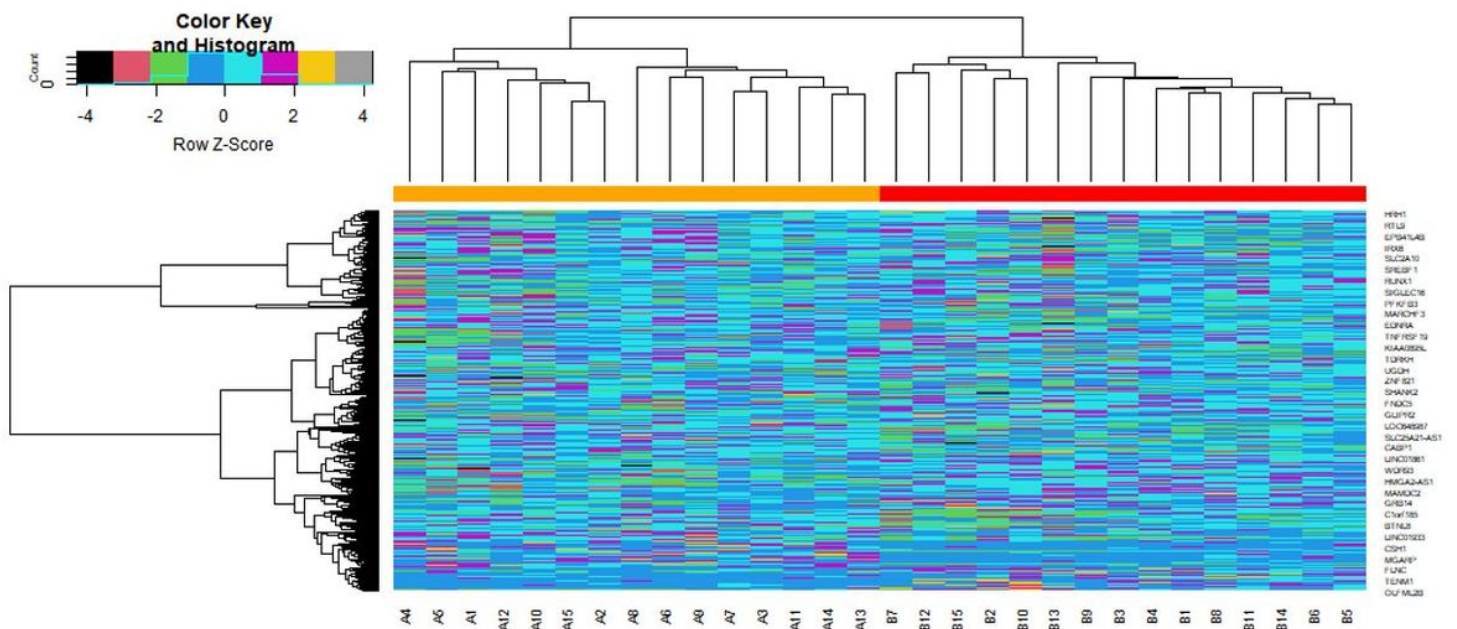
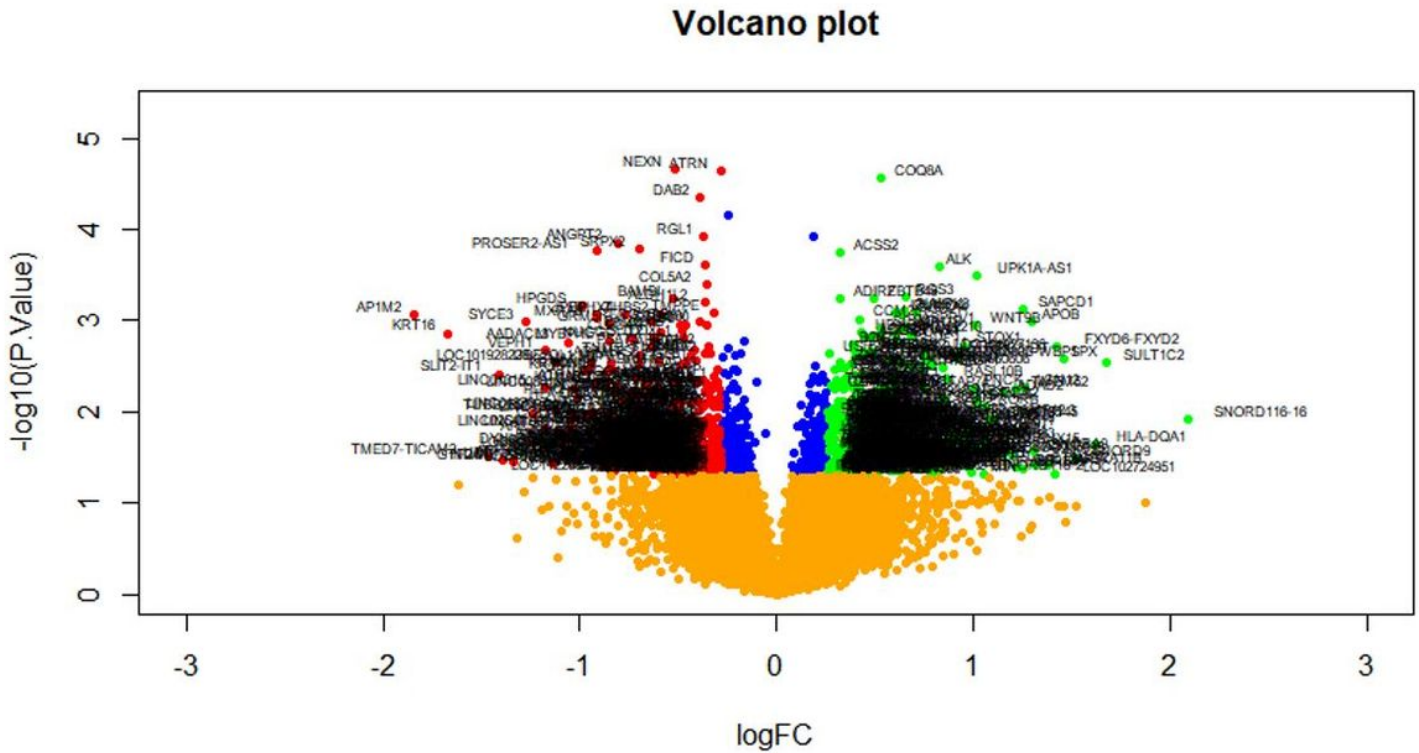


Figure 1

Heat map of differentially expressed genes. Legend on the top left indicate log fold change of genes. (A1 – A15 = metabolically healthy obese samples; B1 – B15 = metabolically unhealthy obese samples)

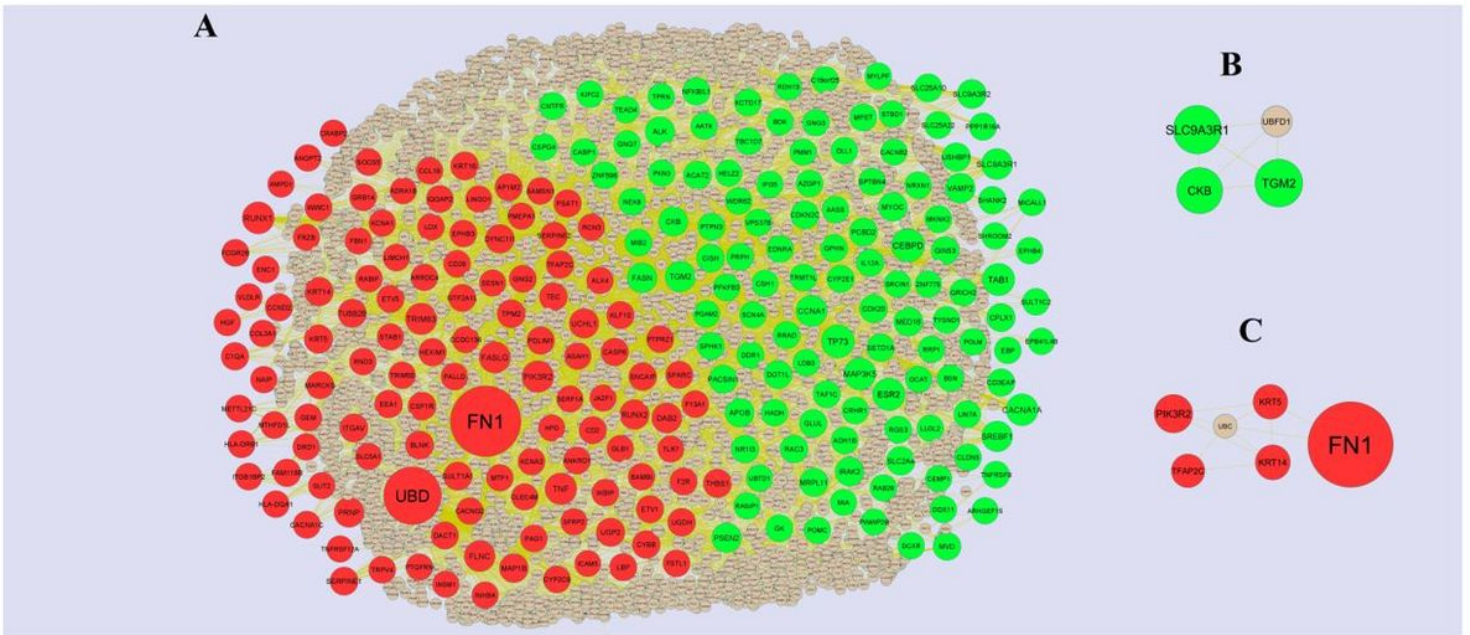


Volcano plot of differentially expressed genes. Genes with a significant change of more than two-fold were selected. Green dot represented up regulated significant genes and red dot represented down regulated significant genes.



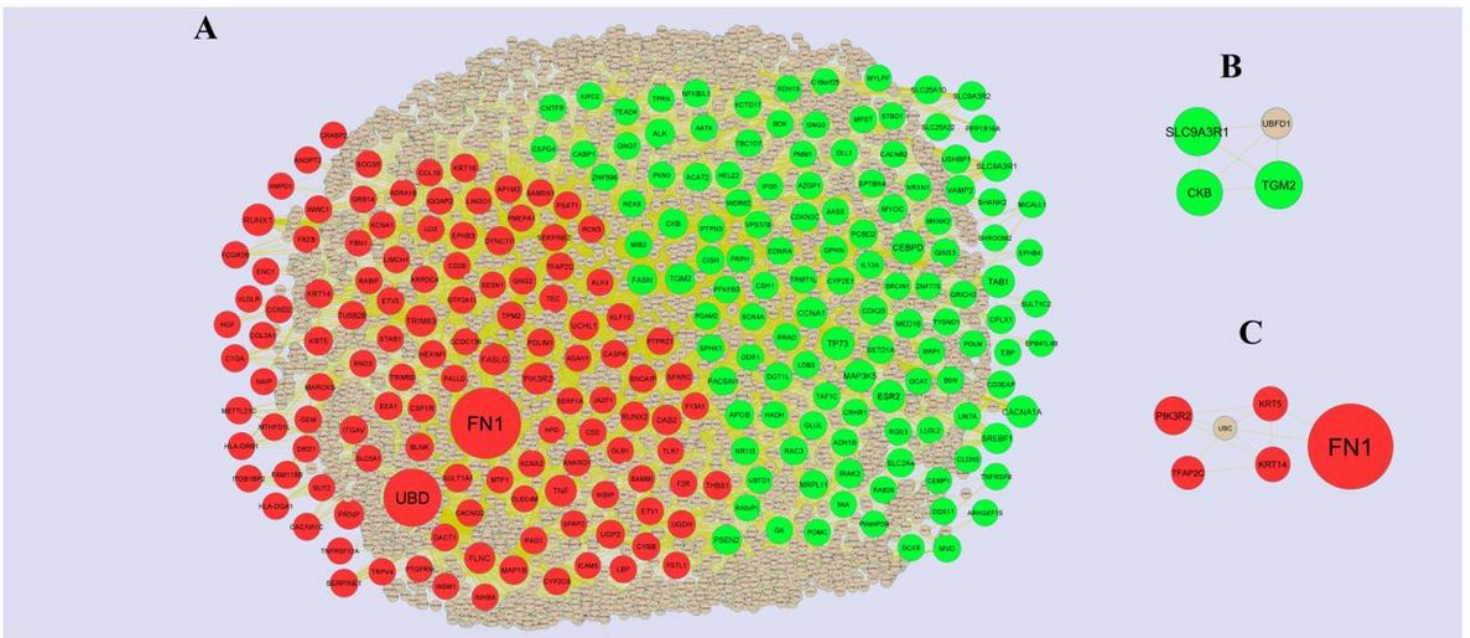
**Figure 2**

Volcano plot of differentially expressed genes. Genes with a significant change of more than two-fold were selected. Green dot represented up regulated significant genes and red dot represented down regulated significant genes.



**Figure 3**

PPI network and the most significant modules of DEGs. (A) The PPI network of DEGs was constructed using Cytoscape (B) The most significant module was obtained from PPI network with 4 nodes and 6 edges for up regulated genes (C) The most significant module was obtained from PPI network with 6 nodes and 10 edges for down regulated genes. Up regulated genes are marked in green; down regulated genes are marked in red



**Figure 3**

PPI network and the most significant modules of DEGs. (A) The PPI network of DEGs was constructed using Cytoscape (B) The most significant module was obtained from PPI network with 4 nodes and 6 edges for up regulated genes (C) The most significant module was obtained from PPI network with 6 nodes and 10 edges for down regulated genes. Up regulated genes are marked in green; down regulated genes are marked in red

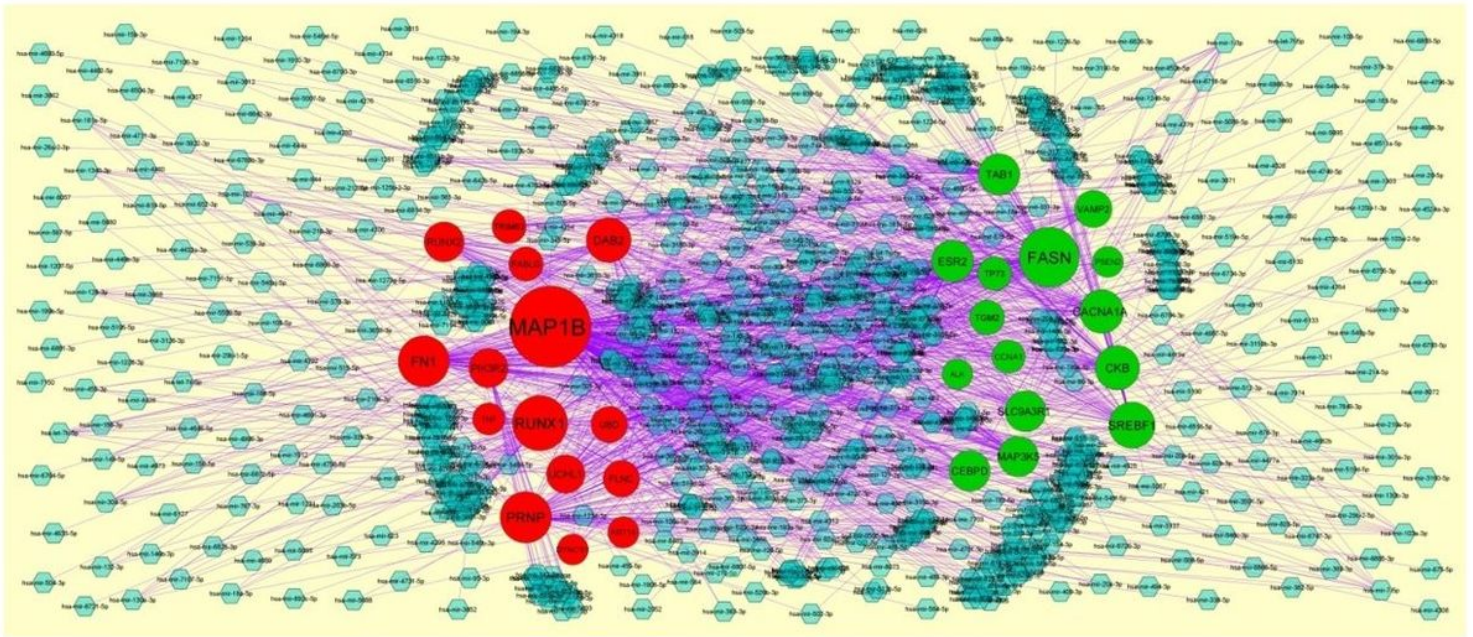


Figure 4

Target gene - miRNA regulatory network between target genes. The blue color diamond nodes represent the key miRNAs; up regulated genes are marked in green; down regulated genes are marked in red.

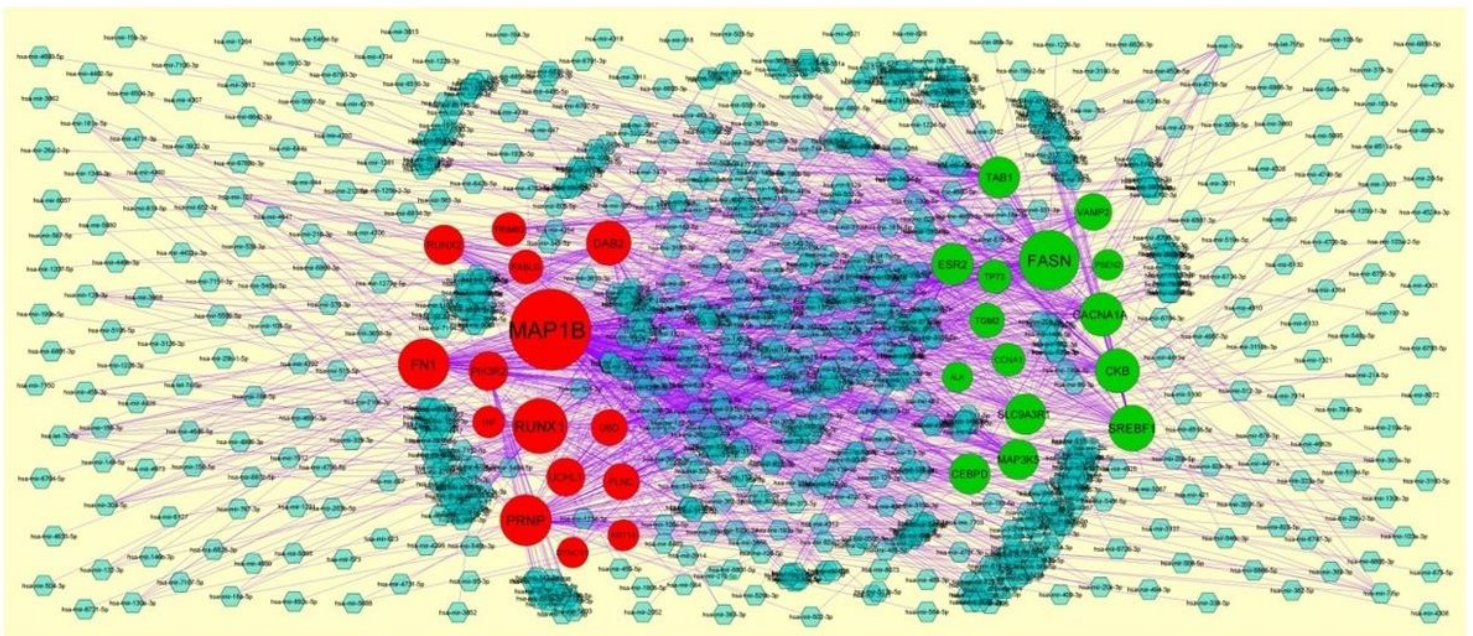


Figure 4

Target gene - miRNA regulatory network between target genes. The blue color diamond nodes represent the key miRNAs; up regulated genes are marked in green; down regulated genes are marked in red.

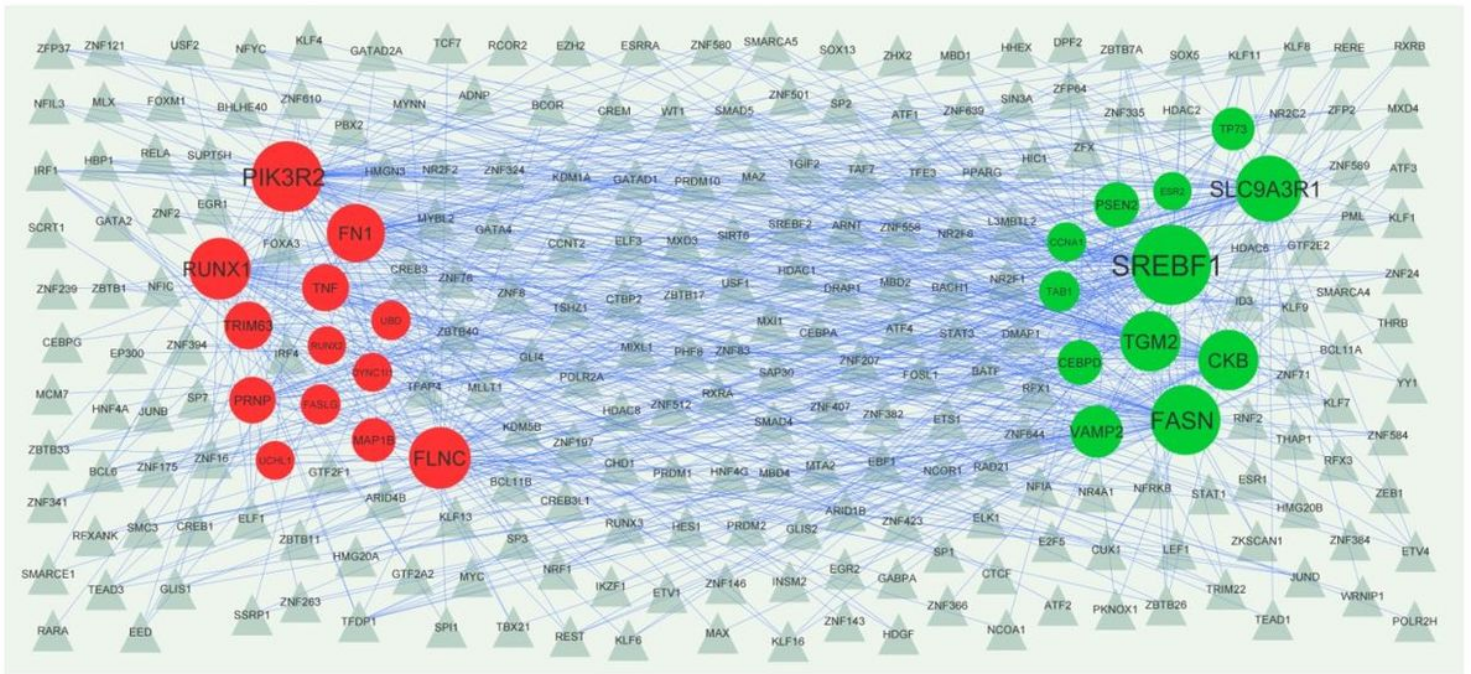


Figure 5

Target gene - TF regulatory network between target genes. The gray color triangle nodes represent the key TFs; up regulated genes are marked in green; down regulated genes are marked in red.

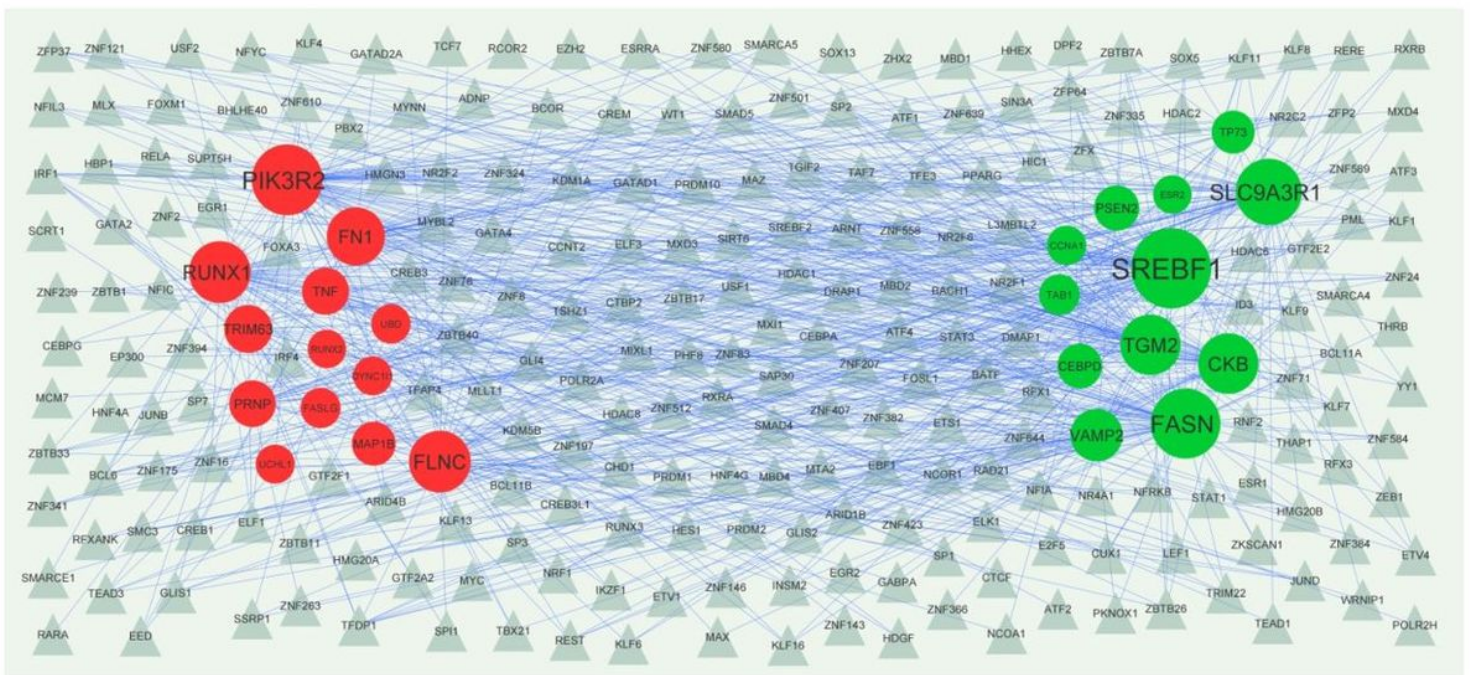
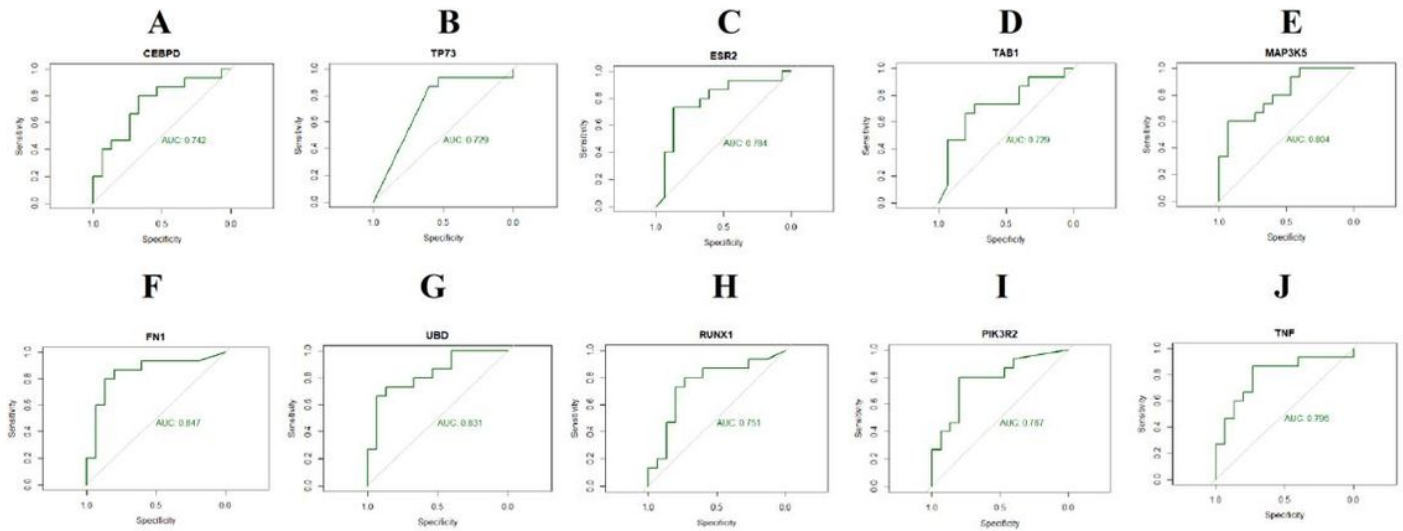


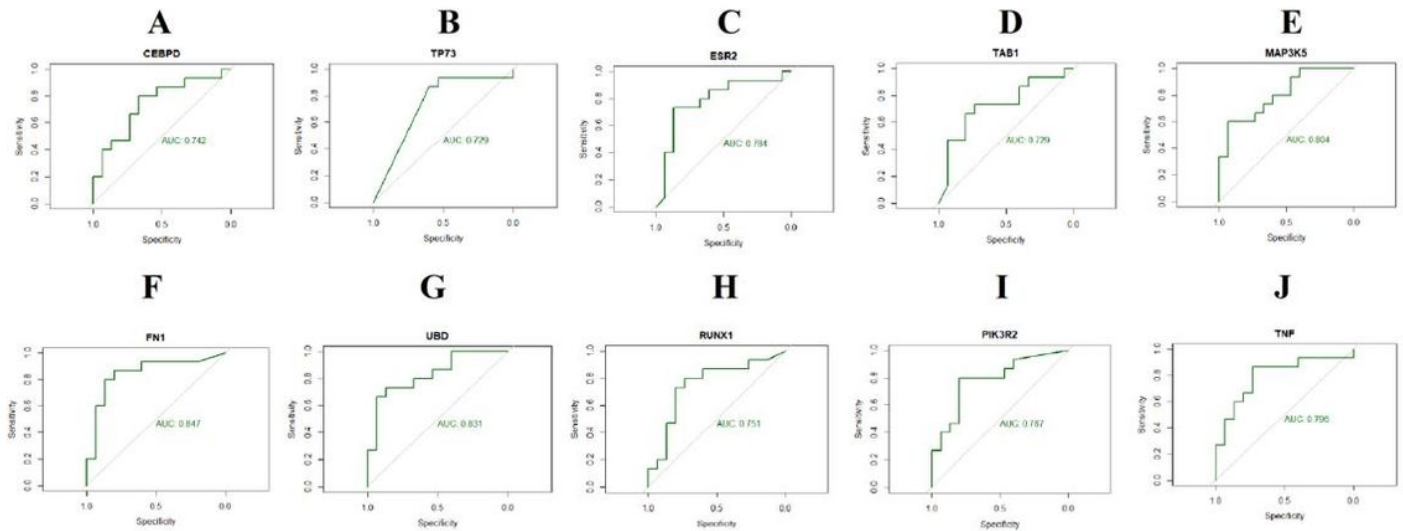
Figure 5

Target gene - TF regulatory network between target genes. The gray color triangle nodes represent the key TFs; up regulated genes are marked in green; down regulated genes are marked in red.



**Figure 6**

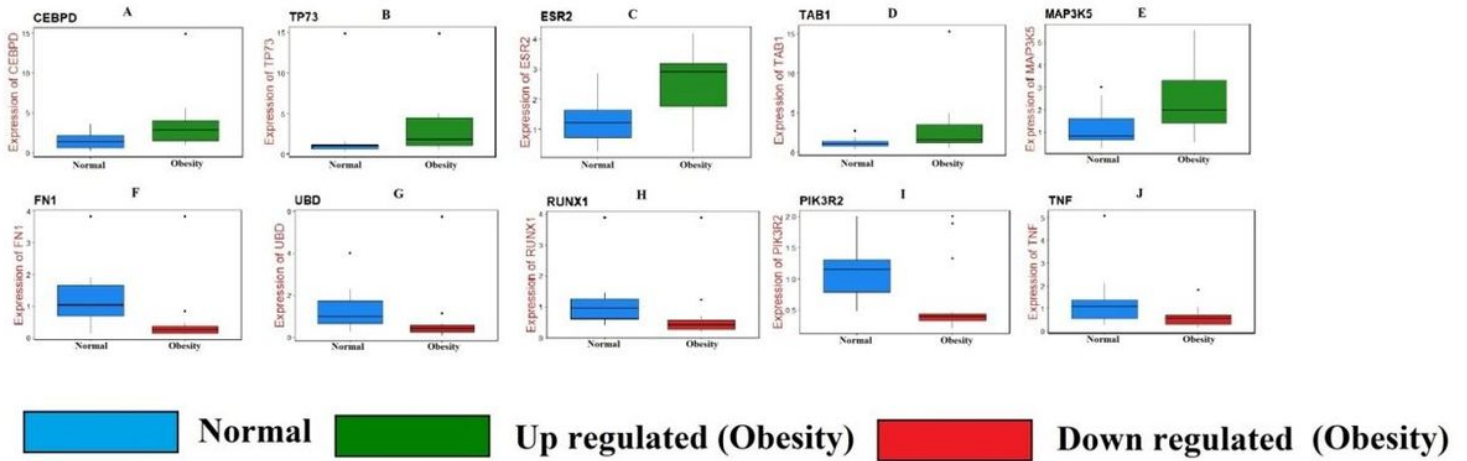
ROC curve analyses of hub genes. A) CEBPD B) TP73C) ESR2 D) TAB1 E) MAP3K5 F) FN1 G) UBD H) RUNX1 I) PIK3R2 J) TNF



**Figure 6**

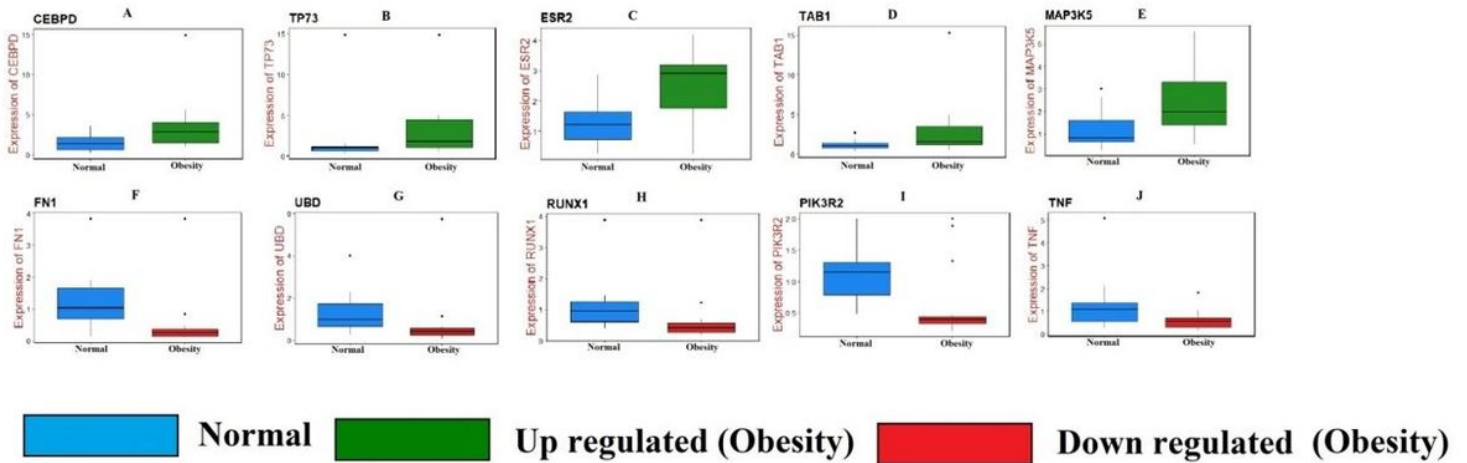
ROC curve analyses of hub genes. A) CEBPD B) TP73C) ESR2 D) TAB1 E) MAP3K5 F) FN1 G) UBD H) RUNX1 I) PIK3R2 J) TNF





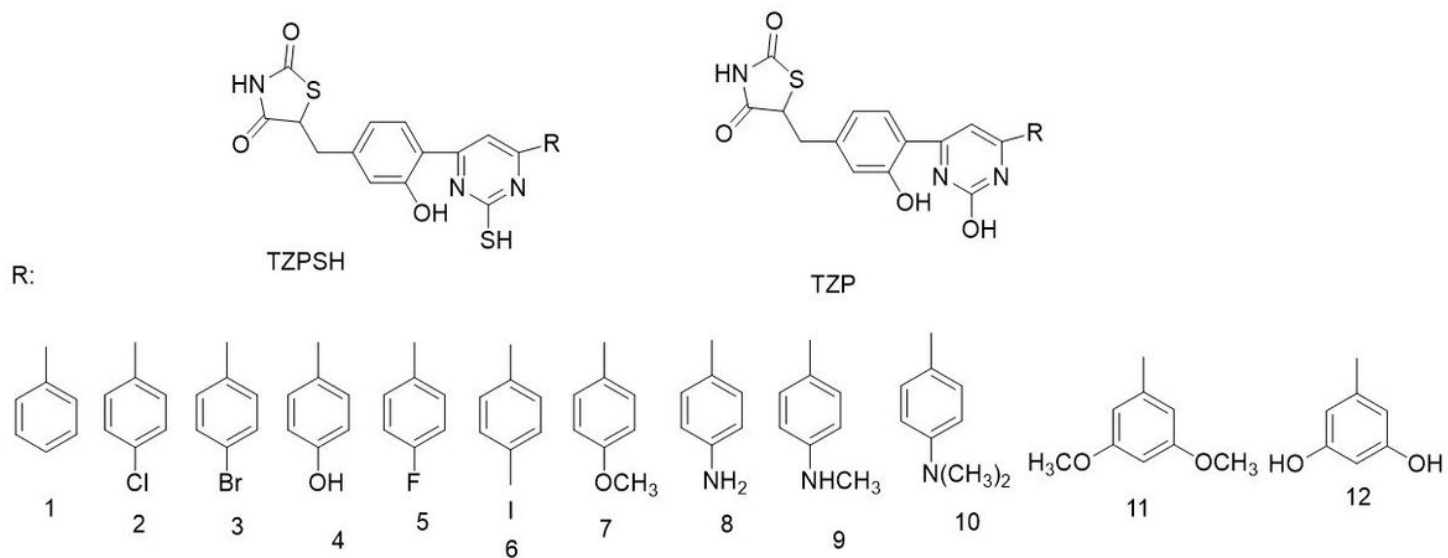
**Figure 7**

RT-PCR analyses of hub genes. A) CEBPD B) TP73C) ESR2 D) TAB1 E) MAP3K5 F) FN1 G) UBD H) RUNX1 I) PIK3R2 J) TNF



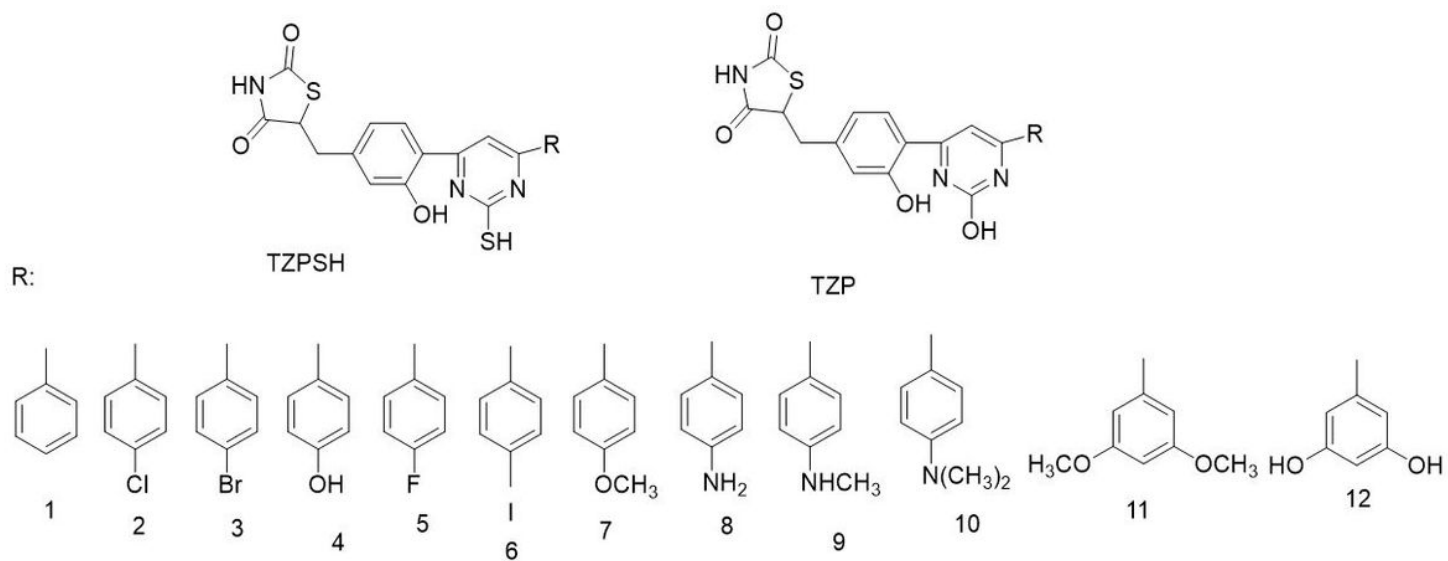
**Figure 7**

RT-PCR analyses of hub genes. A) CEBPD B) TP73C) ESR2 D) TAB1 E) MAP3K5 F) FN1 G) UBD H) RUNX1 I) PIK3R2 J) TNF



**Figure 8**

Structures of designed molecules



**Figure 8**

Structures of designed molecules

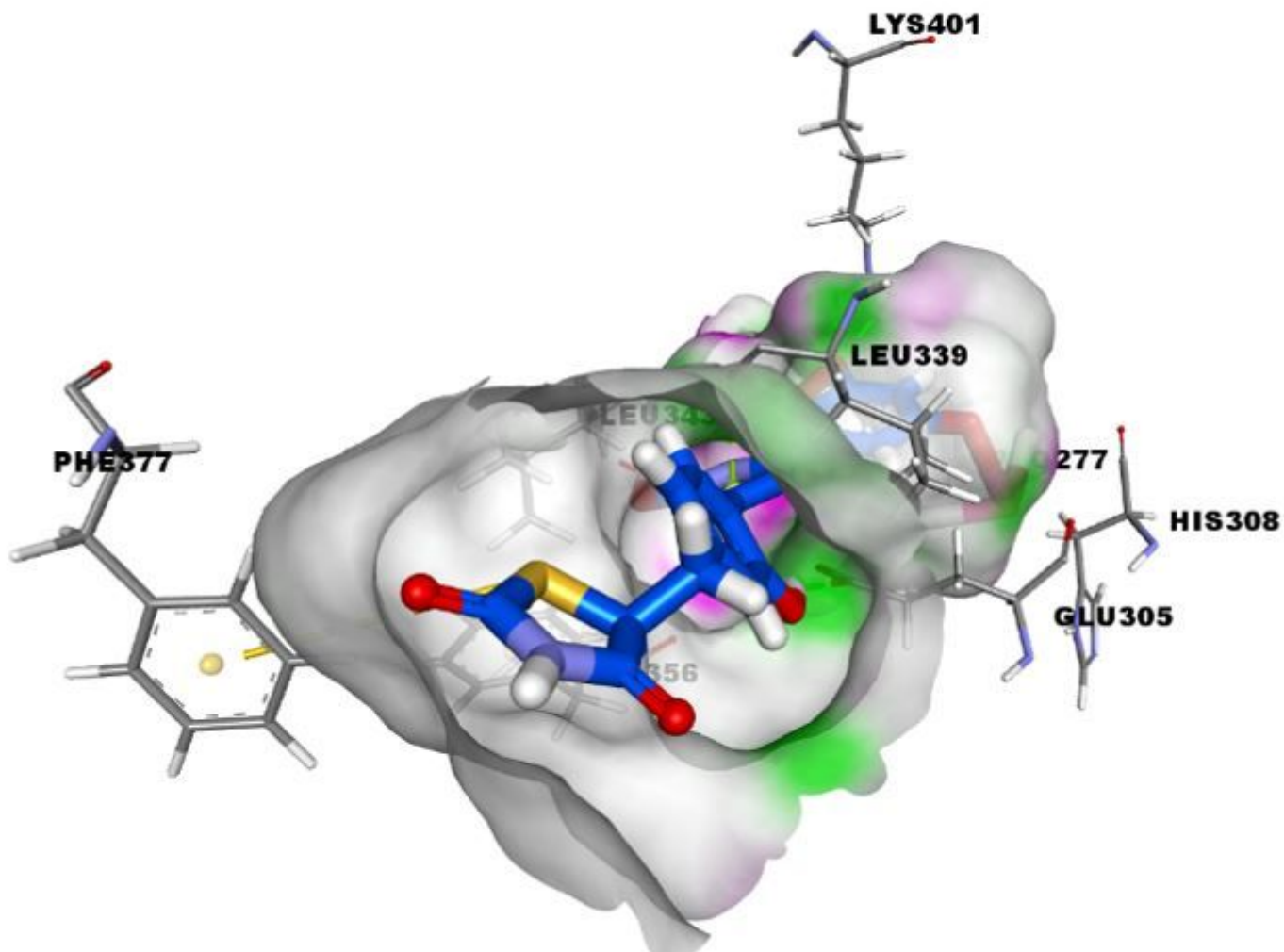


Figure 9

3D Binding of molecule TZP22with 2IOG

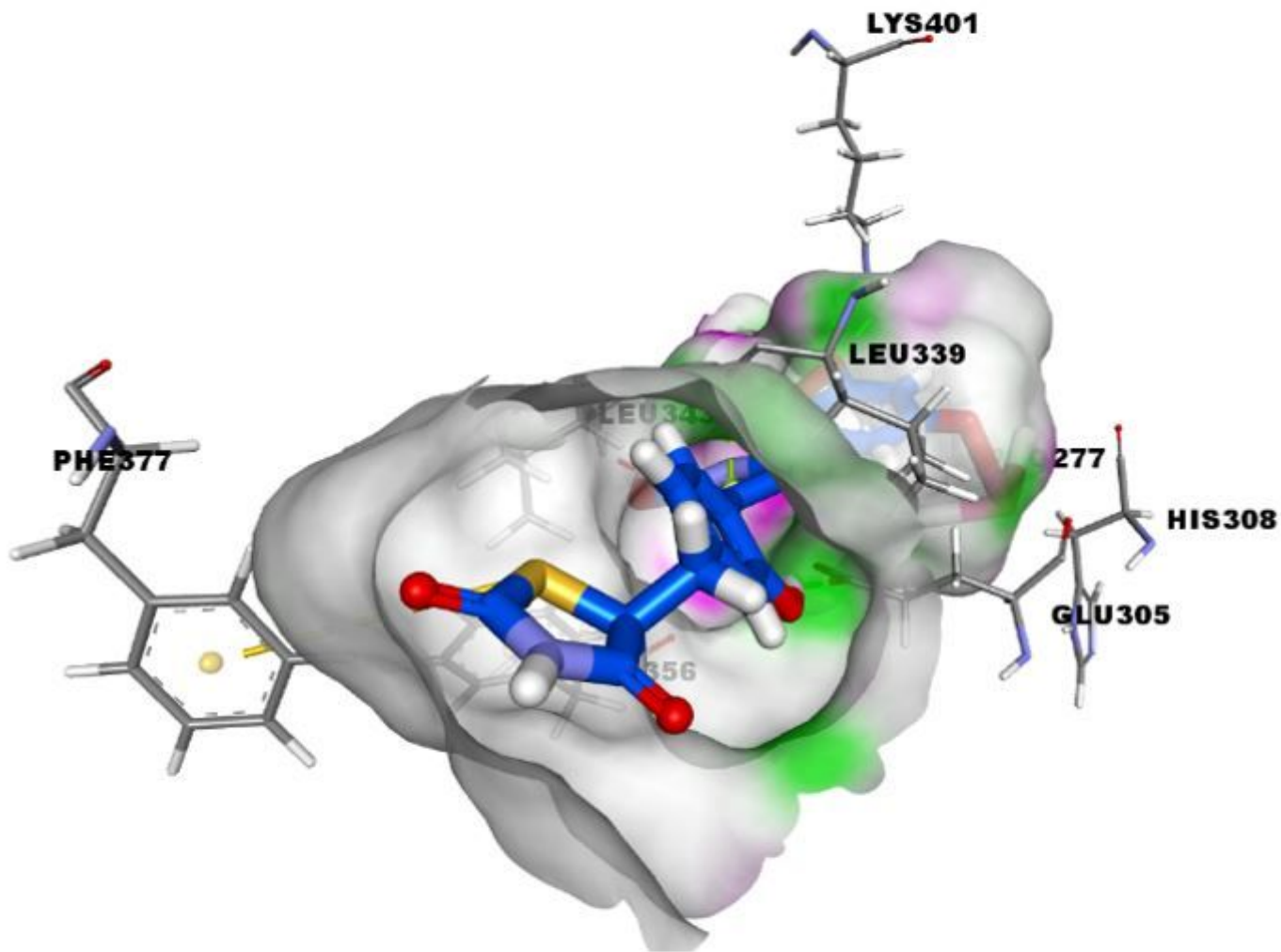


Figure 9

3D Binding of molecule TZP22with 2IOG

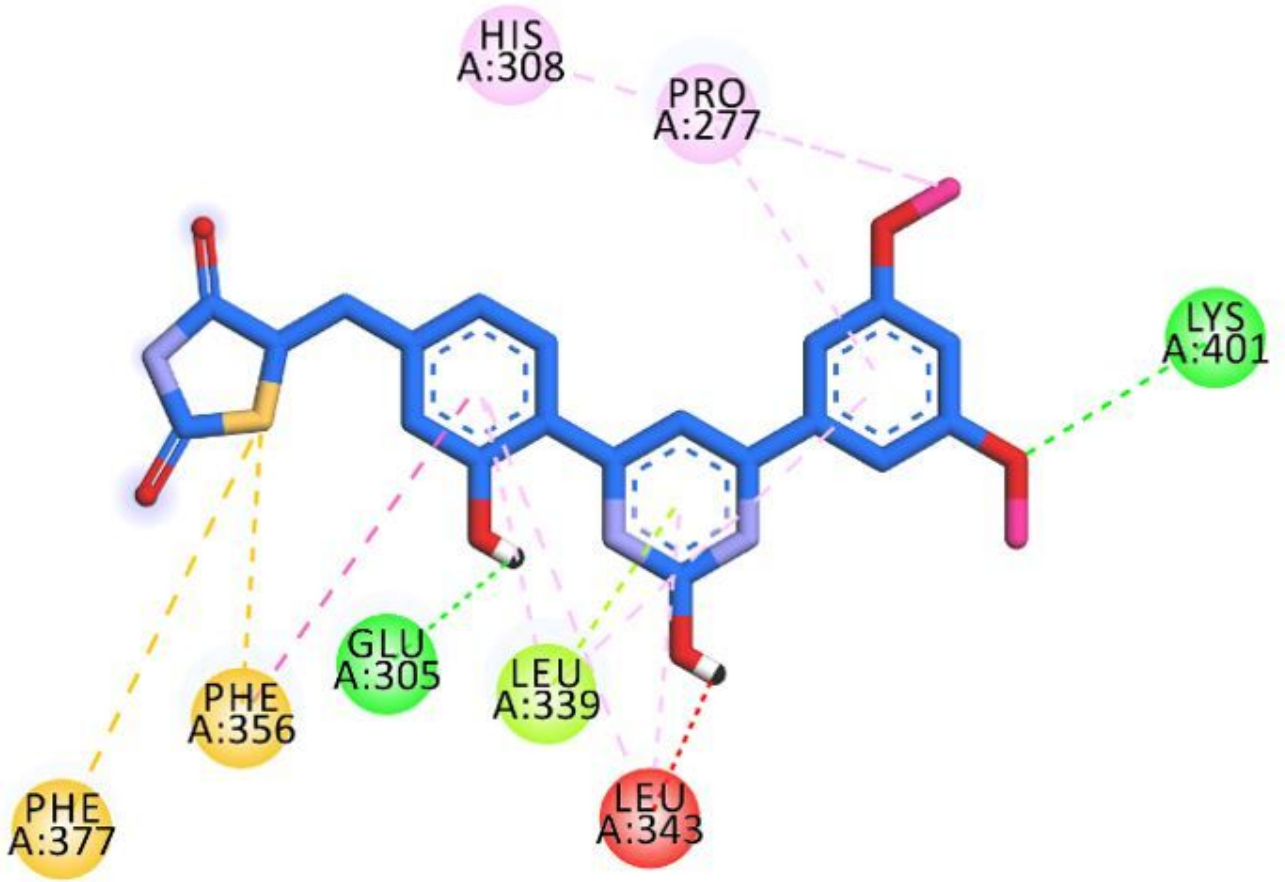


Figure 10

2D Binding of molecule TZP22with 2IOG

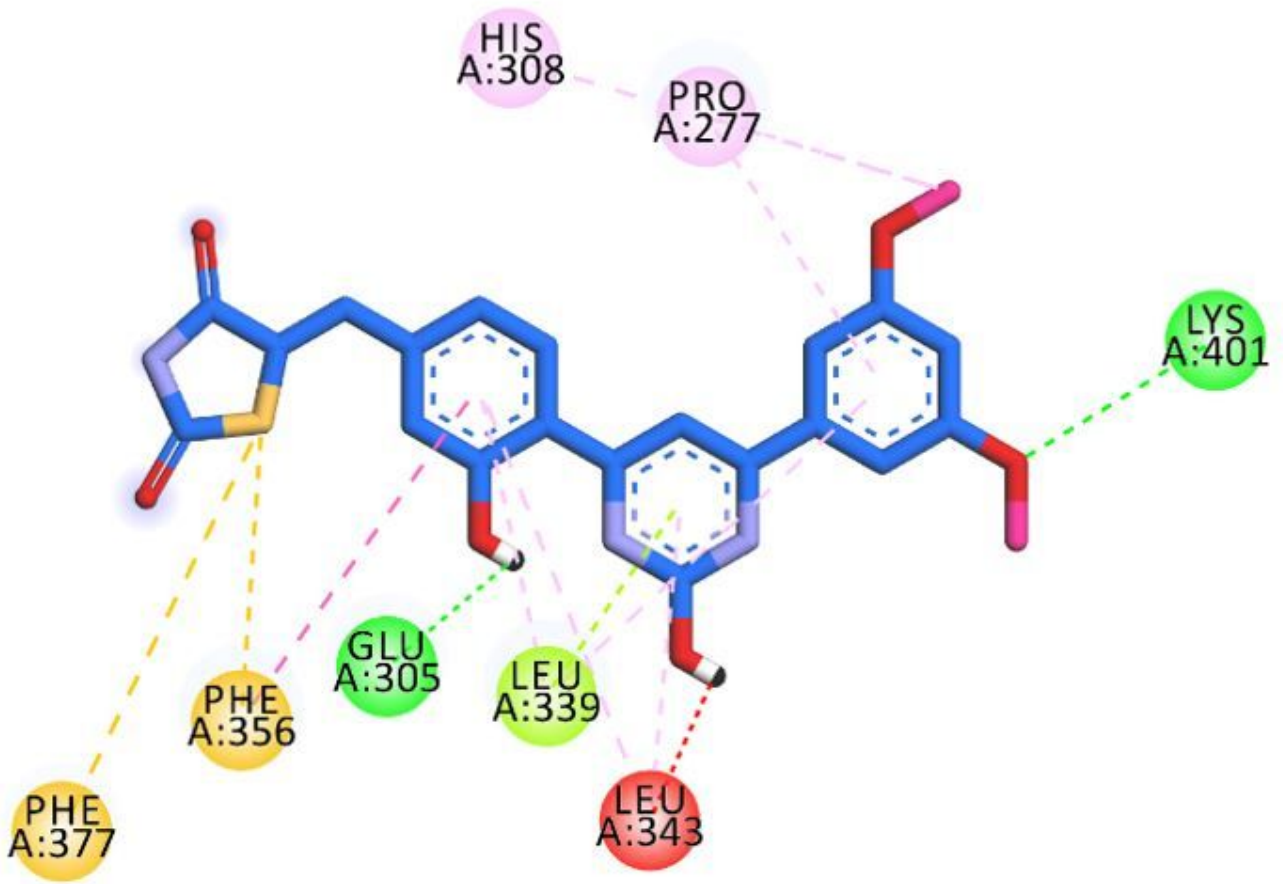


Figure 10

2D Binding of molecule TZP22with 2IOG

## Supplementary Files

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