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Research Article

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Pre-Clinical Computational Screening of New 3-(2-Oxo-2H-Chromen-3-yl)-5-(p-Substituted Phenyl)-4,5-Dihydro-Pyrazole-1-Carbothioic Acid Amide as a Novel Class of Potential Antidiabetic Derivatives

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ABSTRACT

The ten different functional group of 3-(2-Oxo-2H-chromen-3-yl)-5-(p-substituted phenyl)-4,5-dihydro-pyrazole-1-carbothioic acid amide was synthesized by the reaction of 3-(3-substituted phenyl-acryloyl)-chromen-2-one with thiosemicarbazide. Pre clinical screening was done using bioinformatics structure based drug designing technique to reduce the time and money investments. Common method of Insilco screening based on structure scaffold is molecular docking; it is iterative process to virtually screen the compounds binding to the active site of the drug target protein. Among, ten compounds the compound 6 shows negative free energy binding with active site amino acid H-Bond interaction is crucial. Thus, in future this compound 6 as a drug candidate can be used for down regulation of insulin receptor through binding to PTP1B enzyme.

Keywords: 3-(3-substituted phenyl-acryloyl)-chromen-2-one; Thiosemicarbazide; Molecular docking; Drug candidate, PTP1B

INTRODUCTION

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin [1] .Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar .Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaires function or serious damage to many of the body systems like eyes, kidneys nerves, heart and blood vessels[2-7].

The common signs and systems are excessive thirst and urination, weight loss or gain, fatigue and influenza like symptoms.Early diabetes symptoms can be very mild and often even unnoticeable. Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world[8] .Diabetes mellitus is a group of syndromes characterizes by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases. Most patients can be classified clinically as having either Type I diabetes mellitus

Historically, different substituted pyrazoles[9] were known for their hypoglycemic activity in vivo, but in a search for novel structural classes of drugs inhibiting the activity of the ATP-K+ channel of the beta cell pancreatic membrane, including the production of insulin we turned our attention to substituted pyrazoline derivatives. The pyrazoline ring is a prominent structural moiety found in numerous pharmaceutically active compounds. 2-

pyrazolines display a broad spectrum of potential pharmacological activities and are present in a number of pharmacological active molecules such as phenazone/ amidopyrene/ methampyrone(analgesic and antipyretic), azolid/ tandearil (anti-inflammatory) indoxacarb (insecticide) and anturane (uricosuric).Changes in their structure have offered a high degree of diversity that has proven useful for the development of new therapeutic agents having improved potency and lesser toxicity.

The title compound Pyrazoline is five-membered heterocyclic having two adjacent nitrogen atoms within the ring. It has only one endocyclic double bond and is basic in nature[10]. It plays a crucial role in the development of theory in heterocyclic chemistry and is also extensively used as useful synthons in organic synthesis[11]. Pyrazolines have been reported to show a broad spectrum of biological activities including antibacterial[12] ,antifungal[13], anti-inflammatory[14] ,analgesic[15] , antipyretic[16],insecticidal[17] ,diuretic [18] It was thought of interest to synthesize some new pyrazoline derivatives starting from chalcones and substituted hydrazide. In this current study the synthesized compounds are screened and docked with PTP1B.

MATERIALS AND METHODS

All chemicals were obtained from commercial sources and used without any further purification. All the melting points were determined by digital melting point apparatus.IR spectra were recorded in Shimadzu FT-IR-8400 instrument using KBr pellet method. The ^{1}HNMR spectral data were recorded on Bruker AV 400 MHz in $CDCl_3$ using TMS as an internal standard. The purity of the synthesized compounds was ascertained by TLC using iodine vapors as visualizing agents. Steps for synthesis these compounds were shown in the systematic scheme (Figure 1)

Step-1: 3-Acetyl-chromen-2-one (III)

A mixture of salicylaldehyde (1.88 g, 10mmol, 1eq.), ethyl acetoacetate (1.30g, 10 mmol, 1 eq.) and a few drops of piperidine were mixed for 5 min. at room temperature without any solvent. Reaction was neutralized with HCl (1M) and finally the product was isolated by filtration. The final compound was then recrystallized in ethanol.

Step-2: 3-(3-substituted phenyl-acryloyl)-chromen-2-one (IV a-j)

A mixture of 3-acetyl, 6-methoxy coumarin (1 eq.) and the corresponding Para substituted benzaldehyde (1.2 eq.) in ethanol was stirred with a few drops of piperidine under reflux during 2-12 h. Mixture was cooled and the resulting solid was filtered and purified by flash chromatography using a 8:2 mixture of hexane: ethyl acetate as eluent.

Step-3: 3-(2-Oxo-2H-chromen-3-yl)-5-(p-substituted phenyl)-4, 5-dihydro-pyrazole-1 carbothioic acid amide (Va-j)

In to a clean dry round-bottomed flask containing 25 ml of glacial acetic acid introduced Coumarin-chalcone (0.01 mole) and the contents of the flask were stirred to get homogenous solution. To the above solution, add thiosemicarbazide (0.01 mole) with constant stirring. After complete addition of the thiosemicarbazide the reaction mixture was refluxed for 4-6hrs at refluxing temperature. Then the reaction mixture was cooled and poured on to 200 gm of crushed ice with constant stirring. Then the reaction mixture was neutralized to litmus to get the solid product and was collected by filtration. Wash solid with water, dried and recrystallized from appropriate solvent.

Molecular property calculation

A series of chemical structures(Figure 2) were taken for molecular property calculation using online tool Molinspiration (http://www.molinspiration.com/) before that smiles (small molecular input line entry system) structures were generated using ACD/ChemSketch .Each property of molecules plays an vital role in biological process, properties likes AlopP, Molecular weight, hydrogen bond acceptors(HBA),hydrogen bond donors (HBD) are four important drug likeness property of Lipinski's rule of 5[19].other property such as TPSA(Total polar surface area) , no of rotatable bonds(nrotb) and volume are important property for drug transportation inside in vivo system.

Computational Pre clinical analysis

Pre-clinical analysis for synthetic compounds are done using admet SAR(http://lmmd.ecust.edu.cn:8000/predict) to predict the pharmacokinetics and pharmacodynamics behavior of the compounds in various models that includes Blood-Brain Barrier, Human Intestinal Absorption ,CYP450 2D6 Inhibitor, Renal Organic Cation Transporter, AMES Toxicity, Carcinogens, Rat Acute Toxicity(LD50),Fish Toxicity(pLC50),Tetrahymena Pyriformis Toxicity(pIGC50).

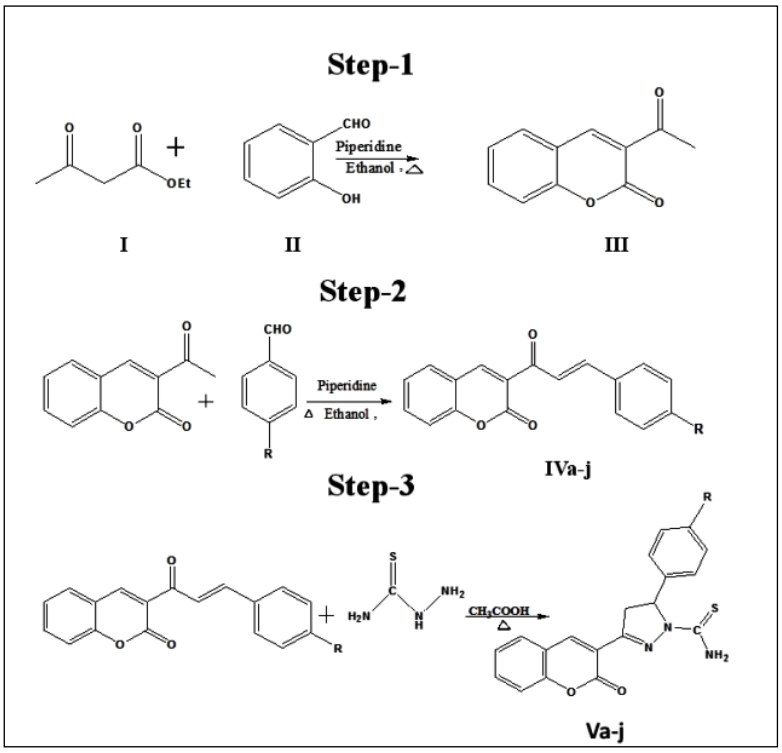

Figure 1: Systematic scheme for synthesis

X-ray crystallography structure of Protein

Down regulation of insulin receptor was activated by an enzyme Protein tyrosine phosphatase 1B of unique UniprotID=P18031, EC=3.1.3.48 and PDB ID of 1NNY with X-ray diffraction resolution of about 2.40Å bound with chemical structure of 3-({5- [(n-acetyl-3- {4-[(carboxycarbonyl) (2-carboxyphenyl) amino]-1-naphthyl}-l-alanyl)amino] pentyl}oxy)-2 naphthoic acid.

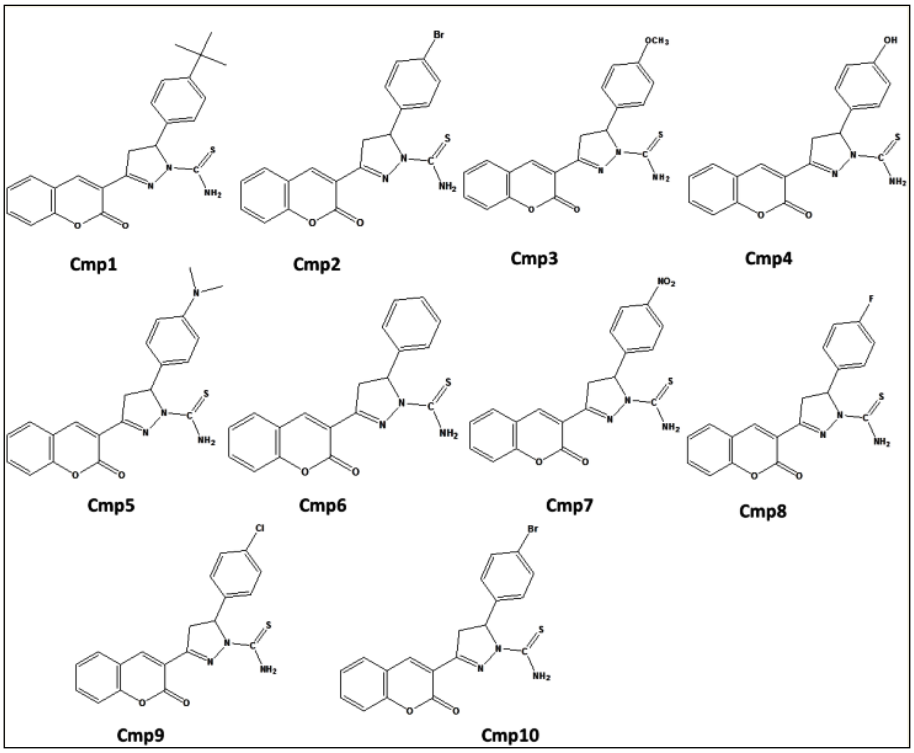

Figure 2: A list of chemical structures

Preparation of protein

The raw protein from protein databank was downloaded and prepared for docking studies initially, all the Hetatms were removed and subsequently all the water molecules were removed and subjected for energy minimization to remove the bad steric clashes using GROMOS96 force filed available through swiss pdb deep view project4.01.

Protein ligand docking

The concept of structure based drug designing (SBDD) is insilico method used in life sciences to screen the set of compounds against drug target protein to check initially the binding interaction between active site protein and ligand functional groups, this concept is commonly used when both protein structure and ligand structure are known for docking .In this current study docking was performed using ARGUS lab software.

RESULTS AND DISCUSSION

Stability of the protein

Initially the energy of the each amino acid residues in the PTP1B enzyme were calculated and found to be -11975.54KJ/mol many amino acid residues shows positive excepted value before optimized with GROMOS96 force filed implemented in Swiss Pdb viewer, after applying force filed to the raw protein the stability of expected value was noted as -15949.93 KJ/mol (Figure 3).

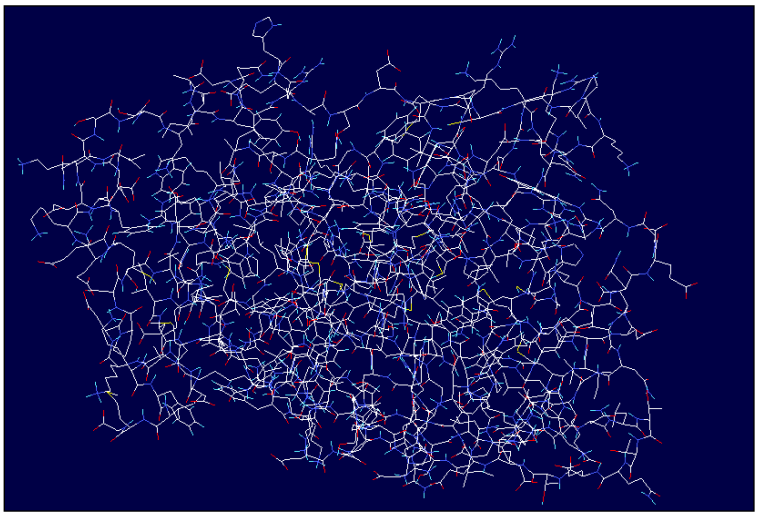

Figure 3: Protein applied with force filed

2D-Property and Pre-Clinical testing

Molecular properties are important for bioavailability and transportation of drug from one part body to another part, any change in drug likeness property of the drug will cause undesirable effects to the in vivo system sometimes it may lead to side effects and toxicity inducing in the body.

Table 1: Molecular property of lead compounds

Compounds	ALogP	PSA	natoms	Molecular Weight	nrotb	volume	HBA	HBD
Cmp1	4.936	81.07	31	435.539	5	389.71	5	1
Cmp2	4.022	81.07	28	393.459	4	340.084	5	1
Cmp3	3.52	90.3	29	409.458	5	349.069	6	1
Cmp4	3.294	101.298	28	395.432	4	331.541	6	2
Cmp5	3.698	84.308	30	422.5	5	369.429	6	1
Cmp6	3.536	81.07	27	379.432	4	323.523	5	1
Cmp7	3.43	126.894	30	424.43	5	346.857	7	1
Cmp8	3.742	81.07	28	397.423	4	328.45	5	1
Cmp9	4.201	81.07	28	413.877	4	337.059	5	1
Cmp10	4.284	81.07	28	458.328	4	341.409	5	1

Hence as per rule of Lipinski's [19]the AlogP<=5, Molecular weight<=500, HBA<=10, HBD<=5, Veber states[20] that rotatable bonds <=10, PSA<=140 from table 1 it shows that compounds taken for this study obeys drug likeness property(Table 1).The pre clinical analysis of compounds using the structure of its 1D property ,is vital for any lead candidate. It is also a kind of virtual screening process to eliminate the large sampling of

compounds. In this work, the selective models are taken and their behavior was studied. Table 2 states the pharmacokinetics and dynamic behavior of the synthesized compounds. The values of each models is probability, BBB(Blood brain barrier) is positive result for all the compound except Cmp4 and Cmp6 , Human Intestinal Absorption of compounds shows HIA+ that indicates it has good absorption property for all the compound . Other parameters such as CYP450 2D6 Inhibitor and Renal Organic Cation Transporter for all the compound are non-Inhibitor .Similarly, AMES Toxicity shows positive effect for Cmp5 and Cmp7,whereas other compounds are nontoxic and Carcinogens shows negative effect on all compounds that indicates compounds are non-Carcinogen.

Table 2: Kinetic and dynamic behavior of the compounds

Compounds	Blood-Brain Barrier	Human Intestinal Absorption	CYP450 2D6 Inhibitor	Renal Organic Cation Transporter	AMES Toxicity	Carcinogens
Cmp1	0.7116	1	0.8968	0.6942	0.5646	0.7922
Cmp2	0.8456	1	0.863	0.6243	0.5096	0.8486
Cmp3	0.7318	0.9925	0.8709	0.6502	0.5	0.847
Cmp4	0.6076	0.9657	0.8356	0.6779	0.5123	0.8423
Cmp5	0.8254	1	0.8856	0.6874	0.5272	0.7489
Cmp6	0.6076	0.9657	0.8356	0.6779	0.5123	0.8423
Cmp7	0.6506	0.9411	0.8989	0.7753	0.712	0.8085
Cmp8	0.8565	1	0.8492	0.6207	0.5361	0.8287
Cmp9	0.8449	1	0.8553	0.5846	0.5541	0.813
Cmp10	0.8348	1	0.8463	0.5839	0.5458	0.839

Lethal concentration (LC50) and its associated parameters are very important during the time of screening drugs in in-vitro lab testing. The three models such as Rat Acute Toxicity, Fish Toxicity and Tetrahymena Pyriformis Toxicity are chosen and their respective values of each compounds are listed in the table 3.

Table 3: Toxicity profile of the compounds

Compounds	Rat Acute Toxicity(LD50) (mol/kg)	Fish Toxicity(pLC50) (mg/L)	Tetrahymena Pyriformis Toxicity(pIGC50) (ug/L)
Cmp1	2.7043	0.9042	0.6795
Cmp2	2.5526	1.1129	0.6178
Cmp3	2.5498	1.0185	0.6436
Cmp4	2.469	1.1965	0.7048
Cmp5	2.6057	0.9978	0.6012
Cmp6	2.469	1.1965	0.7048
Cmp7	2.5146	1.1845	0.6883
Cmp8	2.5426	1.0717	0.7018
Cmp9	2.4842	1.0471	0.7759
Cmp10	2.5303	1.1242	0.8283

Receptor ligand interaction

The ligand and the active site active amino should have binding interaction in between them, functional groups present in the ligand plays important role in interaction any change in functional moiety may or may not interact with binding site of the receptor. A chemist should priory check with active functional groups in the parent structure using cheminformatics tools and software's to produce bioactive compounds to save time and synthesis cost. Receptor can be any type of biomolecule like protein, enzyme, DNA and lipids, preferably proteins and enzyme were used in any type of drug designing most commonly in rational based drug designing. Nearly 97 percent docking was based on structure based drug designing concept in bioinformatics and also we applied the same concept of SBDD. The crucial amino acid in active site were defines as binding site in arguslab docking with grid spacing of 17.25 (X), 16.75 (Y) , 18.75 (Z) in 3D direction respectively with 0.4Å of grid resolution and 157 grid points (Figure 4).An empirical scoring function of Ascore was kept as default optioned ligand type as flexible and rigid docking with high precision for docking and 150 poses for single docking process. Ten compounds were docked to the active site among various functional group of compounds Cmp6 shows least binding energy of -7.94 Kcal/mol and interaction with crucial amino acid Arg221 (Figure 5)

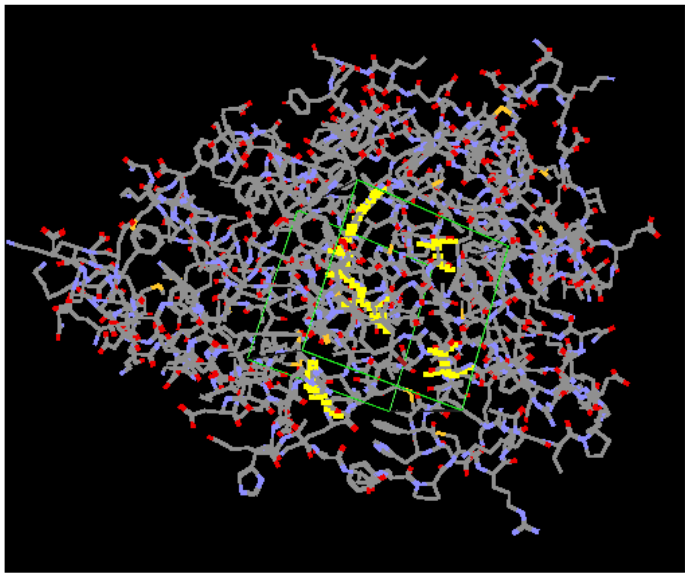

Figure 4: Binding site of protein with grid

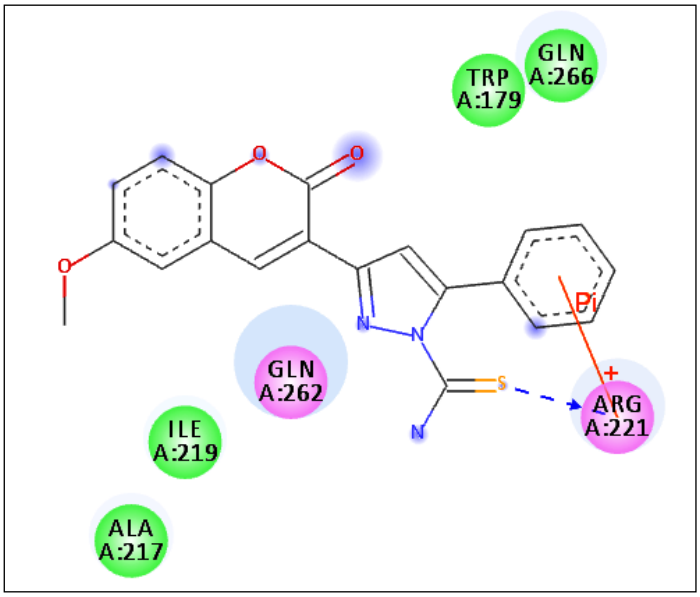

Figure 5: Compound 6 interaction with crucial amino acid

CONCLUSION

We designed and synthesized ten different functional group of compounds and initially screened using bioinformatics tools to check the active pharamacopore and important functional group for the specific binding .The computational pre-clinical testing and drug likeness property are calculated for the compounds, finally the docking was done. Among ten compounds only compound 6 structure is more favorable for binding to active site shape of the drug target protein PTP1B enzyme. Hence, in future the theoretical data of pre clinical testing along with the docked details will do needful for in-vitro and in-vivo clinical trials and the compound6 can be drug candidate for treating the diabetic.

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