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QUINAZOLINE-PURINE DERIVATIVES AS ANTIDIABETICS: SYNTHESIS, *IN-SILICO* AND *IN-VITRO* EVALUATION

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ABSTRACT

The study involved condensation of 8-bromo-7-(but 2yn-1yl)-3-methyl-3,7-dihydro-1H-purine-2,6-dione with 2-(chloro methyl)-4-methyl quinazoline. The compound obtained was assigned 3, on which different substitutions were made at 8th position, to obtain various derivatives of quinazoline. The obtained derivatives were characterized using I.R, ^{1}H-NMR, ^{13}C-NMR and Mass spectra. For finding antidiabetic activity of all synthesized compounds, they were subjected for their inhibitory activity on α-amylase, using Acarbose as standard and DPP-4 using Metformin as standard. The results obtained, from the activity performed was nearly equal with that of the standards used and synthesized compound 3B was found to possess inhibitory activity. It has also been confirmed by docking studies, that Compound 3B and Metformin when docked on 3 proteins 2ONC, 5Y7J and 2OQV, Compound 3B was found to be most potent drug candidate against DPP-4.

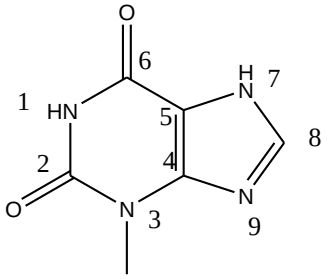
KEY WORDS: Antidiabetic activity, *In silico* Docking, *In vitro* DPP-4, *In vitro* α-amylase, Quinazoline derivatives.

RUNNING TITLE: Quinazoline-Purine derivatives for Antidiabetic activity.

INTRODUCTION:

The basic nucleus involved in the synthesis of the derivatives of quinazoline mentioned in the scheme is purine. Purines mainly consist of pyrimidine ring fused with imidazole[i].

Quinazolines are benzo fused pyrimidine compounds (Figure 1), which have been synthesized since long time, using various reaction conditions and various starting materials. Quinazolines are also used as building blocks for many naturally occurring alkaloids from plants, animals and microorganisms[i]. Majority of the synthesized quinazoline derivatives have been used for their antidiabetic[ii], analgesic[iii-v], antioxidant[vi-viii], anticancer[ix-xiii], anti-inflammatory[xiv-xxii], anticonvulsant[xxiii], antibacterial[xxiv], antifungal[xxv], anti-mycobacterial[xxvi-xxvii], farnesyl transferase, gastric H^+/K^+-ATPase and MAP-kinase p38 inhibitory[xxviii-xxix] activity.



3-methyl-3,7-dihydro 1H-purine-2,6-dione

Figure 1: Basic nucleus of purine

Diabetes mellitus, a metabolic disorder with varied etiologies, and is identified as hyperglycemia. The disorder results from abnormal metabolism carbohydrate, protein and fat, ultimately resulting in diabetes[xxx].

Dipeptidyl peptidase-4 (DPP4), is a protein, which is also known as adenosine deaminase complexing protein 2 or CD26 (cluster of differentiation 26). In humans, this protein was encoded as DPP-4 gene and plays a major role in glucose metabolism. DPP-4 is responsible for degradation of Glucigon like peptide (GLP-1) and Glucose-dependent insulinotropic peptide (GIP)[xxxi-xxxiii]. Oral hypoglycemics, act by inhibiting DPP-4 enzyme, there by prolongs the effect of incretin *in vivo*. Oral hypoglycemic, while inhibiting DPP-4, preserve the GLP, GIP so that incretins were reduced and hence successful in treatment of diabetes. Based on these aspects many drugs are being explored for treatment and management of type 2 diabetes[xxxiv-xxxix]. The article provides information regarding, the design of potent quinazoline derivatives, toxicity and molecular properties, including docking studies and *in vitro* activity of the synthesized molecules for their inhibitory activity on DPP-4[xl-xlii].

EXPERIMENTAL SECTION:

Instruments and chemicals:

The chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), Rankem (Gurgaon, India), and S.D. Fine Chemicals Pvt Ltd. (Mumbai, India). Melting points was measured by open capillary method in ^oC and the information was considered as uncorrected. Infrared spectra (IR) recorded in FT-IR instruments in cm^{-1}. ^1H-NMR and ^{13}C-NMRspectra was recorded Bruker UX-NMR instrument. Mass spectra recorded on Mass spectrometer. Elemental analysis was carried out on VARFIO EL, se Elementor. Thin-layer Chromatography (TLC) was performed using pre coated plates. Visualization of the spots on

TLC plates was using iodine vapor or UV light. All compounds were extracted using Ethyl aceto acetate and water, concentrated using Rotary evaporator.
The Scheme followed was represented in (Figure2)

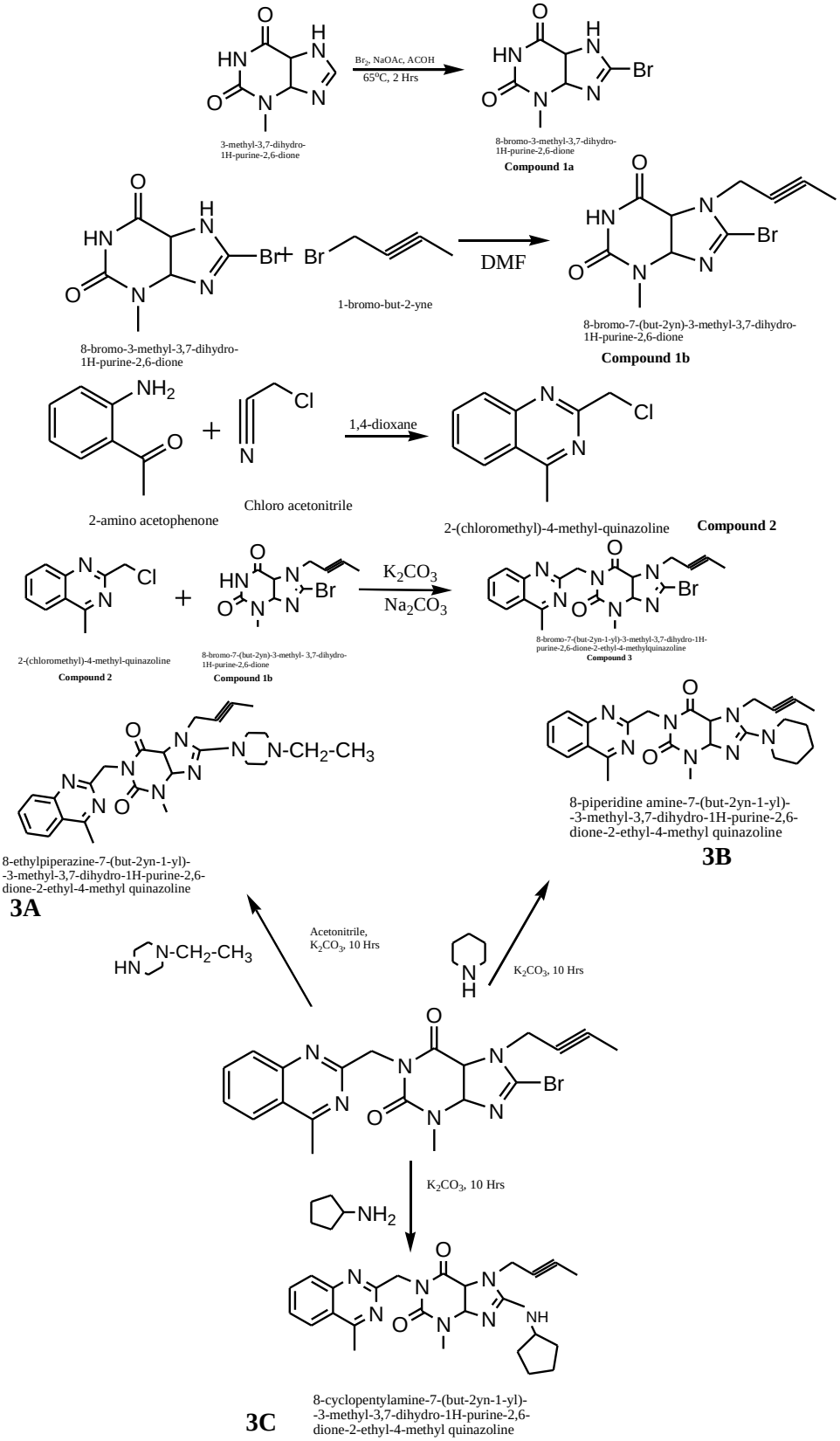


Figure 2: Scheme

Synthesis of (1a):

5 gm of Xanthine, 6.16 gm of Sodium acetate (NaOAc), 40 ml of acetic acid were transferred to round bottom flask (RBF), to this 2.25 ml of Bromine solution was added drop by drop. The temperature was maintained at 65^{o}C with constant stirring and refluxed for 2 hours using oil bath. After the completion, RBF was removed and continue stirring for half an hour on a magnetic stirrer and then poured into a beaker containing crushed ice. The product formation was confirmed by TLC using Hexane: Ethyl acetate (9:1) as mobile phase and observed under U.V light. The product was formed was placed on ice bath and then filtered and Sodium thio sulphate was added to the filtrate and the compound was filtered by using vacuum. Dry the compound in hot air oven at 60^{o}C for 12 hour[xliii]. The product was weighed (6.3 gm). M.F: $C_{26}H_{30}N_8O_2$, M. Wt: 486.21, Yield: 61%, Melting point: 224.8-228.4^{o}C, Appearance: Pale yellow. IR (KBr) cm^{-1}: 2972 (Aliphatic C-H, Str); 1702, 1663 (C=O); 1264 (C-N. Str); 1166 (C-O. Str); 1243 (Aromatic C=C); 3390 (NH); 785 (C-Br); 764 (disubst. benzene), ^{1}H-NMR (δ): 2.8 (Methyl protons); 9.5, 10.2 (Amine protons), ^{13}C-NMR (δ): 33.6 (Methyl carbon); 120.6-158.2 (Aromatic carbons), Mass spectrum (*m/z*): 487 $(M+1)^{+}$.

Synthesis of (1b):

1 gm of 8-bromo xanthine, 0.77 ml of DIPEA (Di isopropyl ethyl amine) and 10 ml of Dimethyl formamide (DMF) were taken in a RBF. Mixture was stirred for 20 mins on a magnetic stirrer until the clear solution persists. To this 1-bromo-2-butyne was added drop by drop and stirring was continued for 20 mins until the solid compound was formed. Formation of the product was confirmed as above. M.F: $C_{10}H_9N_4O_2Br$, M. Wt: 297.03, Yield: 58%, Melting point: 98-100^{o}C, Appearance: Pale yellow. IR (KBr) cm^{-1}: 2865 (Aliphatic C-H, Str); 1703, 1665 (C=O); 1273 (C-N. Str); 1185 (C-O. Str); 1245 (Aromatic C=C); 3385 (NH); 782 (C-Br); 762 (disubst. benzene), ^{1}H-NMR (δ): 1.7, 3.0 (Methyl protons); 2.6 (Methylene protons); 9.8 (Amine protons), ^{13}C-NMR (δ): 3.7, 33.5 (Methyl carbons); 19.6 (Methylene carbons); 78.9, 80.4 (Alkyne carbons); 105-159 (Aromatic carbons), Mass spectrum (*m/z*): 298 $(M+1)^{+}$.

Synthesis of (2):

About 9.09 ml of 2-amino acetophenone, 30 ml of 1, 4-dioxane and 28 ml of 1, 4-dioxane hydrochloride were taken in a three necked RBF. The above mixture was stirred for an hour at room temperature (R.T). Solid compound was formed and to this, 2-chloro acetonitrile was added in portions at R.T. Reaction mixture was heated to 80-90^{o}C and stirred for 12-16 hours. Completion of reaction was confirmed by TLC using Hexane: Ethyl acetate (7:3) as mobile phase and observed in the U.V light.. Reaction mixture was cooled to R.T and basified with aqueous NaOH solution until pH reaches 9-10. Then the compound was extracted with Ethyl acetate and water repeatedly and then finally with NaCl (Brime solution). The compound was concentrated at 50^{o}C by using 75 RPM by adding Sodium sulphate. After concentration, the compound was dried. M.F: $C_{10}H_9N_2Cl$, M. Wt: 192, Yield: 62%, Melting point: 168-170^{o}C, Appearance: Pale yellow. IR (KBr) cm^{-1}: 2820 (Aliphatic C-H, Str); 1285 (C-N. Str); 1166 (C-O. Str); 1246 (Aromatic C=C); 675 (C-Cl); 760 (disubst. benzene), ^{1}H-NMR (δ): 2.4 (Methyl protons); 4.5 (Methylene protons) 7.5-8.2 (Aromatic protons), ^{13}C-NMR (δ): 25.4 (Methyl carbons); 49.5 (Methylene carbons); 127.5-139.9 (Aromatic {CH} carbons); 128.3-176.5 (Aromatic carbons), Mass spectrum (*m/z*): 193 $(M+1)^{+}$.

Synthesis of (3):

About 10 gm of 8-bromo-3-methyl-3,7-dihydro-1H-purine-2,6-dione-pent-2-yne, 6.46 gm of 2-(chloro-methyl)-4-methyl quinazoline and 9.29 gm of Potassium carbonate and 100 ml NMP (N-methyl-2-pyrrolidone) solvent were taken in RBF and maintained at RT. The

mixture was stirred for 15 hours at 100°C on magnetic stirrer. Completion of reaction was confirmed by TLC using Hexane: Ethyl acetate (7:3) as mobile phase and observed in the U.V light. The reaction mixture was cooled. To this, water was added and stirred on magnetic stirrer. The compound was filtered under vacuum. M.F: $C_{20}H_{17}N_6O_2Br$, M. Wt: 453, Yield: 56%, Melting point: 155-157°C, Appearance: Pale yellow, IR (KBr) cm^{-1}: 2969 (Aliphatic C-H, Str); 1619, 1651 (C=O); 1230 (C-N. Str); 1151 (C-O. Str); 1243 (Aromatic C=C); 767 (disubst. benzene), ^{1}H-NMR (δ): 1.5, 1.8, 2.8 (Methyl protons); 3.6 (Methylene protons); 7.5-8.1 (Aromatic protons); 5.2 (Alkyne protons), ^{13}C-NMR (δ): 3.5, 15.8, 24.8, 33.8 (Methyl carbons); 19.6, 34.2 (Methylene carbons); 74.5-78.1 (Alkyne carbons); 5.2 (Alkyne protons); 123.6-140.5 (Aromatic {CH} carbons); 108-176.8 (Aromatic carbons), Mass spectrum (*m/z*): 454 $(M+1)^+$.

Synthesis of (3A):

1 gm of quinazoline derivative prepared in step (3), 0.346 ml of N-ethyl piperazine and 10 ml of acetonitrile were taken in RBF and refluxed for 10 hours. To this 0.78 gm of Potassium carbonate was added and the reflux was continued. The reaction mixture was cooled to R.T and stirred with water. Solid compound formed, was filtered and dried under vacuum. Completion of reaction was confirmed by TLC using Methanol: Dichloromethane, (1:9) as mobile phase and observed in the UV light. M.F: $C_{26}H_{30}N_8O_2$, M. Wt: 486, Yield: 56%, Melting point: 226-228°C, Appearance: Pale yellow, IR (KBr) cm^{-1}: 2969 (Aliphatic C-H, Str); 1619, 1651 (C=O); 1230 (C-N. Str); 1151 (C-O. Str); 1243 (Aromatic C=C); 767 (disubst. benzene), ^{1}H-NMR (δ): 1.2, 1.6, 2.5 (Methyl protons); 2.4-2.9 (Methylene protons); 7.4-8.1 (Aromatic protons); 4.8 (Alkyne protons), ^{13}C-NMR (δ): 3.6, 14.2, 15.9, 23.6, 32.5 (Methyl carbons); 19.4, 34.4-52.9 (Methylene carbons); 74.4-78.1 (Alkyne carbons); 124.8-138.5 (Aromatic {CH} carbons); 109.4-170.3 (Aromatic carbons), Mass spectrum (*m/z*): 487 $(M+1)^+$.

Synthesis of (3B):

2.0 gm of quinazoline derivative prepared in step (3), 0.48 ml of Piperidine were taken in RBF and stirred for half an hour. To this 1.52 gm of Potassium carbonate was added and refluxed for 10 hour. Reaction mixture was cooled to RT and stirred with water. Formed solid compound was filtered using water and dried under vacuum. Completion of reaction was confirmed by TLC using Ethyl acetate: Hexane (8:1) as mobile phase and observed in the U.V light. M.F: $C_{25}H_{27}N_8O_2$, M. Wt: 471.24, Yield: 57%, Melting point: 196-198°C, Appearance: Pale yellow, IR (KBr) cm^{-1}: 2937 (Aliphatic C-H. Str); 1660, 1702 (C=O); 1231 (C-N. Str); 1131 (C-O Str); 1253 (Aromatic C=C); 760 (Disubst benzene), ^{1}H-NMR (δ):1.6, 1.8, 2.8 (Methyl protons); 3.4, 3.6 (Methylene protons); 7.4-8.1 (Aromatic protons); 4.8 (Alkyne protons), ^{13}C-NMR (δ):3.8, 14.8-32.9 (Methyl carbons); 13.5, 26.4-53.6 (Methylene carbons); 76.8-81.1 (Alkyne carbons); 123.4-150.9 (Aromatic {CH} carbons); 115.2-171.9 (Aromatic carbons), Mass spectrum *m/z*: 472 $(M+1)^+$.

Synthesis of (3C):

2 gm of quinazoline derivative prepared in step (3), 0.652 ml of Cyclopentylamine were taken in RBF and stirred for half an hour. To this 1.52 gm of Potassium carbonate was added and refluxed for 10 hour. Reaction mixture was cooled to RT and stirred with water. Formed solid compound was filtered by using water and dried under vacuum. Completion of reaction was confirmed by TLC using Ethyl acetate: Hexane (5:5) as mobile phase and observed in the U.V light. M.F: $C_{25}H_{27}N_7O_2$, M. Wt: 457, Yield: 57%, Melting point: 237-239°C, Appearance: Pale yellow. IR (KBr) cm^{-1}:3335 (2° NH); 2954 (Aliphatic C-H. Str); 1702, 1652 (C=O); 1227 (C-N. Str); 1130 (C-O Str); 1293 (Aromatic C=C); 761 (Disubst benzene), ^{1}H-NMR (δ): 1.8, 2.2 (Methyl protons); 2.5, 2.6, 2.9, 3.5, 3.8 (Methylene protons); 7.5-8.1

(Aromatic protons); 5.1 (Alkyne protons); 7.4 (2^{o} NH), ^{13}C-NMR (δ): 3.8, 16.2-34.6 (Methyl carbons); 18.5, 25.8-36.4 (Methylene carbons); 72.5-78.1 (Alkyne carbons); 55.1 (Aliphatic carbon); 126.4-140.9 (Aromatic {CH} carbons); 100.6-166.3 (Aromatic carbons), Mass spectrum *m/z*: 458 $(M+1)^{+}$.

Protocol of Molecular docking:

The software used for finding whether a molecule can be a drug or not is by Lipinski rule of five. It gives information about Molecular weight, Hydrogen bond donor, hydrogen bond acceptor, logP value and Molar refractivity. Molsoft L.L.C Drug Likeness and Molecular Property Prediction gives information about a molecule i.e Molecular formula, Molecular weight, Hydrogen bond acceptor, Hydrogen bond donor, MolLogP, MolLogS, MolPSA, MolVol, pKa, BBB Score, Number of stereo centers and Drug Likeness Score. OSIRIS Property explorer provides information whether a molecule, if synthesized causes any toxicity effect by showing in the window on the screen, Green color indicates non- toxic whereas Red color toxic. Apart from this other information like solubility, TPSA, clogP, Drug Likeness and Drug Score are available. PASS (Prediction of Activity Spectra for Substances) online software predicts whether a molecule is biologically active, as value Pa and if inactive as Pi. Drug activity ADME, Physicochemical parameters, GI Absorption, Drug likeness, GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor and Bioavailablity score were found out using Molinspiration and SWISS ADME softwares[xl-xlii, xliv-lxiv]. For, all the above activities, internet is required.

For molecular docking, the software used was Autodock 4.0/4.2, as it was user compatible software which was most widely used for Protein-Ligand binding. It can be used in any of the three methods like Rigid Body Docking, Flexible Ligand Docking and Flexible ligand and protein respectively[xl-xlii, xliv-lxiv].

Molecular properties, Druglikeness, Bioactivity with respect to GPCR ligand, Ionchannel modulator, Kinase Inhibitor, Nuclear receptor ligand, Protease inhibitor and Enzyme inhibitor, were found out by using Molinspiration software[xlvi-li].

Physicochemical properties like GI Absorption, Solubility, Inhibitor, Bioavailability and ADME properties were found by SWISS ADME software[xlvi-li].

All allowed torsions, for the ligands were set as flexible. Molecular docking study was executed to understand the probable binding interactions of the synthesized compounds (ligands) onto the active site of the receptors 2ONC, 2OQV and 5Y7J, respectively.

All the hetero atoms including water molecules and bound ligands in PDB crystal structures were removed from the receptors. After adding polar hydrogen and charges, the receptor was set as rigid with no flexible bonds.

The docking glide score, free binding energy (*using Prime MM-GBSA method*), hydrogen bonding and π–π interactions with the surrounding Amino acids were studied to elucidate the binding affinities and appropriate alignment of all the ligands onto the active site of 2ONC, 2OQV and 5Y7J, respectively. The best-suited conformations of ligands, which were successful in reversing the protein in its original conformation and produced maximum dock score, were studied precisely[xl-xlii, xliv-lxiv].

Biological activity:

Antidiabetic Assays

DPP-4 Inhibitory Assay:

DPP-4 inhibitory activity of synthesized compounds was done by using DPP-4 inhibitor screening kit. Initially, 30 µL of diluted assay buffer and 10µL of diluted human-recombinant

DPP-4 enzyme solution were pipetted out and placed in each of the well of a 96-well plate containing 10 µL of samples with different concentrations prepared by using Dimethyl sulfoxide. Then, 50 µL of the diluted fluorogenic substrate, Gly-Pro-Amino Methyl Coumarin (AMC), was added to initiate the reaction. Then the plate was incubated at 37°C for 30 mins. After incubation, the excitation and emission fluorescence of free AMC was measured at 350-360 nm and 450-465 nm, respectively, using a microplate reader (BioTek Instruments, Inc., Winooski,VT, USA). For negative and positive control wells, Dimethyl sulfoxide (solvent) and Metformin standard were used, respectively[lxiii-lxv]. The percentage of inhibition was calculated using the Equation (1):

$$\% \text{ Inhibition} = \frac{\text{OD initial activity - OD inhibitor}}{\text{OD initial activity}} \times 100 \quad (1)$$

α-Amylase Inhibitory Assay:

Assay was done with a slight modification of the method described [64]. Concentrations of the synthesized compounds (0.078-5 mg/mL) were mixed with 30 µl of 0.1 M Sodium phosphate buffer and placed in a 96-well microplate, prior to addition of 10 µl of α-Amylase (1 U/ml) in the wells. The plate was then incubated at 37°C for 15 mins, followed by addition of 30 µL of soluble starch (1.0%) and re-incubated at 37°C for 30 mins. The reaction was stopped by addition of 30 µl Hydrochloric acid (1.0 M) and 30 µl of Iodine reagent. The absorbance was measured at wavelength of 620 nm. Acarbose and Phosphate buffers were used as the positive and negative controls, respectively[lxiii-lxv]. The α-Amylase inhibitory activity was calculated using the Equation (2)

$$\% \text{ Inhibition} = \frac{\text{OD test - OD control}}{\text{OD test}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION:

Chemistry:

Synthesis of new substituted quinazolines comprised of four steps, followed by *In silico* docking studies, and antidiabetic activities.

The first step of synthesis involved preparation of the desired *8-bromo-3-methyl-3, 7-dihydro1H-purine-2, 6-dione* (1a) then converting into compound (1b). Condensation1b and 2, compound 3 was obtained. Finally the derivatives were synthesized according to scheme.

In silico Docking Studies:

All the synthesized compounds were subjected to Lipinski rule of five, all the compounds along with standard, was following the above rule and tabulated in (Table 1). Number of hydrogen bond donors was constant for all of the compounds, and number of hydrogen bond acceptors varied from 4 to 6. But compounds 3B, 3C and standard varied with respect to number of hydrogen bond acceptors from 4-5. Investigation of Lipinski parameters of the synthesized compounds showed that all quinazoline derivatives might be considered drug-like candidates for novel anti-diabetic agents, as they obeyed the rule of five without violating more than one of them.

A computational study for prediction of ADME properties of the molecules was performed by determination of Lipophilicity, Topological Polar Surface Area (TPSA) and simple molecular descriptors used by Lipinski in formulating “rule of five”. (Table 1) represents a calculated, TPSA and Lipinski parameters for the compounds 3A–3C. Too high TPSA values give rise to poor bioavailability and absorption of the drug. So compound 3B has the lower TPSA value (87.46), hence the bioavailability and absorption of the drug will be more.

Table 1: Predicted Lipinski Parameters and Molecular Properties of the Synthesized Compounds

Sl.No	Parameter	3A	3B	3C	Metformin
1	Mol. Wt	456	430	430	118
2	HBD	0	0	0	0
3	HBA	6	5	5	4
4	Log P	-0.734410	-0.497000	-0.091170	-0.513690
5	MR	114.915	109.833900	108.626892	23.128000
6	TPSA	90.7	87.46	96.25	91.49

[HBD: Hydrogen bond donor; HBA: Hydrogen bond acceptor, MR: Molar refractivity, TPSA: Topological Polar Surface Area]

The blood-brain barrier (BBB) has an arbitrate relation between the periphery and the central nervous system (CNS), which protects the brain from toxic adverse effects of the drugs and exogenous molecules. An algorithm, designated 'BBB Score', is used for predicting BBB penetration i.e, the scores between 0-4 are classified as non-CNS drugs and the scores ranging 4-6 are classified as CNS drugs. The BBB Scores for the compounds were given in the (Table 2). The BBB Score for any molecule is calculated by the presence of number of aromatic rings, heavy atoms, molecular weight, hydrogen bond donors, hydrogen bond acceptors, TPSA and pKa of the molecule.

Drug likeness is a qualitative concept used in drug design for how 'a molecule can become drug like is with respect to factors like bioavailability. The activity scores for the four synthesized compounds along with standard were compared and tabulated in (Table 2). The larger the value of score is, higher is the probability that a particular molecule will be active, but as the drug score increases, lipophilicity also increases and hence compound 3A and 3C even though have high score may not become drugs.

Table 2: Estimation of Blood-brain barrier score and Drug-likeness model score for standard and synthesized compounds

Properties	3A	3B	3C	Std.
Molecular formula	C_{26} H_{32} N_8 O_2	C_{25} H_{27} N_7 O_2	C_{25} H_{27} N_7 O_2	C_4 H_{11} N_5
Molecular weight	488.26	457.22	457.22	129.10
Number of HBA	6	5	5	2
Number of HBD	0	0	1	5
Mol LogP	1.48	3.65	3.76	-1.00
Mol LogS [Log (moles/L),(mg/L)]	1.90, 6084.23	3.60, 114.53	3.87, 62.10	-0.54, 37381.02
Mol PSA [A^2]	71.05	65.76	75.00	69.77
Mol Vol [A^3]	522.48	489.23	484.03	119.98
pKa of most Basic/ Acidic group	8.65 / 13.59	2.99 / 19.68	2.29 / 17.96	15.68 / 15.79
BBB Score	3.92	3.47	3.08	1.92
Drug-likeness score	1.14	0.89	1.11	-0.82

The results obtained after entering canonical smiles format for the synthesized compounds along with Metformin were in Molinspiration software was recorded and tabulated in (Table 3).

Table 3: Bioactivity of Synthesized Quinazoline Derivatives and Metformin

Sl. No	Compound	GPCR Ligand	Ion Channnel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
1	3A	0.19	-0.59	-0.31	-1.06	-0.18	-0.09
3	3B	-0.06	-0.36	-0.36	-1.47	-0.84	0.06
4	3C	0.15	-0.83	-0.15	-1.20	-0.17	0.05
5	Metformin	-1.82	-0.84	-2.53	-3.31	-1.43	-1.51

Based on the results obtained the drug likeness was calculated that 3A posses higher score indicates that, probability of the molecule is higher than other compounds.

The synthesized compounds and Metformin structures were converted to smiles format and then, pasted in SWISS ADME and it was Run, the results obtained were tabulated in (Table 4).

Table 4: Absorption and Bioavailability of synthesized Quinazoline derivatives and Metformin

Sl. No	Compound	GI Absorption	Inhibitor	Solubility	Bioavailability Score
1	3A	High	CYP2C9	Low	0.55
3	3B	High	CYP2C9	Soluble	0.55
4	3C	High	CYP2C9	Low	0.55
5	Metformin	Low	No Inhibition	Soluble	0.55

The bioactivity score > 0.00, the compound has considerable bioactivity, if the activity ranges from 0.00-0.50, then moderate activity and if activity is < -0.50, then compound is inactive.

As per the obtained results for the synthesized compounds, 3Bwas found to be possessing higher solubility, higher GI absorption and similar bioavailability score.

The synthesized compounds along with Metformin were evaluated for prediction of Toxicity and Solubility studies by OSIRIS. The possibility for compound 3B to be a drug, is high as compared to other compounds. Potential toxicity and solubility of synthesized quinazolines 3A–3C were estimated by OSIRIS Property Explorer.

The results obtained by OSIRIS indicate that the target compounds 3A-3C **we**re free from mutagenicity, tumorigenicity, reproductive toxicity and irritating effects.

PASS is an online program which provides 'two parameters as a list of predicted types of activity: the probability "to be active" (*Pa*) and the probability "to be inactive" (*Pi*), which vary from zero to one.' The possibility of determining experimentally a certain kind of activity increases with increasing value of *Pa* and decreasing value of *Pi* .

There are many examples for successful usage of PASS approach to find new pharmacologically active agents. Anti-diabetic activity was predicted for synthesized compounds 3A–3C and were found within the range of 0.059 <*Pa* <0.119. It was notable that most active compound, 3B had good *Pa* scores (*Pa*>0.1), as expected. Metformin had no activity on DPP-4.

By comparing the results obtained for PASS (Prediction of Activity Spectra for Substances), Metformin has no activity on DPP4 inhibitor, whereas compound **3B** has high probability of activity and less probability to be inactive on DPP4 inhibitor activity (Table 5).

Table 5: Prediction of in-vitro Antidiabetic Activity of Compounds 3A-3D (Pa: probability of activity; Pi: probability of inactivity)

Compound	Pa	Pi	Activity
3A	0.101	0.004	Dipeptidyl Peptidase IV inhibitor
3B	0.119	0.004	Dipeptidyl Peptidase IV inhibitor
3C	0.059	0.007	Dipeptidyl Peptidase IV inhibitor
Metformin	----	-----	-----

Compounds 3A, 3B, and 3C along with Metformin were docked on 2ONC, 2OQV and 5Y7J (Table 6).The results obtained were most promising for the quinazoline derivatives which were found to be highly potent and active towards diabetes and revealed, that the derivatives had bound to targeted proteins (PDB: 2ONC, 2OQV and 5Y7J).

Table 6: Binding energies of compounds 3A, 3B, 3C and Metformin on 2ONC, 5Y7J and 2OQV

Sl. No	Compound	Rank Grep Pattern	Sub Rank	Run	Binding Energy(kcal/mol)	Cluster RMSD	Reference RMSD
1	3A (2ONC)	1 Ranking	1	3	-8.36	0.00	83.56
2	3B (2ONC)	1 Ranking	1	3	-7.92	0.00	87.68
3	3C (2ONC)	1 Ranking	1	10	-8.28	0.00	82.41
4	3A (5Y7J)	1 Ranking	1	2	-7.90	0.00	38.86
5	3B (5Y7J)	1 Ranking	1	8	-7.65	0.00	38.72
6	3C (5Y7J)	1 Ranking	1	7	-7.78	0.00	27.98
7	3A (2OQV)	1 Ranking	1	2	-7.80	0.00	48.38
8	3B (2OQV)	1 Ranking	1	1	-8.19	0.00	76.05
9	3C (2OQV)	1 Ranking	1	3	-7.65	0.00	78.93
10	Metformin (2ONC)	1 Ranking	1	4	-5.44	0.00	65.82
11	Metformin (5Y7J)	1 Ranking	1	10	-5.70	0.00	49.12
12	Metformin (2OQV)	1 Ranking	1	5	-5.73	0.00	80.18

DISCUSSION:

Compound 3A

Compound **3A** had higher binding energy against 2ONC, 5Y7J and 2OQV were found to be -8.36, -7.90 and -7.80 kcal/mol, respectively as compared to the Metformin as well as RMSD values was also higher, which indicated non binding pose or very less binding affinity towards the receptor binding site.

Compound 3B **(**Figure 3)

Compound 3B was the only compound among the quinazoline derivatives synthesized, to have nearly equal binding energy as that of Metformin on 5Y7J and has lower RMSD value (i.e. 38.72). In this, case a lower RMSD value indicates true binding pose of compound 3B.

***Interacting Amino acids for 3B**: **2ONC**-* LEU366, THR365, PHE364, TRP215, TRP205, LEU214, ALA306, TRP216, PRO218, PRO159 where as for Metformin, the interacting Amino acids were GLU191, TRP124, ASP192 (Figure A).

Furthermore, compound **3B: 5Y7J** forms hydrophobic cloud with TRP157, THR156, TRP154, TRP216, LEU214, SER212, TRP215, PRO159, TRP305 and THR304, where as interacting amino acids for Metformin were GLU191, GLN123, TRP124, TYR195, ASP192 and VAL254 (Figure B).

Moreover, the compound **3B: 2OQV** showed hydrophobic interactions with THR156, TRP216, TRP215, TRP305, THR304, ALA306 and PHE364, while the interacting amino acids for Metformin, were GLN123, TRP124, ASP192, TYR195 and VAL254 (Figure C).

Compound 3C

Though compound 3C had higher energies in all the proteins, but had higher RMSD value. Higher RMSD value indicates non binding pose of compound 3C, where as it had higher binding energy against than Metformin, however, it showed better affinity towards 5Y7J.

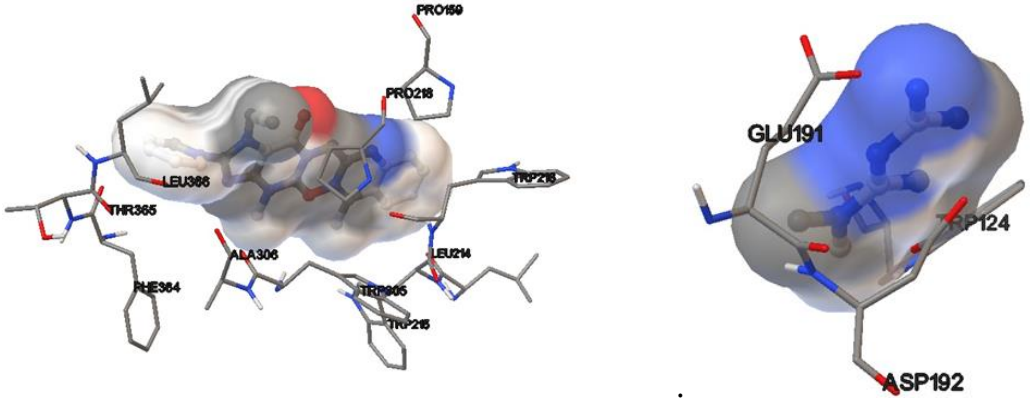


Figure A: Compound 3B and Metformin (2ONC)

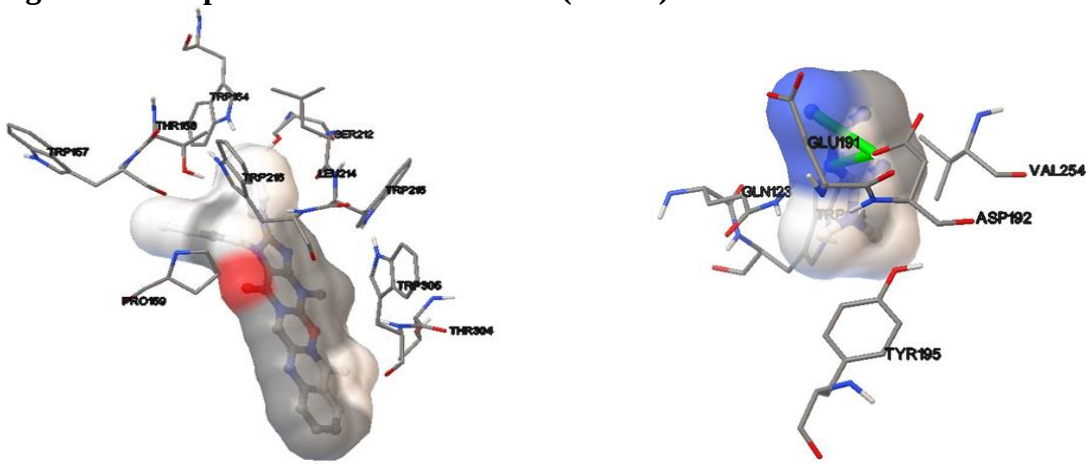


Figure B: Compound 3B and Metformin (5Y7J)

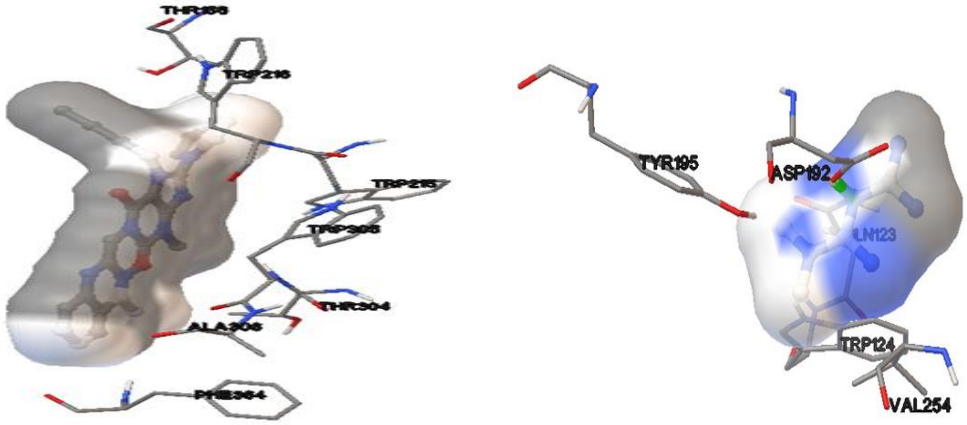


Figure C: Compound 3B and Metformin (2OQV)

Figure 3: Binding mode of compound 3B and Metformin on the active sites of 2ONC, 5Y7J and 2OQV along with interacting amino acids from their respective regions of active site.

Dipeptidyl Peptidase-4 Inhibitory Activity:

The DPP-4 inhibitory potential of quinazoline derivatives were tabulated in (Table 7). Compound (3B) ($p< 0.05$) possess most inhibitory activity against DPP-4 with an IC_{50}: 241.76±0.65 µg/mL than compound 3C and 3A. Preliminary structure-activity relationship revealed that compound **3B** with saturated piperidine moiety was found to possess excellent activity as compared to standard drug, Metformin (IC_{50}: 246.24±0.01). Compound **3B** had exhibited similar DPP-4 activity as that of Metformin. Figure 4, indicates inhibitory activity of Metformin, synthesized quinazoline derivatives, compound 3B (27%) inhibitory activity is similar to that of Metformin (27%).

Table 7: DPP-4 and α-Amylase inhibitory activities of various quinazoline derivatives

COMPOUND	IC_{50} values (µg/ml)	
	DPP-4	α-Amylase
3A	201.22±0.09*	198.81±0.34*
3B	241.76±0.65**	230.23±0.67**
3C	210.33±0.04**	207.32±0.24**
Metformin	246.24±0.01*	--------
Acarbose	--------	235.43±0.04*

[*, ** Significant difference from negative control, Metformin and Acarbose (standard), respectively at P<0.005, using Tukey's test as post ANOVA test. All values are expressed as mean ±standard deviation of triplicates]

α-Amylase Inhibitory Activity:

The α-Amylase inhibitory activity of the quinazoline derivatives was tabulated in (Table 7). All the derivatives had shown inhibitory activity against α-Amylase, with compound 3B being the highest IC_{50} value of 230.23±0.67µg/mL ($p < 0.005$), which was comparable to the positive control, Acarbose (IC_{50}: 235.43±0.04), may be due to presence of piperidine ring and electron donating moiety ($-CH_3$), was found to possess excellent activity. The other compounds 3A, and 3C have shown lower inhibitory activity with IC_{50} values ranging from 177.32µg/mL - 207.32µg/mL. Acarbose and synthesized quinazoline derivatives, compound 3B (26%) has inhibitory activity similar to that of Acarbose (27%) as in (Figure 4).

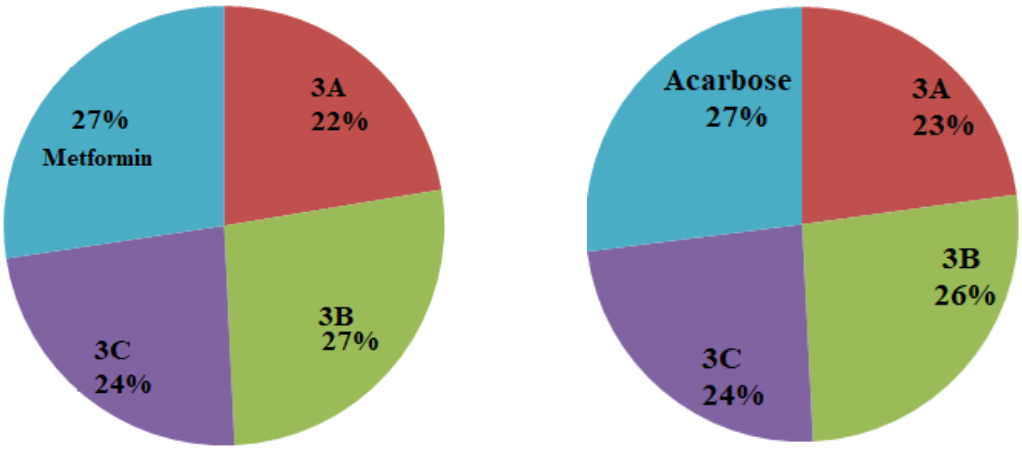


Figure 4: DPP-4 and α-Amylase inhibitory activities.

CONCLUSION:

It can be concluded, based on the results obtained for the synthesized quinazoline derivatives being characterized by I.R, ^{1}H-NMR, ^{13}C-NMR and mass for structural confirmation. Among the synthesized compounds 3B, had exhibited promising Antidiabetic activity, which was comparable with the standard drug Metformin. Even *In Silico*, studies revealed that binding energies and RMSD values for compound3C was lower on all PDBs (2ONC, 5Y7J and 2OQV). Compound 3B had much higher inhibitory activity on DPP-4 and such class of molecules still had to be explored for their other activities as potent compounds.

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CONFLICTS OF INTEREST

The authors declare no Conflict of Interest in publishing the research article.

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