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Graph Theoretical Analysis and In Silico Modeling and Molecular Dynamic Studies of 1,3-Thiazole Derivatives for the Modulation of Glucose Metabolism in 3T3-L1 Adipocytes

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ABSTRACT
1-(2-(dimethylamino)-2-(4-substitutedphenyl) vinyl) (methyl) amino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one derivatives were synthesized and characterized by elemental analysis, IR, [1]H-NMR, [13]C-NMR and Mass spectral studies.In silico studies revealed a good interaction between thiazole derivatives and AMPK binding sites. The analogs were tested for their in vitro cellular viability and glucose uptake on 3T3-L1 adipocytes based on the significant report of in silico screening. The findings showed that 1-(2-((1-(Dimethylamino)-2-(2-nitrophenyl)vinyl)(methyl)amino)-4-(trifluoromethyl) thiazol-5-yl)ethan-1-onederivative greatly improved glucose utilization efficiency and protected cells from insulin resistance.

Keywords*: Thiazole derivatives, 3T3-L1 adipocytes, glucose uptake, in silico modeling, in vitro studies.*

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I. INTRODUCTION

Thiazole is a 5 membered unsaturated heterocyclic system with one Nitrogen and Sulphur atoms present in the ring. It is identified as apotent pharmacophore nucleus due to its various pharmaceutical applications. Its derivatives have a wide range of biological activities such as antioxidant[1], analgesic[2], antibacterial[3], anticancer[4], anti-inflammatory[5], antimalarial[6], antifungal[7], and antipsychotic[8]. Recently thiazoles have found application in drug development for the treatment of Antidiabetic activity[9], Antihyperglycemic[10],Apoptosis activity[11], HIV infections[12], Hypertension[13], Allergies[14], CNS activity[15], antituberculosis[16], antischistosomiasis[17], antitrypanosomal[18], anticonvulsant[19] and carbonic anhydrase inhibitor[20]. In the disciplines of pharmaceutical chemistry and drug discovery processes, thiazole derivatives are one of the most prominent and widely used heterocycles. The distinctive structural features exhibited by thiazoles place them in the special category of molecules that are essential in biological and medicinal chemistry fields. The thiazole ring with various substituents exhibits a broad spectrum of pharmacological and biological activities using weak interactions with receptors and enzymes in the biological system. In particular, several thiazole derivatives with immense therapeutic potency have been well explored as clinical drugs to treat different types of diseases. Based on the biological significance of thiazoles, the current study aimed to investigate anti-diabetic activity of 1-(2-(dimethyl amino)-2-(4-substituted phenyl) vinyl) (methyl) amino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one heterocyclic derivatives.

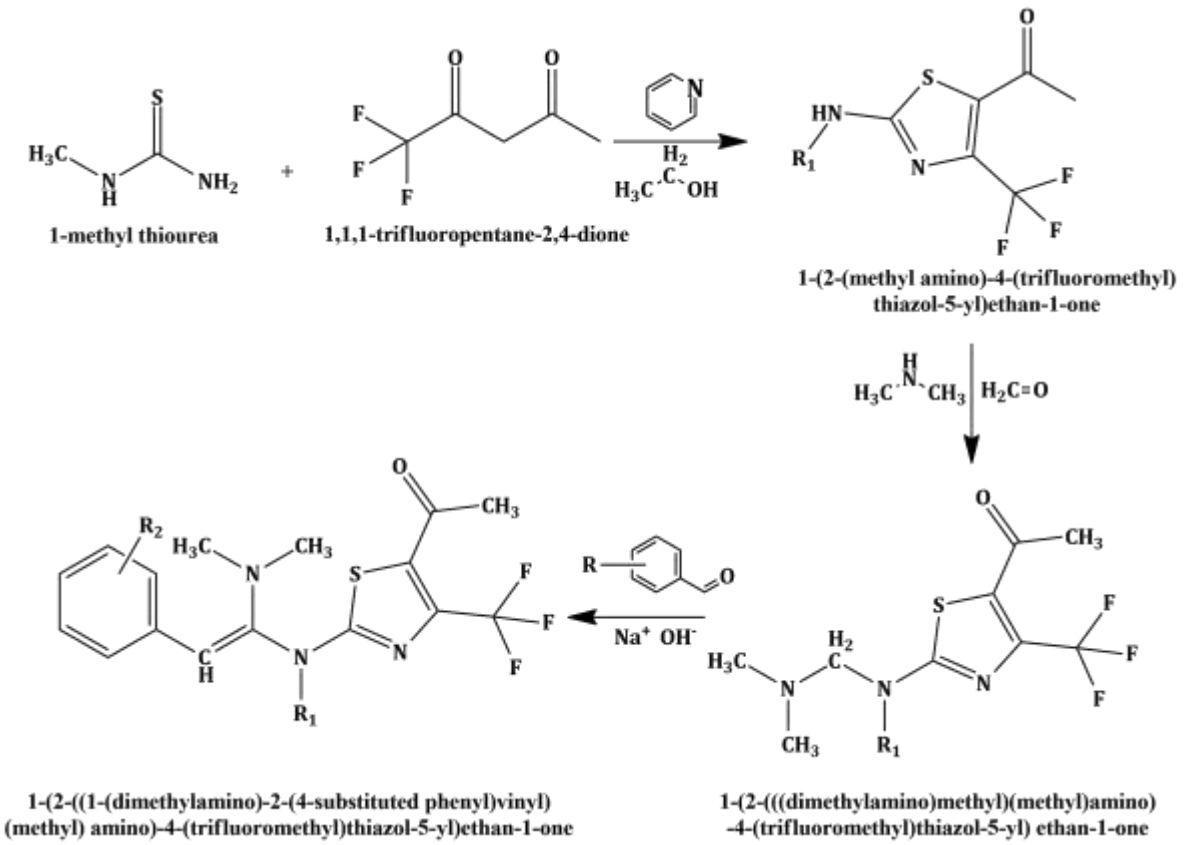
Diabetes mellitus (DM) is a clinical syndromecharacterized by hyperglycemia due to absolute or relative deficiency of insulin. Diabetes comes in many forms, including type 1, type 2, young-onset diabetes, gestational diabetes, neonatal diabetes and secondary causes such as endocrinopathies, steroid use and other variables. Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) are the two most common types of diabetes, both caused by insufficient insulin secretion (T1DM) and/or insulin action (T2DM). T1DM effects children and adolescents whereas T2DM effects middle-aged and older people who have long-term hyperglycemia due to poor lifestyle and nutritional choices. T1DM and T2DM have different pathophysiology, therefore each kind has its own set of etiologies, symptoms and treatments. The serine/threonine kinase [5-adenosine monophosphate (AMP)-activated protein kinase (AMPK)] was first recognized as a vital factor in regulating cellular energy balance. The AMPK pathway has gained a reputation as a master metabolic regulator.

Increased diacylglycerol buildup and activation of PKC(Protein Kinase C), which alters insulin signaling by phosphorylating IRS-1/2(Insulin Receptor Substrate 1 &2), thus the insulin receptor substrate in skeletal muscles, adipocytesand liver can promote insulin resistance. Furthermore, high amounts of amino acids especially BCAAs and insulin produce mTORC1 hyperactivity, which suppresses insulin signaling by activating IRS-1/2 via p70-S6Kinase. mTORC1 hyperactivity also leads to insulin resistance via ER (Endoplasmic Reticulum) stress, which results in the formation of reactive oxygen species (ROS) and chronic inflammation. FFAs(Free Fatty Acids)in particular have the potential to promote chronic inflammation both directly and indirectly via activating TLR4 signaling. AMPK activation improves insulin sensitivity by inhibiting lipogenesis (ACC1, SREBP1c), protein synthesis (mTORC1), and lipolysis (HSL) while also activating glucose transporters. These pathways are linked to decreased IRS-1/2 inhibitory phosphorylation, ER stress/ROS, FFAs, and chronic inflammation. GULT vesicles are a collection of membrane proteins that allows glucose diffuse more easily across the plasma membrane. Each glucose transporter has a unique function in glucose metabolism, which is determined by tissue expression patterns, substrate selectivity, transport kinetics, and regulated expression under diverse physiological conditions. Signaling networks, such as genes, proteins, and enzymes, are shown in this perspective utilizing graph theoretical network analysis based on the impact of introduction about AMPK. The stability and binding mechanisms of selected bioactive chemicals with an appropriate diabetes receptor protein were explored using molecular dynamics modeling. In addition, the selected drugs expected ADMET (absorption, distribution, metabolism, excretion, and toxicity) characteristics were also studied.

II. MATERIALS AND METHOD

The chemicals and reagents used were obtained from various chemical units Qualigens, E. Merck India Ltd., CDH, and SD Fine Chem. The solvents used were of LR grade and purified before their use. The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd.

The equimolar quantity (0.002 mol) of 1-methyl thiourea reacts with 1,1,1-trifluoropentane-2,4-dione in presence of 3 mL of pyridine and 10 mL of ethanol, the reaction mixture was heated at 80^0C for 4hr. to yield 1-(2-(methylamino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one. The mixture of equimolar quantity (0.002 mol) of 1-(2-(methylamino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one (Comp-1) and dimethylamine and formaldehyde (0.0015 mol) in 10 mL ethanol was stirred for 2 hr. on magnetic stirrer. Then the resulting mixture was refluxed on water bath for 6 hr. The above mixture was poured on crushed ice and mixed well to yield 1-(2-(((dimethyl amino) methyl) (methyl)amino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one (Comp-2). Further the equimolar quantities (0.002 mol) of **(2)** and substituted benzaldehyde (0.002 mol) were taken in a beaker.To this 10 mL sodium hydroxide solution was added to make the solution alkaline. The reaction mixture was shaken and kept aside. The progress of the reaction was monitored by TLC. The solid product 1-(2-((1-(dimethyl amino)-2-(4-substituted phenyl) vinyl) (methyl) amino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one obtained were washed with water and recrystallized from absolute ethanol.The purity of the compounds was checked by TLC and melting points were determined.



IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkin Elmer model 2400C analyzer and were within ± 0.4 % of the theoretical values. ^{1}H-NMR and ^{13}C-NMR spectra was recorded at 500 MHz on Bruker Avance-500 NMR spectrometer in $CDCl_3$ using tetra methyl silane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization.

In the present work, novel thiazole derivativeswere prepared from 1,1,1-trifluoropentane-2,4-dione and 1-methyl thiourea as starting materials. Initially, 1-(2-(methylamino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one**(1)** was obtained by the reaction of 1,1,1-trifluoropentane-2,4-dione with 1-methyl thiourea through cyclization leading to the formation of thiazole ring. In the succeeding step, compound (**1)** undergoes Mannich reaction by reacting with formaldehyde and dimethylamine and produced 1-(2-(((dimethyl amino) methyl) (methyl)amino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one**(2)**. Finally, compound (**2)**undergoescondensation reaction by reacting with various aromatic aldehyde and produced 1-(2-((1-(dimethyl amino)-2-(4-substituted phenyl) vinyl) (methyl) amino)-4-(trifluoromethyl) thiazol-5-yl) ethan-1-one.

III. RESULTS AND DISCUSSION

Spectral data:

The appearance of absorption peak in IR at 3373 cm^{-1}& 1715 cm^{-1}corresponds to NH & C=O stretching respectively confirms the formation of 1-(2-(methylamino)-4-(trifluoro methyl)thiazol-5-yl)ethan-1-one **(1)**. This is further supported by the presence of one proton singlet at δ 4.38 ppm corresponds to NH proton and three protons singlet in ^{1}H-NMR spectra at δ 2.15 and 2.92 ppm for N-CH_3 and $COCH_3$ protons, respectively. The disappearance of absorption peak in IR around 3300 cm^{-1} corresponds to NH stretching and the disappearance of singlet around δ 4.30 ppm corresponds to one proton of NH in ^{1}H-NMR spectra approves the formation of Mannich base i.e., 1-(2-(((dimethylamino)methyl) (methyl)amino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one**(2)**. This is further supported by the appearance of two protons singlet for methylene linkage at δ 4.51 ppm.

The appearance of absorption peak around 3030 cm^{-1} corresponds to aromatic CH stretching in IR, the disappearance of two protons singlet for methylene linkage around δ 4.50 ppm in ^{1}H-NMR spectra and appearance of peak around δ 5.00 ppm corresponds to one proton of =CH confirms the formation of 1-(2-((1-(dimethylamino)-2-(4-substituted phenyl)vinyl) (methyl) amino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one. Additionally in IR & NMR spectroscopy emergence of a variety of other peaks for assigned structure confirms

the chemical structure of target derivatives. The molecular weight and purity of prepared analogs were confirmed from their massspectrum.

1-(2-(Methylamino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one (1)

M.P: 185-187 °C; Yield: 84 %. IR (cm^{-1}): 3373 (NH), 2928 (CH_3-CH), 1715 (C=O), 1632 (C=N), 1604 (C=C), 1270 (C-F). ^{1}H-NMR (δ: ppm): 4.38 (1H, s, NH), 2.92 (3H, s, $COCH_3$), 2.15 (3H, s, N-CH_3). ^{13}C-NMR (δ: ppm): 199.2 (C=O), 160.5 (C-2 of thiazole), 156.7 (C-4 of thiazole), 124.9 (CF_3), 104.1 (C-5 of thiazole), 33.4 (N-CH_3), 30.8 (CO<u>C</u>H_3). MS (EI) *m/z*: 224 (M^+). *Anal*. Calcd for $C_7H_7F_3N_2OS$: C, 37.50; H, 3.15; N, 12.49. Found: C, 37.62; H, 3.14; N, 12.45.

1-(2-(((Dimethylamino)methyl)(methyl)amino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one (2)

M.P: 149-151 °C; Yield: 79 %. IR (cm^{-1}): 2969 (CH_3-CH), 1703 (C=O), 1677 (C=N), 1632 (C=C), 1235 (C-F). ^{1}H-NMR (δ: ppm): 4.51 (2H, s, CH_2), 2.64 (3H, s, $COCH_3$), 2.23 (3H, s, N-CH_3), 2.08 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (δ: ppm): 195.7 (C=O), 159.3 (C-2 of thiazole), 152.9 (C-4 of thiazole), 119.6 (CF_3), 106.1 (C-5 of thiazole), 87.4 (CH_2), 45.2 ($N(CH_3)_2$), 35.0 (N-CH_3), 32.8 (CO<u>C</u>H_3). MS (EI) *m/z*: 281 (M^+). *Anal*. Calcd for $C_{10}H_{14}F_3N_3O_S$: C, 42.70; H, 5.02; N, 14.94. Found: C, 42.45; H, 5.00; N, 14.98.

1-(2-((1-(Dimethylamino)-2-*o*-tolylvinyl)(methyl)amino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one (3a)

M.P: 221-223 °C; Yield: 75 %. IR (cm^{-1}): 3019 (Ar-CH), 2930 (CH_3-CH), 1727 (C=O), 1665 (C=N), 1606 (C=C), 1281 (C-F). ^{1}H-NMR (δ: ppm): 7.15-7.59 (4H, m, Ar-H), 5.35 (1H, s, =CH), 2.82 (3H, s, $COCH_3$), 2.31 (3H, s, N-CH_3), 1.76 (6H, s, $N(CH_3)_2$), 1.64 (3H, s, Ar-CH_3). ^{13}C-NMR (δ: ppm): 198.1 (C=O), 165.4 (C-2 of thiazole), 155.7 (<u>C</u>=CH), 149.2 (C-4 of thiazole), 140.6 (C-1), 138.0 (C-2), 129.3 (C-3), 128.5 (C-4), 125.9 (C-6), 125.2 (C-5), 117.6 (CF_3), 107.9 (C-5 of thiazole), 80.5 (C=<u>C</u>H), 44.1 ($N(CH_3)_2$), 32.8 (N-CH_3), 25.4 (CO<u>C</u>H_3), 15.7 (Ar-CH_3). MS (EI) *m/z*: 383 (M^+). *Anal*. Calcd for $C_{18}H_{20}F_3N_3OS$: C, 56.38; H, 5.26; N, 10.96. Found: C, 56.22; H, 5.24; N, 10.99.

1-(2-((1-(Dimethylamino)-2-*m*-tolylvinyl)(methyl)amino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one (3b)

M.P: 247-248 °C; Yield: 78 %. IR (cm^{-1}): 3050 (Ar-CH), 2956 (CH_3-CH), 1718 (C=O), 1664 (C=N), 1602 (C=C), 1257 (C-F). ^{1}H-NMR (δ: ppm): 6.78-7.23 (4H, m, Ar-H), 5.06 (1H, s, =CH), 2.92 (3H, s, $COCH_3$), 2.64 (3H, s, N-CH_3), 1.91 (6H, s, $N(CH_3)_2$), 1.70 (3H, s, Ar-CH_3). ^{13}C-NMR (δ: ppm): 197.5 (C=O), 155.2 (C-2 of thiazole), 154.0 (<u>C</u>=CH), 152.9 (C-4 of thiazole), 141.3 (C-3), 136.8 (C-1), 130.2 (C-5), 129.5 (C-4), 128.9 (C-2), 125.1 (C-6), 122.6 (CF_3), 107.4 (C-5 of thiazole), 78.5 (C=<u>C</u>H), 38.9 ($N(CH_3)_2$), 30.2 (N-CH_3), 23.7 (CO<u>C</u>H_3), 21.4 (Ar-CH_3). MS (EI) *m/z*: 383 (M^+). *Anal*. Calcd for $C_{18}H_{20}F_3N_3OS$: C, 56.38; H, 5.26; N, 10.96. Found: C, 56.60; H, 5.28; N, 10.94.

1-(2-((1-(Dimethylamino)-2-(2-methoxyphenyl)vinyl)(methyl)amino)-4-(trifluoromethyl) thiazol-5-yl)ethan-1-one (3d)

M.P: 234-236 °C; Yield: 81 %. IR (cm^{-1}): 3034 (Ar-CH), 2940 (CH_3-CH), 1722 (C=O), 1657 (C=N), 1625 (C=C), 1248 (C-F), 1025 (C-O-C). ^{1}H-NMR (δ: ppm): 6.91-7.40 (4H, m, Ar-H), 5.12 (1H, s, =CH), 3.34 (3H, s, OCH_3), 2.56 (3H, s, $COCH_3$), 2.19 (3H, s, N-CH_3), 1.83 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (δ: ppm): 189.4 (C=O), 165.7 (C-2 of thiazole), 156.2 (<u>C</u>=CH), 154.9 (C-2), 153.6 (C-4 of thiazole), 135.1 (C-4), 131.8 (C-6), 126.5 (C-5), 122.7 (CF_3), 115.4 (C-1), 112.0 (C-3), 108.3 (C-5 of thiazole), 74.2 (C=<u>C</u>H), 59.8 (OCH_3), 40.5 ($N(CH_3)_2$), 29.8 (N-CH_3), 25.3 (CO<u>C</u>H_3). MS (EI) *m/z*: 399 (M^+). *Anal*. Calcd for $C_{18}H_{20}F_3N_3O_2S$: C, 54.13; H, 5.05; N, 10.52. Found: C, 53.95; H, 5.07; N, 10.56.

1-(2-((2-(4-Aminophenyl)-1-(dimethylamino)vinyl)(methyl)amino)-4-(trifluoromethyl) thiazol-5-yl)ethan-1-one (3i)

M.P: 184-186 °C; Yield: 73 %. IR (cm^{-1}): 3356 (NH), 3015 (Ar-CH), 2881 (CH_3-CH), 1738 (C=O), 1650 (C=N), 1615 (C=C), 1264 (C-F). ^{1}H-NMR (δ: ppm): 6.94-7.32 (4H, m, Ar-H), 5.30 (1H, s, =CH), 4.47 (2H, s, NH_2), 2.96 (3H, s, $COCH_3$), 2.38 (3H, s, N-CH_3), 1.74 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (δ: ppm): 195.9 (C=O), 156.5 (C-2 of thiazole), 155.4 (<u>C</u>=CH), 148.2 (C-4 of thiazole), 145.7 (C-4), 130.3 (C-2 & C-6), 128.5 (C-1), 120.2 (CF_3), 114.0 (C-3 & C-5), 108.6 (C-5 of thiazole), 79.1 (C=<u>C</u>H), 38.4 ($N(CH_3)_2$), 30.5 (N-CH_3), 22.7 (CO<u>C</u>H_3). MS (EI) *m/z*: 384 (M^+). *Anal*. Calcd for $C_{17}H_{19}F_3N_4OS$: C, 53.11; H, 4.98; N, 14.57. Found: C, 52.95; H, 4.99; N, 14.63.

1-(2-((1-(Dimethylamino)-2-(2-nitrophenyl)vinyl)(methyl)amino)-4-(trifluoromethyl) thiazol-5-yl)ethan-1-one (3j)

M.P: 249-251 °C; Yield: 76 %. IR (cm^{-1}): 3026 (Ar-CH), 2969 (CH_3-CH), 1720 (C=O), 1657 (C=N), 1605 (C=C), 1519 & 1324 (NO_2), 1238 (C-F). ^{1}H-NMR (δ: ppm): 7.21-7.60 (4H, m, Ar-H), 5.54 (1H, s, =CH), 2.63 (3H, s, $COCH_3$), 2.32 (3H, s, N-CH_3), 1.95 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (δ: ppm): 199.5 (C=O), 160.0 (C-2 of thiazole), 159.9 (<u>C</u>=CH), 151.2 (C-4 of thiazole), 146.5 (C-2), 132.7 (C-5), 129.9 (C-1), 127.1 (C-4), 125.4 (C-6), 119.2 (C-3), 117.8 (CF_3), 110.3 (C-5 of thiazole), 82.7 (C=<u>C</u>H), 35.9 ($N(CH_3)_2$), 29.2 (N-CH_3), 27.6 (CO<u>C</u>H_3). MS (EI) *m/z*: 414 (M^+). *Anal*. Calcd for $C_{17}H_{17}F_3N_4O_3S$: C, 49.27; H, 4.13; N, 13.52. Found: C, 49.43; H, 4.14; N, 13.48.

1-(2-((1-(Dimethylamino)-2-(4-nitrophenyl)vinyl)(methyl)amino)-4-(trifluoromethyl) thiazol-5-yl)ethan-1-one (3l)

M.P: 188-190 °C; Yield: 80 %. IR (cm^{-1}): 3059 (Ar-CH), 2962 (CH_3-CH), 1715 (C=O), 1674 (C=N), 1620 (C=C), 1524 & 1308 (NO_2), 1256 (C-F). ^{1}H-NMR (δ: ppm): 7.04-7.47 (4H, m, Ar-H), 5.20 (1H, s, =CH), 2.79 (3H, s, $COCH_3$), 2.46 (3H, s, N-CH_3), 1.62 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (δ: ppm): 191.8 (C=O), 164.7 (C-2 of thiazole), 158.5 (C=CH), 150.9 (C-4 of thiazole), 147.0 (C-4), 144.6 (C-1), 129.8 (C-2 & C-6), 127.2 (C-3 & C-5), 118.4 (CF_3), 107.5 (C-5 of thiazole), 79.7 (C=CH), 48.1 ($N(CH_3)_2$), 36.3 (N-CH_3), 20.9 ($COCH_3$). MS (EI) *m/z*: 414 (M^+). *Anal*. Calcd for $C_{17}H_{17}F_3N_4O_3S$: C, 49.27; H, 4.13; N, 13.52. Found: C, 49.41; H, 4.12; N, 13.49.

Molecular docking studies

Molecular docking studies[21]was carried out with the synthesized 1-(2-((1-(dimethylamino)-2-(4-substituted phenyl)vinyl)(methyl) amino)-4-(trifluoro methyl) thiazol-5-yl)ethan-1-one test compounds,reference AMP, Metformin against AMPK protein (PDB: 2UV7). Among tested ligands **3a, 3b, 3d, 3i, 3j, and 3l**showed high amino acid interaction and significant binding affinity towards AMPK protein with the binding energy of -7.1, -6.7 and -6.9 Kcal/mol respectively, compared with reference AMP -6.6 Kcal/mol,standardsMetformin-7.2 Kcal/mol.

The highest 18 amino acids interaction was observed with **3a** that areALA227[4.13 Å, 5.32 Å], ILE240[4.98 Å], ASP245 [3.17 Å, 3.40 Å], ARG269[4.51 Å, 4.83 Å] HSD271 [2.57 Å , 5.15 Å], GLU274 [3.15 Å, 3.44 Å, 3.58 Å], ALA295[3.34 Å, 3.80 Å]GLU296[3.32 Å], VAL297[4.74 Å], HSD298[2.61 Å, 5.27 Å],**3p** was 15 that areILE240[4.45 Å], PHE244[3.76 Å],ARG269[3.70 Å, 4.28 Å] HSD271 [2.68 Å, 4.80 Å, 5.08 Å],GLU274 [2.90 Å, 3.17Å],ALA295[3.64 Å, 4.00 Å, 8.55Å],GLU296[3.46 Å], VAL297[4.11 Å], HSD298[5.19 Å],**3l** was 14 that areILE240[5.26 Å], ASP245[3.22 Å, 3.50 Å], ARG269[4.54 Å, 4.92 Å] HSD271 [2.66 Å , 5.28 Å], GLU274 [3.09 Å, 3.44 Å, 3.52 Å], ALA295[3.41 Å, 3.83 Å], VAL297[4.65 Å], HSD298[2.49 Å] and standard Metformin was 9 that are ILE240[2.05 Å], ASP245[2.46 Å, 2.60 Å, 2.62 Å, 4.96 Å, 5.47 Å,], ARG269[2.61 Å], HSD271[4.79 Å], GLU296[3.43 Å].

Among the test compound, the amino acid interaction of test **3a** compound with the following amino acids ILE240, ASP245, ARG269, HSD271 and GLU296 were matching with the standard Metformin. The observed results indicated that the test **3a, 3b, 3d, 3i, 3j, and 3l** compounds showed significance towards the inhibition of AMPKprotein. The binding interactionsof the test compounds and standard are shown in table-1 and Figure 1 & 2.

Table -1: The docking score and amino acid interaction

Name of the Compound	Docking score	Name of the Compound	Docking score
3a	**-7.1**	**3j**	**-6.2**
3b	**-7.3**	**3k**	**-7.1**
3d	**-6.4**	**3l**	**-6.9**
3e	**-6.5**	**AMP**	**-6.6**
3i	**-7.0**	**Metformin**	**-7.2**

Figure – 1: The binding interactions, 2D and 3D model of test compound **3b**

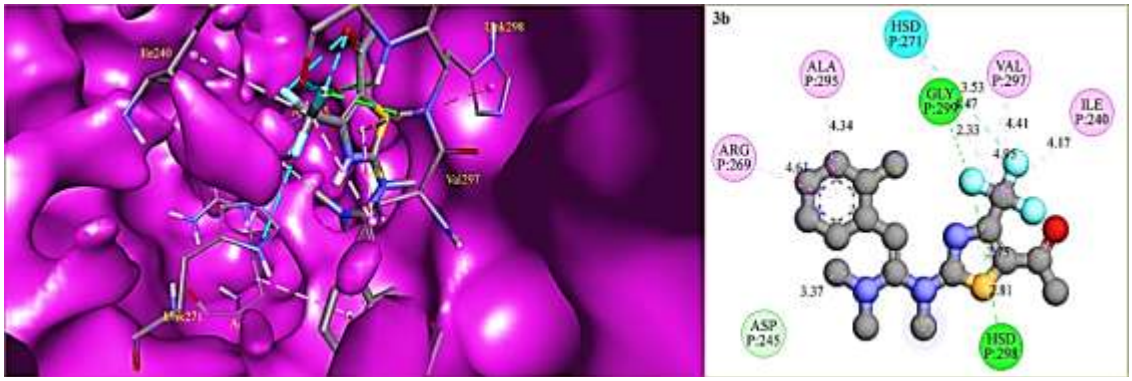
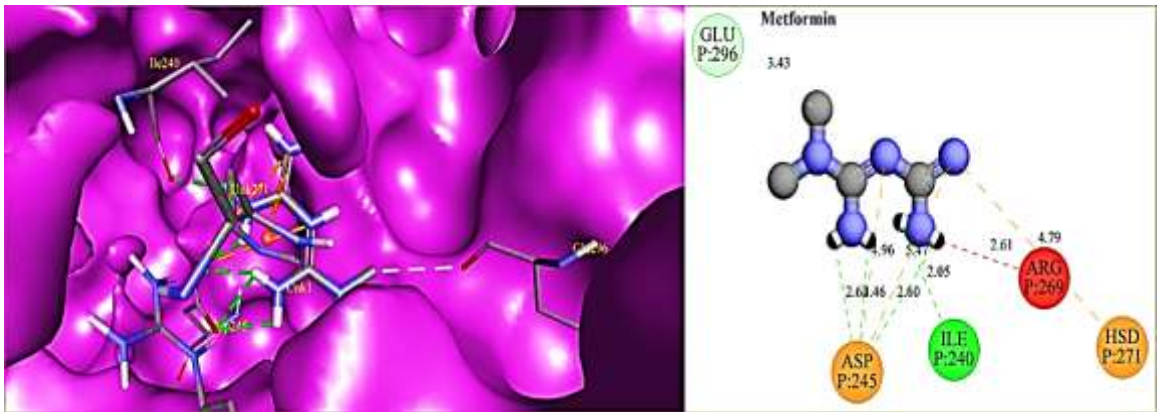


Figure – 2: The binding interactions, 2D and 3D model of standard Metformin



Pharmacokinetic and physicochemical properties prediction analysis

The ADME and physicochemical properties of selected **3a, 3b, 3d, 3i, 3j,3l** and standard were assessed through SwissADME (http://www.swissadme.ch/) webserver and these are presented in **Table 6**. From the assessed data in **Table 2**, the test compounds **3a, 3b, 3d, 3i, 3j, and 3l** and standard was found not to violate Lipinski's rule of five. The polar surface area of test compound **3b** was 64.68 Å², **3i** was 90.70 Å² and **3j**was 110.50 Å² and standard Metformin 88.99Å². The findings also showed thatthe **3b**, **3i**and standard molecule (metformin) had high human gastro intestinal (GI) absorption, **3j** lower GI absorption. In general, increased GI absorption leads to increased chemical bioavailability. As a result, oral administration of the test substance **3b** and **3i** may result in improved absorption from the gastrointestinal system.

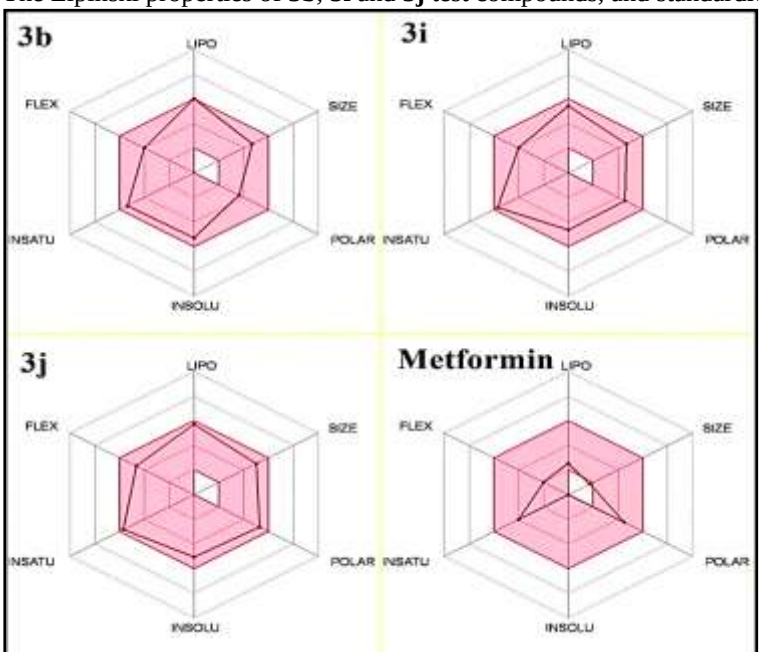
Table 2: The ADME and physicochemical properties of **3b**, **3i**, **3j** test compounds, and standard Metformin

Name of Compounds	Violation of Lipinski's rule	The polar surface area (TPSA)	Gastro intestinal (GI) absorption	bioavailability score	Synthetic accessibility
3b	No	64.68 Å²	High	+0.55	3.66
3i	No	90.70 Å²	High	+0.55	3.57
3j	No	110.50 Å²	Low	+0.55	3.70
Metformin	No	88.99 Å²	High	+0.55	3.11

The higher numbers of H-bonds are possibly measured to be involved during protein ligand binding. From the result, the bioavailability score of three compounds showed better results +0.55 for **3b**, **3i**, **3j**and the bioavailability score +0.55 were observed for standard.Thus, relating with molecular properties **3b**, **3i**, and **3j**was predicted to have better chances as a possible drug-relevant candidate with anti-diabetes potential.

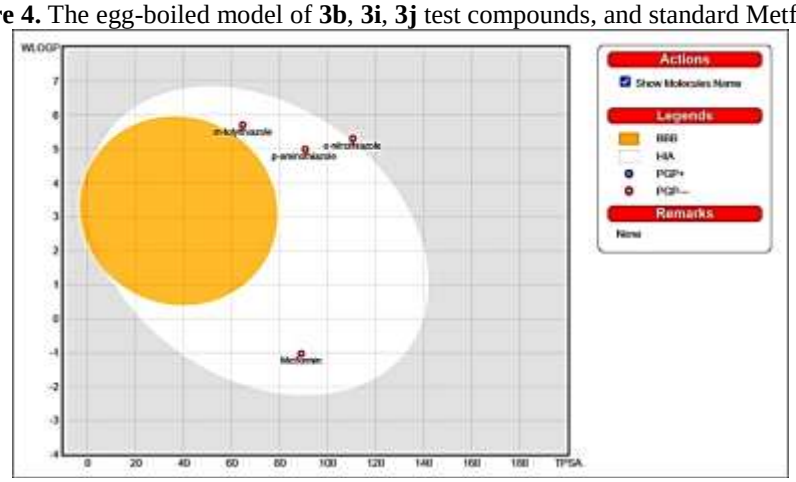
The graphical representations of lipinski rule of selected compounds are presented in the **Figure 3**. The pink area within the hexagon represents the optimal range of the compounds. The recommended range for drug-like compound was in saturation (INSATU): fraction of carbons in the sp^3 hybridization not less than 0.25, insolubility (INSOLU): log S not higher than 6, hydrophobicity (LIPO): between -0.7 and +5.0, rotatable bonds (FLEXI): no more than 9 rotatable bonds, molecular weight (SIZE): between 150 and 500 $g.mol^{-1}$, polar surface area (POLAR): The red slanted hexagon inside the pink tint represents the reference compounds, which have drug-like characteristics. Except for the INSATU characteristic, the **3b**, **3i**, **3j** and standard had drug-like qualities.

Figure 3. The Lipinski properties of **3b**, **3i** and **3j** test compounds, and standardMetformin



Furthermore, the pharmacokinetic parameters of the chosen chemical, and standard, were studied using the egg-boiled model represented in **Figure 4**. Predicting passive gastrointestinal absorption and BBB penetration using the egg-boiled model is useful because it takes into account two important pharmacokinetic features at once. Egg-shaped plot demonstrates that chemical in yolk (i.e., yellow area) represents very possible BBB permeability and albumin (i.e., white region) represents highly probable human intestine absorption, as shown by the egg-shaped plot of organizational structure. From the Figure 10, the **3b**, **3i**, **3j** and standard found in albumin (white region) elucidated the good absorption in gastrointestinal region. From the above observed results, it can be interpreted that the **3b**, **3i**, and **3j** compounds have sufficient potential to be drug.

Figure 4. The egg-boiled model of **3b**, **3i**, **3j** test compounds, and standard Metformin



***In-vitro* cytotoxicity assay**

The synthesized three compounds were subjected to MTT assay to evaluate the cytotoxic concentration of synthesized compounds. The results revealed that cytotoxic concentration of the synthesized compounds ranges from 16.00 to 251.67µg/ml in comparison to the standard metformin (216.67µg/ml) **Table 3**.

Table 3: *In vitro* cytotoxic data of the synthesized compounds against L6 myocytes

Name of Compounds	**CTC_{50} (µg/ml)**
3b	$64.67 \pm 5.46^{***}$
3i	$16.00 \pm 1.53^{***}$
3j	251.67 ± 21.09
Metformin	216.67 ± 12.35

***In-vitro* glucose uptake assay**

The impairment of glucose uptake is the major cause of type II diabetes, about 80% of the insulin stimulated glucose uptake occurs in the skeletal muscles. This uptake is mainly depending on the glucose transporter (GLUT) protein present in the plasma membrane, GLUT4 is the major glucose transporter in skeletal muscle. Hence in this study the percentage of glucose uptake in specific GLUT4 mediated transport system was evaluated in the rats L6 skeletal muscle cells. The *in vitro* glucose uptake assay indicates that the percentage of glucose uptake was almost identical and slightly higher in the **3j,** (94%) than the standard metformin (92%). Other synthesized compounds like **3b** and **3i** have low (34%) to moderate (56%) glucose uptake **Table 4**. Based on this result **3j** was further selected for *in vivo* anti-diabetic activity.

Table 4: Glucose uptake assay of the synthesized compounds on L6 myocytes

Name of Compounds	**CTC_{50} (µg/ml)**
3b	56
3i	34
3j	94
Metformin	92

Acute oral toxicity study of the synthesized compound

Acute oral toxicity study results of the selected **3j** showed that there were no significant changes in the observation and behavioral parameters and no mortality up to the dose level of 300mg/kg **Table 5**. Hence the LD50 of the **3j** was found to be 300mg/kg, besides 1/10of the LD_{50} dose 30mg/kg was further selected as therapeutic dose for *in vivo* anti-diabetic activity.

Table 5: Acute oral toxicity study of synthesized compounds

No. of animals used	**Dose**	**Alive**	**Death**
3 female Mice	5 mg/kg	3	0
	50 mg/kg	3	0
	300 mg/kg	3	0
	2000mg/kg	1	2

***In vivo* anti-diabetic activity of the synthesized compound**

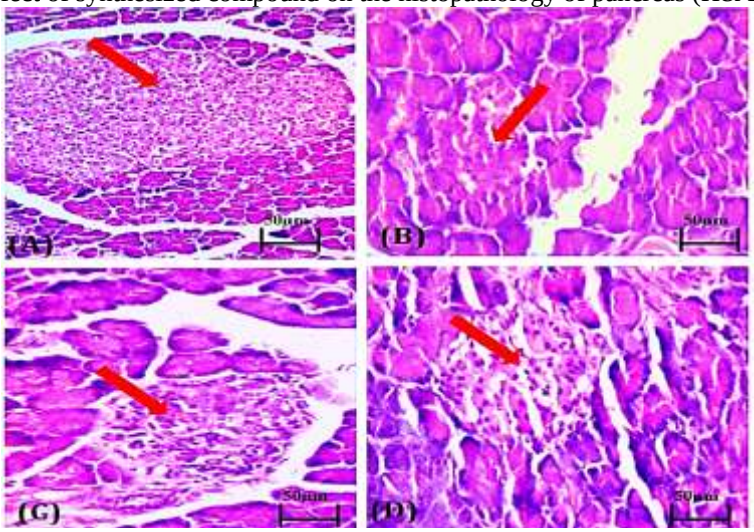
The fasting blood glucose level was significantly (P<0.001) higher on day in the Streptozotocin and Nicotinamide treated groups II-IV than the normal control rats indicate the development of diabetes mellitus **Table 6**and**Figure 5**. On the day 14 diabetic control showed significantly (P<0.001) higher blood glucose level as compared to normal control. The treatment of Metformin and synthesized compound **3j** (30mg/kg) significantly (P<0.001) decreased the elevated blood glucose level when compared to diabetic control. Histopathological studies of the pancreas also confirm the anti-diabetic potential of synthesized compounds, it shows increased cluster of β islet cells with few reactive lymph nodes which is nearer to the normal pancreatic structure. Diabetic control rat pancreas showed few necrotic islet cells.

Table 6: Effect of synthesized compound in the fasting blood glucose level in the diabetic rats

Treatment	**Fasting blood glucose level (mg/dl)**			
	Day 0	**Day 3**	**Day 7**	**Day 14**
Group-I (Normal Control)	85.00±2.89	82.00±3.34	85.25±3.25	84.25±3.04
Group-II (Diabetic Control)	83.50±3.59	223.00±6.14^{c}	238.75±5.94^{c}	301.50±10.97^{c}
Group-III (Metformin-150mg/kg b. w. *p. o.*)	84.00±3.14	219.50±6.74^{c}	177.00±9.33^{cf}	144.75±8.62^{cf}
Group-IV (Compound **3i** 30mg/ kg b. w. *p. o.*)	84.50±3.59	220.00±5.18^{c}	175.75±5.89^{cf}	135.00±7.54^{cf}

Values are expressed as mean ± SEM, n=6. Symbols represent statistical significance ^{a} P<0.05; ^{b} P<0.01; ^{c} P<0.001 Vs Group I. ^{d} P<0.05; ^{e} P<0.01; ^{f} P<0.001 Vs Group II. Data were analyzed by one way ANOVA followed by post hoc Dunnett's multiple comparisons test

Figure 5. Effect of synthesized compound on the histopathology of pancreas (H& E stain 45x)



(A) Normal control; (B) Diabetic control; (C) Metformin (150 mg/kg) treatment; (D) Synthesized Compound (30 mg/kg) treatment.

Cell culture

The rat L6 myocytes cell line was obtained from the National Center for Cell Science (NCCS), Pune and utilized for cytotoxicity assay and glucose uptake assay. The L6 cells were maintained in the RPMI-1640 media (Himedia, India) with 1% antibiotic mixture solution (Penicillin & Streptomycin) and 10% fetal bovine serum (Himedia, India). They were incubated in a humidified atmosphere with 5% CO_2 at 37^0C and this culture was used for further *in vitro* assay.

MTT Cell viability assay

The L6 cells were plated in the 96 well plats at a concentration of 1 x 10^4cells/well for cell viability assay. They were incubated in the RPMI media for 72hrs and allowed to mature[23]. The mature cells were incubated with 0.2 % dimethyl sulfoxide (DMSO) or the test samples (200 - 3.125 µg/ml) for 48 hrs. A total volume of 10 µl of 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT -5mg/ml) solution was added and further incubated for 4 hrs at 37^0C. After that the 200 µl of DMSO was added to solubilize the formazan dye. The optical density absorbance was read at 560 nm using micro plate reader to determine the formazan concentration, which is proportional to live cell numbers[23]. The assay was performed in triplicate. Cell viability percentage was calculated based on the following equation.

$$\% \text{ Cell Viability} = \frac{\text{Mean OD}_{\text{Sample}}}{\text{Mean OD}_{\text{Control}}} \text{ X } 100$$

Where, OD_{Sample} is the Optical density of the sample and $OD_{Control}$ is the Optical density of control, and the cytotoxicity is expressed as a percentage relative to control cells.

Glucose uptake assay

The L6 cells were maintained at sub confluent in Dulbecco's Modified Eagle's (DMEM) growth media with 4.5 g/l glucose, 100 U/ml penicillin, 100-µg/ml streptomycin, and 10% fetal bovine serum. The cells were maintained in continuous passage by trypsinization using Trypsin Phosphate Versene Glucose (TPVG) solution[23].

The cells were cultured in 24 well plates and incubated for 48 hr at 37^0C in CO_2 incubator. When semi confluent monolayer was formed the culture were renewed with serum free DMEM containing 0.2% Bovine serum albumin (BSA) and incubated for further 18 hr. at 37^0C in CO_2 incubator. After 18 hr. media was discarded and cells were washed with buffer solution. Then the cells were treated with Insulin, metformin and test compounds at CTC_{50} concentration individually. Consequently, glucose solution (1 M) was added and incubated for half an hour. Supernatant was collected for glucose estimation and glucose uptake was terminated by washing the cells three times with 1 ml ice-cold KRP buffer. The subsequent freezing and thawing were done three times to lyses all the cells. The cell lysate was collected for glucose estimation. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium by GOD-POD

method[22,23]. The absorbance of standard ($A_{Control}$) and test compounds (A_{Sample}) against blank was measured at 505 nm. Formula for percentage glucose uptake is;

$$\% \text{ Glucose uptake} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \text{ X } 100$$

Acute oral toxicity study

Acute oral toxicity study of the chosen compounds was evaluated by OECD guide line 423 in healthy female rats (150-180gm body weight). In this method compound was administered and tested in a stepwise graded dosing (5mg/kg, 50 mg/kg, 300mg/kg, and 2000mg/kg); each step three female rats were used. Before administration of test compound animals were fasted, after administration the changes in body weight, behavioral changes, and mortality was observed for 14 days, special attention was given on first 4 hours.

Anti-diabetic activity

The synthesized compound was screened for anti-diabetic activity in streptozotocin and nicotinamide induced diabetic rats [24]. Twenty-four albino wistar rats of either sex with an average weight of 180-220 gm were selected and grouped in to four groups of each six animals. Group1 rats were treated with normal saline and served as vehicle control, Group 2-4 were treated by a single intraperitoneal injection of nicotinamide (110 mg/kg; prepared in normal saline) followed by 15 min after that streptozotocin (STZ; 45 mg/kg; prepared in 0.1 M citrate buffer, pH 4.5) administration in order to induce diabetes mellitus[7]. The induction of diabetes was confirmed by measuring blood glucose levels after 72hrs. The rats with elevated fasting blood glucose level (FBG) $\geq$ 200 mg/dl were considered diabetic rats and included in further studies. After confirmation of induction of diabetes group 2 was designated as diabetic control received 1 ml of 0.5% CMC solution *p. o.* for 14 days, group 3 served as standard drug treatment group received metformin (150mg/kg *p. o.*) for 14 days, and group 4 received synthesized compound 30 mg/kg *p. o.* for 14 days

The anti-diabetic efficacy of the chosen synthesized compound was evaluated by measuring fasting serum blood glucose level on day 7 and 14, through tail vein blood samplings using a single touch glucometer (ACCU-CHECK, Active, Roche diabetes Care GmbH, Germany). Further treatment efficacy was confirmed by histopathological analysis of pancreas in each treatment groups.

IV. CONCLUSIONS

We have synthesized a series of 1-(2-((1-(dimethylamino)-2-(4-substituted phenyl)vinyl) (methyl) amino)-4-(trifluoromethyl)thiazol-5-yl)ethan-1-one derivatives and described them. Investigation of the interaction energies between the hybrids and AMPK binding sites using *Insilco* docking. The synergism of the thiazole moieties was clearly recognized as a result of this by the increased bind affinity of the complex generated by contacts between candidates 3j and biological receptors that were evaluated. Due to the existence of hydrogen bonds and glide energy, we were able to locate the binding site of the enzyme AMPK using ligands 3j. This location has a high binding energy (-7.2 kcal/mol), which indicates that it is associated with protein. The high interaction of hydrogen bonds is responsible for the majority of the observed binding energy (-7.2 kcal/mol) with the ligand 3j for the AMPK receptor. Newly synthesized thiazole compounds have good physical qualities, which qualify them to have improved pharmacokinetics and drug bioavailability. The pharmacodynamic property has been investigated and carried out. The anti-diabetic efficacy of the produced compounds was investigated in vitro and in vivo, and the results showed that the compounds had outstanding activity. The newly synthesized 1-(2-((1-(Dimethyl amino)-2-(2-nitro phenyl) vinyl)(methyl) amino)-4-(trifluoromethyl) thiazol-5-yl)ethan-1-one (**3j**) has the potential to be a promising compound due to the fact that it increases bioavailability, minimizes the dose concentration, and reduces the number of adverse effects.

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