Original Article

Molecular Docking Study For Adenosine Monophosphate-Activated Protein Kinase Agonist From Syzygium Cumini For Treatment Of Diabetes Mellitus-Type 2

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Abstract

Diabetes mellitus has been one of the most frequent diseases in both industrialised and developing countries and it is spreading fast over the globe. It is believed that nearly 30 percentage of diabetic patients depends on alternative or traditional therapy in some manner. The AMP-activated protein kinase (AMPK) is a conserved cellular energy sensor that seems to have evolved early in the evolution of eukaryotes. Metformin, the most frequently used to treat type 2 diabetes, was discovered to activate it in 2001. Even though the metabolic consequences of AMPK activation were similar to the concept that mediates part of metformin's medicinal effects, it currently looks doubtful that AMPK is the drug's primary target, as explained below. The ways by which natural plant compounds obtained from traditional plant Syzygium cumini activate AMPK are explored. From the study, it was noted that 1,2-Benzisothiazol-3-amine tbdms met the AMPK agonist criteria. The 1,2-Benzisothiazol-3-amine tbdms prodrug was already demonstrated to enhance metabolic activities in people with insulin resistance and prediabetes, and it's being debated if this is mediated in partly by AMPK.

KEYWORDS: AMPK, Diabetes mellitus, insulin, Syzygium cumini, docking

INTRODUCTION

Worldwide, Diabetes mellitus is among the top five major causes of death[1]. Diabetes is a serious public health issues, with prevalence predictable to climb from 171 million in 2000 to 366 million in 2030[2]. It is a metabolic condition that results in unusually high blood sugar levels (hyperglycemia)[3]. This is caused by a mix of inherited and environmental factors. Diabetes is a serious degenerative disease that affects people all over the world, and that has quickly becoming the 3rd deadliest disease in the world[4] . Diabetes is a clinical paradigm for other medical conditions[5]. Herbs with other nutritional supplements are used in complementary therapies as an alternative to the conventional western medical therapy. According to a recent survey, up to 30 percent of diabetic patients use complementary / alternative therapy[6].

AMPK has been in thrust area of research during the last decade since it provides a vital function in monitoring energy to cells or nutrition[7]. AMPK can be found in skeletal muscles, the brain, and the liver [8]. When the skeletal muscles are working for a long time, active AMPK assists the cells in adapting to the energy challenge by boosting uptake of glucose[9]. The master modulator of metabolic homeostasis is AMPK[10]. While AMPK is turned on, it activates the catabolic networks of cellular glucose absorption, triglycerides and cholesterol reduction, and biogenesis. Adiponectin or another upstream signal molecule, promotes AMPK to use glucose and fatty acids. Hypothalamus's active AMPK promotes appetite or food consumption desire[11]. Adiponectin activates AMPK, a protein that protects the heart from myocardial

ischemia. Leptin, an upstream signal molecule, initiate fatty acid oxidation by activating AMPK. Thyroid hormone is also facilitated by AMPK in the hypothalamus, which regulates energy balance [12].

Blood glucose is partially controlled by AMPK. Insulin resistance caused by obesity is reduced when AMPK is activated by its agonist. The development of an AMPK activator drug offers hope for the betterment of type 2 diabetes[13]. For the treatment of metabolic disease, AMPK is appeared as a prospective target for the treatment. Metformin is a well-known diabetes medication that works by activating AMPK. AMPK research has recently yielded fresh insights. By simulating a condition of pseudostarvation, activated AMPK exerts an anti-inflammatory effect. By integrating signals, AMPK can control cell development. Phosphorylation of p27kip1 by the LKB1-AMPK pathway can cause autophagy or death in cells. In the realm of tumour suppression, the LKB1-AMPK pathway has therefore been validated. In an energy fatigue state, autophagy can be induced via AMPK-induced phosphorylation of Unc51-like kinase 1 (ULK1). ULK1 is a protein kinase that phosphorylates serine and threonine.

The binding events of protein dynamics motion and structure changes may now be investigated using computational simulation[15]. In silico biology or Computational systems biology can be used to investigate protein interaction or protein-molecule interactions. This techniques allow people to take part in computer-assisted drug design (CADD). When compared to conventional one-by-one biochemistry, CADD approaches save us time by allowing us to quickly pick the most appropriate therapeutic component. Virtual screening, confirmations, and evaluation are all part of the CADD operations. Docking and Molecular Dynamics (MD) simulation are used in virtual screening and assessment. MD can estimate how long it will take for the drug to develop a stable complex structure with the target protein[16]. Docking and MD efficiency are aided by a set of statistics or score systems. The best virtual screening and MD candidates could be chosen as prospective therapeutic medicines[17].

It's currently debatable if AMPK has the metformin focus that fully accounts for their medicinal benefits, at least when it comes to its impacts on hepatic glucose production. The gluconeogenesis enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase were negatively regulated by the pharmacological AMPK activator 5-aminoimidazole-4-carboxamide riboside (AICAR) (G6Pase), while metformin's hypoglycemic impacts were lost in mice with a liver-specific knockout of LKB1. Although metformin significantly decreasing G6Pase expression and the production of gulcose, and AICAR diminishing the expression of PEPCK and G6Pase in isolated hepatocytes derived from the animals, mice with both AMPK catalytic subunit isoforms knocked out in the liver maintained normal sensitivity to insulin. It was found that, not only metformin, but AICAR can also decrease the expression of gluconeogenic enzymes and glucose production in the liver via AMPK-independent mechanisms[16].

Medicinal plants play a major part in daily life because they are natural factories for the creation of a variety of useful substances that aid in the treatment of various infections and disorders[3]. Numerous studies have been conducted on most medicinal plants with the goal of boosting commercial and pharmaceutical production of active phytochemicals (such as phenolic compounds, alkaloids, terpenoids, tannins, steroids, flavonoids, and glycosides). The gas chromatography (GC-MS) method is one of the most modern and successful methods for screening and detecting active secondary metabolites in any plant sample without the use of reference chemicals. It includes a library with a variety of mass spectrometers for chemicals that may be compared to raw materials extracted from plant samples. The major goal of this work is to uncover chemicals from the plant Syzygium cumini that can activate the function of AMPKs and inhibit diabetes 2 mellitus illness.

Materials and Methods

Structure retrieval

3D structure of AMP-activated protein kinase bound to pharmacological activator R734 was downloaded from RCSB with PDB ID: 6C9F with the resolution 2.92 Å[18] and chain B which has 5'-AMP-activated protein kinase subunit beta-1 structure is further used for the study. The ligand structure for Syzygium cumini were derived from GC-MS analysis and the control metformin, a commercial drug is taken from pubchem[19].

GC-MS analysis

GC-MS was used to tentatively identify the number of components present in the plant extracts. GC-MS was done with GC Clarus 500 Perkin Elmer equipment. On a capillary column the compounds were separated. The split ratio of the samples was 50:1, and helium was used as the carrier gas.

Protein and ligand preparation

Removing the macromolecule from the solvent and non-standard residue, providing a polar hydrogen atom, and restoring the force field with a Gasteiger charge were all used in the optimization process. Gypsum-DL, a free accessible tool for

building small-molecule libraries, was used to create the ligands[20]. This results in molecular models of various ionisation states, tautomeric and isomeric states, and non-aromatic ring conformations.

Active site prediction

After protein preparation the pdb structure was subjected to active site prediction in CASTp 3.0[21] and the top active site was further considered for the studies.

Grid based rigid docking

The compound from GC-MS analysis along with the control Metformin was further subjected to grid based molecular docking using AutoDock[22]. The grid boxes were constructed encapsulating the active site region calculated from CASTp with dimension of -17.68 x 34.54 x -47.86 incorporating the residues from 74 to 164 were set. For 100 iterations, the Lamarckian genetic method was used to simulate docking. One best conformation from 10 different conformations generated by autodock was considered. The complex structures showing least binding energy, ligand efficiency, with more number of hydrogen bonds were selected for proficient results. The study of protein-ligand complexes and its amino acid positions in relation to bond distances and the types of bonds involved were analysed and visualized through Maestro academic version[23].

Pharmacokinetic studies

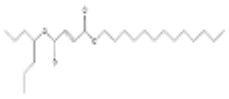
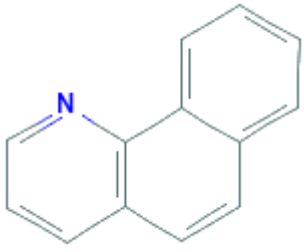
The pharmacokinetic study of the best compound melezitose was estimated by using online SwissADME program[24]. Significant ADME (Adsorption, Distribution, Metabolism, and Excretion) linked features were studied, including Lipinski's rule of five, pharmaceutical solubility's, and drug similarity.

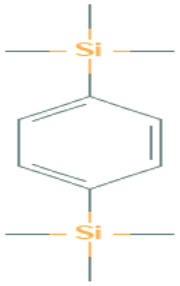
Results and Discussion

GC-MS analysis - Syzygium cumini

The presence of compounds in Syzygium cumini was confirmed by GC-MS analysis which showed the presence of Fumaric acid, 4-heptyl tridecyl ester, Benzo[h]quinoline, 2,4-dimethyl-, 1,2-Benzisothiazol-3-amine tbdms, and 1,4-Bis (trimethylsilyl) benzene. Results are shown in Table 1.

Table 1. The phytochemicals of Syzygium cumini analysed with GC-MS

Sl No.	Name	Retention time	Molecular formula	Molecular weight	Structure
1	Fumaric acid, 4-heptyl tridecyl ester	15.79	$C_{24}H_{44}O_4$	396.6	
2	Benzo[h]quinoline, 2,4-dimethyl-	16.99	$C_{13}H_9N$	179.22	
3	1,2-Benzisothiazol-3-amine tbdms	17.08	$C_7H_6N_2S$	150.2	

4	1,4-Bis (trimethylsilyl) benzene	17.54	$C_{12}H_{22}Si_2$	222.47	

In Germany during 1950s, Fumaric acid was used to treat and cure the autoimmune disease psoriasis. Fumaric acid was taken as a tablet contains 3 esters, chiefly dimethyl fumarate, and marketed as Fumaderm in Europe by Biogen Idec. Biogen then later developed dimethyl fumarate, the primary ester, as a therapy for multiple sclerosis. In a phase 3 trial, the ester dimethyl fumarate (BG-12, Biogen) reduced significantly recurrence and impairment progression in patients with relapsing-remitting sclerosis. It triggers the Nrf2 antioxidant response pathway, which is the major cellular defence against oxidative stress's harmful effects[25]. Benzo[h]quinoline, 2,4-dimethyl-, 1,2-Benzisothiazol-3-amine tbdms and 1,4-Bis (trimethylsilyl) benzene compounds have anticancer, antibacterial and antioxidants activities[26].

Structure of AMPK

AMPK seems to be a 3-subunit protein kinase with 2 alternative kinase domain containing α-subunits (α1 and α2), two alternative carbohydrate-binding module–containing β -subunits (β1 and β2), and three alternative AMP/ADP/ATP-binding γ -subunits (γ1, γ2, and γ3) [27, 28]. It has a disordered region from 1- 43 amino acid and Glycogen-binding domain region from 68 – 163. The glycogen-binding domain may guide AMPK activity to glycogen, allowing additional variables such as glycogen-bound debranching enzymes or protein phosphatases to have an impact.

AMP-activated protein kinase (AMPK), an energy sensor protein kinase that regulates cellular energy metabolism, has a non-catalytic component. AMPK stimulates energy-producing pathways and suppresses energy-consuming activities in result of low intracellular ATP levels: it suppresses protein, glucose, and lipid manufacture, and also cell growth and proliferation. AMPK works by phosphorylating metabolic enzymes directly and via phosphorylating transcription regulators for longer-term impacts. Works as a polarity regulator by modifying the actin cytoskeleton, most likely by stimulating myosin indirectly. Through its C-terminus, which connects alpha (PRKAA1 or PRKAA2) and gamma subunits, the beta non-catalytic subunit serves as a scaffold on which the AMPK complex assembles (PRKAG1, PRKAG2 or PRKAG3).

Molecular docking analysis of ligand against AMPK

Metformin seems to be a biguanide-class antihyperglycemic medication for type 2 diabetes. Metformin is classified as an antihyperglycemic medicine since it decreases blood glucose levels without inducing hypoglycemia. Metformin, type of insulin sensitizer that lowers insulin resistance and lowers plasma fasting insulin levels by a clinically relevant amount [29]. Metformin has long been known to reduce mitochondrial complex I function, and has been assumed that this is how it achieves its effective anti-diabetic effects[30, 31].

The organic cation transporter-1 (OCT1) is important for metformin absorption into hepatocytes after consumption. Due to the obvious membrane permeability across the cell membrane and the mitochondrial inner layer, this positively charged medication aggregates in cells and mitochondria. Metformin affects mitochondrial complex I, inhibiting mitochondrial ATP synthesis and resulting in an increase in cytoplasmic ATP[30]. These modifications activate AMPK, an enzyme involved in glucose metabolism regulation[30]. AMPK can also be triggered by a lysosomal mechanism that involves other activators. AMP levels rise as a result of this process: The ATP ratio also inhibits fructose-1,6-bisphosphatase, which inhibits gluconeogenesis, as well as adenylate cyclase, which inhibits the generation of cyclic adenosine monophosphate (cAMP) [30]an ATP derivative used only for cell signalling[32.] Activated AMPK phosphorylates two isoforms of the Acetyl-CoA carboxylase enzyme, which inhibits fat production and promotes fat oxidation, lowering hepatic lipid storage and improving insulin sensitivity[30].

Metformin promotes anaerobic glucose metabolism in enterocytes (intestinal cells) in the intestines, resulting in decreased net glucose absorption and enhanced lactate transport to the liver. Recent research has also linked the gut as a main site of metformin activity, implying that the liver may not be as significant for metformin action in type 2 diabetic patients. Metformin may have an effect on the intestines via boosting glucose metabolism by raising glucagon-like peptide I (GLP-1), and also enhancing glucose consumption in the gut[30.]

The AMPK has a glycogen-binding domain and the predicted active site lies in this region. Hence for AMPK, the grid box was generated around the active site region. Autodock generated parameters like binding energy, ligand efficiency and number of hydrogen bonds were analysed. Rigid docking showed good binding affinity of the compounds with AMPK than the commercial compound metformin is depicted in Table 1. The natural compounds showed binding affinity in the range from –2.78 kcal/mol to -0.84 kcal/mol. The residue VAL81 had formed hydrogen bonding with our target and ligand in almost all complex, which showed its major role in forming strong interaction than the other residues. Binding affinity, the number of hydrogen bonds and the interactive residues are shown in Table 1 and the top interacted complex is shown in Figure 1. Notably, the compounds 1,2-Benzisothiazol-3-amine TBDMS, Cyclobarbital, and 4-cyclohexene-1,2-dicarboximide,N-butyl-cis showed good binding affinity than the control drug Metformin. Metformin showed three hydrogen bonds with the target, whereas the top compounds also showed a reasonable h-bond of two.

Table 1. Binding energy for the interaction of the compounds with ADMPK

Sl. No	Ligand	Binding Affinity (Kcal/Mol)	Hydrogen bond forming residue	Other active site residues
1	METFORMIN	-2.09	Arg83, Val81, Glu139	Ile153, Thr80, Phe82, trp84, Tyr125, Phe127
2	1,2-BENZISOTHIAZOL-3-AMINE TBDMS	-2.78	Val 81 (2)	Ile153, thr80, Pro79, Phe160, Asp159
3	CYCLOBARBITAL	-2.75	Val81, Glu139	Thr80, Phe82, Tyr125, Phe160, Asp159, Lys156, Val155, Gln154, Ile153
4	4-CYCLOHEXENE-1,2-DICARBOXIMIDE, N-BUTYL-, CIS-	-2.55	Val81	Thr80, Pro79, Arg83, Phe160, Asp159, Lys156, Val155, Gln154, Ile153
5	EUGENOL	-1.65	Arg83	Ile153, Thr80, Val81, Phe82, Trp84, Thr85, Tyr125, Phe127, Asp136, Glu139
6	1,4-BIS(TRIMETHYLSILYL)BENZENE	-1.64	Nil	Val81, Thr80, Pro79, Val155, Ile153, Asp159, Phe160
7	BENZO[H]QUINOLINE, 2,4-DIMETHYL-	-1.17	Nil	Ile153, Thr80, Val81, Phe82, Arg83, Trp84, Phe127, Tyr125, Glu139, Asp136
8	CARYOPHYLLENE	-0.84	Nil	Glu139, Pro140, Tyr125, Ile153, Phe127, Thr80, Val81, Phe82, Arg83

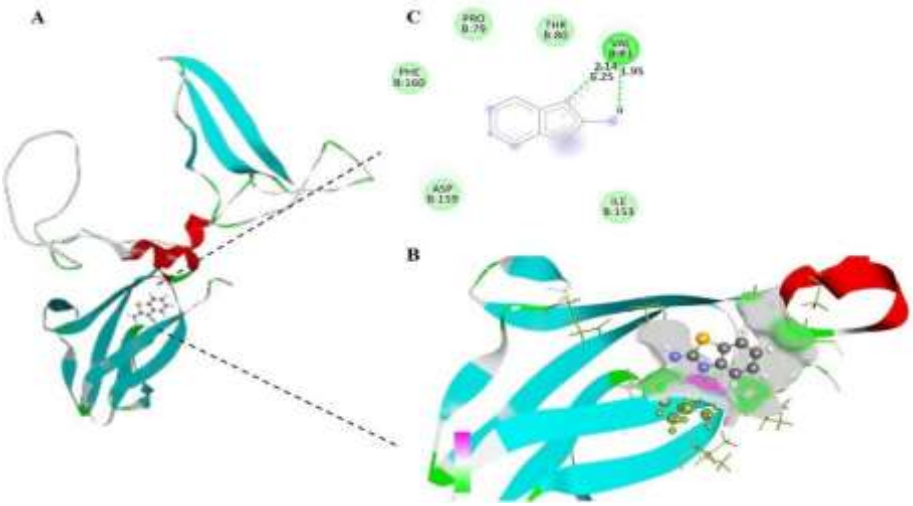


Figure 1. Molecular interaction of 1,2-Benzisothiazol-3-amine tbdms with ADMPK A) Docked Complex. B) 3D interaction of docked complex. C) 2D interaction of docked complex

ADME Screening

Pharmacokinetic studies found that 1,2-Benzisothiazol-3-amine tbdms contains 0 rotatable bonds, has 1 H-bond acceptor and donor, molecular weight lie in the acceptable range of 150.2 g/mol, has 10 heavy atoms, and solubility analysis revealed the compound is soluble. Gastrointestinal absorption is high, according to the yolk of boiled egg the brain barrier permanent happens, the compound follows Lipinski rule of five without violating any rule. The control compound metformin has a molecular weight of 129.16 g/mol, number of heavy atoms is 9, has rotatable bonds 2, H-bond acceptor and donor 3, follows Lipinski rule with 0 violation, its highly soluble, it doesn't cross blood brain barrier. It was noted that, all the compounds possess an acceptable range of druggable properties as shown in table 2.

Table 2: Physicochemical properties of the compounds

Compound Name	Molecular weight (50-500)	LopgPo/w (2-10)	HBA (0-10)	HBD (0-5)
METFORMIN	467.25	3.06	8	1
1,2-BENZISOTHIAZOL-3-AMINE TBDMS	150.19	1.84	2	2
CYCLOBARBITAL	236.27	1.32	4	2
4-CYCLOHEXENE-1,2-DICARBOXIMIDE, N-BUTYL-, CIS-	207.27	2.32	3	0
EUGENOL	164.20	2.66	1	4
1,4-BIS(TRIMETHYLSILYL)BENZENE	222.47	6.44	0	0
BENZO[H]QUINOLINE, 2,4-DIMETHYL-	207.27	4.10	1	0
CARYOPHYLLENE	204.35	5.88	0	0

CONCLUSION

It is essential to monitor the cellular energy or nutrient status, AMPK has been a hot topic in the last decade. As a result, AMPK helps in diabetic inhibition medication development. The activation of AMPK was triggered by a conformational change in the protein. We used Grid-based rigid docking of the plants Syzygium cumini to look for possible chemicals that could bind to AMPK and stimulate its function. 1,2-Benzisothiazol-3-amine tbdms were chosen as candidate for further research. These chemicals were also able to modify the structure of AMPK. From this study, its ccan be concluded that 1,2-Benzisothiazol-3-amine tbdms can act as a diabetes regulating agent since it is less toxic and follows drug similarity requirements.

REFERENCES

1. Joseph B, Jini D. Insight into the hypoglycaemic effect of traditional Indian herbs used in the treatment of diabetes. Res J Med Plant 2011a; 5(4): 352-376.
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87: 4-14.
3. Patel DK, Prasad SK, Kumar R, Hemelatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2012; 2: 320-330.
4. Zimmet, P.Z. Diabetes and its drivers: the largest epidemic in human history?. Clin Diabetes Endocrinol 2017; 3, 1 https://doi.org/10.1186/s40842-016-0039-3.
5. Sharma AK, Aggarwal A, Singhal VK. Treatment of diabetes mellitus with indian herbal Drugs. IJARPB 2012; 1(2): 145-153.
6. Raman BV, Krishna NV, Rao NB, Saradhi PM, Rao BMV. Plants with antidiabetic activities and their medicinal values. Int Res J Pharm 2012; 3(3): 11-15.
7. Daniel Garcia and Reuben J. Shaw AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. Mol Cell. 2017; Jun 15; 66(6): 789–800. doi: 10.1016/j.molcel.2017.05.032.
8. D. Carling, F. V. Mayer, M. J. Sanders, and S. J. Gamblin, "AMP-activated protein kinase: nature's energy sensor," Nature Chemical Biology, 2011; 7(8):512–518,.
9. Day, E. A., Ford, R. J., and Steinberg, G. R. AMPK as a therapeutic target for treating metabolic diseases. Trends Endocrinol. Metabol 2017;28, 545–560. doi: 10.1016/j.tem.2017.05.004.
10. Fullerton, M. D., Galic, S., Marcinko, K., Sikkema, S., Pulinilkunnil, T., Chen, Z. P., et al. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. Nat. Med. 2013;19, 1649–1654. doi: 10.1038/nm.3372
11. Stern, J. H., Rutkowski, J. M., and Scherer, P. E. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. Cell Metab. 2016; 23, 770–784. doi: 10.1016/j.cmet.2016.04.011.
12. Baile Wang and Kenneth King-Yip Cheng. Hypothalamic AMPK as a Mediator of Hormonal Regulation of Energy Balance. Int J Mol Sci. 2018; 19(11): 3552.
13. Kimberly A Coughlan, Rudy J Valentine, Neil B Ruderman, and Asish K Saha. AMPK activation: a therapeutic target for type 2 diabetes? Diabetes Metab Syndr Obes. 2014; 7: 241–253.
14. J. Kim, M. Kundu, B. Viollet, and K.-L. Guan, "AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1," Nature Cell Biology, 2011;13(2): 132–141.
15. Chia-en A. Chang., Yu-ming M. Huang., Leonard J. Mueller and Wanli You. Investigation of Structural Dynamics of Enzymes and Protonation States of Substrates Using Computational Tools. Catalysts 2016; 6(6), 82; https://doi.org/10.3390/catal6060082
16. Mayasah Al-Nema Anand Gaurav and Vannajan Sanghiran Lee. Docking based screening and molecular dynamics simulations to identify potential selective PDE4B inhibitor. 2020;6(9), e04856. doi.org/10.1016/j.heliyon.2020.e04856
17. Luca Pinzi and Giulio Rastelli. Molecular Docking: Shifting Paradigms in Drug Discovery. Int J Mol Sci. 2019; 20(18): 4331. doi: 10.3390/ijms20184331
18. Y. Yan, X. Edward Zhou, S.J. Novick, S.J. Shaw, Y. Li, J.S. Brunzelle, Y. Hitoshi, P.R. Griffin, H. Eric Xu, K. Melcher, Structures of AMP-activated protein kinase bound to novel pharmacological activators in phosphorylated, non-phosphorylated, and nucleotide-free states, Journal of Biological Chemistry. 2019; 294 :953–967.
19. S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P.A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E.E. Bolton, PubChem in 2021: New data content and improved web interfaces, Nucleic Acids Research. 2021;49: D1388–D1395.
20. P.J. Ropp, J.O. Spiegel, J.L. Walker, H. Green, G.A. Morales, K.A. Milliken, J.J. Ringe, J.D. Durrant, GypSUm-DL: An open-source program for preparing small-molecule libraries for structure-based virtual screening, Journal of Cheminformatics. 2019;11: 1–13.
21. W. Tian, C. Chen, X. Lei, J. Zhao, J. Liang, CASTp 3.0: computed atlas of surface topography of proteins, Nucleic Acids Research. 2018;46: W363–W367.
22. Surbhi Pradhan and R C Dubey. GC-MS analysis and molecular docking of bioactive compounds of Camellia sinensis and Camellia assamica. Arch Microbiol . 2021 ;203(5):2501-2510. doi: 10.1007/s00203-021-02209-6.
23. Rai, H., Barik, A., Singh, Y.P. et al. Molecular docking, binding mode analysis, molecular dynamics, and prediction of ADMET/toxicity properties of selective potential antiviral agents against SARS-CoV-2 main protease: an effort toward drug repurposing to combat COVID-19. Mol Divers 2021;25, 1905–1927. https://doi.org/10.1007/s11030-021-10188-5
24. A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Scientific Reports 2017; 7(1): 7 1–13.
25. Gold R.; Kappos L.; Arnold D.L. "Placebo-Controlled Phase 3 Study of Oral BG-12 for Relapsing Multiple Sclerosis". N Engl J Med. 2012; 367 (12): 1098–1107.
26. Li H, Wang X, Xu G, Zeng L, Cheng K, Gao P, Sun Q, Liao W, Zhang J. Synthesis and biological evaluation of a novel class of coumarin derivatives, Bioorg Med Chem Lett 2014; 24: 5274–5278

27. Steneberg, P. et al. PAN-AMPK activator O304 improves glucose homeostasis and microvascular perfusion in mice and type 2 diabetes patients. JCI Insight 2018;3, 99114.
28. Steinberg, G.R., Carling, D. AMP-activated protein kinase: the current landscape for drug development. Nat Rev Drug Discov 2019;18, 527–551. https://doi.org/10.1038/s41573-019-0019-2
29. B. Viollet, B. Guigas, N. Sanz Garcia, J. Leclerc, M. Foretz, F. Andreelli, Cellular and molecular mechanisms of metformin: an overview, Clinical Science (London, England: 1979). 2012;122 :253–270.
30. G. Rena, D.G. Hardie, E.R. Pearson, The mechanisms of action of metformin, Diabetologia. 2017;60:1577–1585.
31. G. Rena, E.R. Pearson, K. Sakamoto, Molecular mechanism of action of metformin: old or new insights? Diabetologia. 2013;56: 1898–1906.
32. F. Valsecchi, L.S. Ramos-Espiritu, J. Buck, L.R. Levin, G. Manfredi, cAMP and Mitochondria, Physiology. 2013;28: 199.