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Computational Design of Bioactive Epigallocatechin Gallate (EGCG) Analogues Targeting Heme Oxygenase-1 (HO-1) Pathway for Metabolic Regulation

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Abstract

Epigallocatechin-gallate (EGCG) is a flavone-based natural product that has a more significant impact on Diabetes and other cardiometabolic complications. This in silico based computer-aided drug design ensures the drug's Pharmacokinetic parameters and particular compounds towards the precise target. Based on the network data designed, EGCG were docked against PDB: 1N3U, using Dimethyl fumarate as the standard reference. Such desirable quality of modified EGCG will create a spark in the novel drug discovery of a bioenhancer. This slight evidence will support a higher quantum of drug discovery in semisynthetic chemistry toward Metabolic Complications.

Keywords: *Bioinformatics, Docking, Epigallocatechin-gallate, Halogen, Nitrogen, Pharmacokinetics.*

Introduction

According to the International Diabetes Federation, Diabetes is considered a multifactorial metabolic complication with frightening public health issues due to the persistent elevation of blood glucose leading to major microvascular and macrovascular complications [1]. Type 1 diabetes mellitus is an autoimmune condition where the destruction of pancreatic beta cells leads to insulin deficiency. Secondary complications like dyslipidemia, excessive glucose-mediated oxidative stress, endothelial cell dysfunction, and apoptosis. The pancreatic beta cell destruction is mediated by T cells [2]. The onset of type 1 diabetes mellitus is characterized by a normal glucose profile, and it is asymptomatic with more than 2 pancreatic antibodies. Then, in the next stage, abnormal glucose (dysglycemia) profiles with more than 2 pancreatic antibodies are observed. In the later stages, hyperglycemia and its related symptoms are observed with more pancreatic antibodies [3, 4]. Most type 1 diabetes mellitus patients (children) present with diabetic ketoacidosis as a complication. The major factors contributing to type 1 diabetes mellitus are genetic, environmental (viruses, vaccinations, diet, gut microbiota), epigenetic and immunological factors. The human leukocytes antigen, insulin-Variable Numbers of Tandem Repeats (VNTRs), Cytotoxic T lymphocyte Antigen-4 (CTLA-4 gene), FoxP3, STAT3, and ERBB3 play a major role in enhancing the process of apoptosis, cytokine release mediated inflammatory process [5]. The immunological responses constitute immune tolerance where AIRE gene mutation results in an activity of self-reactive lymphocytes in the peripheral tolerance by escaping from central tolerance. In the case of cellular immunity, the increase in production of pro-inflammatory cytokines like

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IL-1, TNF- alpha, INF- gamma. The above process is induced by auto-reactive T lymphocytes in the islet of Langerhans through Fas/Fas ligand interaction. In the type 1 diabetes mellitus population, chronic aortic inflammation involving T lymphocytes, macrophages, B lymphocytes, and dendritic cells is observed before the onset of the disease [6-8]. Apoptosis is triggered by extrinsic and intrinsic pathways. In the extrinsic pathway, the apoptosis process observed in type 1 diabetes mellitus in the beta cell environment is run by Fas (CD 95)-Fas L (CD 178) which is mediated by caspase-3-like activity in *in vitro* studies. The intrinsic pathway involves a mitochondrial-driven pathway where the balance between anti-apoptotic Bcl-2 and Bcl-xL and pro-apoptotic proteins [9, 10]. Type 2 diabetes mellitus is a heterogenous metabolic disorder influenced by environmental, environmental, genetic, and immunological factors in the pathogenesis. Type 2 diabetes mellitus is characterized by hyperglycemia accompanied by obesity, cardiovascular complications, renal and biliary dysfunction (macrovascular complications), and microvascular complications [11, 12]. The inflammation in the type 2 diabetes mellitus population is driven by weight gain and obesity. Activation of Jun N-terminal kinases (JNK) and the transcription factor NF-kappaB under conditions of cellular stress. This above process results in the production of pro-inflammatory cytokines, aggravated by the adipokines, and strongly correlates with the risk of other cardiometabolic disorders [13, 14]. In Previous *in vivo* studies, the role of brown adipose tissue (BAT) in glucose homeostasis and energy regulation is clearly established. Its involvement in peripheral insulin resistance is also demonstrated [15]. Several cytokines and inflammatory biomarkers(TNF-alpha, IL-1, IL-6, IL-10, leptin, adiponectin, MCP, chemokines, and amyloid protein) essential for inflammatory pathways are produced by the white adipose tissues (WAT) [16] .In normal conditions, glucose induces insulin secretion with the help of glucose sensors and metabolism. Insulin regulates glucose metabolism. This process involves glycolytic reactions that take place in mitochondria with the production of ATP. Plasma membrane depolarization due to the closing of the potassium channel by ATP and opening of voltage-dependent calcium channel results in an increase of free cytosolic calcium concentration, which gives way to increased insulin secretion. If there is excessive production of glucose leading to hyperglycemia, glucose toxicity and beta cell apoptosis are observed due to the induction of a myriad of biological reactions and mitochondrial dysfunction. Like type 1 diabetes mellitus, the imbalance in the Bcl family and activation of the apoptotic gene is also responsible for apoptosis in type 2 diabetes mellitus [17]. In mammals, regulation of cellular redox balance, past II detoxification responses and antioxidant properties are managed by the Nrf2, encoded by *the Nfe212* gene, a transcriptional factor [17, 18]. Glutamate-cysteine ligase (GCL), thioredoxin reductase 1 (Txnrd1), NADPH-quinone oxidoreductase 1 (NQO1), and heme oxygenase (HO)-1 are the factors responsible for the antioxidant response element (ARE) are regulated by Nrf2 activation. Nrf2 binds to the ARE regulatory genes, Kelch ECH associating protein-1 (Keap-1), which is a repressor protein rich in cysteine. Keap-1 is present in the skeletal muscle, anchored to actin. Keap-1 regulates the cytosolic sequestration of Nrf2. Normally Nrf2 via ubiquitination undergoes degradation under the control of Keap-1 with a short half-life of 20 minutes [19, 20]. If there is cellular stress or abnormal cellular response, the oxidants induce the Nrf2-dependent cellular defense mechanism. The Nrf2 released from Keap-1 binds to the conserved ARE sequence. The above process leads to the inactivation of Keap-1 via modification of electrophiles of reactive cysteines and stabilization of Nrf2 with

a half-life of 200 minutes[21]. Followed by activation of cytoprotective genes. Out of the 27 cysteines, Cys151 is predominant in control of Nrf2 in basal and stress conditions [22]. As mentioned earlier, the Keap-1 Nrf2 pathway regulates various cytoprotective genes to exert antioxidant properties, which participate in the synthesis and regeneration of glutathione responsible for regulating transcription and growth factors. And one such gene is HO-1, an inducible 32-kDa protein. This is activated by heme, nitric oxide, heavy metals, growth factors, and cytokines during the inflammatory process or under stressful conditions [23].

A combination of systemic and local effects is exerted by H0-1, which involves macrophage differentiation and polarisation with the help of enzymatic byproducts. The production of biliverdin, carbon monoxide (CO), and ferrous ion release [24]. The oxidation process is catalyzed by NADPH-Cytochrome P450 Reductase leading to the generation of biliverdin, CO, and free ion from heme. The free iron ions released are majorly involved in the regulation of iron homeostasis, CO acts as a gaseous signaling molecule similar to nitric oxide, which regulates vascular tone, vascular blood flow, and also mitochondrial oxygen consumption by inhibiting cytochrome c oxidase [25]. The produced biliverdin is converted into bilirubin by biliverdin reductase-A (BVR-A) [26]. The antioxidant and anti-inflammatory property of bilirubin is exerted by its action against lipid peroxidation of cell membranes as bilirubin is lipophilic in addition to the protection of water-soluble proteins by glutathione [27].

Catechin Overview

Basic physicochemical parameters were calculated for various natural flavonoid products using Swiss ADME to analyse the structure-activity relationship. Additionally, each natural flavonoid product's side chains and core scaffold were created based on their respective chemical structures. Due to the shared core scaffold of flavones, flavonols, flavanones, flavanonols, and flavan-3-ols, the structure-activity connections of the aforementioned five subclasses were examined. The hydroxyl group present in flavonol at position 3 increases the activity. Epicatechin is a powerful anti-ageing, antioxidant, and radical scavenger due to the five hydroxyl groups that are connected to it. The chemical structures and substituents where hydroxyl group quantity and configuration are related to bioactivity [28]. Additionally, it was found that the arrangement of hydroxylation, rather than the number of hydroxyl groups, caused the increase in activity. The catechol structure or 4-hydroxyl group in ring B and the hydroxyl groups in ring A appear to increase the anti-aging and radical-scavenging properties. The compounds' total quantity and hydroxyl group arrangement boosted the flavonols' anti-diabetic benefits. Flavonols' catechol-containing dihydroxyl groups at positions C-3 and C-4 are successfully attached to alpha-glucosidase active-site residues. The presence of the catechol system on the B ring of flavonoids is anticipated to help distribute the electron cloud, which becomes available to donate hydrogen atoms and establish hydrogen bonds with the active-site residues of alpha-glucosidase, playing a significant role in suppressing its activity [29, 30]. The chemical structure of EGCG is illustrated in Figure 1.

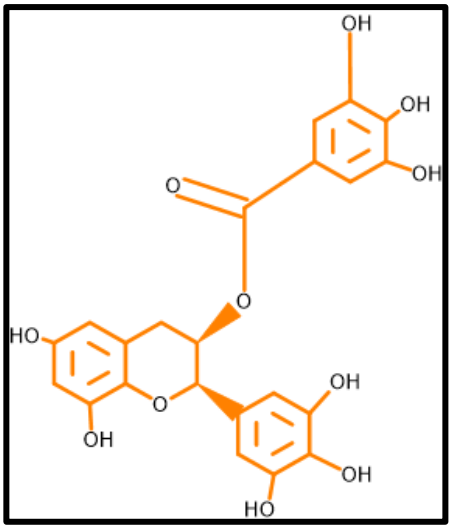

Figure 1. Structure of EGCG

Chemistry Aspects of Drug Repurposing

As per the International diabetes federation (IDF) statistics, the incidence of Diabetes has been rising in disproportionate numbers in recent times; presently, 380 million people are reported, which is expected to reach 592 million in the year 2035. Since Diabetes is a multifactorial metabolic complication, there is an imperative demand for a targeted therapeutic regimen. Various medications have been used in clinical settings to treat glucose homeostasis. However, few of the drugs are exhibiting additional undesirable consequences [31]. Repurposing plant-based medicinal products help to treat both common and rare diseases. Repurposing of bioactive compounds offers a strategy for the discovery of novel drugs in uncommon diseases that has benefits over conventional drug development due to cost-effectiveness and a shortened timetable.

Materials and Methods

Drug-likeness Property Estimation

The drug-likeness properties were investigated using a set of rules, including Molecular weight, H-bond donor, H-bond acceptor, QPlogPo/w, and Total polar surface area. The frequency of violations represents the impact of the compound's druggability, and these fundamental characteristics aid in detecting the drug's essential pharmacokinetic attributes.

Pharmacokinetic Parameters Estimation

Unsatisfactory pharmacokinetic and safety characteristics are a major barrier to drug development, with a high attrition rate. This graph-based online server helps assess the compounds' absorption, distribution, metabolism, excretion, and toxicity properties, illustrating radar images in supplementary files.

Molecular Docking Analysis

Molecular Docking was carried out in CB-Dock is a protein-ligand docking method. In this current study, PDB: 1N3U was chosen to identify the pharmacotherapeutic profile of EGCG towards the Nr-f pathway. PDB:1N3U is the Crystal structure of human heme oxygenase 1 (HO-1) in complex with its substrate heme, crystal form B with: 2.58 Å Resolution and non-mutant properties. To compare the docking score, two (Dimethyl fumarate & vitexin) positive controls were employed in this study. Dimethyl fumarate (DMF) is in a class of medications called Nrf2 activators. It decreases inflammation, and Vitexin has antioxidant, anti-inflammatory, anticancer, antinociceptive, neuroprotective effects, etc. DMF is considered a well-known HO-1 inducer due to its peculiar action on

cysteine by covalently binding ability at the active site.

Results

In this study, we employed a computational approach to determine the impact of Halogens and Nitrogen based (Br, Cl, F, I, NH_2, and NO_2) substitutions in the C-6 & c-8th position in Ring A of EGCG. This study compared the binding scores with actual EGCG, dimethyl fumarate, and Vitexin. The crucial score of EGCG is -9.2Kcal/mol, DMF is -4.4Kcal/mol, and Vitexin is -8.5Kcal/mol. The molecular docking score is given in Table 1.

Table 1. Molecular Docking Analysis of Designed Compounds Towards PDB:1N3U

Compound Code	Docking score (Kcal/mol) PDB:1N3U	Reported Amino acid Residue
EGCG	-9.2	HIS25 ALA28 GLU29 MET34 PHE37 GLN38 VAL50 LEU54 TYR114 THR135 ARG136 TYR137 GLY139 ASP140 GLY143 VAL146 LEU147 ILE150 PHE166 PHE16 7 ALA206 PHE207 ASN210 PHE214
DMF	-4.4	SER14 LYS18 THR21 HIS25 TYR134 THR135 LEU138 GLY139 SER142 LYS179 ARG183 PHE207
Vitexin	-8.5	HIS25 ALA28 GLU29 MET34 TYR134 THR135 ARG136 LEU138 GLY139 ASP140 SER142 GLY143 GLY144 LEU147 PHE207 ASN210 PHE214

The molecular docking images of EGCG is illustrated in Figure 2.

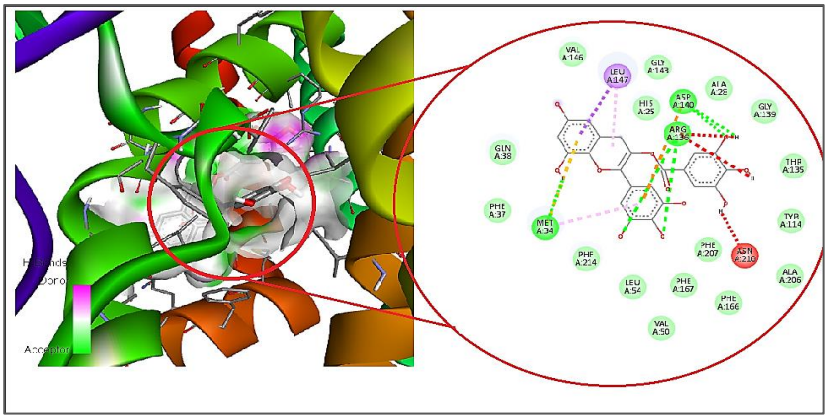

Figure 2. Molecular Docking Image of EGCG Towards PDB:1N3U

The Pharmacokinetic profile of designed compounds were given in Table 2.

Table 2. Pharmacokinetics (SWISSADME) Profile of EGCG

Compound code	**GI absorption**	**BBB permeant**	**P-gp substrate**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**	**Log Kp (skin permeation)**
EGCG	Low	No	No	No	No	No	No	No	-8.27 cm/s

Discussion

Previous research has shown that the Nrf2-HO-1 pathway and cardiometabolic disorders are interconnected. This pathway has promising therapeutic potential in Diabetes, where it regulates glucose homeostasis and improves insulin sensitivity [32-35]. In an *in vitro* study, upregulation of HO-1 expression through

activation of Nrf2 was observed in hydrogen peroxide-induced toxicity in PC12 cells when the administration of resveratrol. Resveratrol exerted its antioxidant and anti-inflammatory properties by activation of transient activation of Akt/protein kinase B and extracellular signal-regulated protein kinase 1/2 (ERK1/2) and also induction of HO-1 via Nrf2 -ARE signalling [36]. Chia FL et al. observed that induction of Diabetes Mellitus by administering streptozocin was performed in diabetic rats. The reactive oxygen species production in the bladder increased Keap-1, decreased Nrf2 expression with ROS in the bladder, mitochondrial Bax translocation, cytosolic cytochrome c release, and caspase-3/PARP apoptosis. On administration of Sulforaphane, there was a significant increase in Nrf2 HO-1 axis expression and a decline in ROS production at the site of the bladder[37]. Most bioactive compounds, such as resveratrol, Sulforaphane, Curcumin, quercetin, and start-Butylhydroquinone, correlate positively with Nrf2 activation, which leads to pancreatic beta cell apoptosis and related oxidative and inflammatory stress. Stimulation of HO-1 enhances insulin sensitivity and also downregulation of the peripheral endocannabinoid system, decreases adipose tissue volume and adipose tissue remodeling [38]. Repurposing plant-based medicinal products to treat both common and rare diseases is increasingly emerging as an attractive proposition because of its therapeutic potential, development costs, and shorter development timelines. Numerous data-driven and experimental strategies have been put forth to discover therapeutic candidates that can be repurposed, but substantial technological and regulatory concerns must be handled during the drug development process. Therefore, we have performed research towards the initiation of bio compounds (EGCG) to establish the viability in this current contemporary state, which was historically used in day-to-day life through food/spices for various chronic disorders [39, 40]. Computational drug design and machine learning techniques would be preferable to achieve selective therapeutic methodological drug repurposing. In green tea, EGCG accounts for about 50% of the catechin content (*Camellia sinensis*). It has significant anti-inflammatory, antihypertensive, and antioxidant properties [41].

Through preliminary research, it has been identified that EGCG has peculiar characteristics such as vasoprotective, increased endothelial function, and lowered blood pressure in hypertensive rats. In patients with coronary artery disorders, EGCG improved endothelial dysfunction and strengthened flow-mediated elongation of the brachial artery [42]. EGCG acts on various pathways such as the NF-kB pathway, MAPK pathway, PI3K/AKT pathway, VEGF pathway, EGFR pathway, etc. Several periclinal studies have confirmed that EGCG plays a critical role in apoptosis and cell death induction. Earlier, invitro/cell model studies described EGCG's tendency to regulate oxidative stress through gene transcription response in the NF-kB pathway [43]. Oxidative stress has been linked to cellular damage in several human diseases, including atherosclerosis, as a significant contributing factor. Recent research suggests that bioactive compounds from plants such as Curcumin, baicalein, quercetin, and catechin may work as HO-1 response protein inducers. Catechin derivatives are known as effective scavengers of Reactive Oxygen Species, which are also involved in the modulation of gene expression. Although EGCG is usually recognized as an antioxidant, it has also been demonstrated to act as a pro-oxidant in specific circumstances. C.C. Wu *et al.* have identified EGCG as an HO-1 inducer and protection against oxidative stress [44]. Zhou T et al. stated that EGCG reduces the levels of expression of inflammatory factors such as tumor necrosis factor-α and interleukin-6 in serum and also acts as neuroprotective in -methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(MPTP)-induced PD mouse model [45]. Currently, numerous life-saving drugs are made from the bioactive compound. The plant's substance has rejuvenated scientific interest in drug discovery and development. Hence, we believe the EGCG derivative may be a significant source of innovative medications. [46-48].

Conclusion

This study spotlights the EGCG and Pharmacokinetic parameters based clinical correlation of halogen and nitrogen-based alterations in the Ring A of EGCGS in cardiometabolic complications through Heme-oxygenase -1 Pathway. In addition, our study emphasizes that alteration in C-8 of Ring A would be much more active in clinical practices. The Heme-oxygenase-1 inducing capability would be much better in S4, S6, S8, and S12. The docking scores of EGCG and designed compounds (S1-S12) have a higher binding value than the HO-1 inducer (DMF). Despite massive investment by pharmaceutical companies toward the discovery of conventional medicines for Diabetes, the new drug has not withstood or reduced the incidence of many diseases, including type 2 diabetes (T2D). Drug repurposing is gaining popularity to overcome not only Diabetes but also various communicable/non-communicable diseases, even where the therapeutic activity is not met by the existing conventional medicine. Repurposing substantially reduces the time needed for medication development by employing the molecular pharmacology data currently known about treatments.

Conflicts of Interest

Authors have nothing to disclose.

Funding Statement

No findings have been raised for this study.

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