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DRUG-REPOSITIONING OF THE GALLATE GROUP FROM EGCG FOR THE REPURPOSING

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ABSTRACT

Context: Recycling the old drugs to a shelved drug, extending the patient's life, and creating drug repositioning in an attractive form in modern drug discovery has been our biggest motivation. The identification and targeting of all potential drugs which play crucial role in reuse of known drugs towards achieving new therapeutic indications is the main motivation. **Objective:** This study aims to bring out drug repositioning and repurposing of EGCG to cure or prevent diseases in large numbers. **Methods:** EGCG structure was designed in six possible directions. 3-chromanyl gallate was further minimized by applying the steepest descent followed by the conjugate gradient, later charm force field was applied and performed the molecular dynamics. **Results:** In this study, changing the configuration of the molecule (3-chromanyl Gallate) from 3rd position to 4th position has accounted for significant changes in logP, ClogP, Lipinski rule of 5 and free energy changes. **Conclusions:** It is from these studies, that this molecule potentially, and predominently leads to its target.

KEYWORDS: EGCG, Gallate Group, Proteasome, Docking, Repositioning, Oxidation.

INTRODUCTION

EGCG or epigallocatechin-3-gallate, is an ester of epigallocatechin and gallic acid, another catechin in occurrence. The International Union of Pure and Applied Chemistry (IUPAC) nomenclature of the compound goes is (2R, 3R)-5, 7-Dihydroxy-2-(3, 4, 5-trihydroxyphenyl)-3-chromanyl gallate.

EGCG occurs as a polyphenolic compound and can be derived from Delisea pulchra algae and green tea. It is found in green tea, white tea and in smaller quantities in black tea[1] besides its presence in various vegetables, nuts as well as carob powder at 109 mg per 100g.[2] EGCG is also a green tea catechin and provides potential health benefits which are namely anti-oxidant, anti-carcinogenic and anti-inflammatory.

EGCG has a positive and significant correlation with antioxidant quality.[3] Antioxidants are substances which may protect cells against the effects of free radicals[4] or damaged cells. In addition to their antioxidant quality and inhibition of cancer development by phenolic compounds, tea polyphenols have the potential to work in a preventive way against many causes of cancer.[5] There have been many clinical and epidemiological studies yielding conflicting results showing the evidence towards the confirmation of variety of cancers involving green tea catechins (EGCG), including breast[6,8], colorectal[9], esophageal[10], lung cancer[11-12] and prostate cancers.[13]

Green tea polyphenols (EGCG) are also believed to possess hypotensive effects. In popular Chinese medicine a large number of studies demonstrating that green tea may affect the cardiovascular function, through mechanisms of action related to LDL-cholesterol oxidation[14] inhibited by green tea due to EC and EGCG antioxidant activity.

High intake of polyphenolic compounds during pregnancy is assumed to increase risk of neonatal leukemia. Bioflavonoid supplements are excluded in medications of pregnant women.[15-18] Maternal consumption of tea or coffee has shown elevated risk of childhood malignant central nervous system (CNS) tumors through mechanisms unknown[19-21]

Divergences from the positive effects were observed in phenolic compounds such as EGCG. These effects are potent inhibitors of iron absorption and are also dose-dependent due to the different content of total polyphenols[22] which interfers with its assimilation by

the complex formation of the gastro-intestinal lumen.[23-25] EGCG is reported to be hepato toxic at higher doses.[26] In addition to this EGCG significantly inhibited trans epithelial transport of heme iron, strengthening the unhealthy effects of EGCG.

EGCG is a polyphenolic natural product offering a wide range of biological activities and unfavorable pharmaceutical properties. A vigilant study on the drug makes it less suitable for consumption in its present form, with the inhibitory concentrations of the drug for colon cancer showing 11 cases for inhibitory effects and 6 no of inhibitory cases which indicates one case out of every 3 cases showing no inhibition on colon carcinoma. Conflicting epidemiological inferences and discrepancies between in vitro and in vivo studies may be due to its erratic bioavailability. Hence there is a need to improve the structure of compound or derivative of the compound to check the group responsible for toxicity and further enhance its activity.

After oral absorption of tea, catechins undergo extensive methylation, glucuronidation, and sulfation. Rapid methylation of EGCG is catalyzed by the liver cytosolic catechol-O-methyltransferase. Hydrophilicity of catecholic compounds gets decreased by Methylation; to nullify this effect it requires the sulfation/glucuronidation of the methylated product so as to effectively eliminate the methylation product from the body. Another derivative namely, O-protected derivatives of (-)-epigallocatechin-3-gallate has also been reported to show decreased antioxidant activity.[15]

We initiated structure-activity investigations using semi-synthetic and synthetic EGCG analogs to identify more drug-like EGCG derivatives which are more effective. The data shows that there are multiple regions in the EGCG structure that contribute to its activity. The gallate ester portion of the molecule seems to be of particular importance as a 3, 4-difluoro analog of EGCG of more potency. This derivative and other active compounds have not proved to be cytotoxic in Huh-7 cell culture. These data suggest that more potent, yet non-cytotoxic EGCG analogs may be prepared in an attempt to identify more drug-like candidates to treat hepatitis C virus (HCV) infection by this mechanism.[27]

MATERIALS AND METHODS

New medicines can be formulated based on the discovery about the molecular basis of the disease and it provides immense opportunities However, developing a new drug takes an enormous amount of time, money and effort due to many bottlenecks during the therapeutic development process. Recent estimates suggest that on average it takes 10 - 12 years and at least $1 billion to bring a new drug into the market. Given such length of time and expense, pharmaceutical companies have shown increased interest in finding new uses for the existing drugs – a process referred to as drug repurposing or repositioning. In some cases where data has already been acquired, repurposing a drug can save time and money when compared to developing a drug de novo. Drug repurposing is one such measure. "Repurposing" generally means studying a compound or biologic (referred to as agents) which treats one disease or condition to see whether it can be safely and effectively employed in treating other diseases.

Main advantage of drug repositioning over traditional drug development is that the repositioned drug has already passed a large number of toxicity and other tests, its safety has already been established and the risk of failure due to adverse toxicology are also reduced. It is advantageous for repurposed drugs to gain market approval quickly in comparison to newly developed drugs as they are already accepted in the market . About 80% of drug approvals fail in phase 2 trials because they don't reach endpoints for efficacy. Out of those drugs that get FDA approval, little is known about their possible applications outside the narrow science of their original indication. Repurposing marketed drugs or rescuing compounds which failed in clinical trials offer entrepreneurs the advantage to replenish pipelines with lesser risk and shorter time in drug development (adopted from http://www.ncats.nih.gov/).

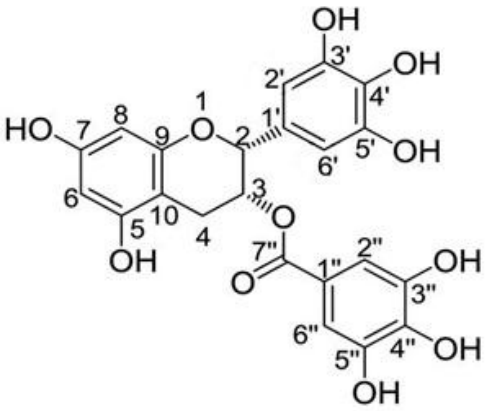

Figure.1. 2D structure of the EGCG
((2R, 3R)-5, 7-Dihydroxy-2-(3, 4, 5-trihydroxyphenyl)-3-chromanyl gallate)

EGCG structure was retrieved from PUBCHEM database (https://pubchem.ncbi.nlm.nih.gov/) and molecule structure was drawn using the chemdraw software[28], EGCG structure was designed in six possible directions (3 - chromanyl gallate to 4th, 6th, 8th, 2, 3, 4- trihydroxyphenyl gallate) as shown in (Figure.1). 3-chromanyl gallate was further minimized by applying the steepest descent followed by the conjugate gradient, later charm force field was applied and performed the molecular dynamics simulations using Discovery Studio 2.1(DS 2.1). This structure has the clinical usage and the reported side effects.[27] that is the reason we chose this topic for our studies than to repositioning the possible group and use the repurposing.

Core 20S Proteasome

Prokaryotic and eukaryotic 20S proteasomes consist of 28 subunits. A prokaryotic proteasome contains 14 copies of identical a-subunits and 14 copies of identical b subunits. A eukaryotic proteasome carries two copies of seven different a-subunits and two copies of seven different b-subunits.[29-30] In addition to the constitutive 20S proteasome, mammals also have an immunoproteasome, the assembly of both within the cell begins after its stimulation by g-interferon. This structure[31] consists of β1, β2, β 3, β 4, β 5, β 6, β7.

The proteasome is a massive multicatalytic protease complex which is responsible for degrading most of the cellular proteins.[32-33] The shape of 20S-core particle of the 26S proteasome is like barrel, and the sites of proteolytic activity reside on the inside. The eukaryotic proteasome has three known activities, which are associated with its β-subunits. These are the chymotrypsin-like (cleavage after hydrophobic residues, β5-subunit), trypsin-like (cleavage after basic residues, β2-subunit), and caspase-like (cleavage after acidic residues, β1-subunit) activities.[34]

The purpose of the present study is to build a mechanistic model to describe how EGCG binds the proteasome before attack and cleavage of its ester bond[35]. In the present study, for the first time, we repositioned the gallate groups of the original compound at different positions in the compound and demonstrated that (_) - EGCG is an irreversible mechanism-based inhibitor of the chymotrypsin- like activity of 20S proteasome. On the basis of this finding, we established a docking model in testing the (_) - EGCG's interaction with the 5-subunits of the 20S proteasome. This model was verified by the application of several other natural and synthetic EGCG analogs, as their docking free energy could be used to predict the actual proteasome-inhibitory activity. Finally, the proteasome inhibition model of (_) - EGCG is further validated by rationally designing and synthesizing two EGCG-amide compounds, followed by comparing their predicted and actual proteasome-inhibitory activities.

Yeast Proteasome structure was retrieved from Protein Data Bank (1RYP.pdb). From that Beta5 subunit was retrieved. This was reduced to minimum using steepest descent and followed by conjugate gradient using Discovery Studio2.1.

Later, the ligands were minimized by the same protocol. It was further docked by the 20s Proteasome receptor with all the six confirmers.

RESULTS

The designed structure of the EGCG as shown in the figure.1 reveals that the native structure of the EGCG is found to be in IUPAC name **((2R, 3R)-5, 7-Dihydroxy-2-(3, 4, 5-trihydroxyphenyl)-3-chromanyl gallate.** Later, while the carbonyl group of 3-chromonyl gallate group was shifted towards the 4th, 6th, 8th position of the phenyl ring and further it was also attached/modeled on 1st and 5th positions of another group (trihydroxy phenyl ring group) as shown in the figure.2. Gallate was observed to be the toxic compound[35] due to which lesser inhibition was observed in the parent compound to cure the diseases and also the bioavailability of the compound was reported to be poor and found to produce the side effects. **Thus repositioning or repurposing of the gallate group is expected to bring positive changes in the compound due to the change in stereochemical and resonance changes in the molecule.** After designed structures we recorded the proton and C13 nuclear magnetic resonances (NMRs) using the Chemdraw software, NMR peak intensity values are shown in table.1.

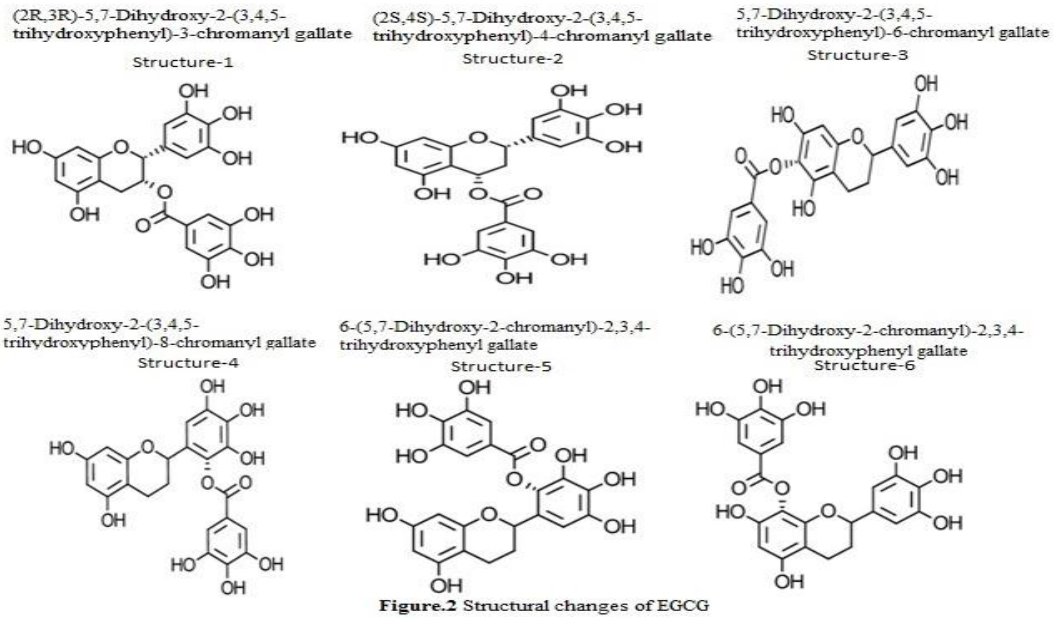

Figure.2 Structural changes of EGCG

Figure.2 structural changes of EGCG

From the NMR studies the native structure was recorded in proton H^1, C^{13} and was also compared with the 4-chromanyl gallate (Structure-2) where the position has changed from 3rd to 4th, which reveals that the ppm values gets changed as shown in the Table.1a and 1b.

These changes are due to the resonance and sterochemical properties of the group when changed from 3rd to 4th position. From our data it is clear that the repositioned 3-chromyl gallate bounded on to the 4th position is more active than the other conformations.

We have changed the 3-chromanyl gallate group from the 3rd position to 4th, 6th, 8th and we found that there were significant changes in the Free energy, LogP, tPSA (topological polar surface area), ClogP and Henry law.

Later, we have verified druggable properties like; LogP, ClogP, AlogP98,Hydrogen bond donors and Acceptors, Bioavailability, Lipinski rule of Five, NC+HET, MR, CMR, Gibbs free energy, Free energy, heat of form, tPSA (topological polar surface area) Wiener Index, Henry's law, BP, MP, Critical Temperature(CT), CP, CV as tabulated in Table.2.

According to the Lipinski rule of five, the molecule has fulfilled the 10 Hydrogen bond acceptors, donors >5, logP<5, MW<500 from these it is observed that it has an absorption, permeability is possible and tPSA is also satisfied with surface contributions of polar fragments.

We could not find any changes in the Lipinski rule of 5, Bioavailability, NC+NHET, CTemp, CPressure, CVolume and Hydrogen bond donors and acceptors. Small fractional variations are noticed from 6th, 8th of 3-chromyl gallate and 1st and 5th positions of another group (trihydroxyphenyl ring group) in MP, Gibbs Energy, CMR, MR and Heat of form were performed.

From the above observations, the end product may act like antagonist for many diseases with reduced side effects.

The yeast 20s proteosome structure was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) database (www.rcsb.org) and visualized with SSViewer as shown in the figure.3.

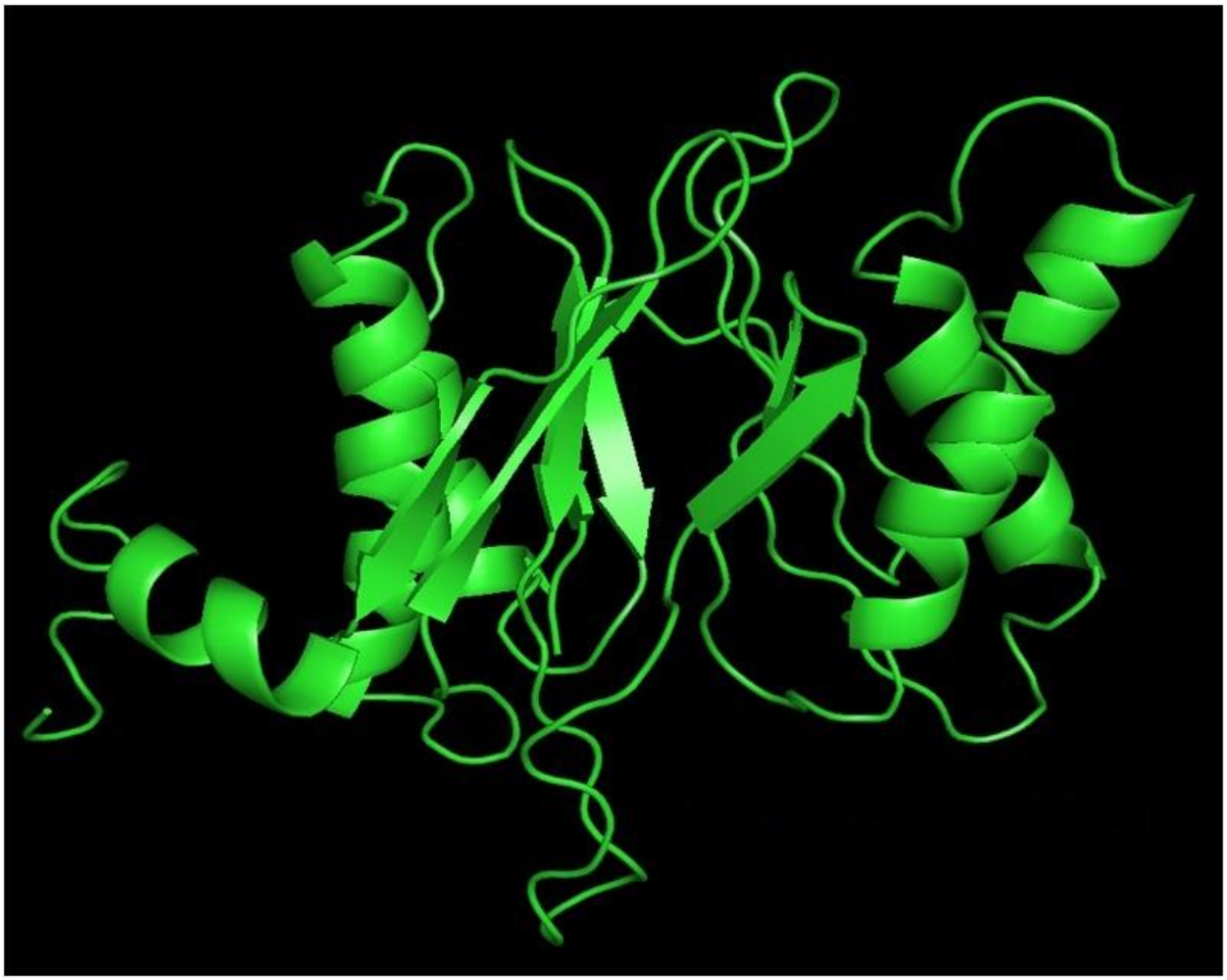

Figure -3. 3D structure of the proteosome β5 receptor structure

The molecular docking studies were carried out using the discovery studio2.1, after completion of the docking studies and the interactions are measured and tabulated in table.3.

All the docked complexes are shown in the figure.4-9. In the docking figures the ligands were shown in the sticks representation and receptor residues(amino acids) were shown in lines and Hydrogen bond interactions are displayed in yellow colored dotted lines.

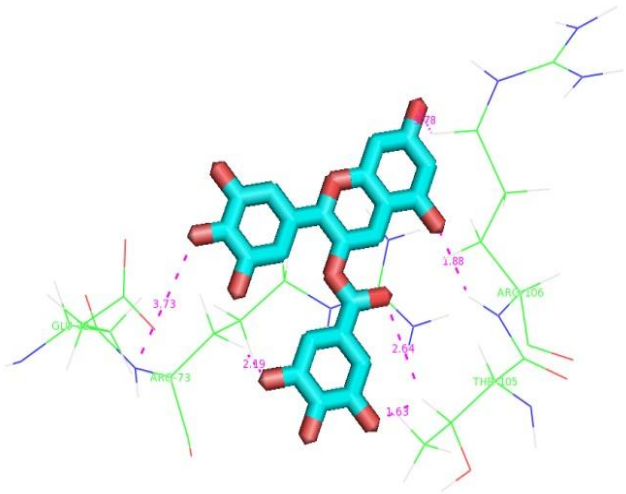

Figure.5 Binding interactions of the native structure of EGCEG with receptor. The ligand EGCG structure was shown in sticks representation and interacting amino acids were shown in lines representation. EGCG binds to the amino acids of ARG106, THR105, and GLU72.

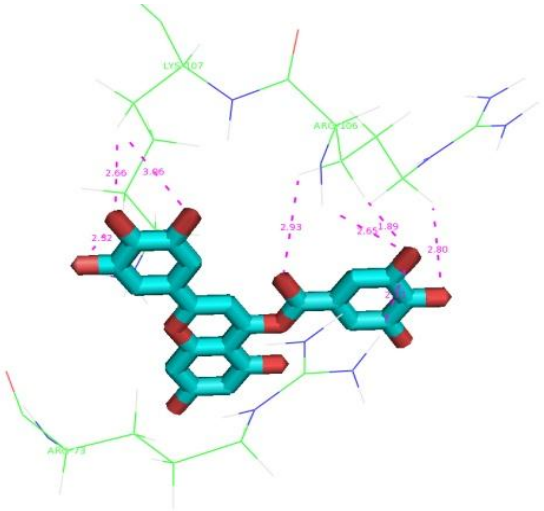

Figure.6. Binding interactions of second conformation of the EGCEG with receptor. ARG73, LYS107, ARG106, GLU182.

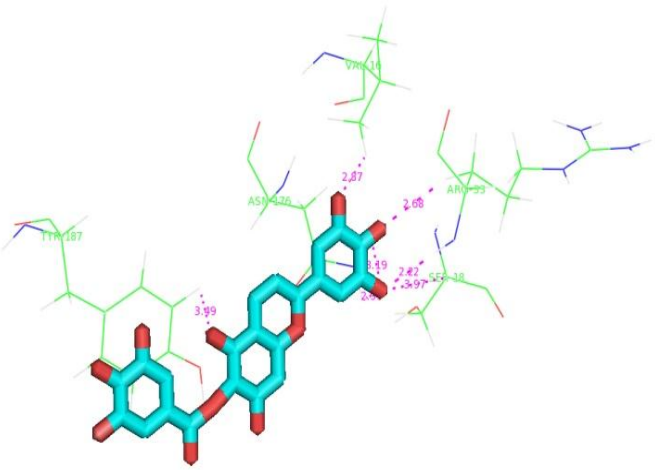

Figure.7. 3rd conformation of the EGCEG with receptor interactions. Interacting partners ARG33, TYR187, ASN176, and VAL162.

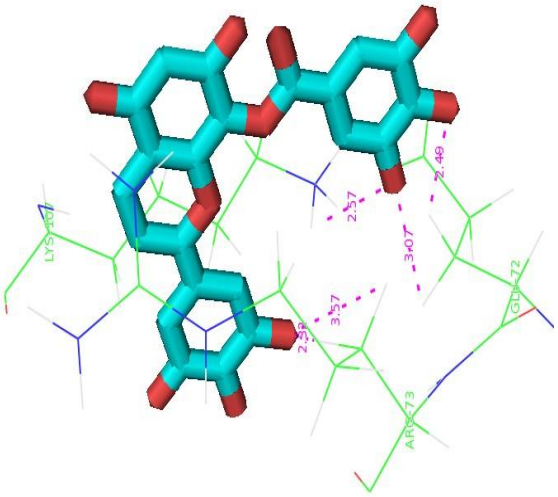

Figure.8. 4th conformation of the EGCEG with receptor interactions. Interacting partners GLU72, LYS107, ARG73,

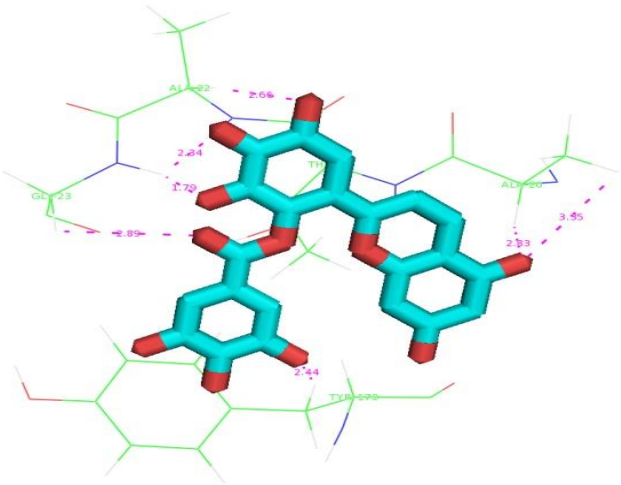

Figure.9. 5th conformation of the EGCEG with receptor interactions. Interacting partners GLY23, ALA20, ALA22, and TYR170.

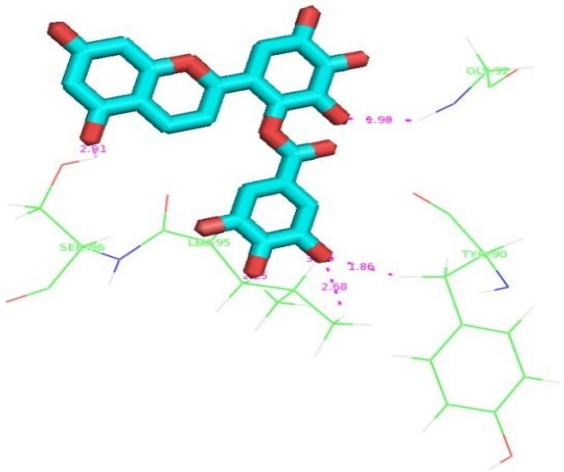

Figure.10. 6th conformation of the EGCEG with receptor interactions. Interacting partners LEU95, GLY92, SER96.

TABLES

Table.1a. Proton (^{1}H) NMR

	Structure-1	Structure-2	Structure-3	Structure-4	Structure-5	Structure-6
2CH	5.56	4.97	4.97	4.97	4.97	4.97
3 CH	5.23	2.88, 2.62	2.46, 2.21	2.46, 2.21	2.46, 2.21	2.46, 2.21
4 CH2	3.10, 2.85	5.24	2.85, 2.75	2.85, 2.75	2.85, 2.75	2.85, 2.75
5 OH (C-OH)	9.68	9.68	9.82	9.68	9.68	9.68
6 CH	5.82	5.80	**N/A**	6.08	5.82	5.82
7 OH (C-OH)	10.29	10.29	9.48	9.48	10.29	10.29
8 CH	5.86	5.84	6.12	**N/A**	5.86	5.86
3G 2CH	6.97	6.95	7.12	7.12	7.12	7.12
3 OH	9.48	9.48	9.48	9.48	9.48	9.48
4 OH	8.73	8.73	8.73	8.73	8.73	8.73
5 OH	9.48	9.48	9.48	9.48	9.48	9.48
6 CH	6.97	6.95	7.12	7.12	7.12	7.12
3PHENYL 2CH	6.49	6.49	6.49	6.49	**N/A**	6.56
3 OH	9.48	9.48	9.48	9.48	8.73	9.48
4 OH	8.73	8.73	8.73	8.73	8.73	8.73
5 OH	9.48	9.48	9.48	9.48	9.48	8.73
6 CH	6.49	6.49	6.49	6.49	6.56	**N/A**

Table.1b. ^{13}C-NMR

	Structure-1	Structure-2	Structure-3	Structure-4	Structure-5	Structure-6
2CH	79.0	74.2	77.5	77.5	72.6	72.6
3 CH	68.6	35.0	25.4	25.4	29.5	29.5
4 CH2	24.8	58.5	25.5	25.5	30.9	30.9
5 C (**C**-OH)	157.2	159.0	153.8	154.0	157.2	157.2
6 CH	95.3	95.3	120.8	94.6	95.3	95.3
7 C (**C**-OH)	157.8	157.8	146.1	146.1	157.8	157.8
8 CH	94.8	94.8	95.2	120.3	94.8	94.8
9 C	157.3	160.1	146.1	156.4	157.3	157.3
10 C	99.4	103.0	103.4	103.4	103.0	103.0
3G C (**C** =OOH)	165.9	165.9	165.2	165.2	165.2	165.2
1C	121.2	121.2	125.6	125.6	125.6	125.6
2CH	109.6	109.6	110.0	110.0	110.0	110.0
3 C	146.1	146.1	146.1	146.1	146.1	146.1
4 C	140.3	140.3	141.2	141.2	141.2	141.2
5 C	146.1	146.1	146.1	146.1	146.1	146.1
6 CH	109.6	109.6	110.0	110.0	110.0	110.0

3PHENYL 1C	130.9	137.9	137.9	137.9	127.6	127.6
2CH	107.8	107.8	107.8	107.8	133.3	106.2
3 C (**C**-OH)	146.3	146.3	146.3	146.3	143.2	143.1
4 C (**C**-OH)	133.3	133.3	133.3	133.3	132.6	132.6
5 C (**C**-OH)	146.3	146.3	146.3	146.3	143.1	143.2
6 CH	107.8	107.8	107.8	107.8	108.2	133.2

Table: 2. Chemical activity of the six molecules.

	Structure – 1	**Structure – 2**	**Structure – 3**	**Structure – 4**	**Structure – 5**	**Structure – 6**
Lipinski's rule of 5 violations count	2	2	2	2	2	2
Bioavailability Score	0.170	0.170	0.170	0.170	0.170	0.170
A log P98	1.909	2.104	2.293	2.292	2.292	2.292
NC + NHET	2.670	2.670	2.670	2.670	2.670	2.670
$\Delta G^0_{f,298}$	-3.5467E2 KJ/mole	-3.5467E2 KJ/mole	-3.6078E2 KJ/mole	-3.6078E2 KJ/mole	-3.6078E2 KJ/mole	-3.6078E2 KJ/mole
Wiener Index	1179	1186	1333	1235	1228	1228
Hydrogen bond donor Count	8	8	8	8	8	8
Hydrogen bond acceptor Count	11	11	11	11	11	11
	Structure – 1	Structure – 2	Structure – 3	Structure – 4	Structure – 5	Structure – 6
Boiling Point	1524.45 [k]	1524.45 [k]	1534.1 [k]	1534.1 [k]	1534.1 [k]	1534.1 [k]
Melting point	1401.82 [k]	1401.82 [k]	1414.58 [k]	1418.58 [k]	1418.58 [k]	1418.58 [k]
Critical Temperature	1217.27 [k]	1217.27 [k]	1221.67 [k]	1221.67 [k]	1221.67 [k]	1221.67 [k]
Critical pressure	92.1 [Bar]	92.1 [Bar]	95.37 [Bar]	95.37 [Bar]	95.37 [Bar]	95.37 [Bar]
Critical Volume	1066.5 [cm^3/mol]	1066.5 [cm^3/mol]	1067.5 [cm^3/mol]	1067.5 [cm^3/mol]	1067.5 [cm^3/mol]	1067.5 [cm^3/mol]
Gibbs energy	-1130.55 [kJ/mol]	-1130.55 [kJ/mol]	-1132.47 [kJ/mol]	-1132.47 [kJ/mol]	-1132.47 [kJ/mol]	-1132.47 [kJ/mol]
Log P	2.07	1.9	2.38	2.38	2.38	2.38
MR	110.79 [cm^3/mol]	110.79 [cm^3/mol]	111.18 [cm^3/mol]	111.18 [cm^3/mol]	111.18 [cm^3/mol]	111.18 [cm^3/mol]
Henry's Law	38.36	38.36	37.65	37.65	37.65	37.65
Heat of Form	-1620.75 [kJ/mol]	-1620.75 [kJ/mol]	-1611.88 [kJ/mol]	-1611.88 [kJ/mol]	-1611.88 [kJ/mol]	-1611.88 [kJ/mol]
tPSA	197.37	197.37	197.37	197.37	197.37	197.37
C log P	1.49069	1.35069	0.9924	1.39056	1.38702	1.38702
CMR	10.9555	10.9555	10.9555	10.9555	10.9555	10.9555

Table: 3 Bonding energies of the 6-Conformations with the 20s proteosome receptor.

Compound	Docking Energy	VDW	HBond
20S_B5-1-0.pdb	-163.714	-137.521	-26.193
20S_B5-2-0.pdb	-143.983	-116.94	-27.0426
20S_B5-3-0.pdb	-146.552	-122.675	-23.877
20S_B5-4-0.pdb	-144.524	-122.445	-22.079
20S_B5-5-0.pdb	-161.357	-124.511	-36.846
20S_B5-6-0.pdb	-138.585	-115.41	-23.1754

Table: 4 interacting amino acids with the ligand atoms and their H-bond distances.

Residues in Receptor Structure	**Atoms in Ligand**	**H-Bond Distance in A^o**
Conformation -1		
ARG 106 NH	24O	1.88
THR 105 HB	71O	1.63

THR 105 HB	31O	2.64
ARG 73 HB2	O	2.19
GLU 72 HB1	O	3.73
Conformation -2		
ARG 73 1H1	O	3.76
LYS 107 HZ3	O	2.99
ARG 106 HB1	O	2.8
GLU 182 HG1	O	2.42
LYS 107 HD1	O	2.32
ARG 106 HB1	2 O	1.89
LYS 107 HB1	O	2.66
ARG 73 2HH1	2 O	2.71
ARG 106 HN	2 O	2.65
LYS 107 HB1	3 O	3.06
ARG 106 HB2	2 O	2.93
Conformation -3		
ARG 33 HN	3 O	2.22
ARG 33 HB1	3 O	2.68
TYR 187 HE1	1 O	3.49
ASN 176 1HD2	3 O	3.19
ASN 176 2HD2	3 O	2.99
SER18AH	3 O	3.97
VAL 16 2HG2	3 O	2.87
Conformation -4		
GLU 72 HB2	3 O	2.49
GLU 72 HB1	2 O	3.07
LYS 107 HZ2	2 O	2.57
ARG 73 HB1	2 O	3.57
LYS 107 HZ3	3 O	2.32
Conformation -5		
GLY 23 HN	3 O	1.79
GLY 23 HN	3 O	2.89
GLY 23 HA1	3 O	2.34
ALA 20 HA	1 O	3.35
ALA 20 HB3	1 O	2.83
ALA 22 HA	3 O	2.66
TYR 170	2 O	3.14
TYR 170 HB1	2 O	2.14
Conformation -6		
LEU 95 HG	3 O	3.63
LEU95 3HD1	3 O	2.68
LEU 95 HD1	3 O	2.25
TYR 90 HB1	3 O	1.86
GLY 92 HN	2 O	1.98
SER 96 HZ2	1 O	2.91

CONCLUSION

From our observations, it is evident that the ligand EGCG ((2R, 3R)-5, 7-Dihydroxy-2-(3, 4, 5-trihydroxyphenyl)-3-chromanyl gallate has shown significant admet properties. Subsequently, when we altered the positions from the carbonyl group of 3-chromonyl gallate group got shifted towards the 4th, 6th, 8th position of the phenyl ring from position-3rd and further it was also attached / modeled on 1st and 5th positions of another group. This has shown a significant contribution to the logP, Lipanski rule of five, H-bond donors and acceptors. From our docking studies it also indicated the variations.

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