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Targeting NRF2 for Relieving Oxidative Stress: In-Silico Analysis of Therapeutic Potential of Some Curcumin Conjugates in Parkinson's Disease

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Abstract

Oxidative stress and depleted levels of cellular antioxidant glutathione (GSH) is a crucial implication during early neurodegeneration in Parkisnson's disease. Nuclear factor-erythroid 2 (NF-E2) related factor 2 (NRF2) and its negative regulator Keap1 have been widely reported as key regulators in antioxidant defense machinery by inducing GSH synthesis. Various experimental studies have suggested curcumin, the turmeric's dietary polyphenol as an excellent anti-oxidant and stress preventing molecule, demonstrating its neuroprotecting activity. We have analyzed the potential of curcumin as a neuroprotective agent in pathophysiology of Parkinson by targeting NRF2 regulatory machinery and using molecular modeling and docking simulation techniques. We have also compared the efficacy and efficiency of eight curcumin conjugates, out of which three have been recently reported to show better performance than curcumin in neuroprotection. Current study highlights the mechanism of action of curcumin and its conjugates in combating oxidative load thus paving way for designing better neuroprotective agents.

Keywords: Curcumin conjugates, Keap1, NRF2, Oxidative stress, Parkinson's disease.

Introduction

Parkinson's disease (PD), characterized by substantial loss of dopaminergic neurons

(DN) mainly in substantia nigra (SN), is a progressive neurodegenerative disorder which impairs mainly motor skills and speech functions of the patients [1]. Direct role of oxidative stress associated with mitochondrial dysfunction in cell death during the Parkinson's disease is variously suggested [1-4]. During early stages of PD, significantly lower concentration of glutathione (GSH) was observed [5] and its depletion has been reported as earliest known oxidative stress indicator and a crucial triggering factor for oxidative load [6-7]. Reports also depict dynamic relationship between metabolism of GSH and dysfunction of mitochondria [8]. Level of GSH is regulated by the rate-limiting enzyme γ-glutamyl cysteine ligase (γ-GCL) and GSH synthetase (GS) [9]. Synthesis of γ-GCL, a phase-2 enzyme is regulated by a dynamic balance between transcription factor, nuclear factor-erythroid 2 (NF-E2) related factor 2 (NRF2) and its negative regulator Keap1. Recent reports also emphasize the importance of NRF2 and its positive role in combating the oxidative stress in PD [10-12]. In normal conditions NRF2 remains in cytosol bounded with Keap1 which promotes its ubiquitination. Keap1 (624 residues) regulates NRF2 functioning via two important domains, viz. Intervening region (IVR, 180-314) and Kelch domain (315-598). IVR is richer in Cys (cysteine) residues and functions to sense the oxidative stress by conformationally changing itself which prevents NRF2 ubiquitination. Kelch domain (315-598) directly binds to NRF2 preventing its movement to nucleus. Under oxidative stress conditions conformational changes in Keap1 result in its permanent binding to NRF2 thus blocking itself which forbids their NRF2 binding and degradation of newly synthesized NRF2. This event results in higher free concentration of NRF2 in cytotsol capable of moving to nucleus, and promoting the transcription of detoxifying phase-II enzymes like hemeoxygenase-1 and GSH synthetase which help in defending stress conditions [13]. This natural mechanism fails to sustain in high oxidative stress load, hence requires some therapeutic supplement for up regulating the antioxidant defense.

Curcumin (diferuloylmethane); is an orange–yellow, crystalline, water–insoluble polyphenol; most-active and non-toxic component of turmeric and has been extensively reported for its excellent anti-oxidant properties [14-15]. Its therapeutic potentials as an anticancer agent and in neurological disorders, showing ability to cross blood brain barrier (BBB) are widely reported [14-16]. Limitation reported for curcumin is low bioavailability due to its rapid metabolism, poor absorption, and quick systemic elimination [17]. However, conjugation of curcumin with different ligands at its phenolic functions has been earlier shown by our research group to improve its therapeutic properties probably due to delayed metabolism resulting in better bioavailability supplemented with enhanced activity on different molecular targets [18-20]. High neuroprotective potentials of curcumin and its three conjugates, viz. diesters of demethylenated piperic acid, valine and glutamic acid, tested on N27 cell-line as a model for GSH depletion associated with PD are recently reported by us and inferred as the result of their target selectivity and high cellular concentration [21]. Better therapeutic potency of curcumin conjugates may be attributed to three reasons; first: due to delayed metabolism as free phenolic groups are not available for sulphonation or glucoronidation; second: due to facilitated cellular transport via natural transporters of conjugated amino acids and third: due to their enhanced

hydrophobicity facilitating their adsorption and dispersion [22]. For investigating these possibilities, interactions of curcumin and its eight heuristically selected conjugates (one reported by Kumar et al [18]., three by Harish et al. [21] and four newly synthesized [unpublished work]) with two important domains of Keap1 were investigated. Out of these eight conjugates, three are reported in literature for their neuroprotective potentials and better systemic delivery, viz., diesters of demethylenated piperic acid, valine, glutamic acid [21], one as antioxidant and antiinflammatory agent i.e. di-O-glycinoyl [18]. While, four conjugates are newly designed and synthesized namely di-O-serinoyl curcumin, di-O-succinoyl curcumin, di-O-cysteinoyl curcumin, di-O-1-carbathiol-1,2,4 triazolyl curcumin (unpublished work). A comparative in-silico profiling of these conjugates is done in this work with the aim of designing better conjugates in terms of bioavailability and efficacy. This study will help in contributing to insight into the mechanism of defense offered by curcumin and its conjugates in neurodegenerative diseases and their comparative efficacy and affinity.

Material and Methods

Human Keap1 (624 residues) is a multidomain protein having five functional domains which regulates the NRF2 concentration and activity by using its IVR domain (180-314) for oxygen sensing and Kelch domain (315-598) for binding NRF2 [31]. The binding affinity of curcumin and its conjugates was analyzed on its both domains. For this purpose first, molecular structure of receptor was prepared followed by docking simulations with curcumin and its aforementioned eight conjugates selected for the study. Software and tools used in the study are Blast server (http://blast.ncbi.nlm.nih.gov/Blast.cgi), MODELLER9v8 [24-26], Chemsketch [27] and Schrödinger suit 2009 [28].

Receptor Structure Preparation and Modeling

Initial coordinates for Kelch domain of Keap1 predicted by X-ray crystallography (PDBId: 2DYH; Resolution: 1.9Å) were retrieved from Protein Data Bank (http://www.rcsb.org/pdb/) and were refined using protein preparation wizard (shipped by Schrödinger). Refinement involved proper bond order was assignment, Hydrogen addition, addition of missing disulfide bonds, proper hydrogen bond assignment, rectification of tautomers and protonation states, water removal and loop filling in available PDB structure. Finally protein was minimized using OPLS2005 force field to resolve steric clashes and poor resolution upto a RMSD of 0.3 Å for non hydrogen atoms.

While, unknown crystal structure of IVR domain of Keap1 was first modeled using MODELLER program [24-26] and then optimized using OPLS_2005 all-atom force field [29-30] available with Prime 2.1 module in Schrödinger suit 2009 [28]. Template sequence for Keap1 was obtained from UniProtKB (http://www.uniprot.org/) having accession number: Q14145. IVR domain along with BTB and NTR domain spanning 1-318 amino acid length was modeled using Modeller9v8 using multiple templates and automodel class. Templates were selected

by submitting protein sequence for 1-318 residues to pBLAST server and running PSI-Blast against Protein Data Bank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Two templates, having PDBId: 3HVEA and 3I3NA, with sequence coverage of 71% and 72% of target and e-value of 7e-20 and 2e-18 respectively were selected for modeling the desired domains. Best model generated by Modelle was refined using OPLS_2005 all-atom force field [29-30] using Prime 2.1 module (shipped by Schrödinger). Finally, validity of the refined model was checked using Procheck [32], ERRAT [33] and Verify_3D [34] available at UCLA Stuctural Analysis and Verification Server (SAVS) (http://nihserver.mbi.ucla.edu/SAVES/).

Ligand Preparation

All nine ligands, viz., curcumin, didemethylenatedpiperioyl curcumin, di-O-valinoyl curcumin, di-O-glutamoyl curcumin, di-O-serinoyl curcumin, di-O-succinoyl curcumin, di-O-cysteinoyl curcumin, di-O-glycinoyl curcumin and di-O-1-carbathiol-1,2,4 triazolyl curcumin, were drawn using ACDLabs Chemktech 12.0 [27] and were saved in mol format as depicted in figure 1 and 2. All ligands were prepared using Ligprep 2.3 (shipped by Schrödinger), in which 2D structures were converted to 3D and optimized to attain proper geometry, correct sterioisomeric groups, proper ionization and tautomeric states with minimized conformations to enhance the accuracy and efficiency of docking simulation. All optimized conformational states for the ligands, thus generated were used for docking simulations to selected receptors.

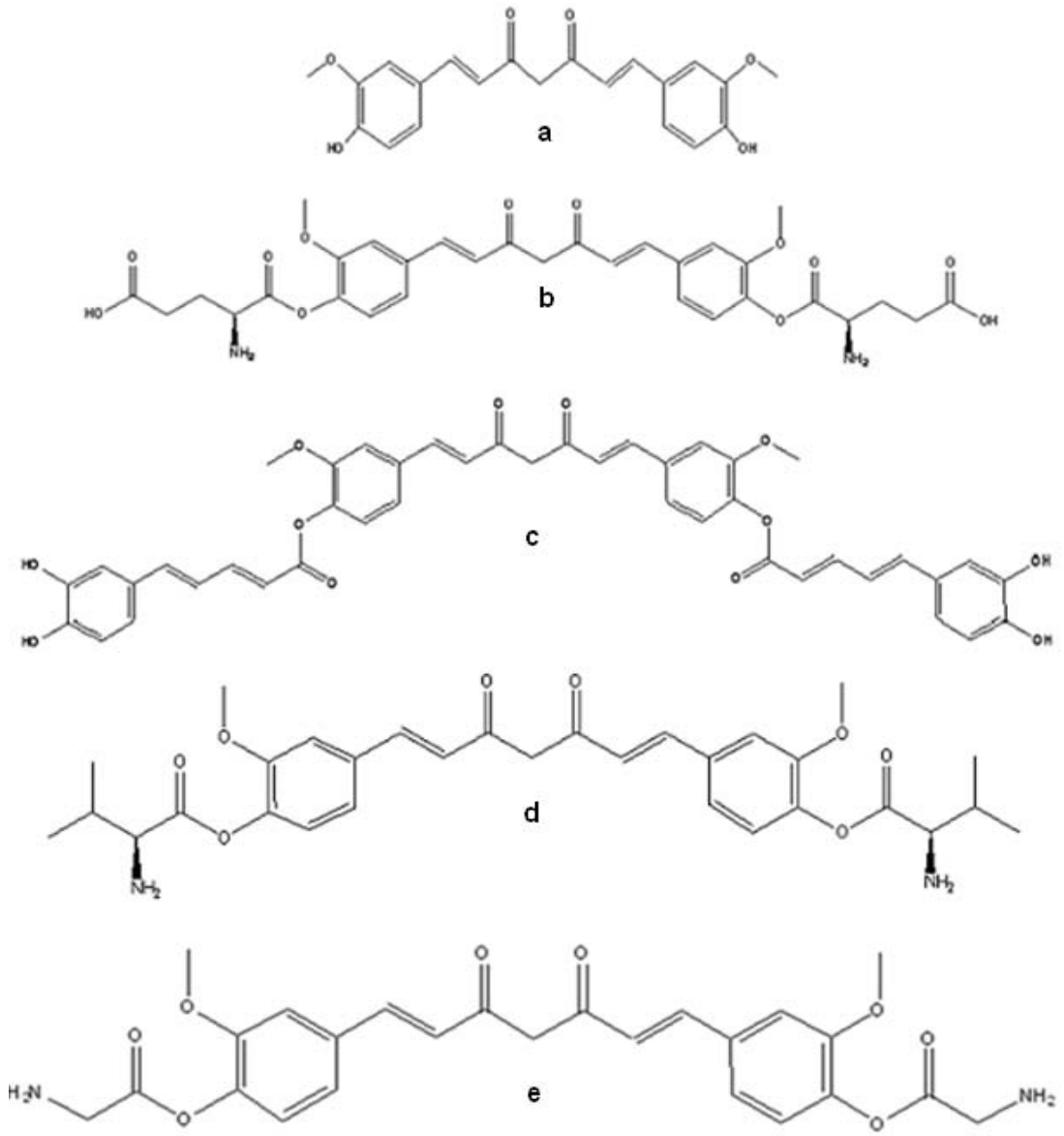

Figure 1: Chemical structures of curcumin (a) and its conjugates; tested by Harish et al. 2010 on N27 dopaminergic neuronal cell lines for their GSH enhancing and neuroprotective effects, viz., di-O-glutamoyl curcumin(b), didemethylenatedpiperioyl curcumin (c) and di-O-valinoyl curcumin (d) respectively and another conjugates prepared and tested by S. Mishra et al. 2005 [19] for its apoptotic potential on tumor cells viz., di-O-glycinoyl curcumin (e) drawn with Chemsketch [27].

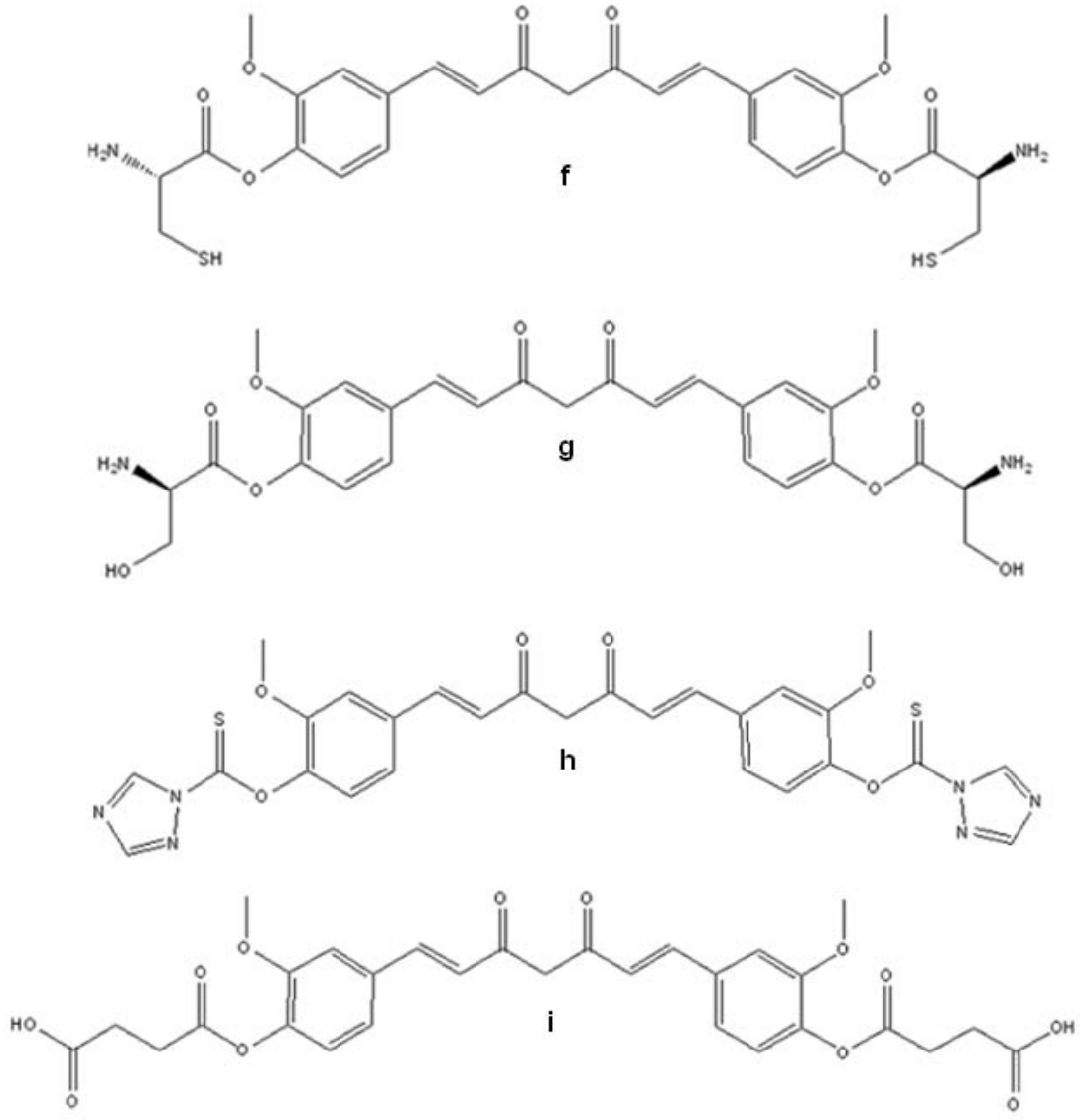

Figure 2: Chemical structures of newly synthesized conjugates of curcumin (unpublished work) viz., di-O-cysteinoyl curcumin (f), di-O-serinoyl curcumin (g), di-O-1-carbathiol-1,2,4 triazolyl curcumin (h) and di-O-succinoyl curcumin (i) drawn with Chemsketch [27].

Docking Simulations

Docking simulations were performed using Glide program (Grid-based Ligand Docking with Energetics) [35] of Schrödinger suit 2009 which uses impact-v55211 for its calculation. Glide performs the exhaustive search employing hierarchical filter for finding most favorable interaction between one or more ligand molecules and a receptor (protein or protein with cofactor). Receptor-grid files were generated after preparing correct forms of proteins and ligands using Receptor-grid generation program (shipped by Schrödinger). For grid generation potential of non-polar parts of

receptor was softened by scaling van der Waals radii of ligand atoms by 1.00 Å with partial charge cutoff of 0.25.

For Keap1(1-318) fragment spanning IVR and BTB domains, grid box of size 20 x 20 x 20 Å with coordinates X:-17.5794, Y: -4.0912, Z: -77.9218 was generated at the centroid of conserved residues reported for oxygen stress sensing: Cys257, Cys273, Cys288 [37-38].

For Keap1(2DYH) spanning Kelch domains bound with NRF2, grid box of size 20 x 20 x 20 Å was generated around the centroid of naturally bound ligand NRF2 fragment at its binding site (treated as active site) with coordinates X: -1.7793, Y: -46.608, Z: 11.0513.

'Xtra precision' Glide algorithm recommended by Schrödinger for obtaining high precision was used for docking using prepared ligands at the active sites of receptor. During docking, potential of non-polar parts of ligands was softened by scaling van der Waals radii of ligand atoms by 0.8 Å with partial charge cutoff of 0.15. During docking, Glide first places the center of ligand at various grid positions of a 1 Å grid, then by rotating ligand in all Euler angles it generates various possible conformations which pass through a filter series composed of initial rough positioning followed by scoring phase. This step, augmented with geometrical filters and crude scoring dramatically narrows the search space. Then non bonded OPLS-AA potential grid is used for optimization of torsionally flexible energy of docking solutions survivals. Next surviving, few very best docking poses are further refined by Monte Carlo pose sampling. Finally, a model energy function named Glide score (Gscore) is used which combines force field and empirical terms for selecting best docking pose which are generated as output (Halgren et al., 2004). Gscore is defined as:

$$GScore = a * vdW + b * Coul + Lipo + Hbond + Metal + BuryP + RotB + Site$$

Where vdW is van der Wall energy, Coul is Coulomb energy; Lipo is lipophilic contact term, HBond represents hydrogen-bonding term, Metal is metal-binding term, BuryP is penalty for buried polar groups, RotB represents penalty for freezing rotatable bonds, Site is polar interactions at the active site and a, b representing coefficients of vdW and Coul are set as: a = 0.065, b = 0.130.

In docking run, 5000 poses per ligand were kept for initial phase of docking; scoring window for keeping initial poses was set to 100.0. Best 800 poses per ligand were kept for energy minimization and expanded sampling was used. For energy minimization, distance-dependent dielectric constant was kept to be 2.0 and maximum number of minimization steps was set to be 100. The final energy evaluation is done with GlideScore and top 5 poses for each ligand were generated as the output.

Results generated by docking simulations were analyzed using Glide XP visualizer, which enables to see ligand- receptor interactions in an interactive manner. Important residues participating in forming hydrogen bond with receptor proteins were analyzed and are reported in the results.

Results

For studying the interactions and binding affinities of selected ligands to the specified target protein, accurate model is utmost requirement. In our study, high accuracy of

modeled target proteins was obtained which assures the authenticity of further docking simulation analysis. Also use of OPLS-AA force field and Monte Carlo simulations for docking analysis increases the reliability of the binding poses and depicted interactions. Detailed description of results obtained via modeling and docking simulations is discussed in following sections.

Molecular Modeling

Accuracy of Modeled proteins was verified using PROCHECK, Errat and Verify_3D scores. Ramchandran plot showed the matched stereochemical spatial arrangement for the amino acid residues of modeled proteins.

The torsion angles of the 3-D structure of modeled fragment of Keap1 showed 90.8 % of amino acid residues in the favored region, 8.8% fall in additional allowed regions and 0.4% generously allowed regions whereas 0.0% of amino acid residues were in the disallowed region. Verify_3D [34] results showed 92.58% of the residues had an averaged 3D-1D score greater than 0.2, showing the high compatibility of an atomic model (3D) with its own amino acid sequence (1D). Further ERRAT [33] score of 65.53 shows the reliability of the modeled structure.

Docking Simulations

The study was aimed at exploring the interactions and binding efficiency of curcumin and its conjugates to the major regulatory proteins involved in combating the oxidative load. Results of docking simulations are summarized as XP Gscore (Glide Score for Xtra precision) and Hbond Energy along with residues involved in Hydrogen bond interactions, for all three identified receptor proteins, viz. IVR domain of Keap1 and Kelch domain of in table 1 and 2 respectively.

Table 1: Xtra Precision Glide Score (XP Gscore), hydrogen bond energy(kcal/mol) and residues of IVR region of Keap1 protein participating in hydrogen bonding with curcumin and its eight conjugates, used as ligands in docking simulations analysis.

S. No.	Ligands	XP GScore	Hydrogen bond Energy (kcal/mol)	Residues of receptor participating in Hydrogen bonding with ligand
1	Di-O-cysteinoyl curcumin	-7.29284	-2.886228	Arg261,Arg269,Val271,Cys273, Gln286, Gln292,Lys303
2	Di-O-glutamoylcurcumin	-5.76064	-2.038206	Asn251,Lys254,Cys257,Arg261, Asn279, Cys288
3	Di-O-serinoyl curcumin	-5.64921	-3.633509	Arg261,Phe262,Gln284,Asp294
4	Di-O-succinylcurcumin	-5.0614	-2.690624	Lys254, Arg261,Glu289
5	Di-O-valinoylcurcumin	-4.81476	-1.056635	Arg261,Gln284,Asp294
6	Di-O-glycinoyl curcumin	-4.78575	-1.975765	Arg261,Gln284,Glu289
7	Curcumin	-3.93136	-0.839318	Asn251,His247,Glu289
8	Di-O-1-carbathiol-1,2,4 triazolyl curcumin	-3.29147	-1.027438	Ser243,Lys254
9	Didemethylenatedpiperoyl curcumin	-1.1488	-1.079463	Glu286,Glu289,Glu306

Table 2: Xtra Precision Glide Score (XP Gscore), hydrogen bond energy (kcal/mol) and residues of Kelch domain of Keap1 protein participating in hydrogen bonding with curcumin and its eight conjugates, used as ligands in docking simulations analysis.

S. No.	Ligands	XP GScore	Hydrogen bond Energy (kcal/mol)	Residues of receptor participating in Hydrogen bonding with ligand
1	Di-O-glutamoyl curcumin	-9.58131	-3.28192	Arg415, Ser508, Tyr525, Gln528, Asp529, Ser555, Ser602
2	Di-O-succinyl curcumin	-9.08343	-3.75283	Ser363, Asn382, Asn414, Arg415, Arg483, Ser508, Ser602
3	Di-O-serinoyl curcumin	-9.07143	-3.57834	Arg380, Asp389, Asn414, Arg415, Ser431, Ser508, Tyr572,Gly574
4	Curcumin	-7.82897	-4.35989	Ser363, Asn382, Asn414, Arg415, Arg483, Ser508, Ser602
5	Di-O-cysteinoyl curcumin	-7.59062	-2.71727	Ser363, Gln530, Gln528, Asp529, Gln530, Ser602
6	Di-O-valinoyl curcumin	-6.61715	-2.32215	Arg415, Ser508, Ser555, Tyr572
7	Di-O-glycinoyl curcumin	-6.50609	-1.89032	Arg415, Ser508, Tyr525, Asp529, Gln530
8	Didemethylenatedpiperoylcurcumin	-5.31996	-2.51117	Asn414, Arg415, Ala510, Ser555, Tyr572
9	Di-O-1-carbathiol-1,2,4 triazolyl curcumin	-4.74153	-1.27495	Ser363, Asn382, Asn414, Arg415, Ser508, Tyr525, Gln530, Ser555, Tyr572, Ser602

Glide docking results at Keap1 IVR region

In IVR region, which is reported to be involved in oxygen load sensing, di-O-cysteinoyl, di-O-glutamoyl, di-O-serinyl and di-O-succinoyl conjugates of curcumin are showing good binding with Gscore of -7.29284, -5.76064, -5.64921, -5.0614 respectively. Di-O-cysteinoyl curcumin residue having best binding in IVR region is showing interactions with Arg261, Arg269, Val271, Cys273, Gln286, Gln292, Lys303 residues and di-O-glutamoyl curcumin, the next scorer shows interactions with Asn251, Lys254, Cys257, Arg261, Asn279, Cys288 residues. These conjugates are showing interaction at conserved cysteine residues i.e. Cys257, Cys273 and Cys288 which are reported to be directly involved in Keap1 functioning to repress NRF2 [38]. These cysteine residues are reported to form covalent bonds with electrophilic groups in vitro with ligands resulting in repression of NRF2 [37]. Results of docking simulation of curcumin and all eight conjugates analyzed in the study are summarized in table1. In addition to this, binding positions of di-O-cysteinoyl and di-O-glutamoyl curcumin showing binding at the adjacent position, but placed opposite to each other in the hinge of IVR region are shown in figure 3.

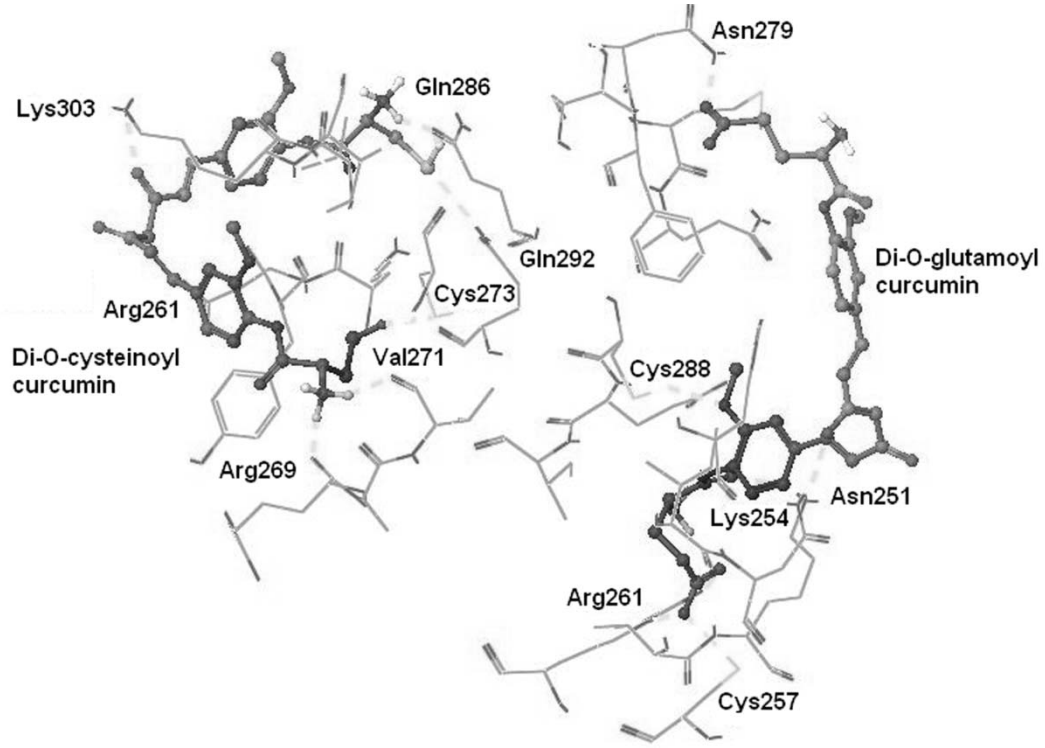

Figure 3: Docked conformation and hydrogen bond interactions of di-O-glutamoyl curcumin and di-O-cysteinoyl curcumin drawn in green backbone with residues constituting IVR domain of Keap1, drawn in gray backbone. Oxygen atoms are depicted by red, nitrogen atoms by blue and sulfur atoms by yellow colour.

Glide docking results at Keap1 Kelch domain

Kelch domain is the binding site of Neh2 domain of NRF2 [39] and is directly involved in inhibition of NRF2 function and facilitates its ubiquitization. Blocking of Kelch domain by any ligand can help to escape newly translated NRF2 molecules directly to the nucleus. Also, high affinity of ligands to Kelch domain may also help in blocking the newly synthesized Keap1 molecules which will further enhance NRF2 availability for nuclear movement. Docking simulation results obtained from Glide show that di-O-glutamoyl, di-O-succinyl , di-O-serinoyl and di-O-cysteinoyl conjugates of curcumin along with curcumin itself are showing good binding affinities for Kelch domain as depicted by Gscore of -9.58131, -9.08343, -9.07143, -7.82897, -7.59062 respectively.

Discussion

Keap1 is reported as cytoplasmic effector of NRF2 and the two together viz. NRF2 and Keap1 are postulated as cardinal cellular sensors for oxidative stress [39]. The role of oxidative stress is well established with initiation and cascade of dopaminergic neuronal degeneration involved in Parkinson's disease (PD) [40]. L-Dopa which is

considered to be a standard remedy for PD has various side effects and limited potential [41]. Curcumin and its conjugates which are reported recurrently for their safer therapeutic potential as antioxidants are analyzed in this study for their neuroprotective potentials and the results show conformity with earlier wet experimentations [21]. The better therapeutic potency of curcumin conjugates vis-à-vis curcumin itself has been attributed to their delayed metabolism, easy transport through cellular membrane due to their recognition by the ligand transporters and their enhanced hydrophobicity [22]. The results obtained in the present study i.e better binding potential of conjugates as compared to curcumin at the target site corroborated the earlier hypothesis. In addition, this is also an indication that probably esterases do not affect the molecules before they reach the targets. Such analysis highlights the actual mechanism played by curcumin for neuroprotection and thus lays foundation for further improvement of its therapeutic candidature, using it as a lead compound. This study considers two important aspects for analysis, first is modification of IVR region which constitute the hinge of Keap1 and plays crucial role in stress signaling; second is blocking of Kelch domain which is the binding site for NRF2. High accuracy obtained in modeled targets for this analysis ensures the better reliability of results and interpretation obtained via docking simulations.

Intervening region (IVR) of Keap1 is reported to play important role in constitutive repression of NRF2 and in this process cysteine residues which are abundant in IVR play cardinal role [37]. In this regard, most important are Cys273 and Cys288 which are conserved and play key role in stress sensing [42]. Modification of these residues by some ligand may help to enhance NRF2 availability which will help to increase phase2 enzymes expression which in turn, will help to alleviate oxidative stress, the root cause of PD via GSH synthesis [43]. Curcumin and it conjugates show good binding affinity as summarized in table 1 at this region and thus show their modification capabilities. Among these conjugates results shown by di-O-cysteinoyl and di-O-glutamoyl conjugates are of higher value, as depicted by Gscore of -7.29284 and -5.76064 respectively. The two molecules face each other in the bound conformation with IVR region of Keap1 as shown in figure 3. Cys288 shows binding with methoxy group of the glutamoyl conjugate, indicating the importance of the presence of methoxy groups ortho to phenolic moieties in curcumin. Interaction of this molecule via the carboxy fuctional group of glutamoyl chain is also important. However, these two bindings would contribute more to the hydrophobicity of the complex rather than actual hydrogen binding since sulfur atoms are involved. In case of di-O-cysteinoyl moiety the interactions via thiol groups (-SH) appear to be more important since it interacts with conserved residue Cys273 probably via a disulfide linkage, which can change the conformation of the complex, making feasible the docking of di-O-glutamoyl in the adjacent position. The interaction with Val271 and Arg269 also may be adding to the total conformational changes, thus accommodating the di-O-glutamoyl residue in the adjacent location. It can be concluded from these results that the presence of both moieties di-O-cysteinoyl and Di-O-glutamoyl may initiate signaling finally resulting in the inhibition of the blocking of NRF2 at Keap1 site. As a result it can be suggested that synergistic effect of the two conjugates can activate the desired therapeutic effect.

NRF2 binds Keap1 at Kelch domain which has function of holding NRF2 for proteosomal degradation and represses NRF2 in a dose-dependent manner [44]. In addition to modification of IVR, blockade of this domain via therapeutic ligands will help in preventing newly synthesized Keap1 molecule to bind the cytoplasmic NRF2. Curcumin and its conjugates also show good binding capacity at Kelch domain and interact with similar residues with which natural binder NRF2 interacts, like Asn382, Arg415, Arg483, Ser508, Ser555, Ser602 [45]. As indicated in table 2, the order of binding energies at the NRF2 binding site at Keap1 Kelch domain is di-O-glutamoyl > di-O-succinyl > di-O-serinyl > curcumin > di-O-cysteinoyl > di-O-valinoyl > di-O-glycinoyl> didemethelenated piperoyl curcumin > di-O-1-carbathiol-1,2,4-triazolyl curcumin. Among these conjugates di-O-glutamoylcurcumin, di-O-succinylcurcumin, di-O-serinoyl curcumin, curcumin, di-O-cysteinoyl curcumin have appreciably higher binding capacities respectively and thus can be suggested as good therapeutic agents for blocking Keap1.

These results also suggest that di-O-glutamoyl and di-O-succinoyl conjugates of curcumin can easily block the NRF2 binding site of Kelch domain of Keap1 and even before a new Keap1 is ready for NRF2 binding, these molecules probably bind the site and thus inhibit further process. In addition, modification in IVR domain of Keap1 prevents ubiquitinization of NRF2 as well as its release from a functional Keap1 preventing it to accept new NRF2 molecule which can escape to nucleus [46]. Thus acting bi-way, these ligands can help in building up concentration of newly synthesized NRF2 resulting in increased GSH synthesis which help in combating oxidative stress in dopaminergic neurons and course of disease.

Conclusion

According to our findings, therapeutic activity of curcumin which is so far well known for having therapeutic potentials for Parkinson's disease can be enhanced with conjugation especially with glutamic acid and cysteine. In one of our previous publication [21] we have reported that glutamoyl conjugate enhances the concentration of cellular GSH and thus controls the oxidative degradation in dopaminergic neurons. It can be concluded that the results obtained in present work are in conformity with the wet experiment results with di-O-glutamoyl curcumin as up regulator of glutathione. However, as inferred from current results it can be suggested that a dose dependent combination of the diglutamoyl and dicysteinoyl derivatives can work as a potential activator for glutathione formation. Additionally, di-O-succinyl and di-O-serinyl conjugates of curcumin are also showing significant potency which may be due to their optimal hydrophobicity and also better transportation. However these results have to be verified by the wet experiments.

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