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in silico Anti-Cholinestarase Activity of Flavonoids: A Computational Approach

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In the present study, a computational approach has been designed to evaluate the potential anti-cholinesterase activity of derivatives of flavonoids. Molecular docking studies is performed for the 9 flavonoids against the human acetylcholine (ACh) enzyme to evaluate their binding affinity for having anti-alzheimer activity. All the 9 flavonoid compounds exhibited strong binding affinity that promises potent inhibition of human acetylcholine enzyme. Potential binding affinity of all the flavonoids against human acetylcholine enzyme confirms their possible mechanism of action by using AutoDock based molecular docking simulation technique. Thus, these flavonoid compounds could be presumed to be potential anti-cholinesterase drugs.

Keywords: Flavonoids, Alzheimer, Anticholinesterase.

INTRODUCTION

Alzheimer's disease is a most common form of dementia and considered as a multifaceted neurodegenerative disorder that is approximately prevalent in 13.8 millions around the world. Further, it has been reported that Alzheimer's disease is highly prevalent in elderly population with of 65 or more years of age. It is observed that at least one new case of Alzheimer's disease is reported in every 33 seconds and almost a million new cases in every year in the elderly population across the world [1-3]. The phenotypic characteristic of Alzheimer's disease is progressive loss of memory which makes a burden of a patient to both family and society. In the pharmacotherapy of Alzheimer's disease the cholinesterase inhibitors are the standard FDA approved drugs. These compounds only potentiate the activity of acetylcholine (ACh) at the neuronal synapse because decline activity of synaptic ACh is partly responsible for pathogenesis of cognitive dysfunction in Alzheimer's disease. Till date, several anti-cholinesterase drugs such as tarcine, donepezil, rivastigmine, galantamine and many more are used in the management of Alzheimer's disease. However, the use of these drugs is restricted due to their several serious side effects [4]. Therefore, new treatment options should be adopted which can be a multi target-directed ligand.

Recently, polyphenolic compounds are considered as potential alternatives in the management of Alzheimer's disease. These drugs gain critical attention because of their promising therapeutic and minimum side effects [5]. It has been well documented that diets such as fruit and vegetables rich in polyphenols are protective against cardiovascular diseases and cytoprotective [6,7]. These protective effects have been attributed to their antioxidant properties.

Plant's flavonoids, phenolic compounds and their derivatives have been explored to have multiple biological and pharmacological activities including free redical scavenging, vasodilatory, immunomodulating, anti-inflammatory, antiviral, anti-allergic, antiviral, estrogenic effects and many more [8-10]. These compounds also act as inhibitors of phospholipase A2, cyclooxygenase and lipoxygenase [11], glutathione reductase [12] and xanthine oxidase [13]. The antioxidant activity of these compounds is reported through scavenging the superoxide radicals [14-16], peroxyl radicals [17,18], lipid peroxidase [19-21]; copper ion- and macrophage-induced LDL oxidation [22,23].

It has been reported that flavones and catechines are considered as most potent flavonoids that protect the human body from reactive oxygen species (ROS) [24]. Further, it has been also documented the several plant flavones can be used against development and progression of chronic revascularization

diseases including solid malignant tumors. A few of them have been reported to have inhibitory effects on cell proliferation and *in vitro* anti-angiogenesis activity [25,26]. It has been suggested the many polyphenols have promising antiviral activity against many viruses including HIV, herpes simplex virus (HSV), influenza virus and rhinovirus. Some polyphenols have been tested as potent inhibitors of cyclin-dependent kinases found in carcinoma cell of breast [27].

Major polyphenols that are the constituents of food include flavonols such as quercetin and kempferol, flavones like luteolin, flavanols including catechins and anthocyanidins for example cyanidin & malvidin and their glycosides have been found to have grater antioxidant efficacy than most of the standard antioxidants likes vitamin C, vitamin E and carotene [28,29]. The aggregation of Aβ, cholinergic dysfunction, excitotoxicity, mitochondrial dysfunction and many more are the contributors in the pathophysiology of Alzheimer's disease. Further, it has been well reported that ROS plays a critical role and can form the basis of the above contributors in pathogenesis and progression of Alzheimer's disease [30,31]. Taken into consideration of the above facts, in the present study, a computational approach has been designed to evaluate the potential anti-cholinesterase activity of flavonoids derivatives.

EXPERIMENTAL

Selection of macromolecule and its preparation: The human acetyl cholinesterase (ACh) bound with ligand asoxime (HI-6) (pdb id-5HF9) was downloaded from the protein data bank (Fig. 1). The ACh was prepared for molecular docking by removing ligand from active site. Thereafter, water molecules were removed to avoid the interaction with the ligand and further the polar hydrogens were added [32,33].

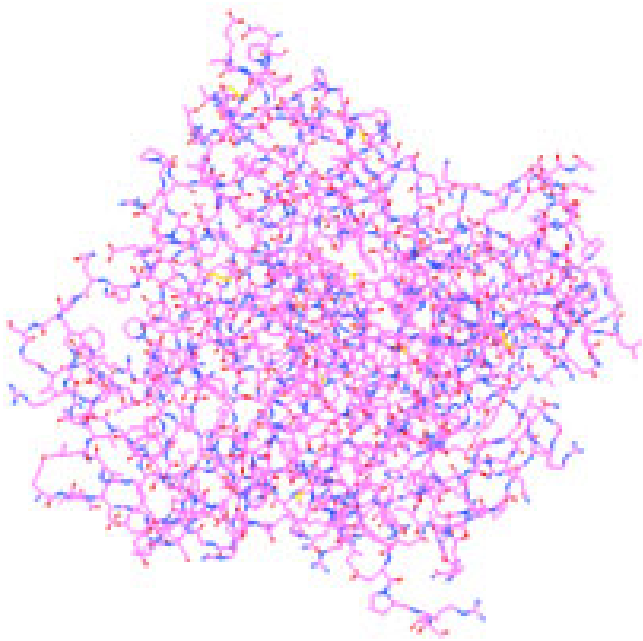


Fig. 1. Crystal structure of the human ACh enzyme (PDB ID-5HF9) acquired from the protein data bank database

Preparation of ligand for molecular docking: The bound ligand HI-6 was prepared for molecular docking simulation by providing the rotatable, non-rotatable as well as unrotatable bonds present in the ligand to the AutoDock software [34].

Identification of binding site: The ligand binding site of the human ACh was identified by exploring the binding interaction of bound ligand HI-6 by using PyMol software. The complexed ligand HI-6 bound in the receptor's active binding site was separated from the complex molecule by using software chimera [35,36].

Molecular docking: The binding site of ACh was identified by using protein visualization PyMol software to enumerate the grid parameter points of grid box required to perform the molecular docking simulation of ligand molecules with human ACh. These grid parameters were utilized for all docking runs. The grid-box was placed by centering the ligand molecule and covering all the residues involved in the binding of ligand to ensure that all the extended conformations of ligand fits within the grid box (Fig. 2).

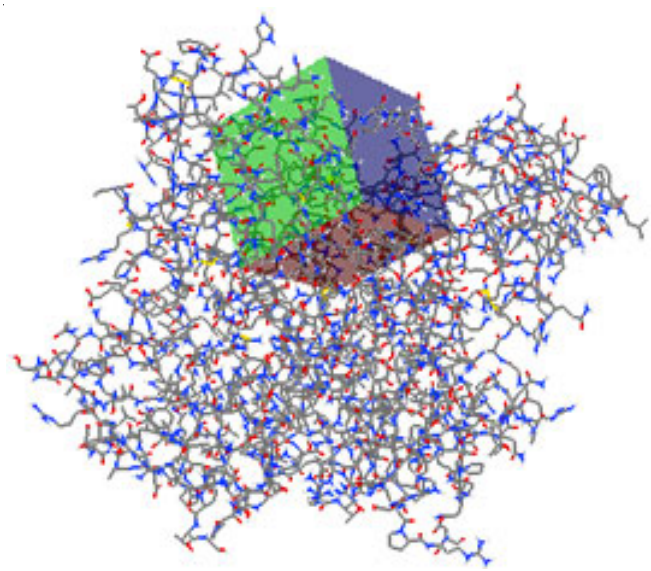


Fig. 2. Three dimensional grid-box covering the ligand binding site of the human ACh enzyme

The separate map files for each of the atom types present in the receptor as well as ligand *viz.* A C HD OA N SA, *etc.* were prepared by running Autogrid utility of the AutoDock suite. These map file prepared by Autogrid was used for carrying out molecular docking simulations by AutoDock.

Lamarckian genetic algorithm (LGA) was one of the primary conformational search approaches employed in AutoDock for molecular docking simulation. A trail population was created for various possible conformations, followed by the mutation, conformational parameters exchange, and compete in a manner kindred to biological evolution in successive generations for eventually selecting individuals with lowest binding energy. The individual conformational search for its local conformational space, discovering local minima and then proceed this information to later generations was performed by "Lamarckian" aspect, which was its additional feature. The binding energy of the small molecules with macromolecular targets was predicted by using semi-empirical force field. The force field allows the assimilation of the intramolecular energies into the predicted binding energy by the evaluation of the energetics for both bound

as well as unbound states based on a comprehensive thermodynamic model. Docking parameter file required for the docking of each ligand molecule was prepared by using the 150 Genetic Algorithm (GA) runs, 250000 maximum numbers of evaluations, 27000 maximum numbers of generations and 0.02 % rate of gene mutation [37].

Validation of docking method: The position and orientations of the ligand obtained after the molecular docking study represented probable binding patterns of the inhibitors. Various docking parameters considered in the docking methods were validated by re-docking individually crystallized ligand HI-6 over ACh.

Overlay methods: The validation of molecular docking method was performed by overlay method. The docked conformation of the bound ligand was impeccably overlaid with reference to the bioactive conformation of the ligand present in the crystal structure of the downloaded protein.

Chemical resemblance: The molecular docking method was validated when the docked ligand was same interactions with the residues of macromolecule as that present in the downloaded crystallized macromolecule.

Selection of ligands: In present study, 9 reported flavonoid molecules which had potential anti-Alzheimer activity were selected from the literature. These reported drugs were utilized for the *in silico* validation of their mechanism of action by inhibition of human ACh.

Molecular docking simulations: The selected ligand molecules were docked against human ACh enzyme by using in-silico molecular docking simulation technique to identify their affinity for the same enzyme. The molecular docking simulation was performed by MGL tools based AutoDock software. MGL-tools was a graphical user interface (GUI) for AutoDock based molecular docking of ligand molecules against a macromolecule. The input filename, coherent format of grid maps, the existence of non-standard atom types and confirming that parameters were evaluated at every step for the validation of the process.

Analysis of molecular docking simulation: After performing molecular docking simulation of the selected ligand molecules against the human ACh enzyme, the best ligand molecule were evaluated on the basis of their binding energy. The Lamarckian Genetic Algorithm used for scoring. All the results obtained by molecular docking simulation were evaluated on the basis of hydrophilic and lipophilic interactions obtained between the binding residues present in the active ligand binding site of the macromolecule and ligand. The empirical range of the free binding energy was considered in the range of -5 to -15 kcal/mol. The mathematical equation to calculate binding affinity of the specific ligand for a particular target was as follows:

$$K_i = e^{[(\Delta G/(RT)]}$$

where, ΔG = change in free energy upon binding, R = gas constant and T = temperature.

RESULTS AND DISCUSSION

Selection and preparation of macromolecule: ACh bound with ligand HI-6 (pdb id-5HF9) was downloaded from protein data bank database. Three dimensional structure model of protein was procured by using X-ray diffraction technique at a resolution of 2.2 Å by using Homo sapiens as an expression system. The 5HF9 protein complex consisted of two identical polypeptide chains of 542 amino acids. The chain B was removed with the help of Chimera software and chain A was selected for the experiment. The receptor molecule was prepared for molecular docking simulation process by adding polar hydrogen bonds, removing redundant water molecules and addition and distribution of charge. After processing the receptor molecule it was saved in *.pdbqt format by using AutoDock software.

Preparation of ligand for molecular docking: Seven rotatable bonds were present in the ligand molecule. All the seven bonds were kept rotatable in the ligand molecule for the current experimental study. The prepared ligand was saved in the *.pdbqt format.

Identification of binding site and grid-box preparation: The amino acid residues Tyr72, Tyr337, Tyr341, Phe295, Trp286, Val282 and Glu285 were involved in the active binding of HI-6 ligand with the human ACh enzyme.

An appropriate grid box was prepared by covering all the macromolecular residues which are involved in the active binding of the bound ligand HI-6 with the human ACh receptor. The coordinates used for the preparation of the grid box are tabulated in Table-1.

Molecular docking simulations and its validation: The results obtained after molecular docking of the bound ligand HI-6 with the human ACh are presented in Table-2. The molecular docking process for docking of particular ligand with a specific macromolecule was performed by considering following parameters:

Overlay method: The molecular docking method was validated as the docked conformation of the ligand was perfectly overlaid with the crystal structure of the ligand present in the downloaded protein. The overlaid conformation of docked ligand with reference to the crystal structure of downloaded ligand is shown in Fig. 3.

Chemical resemblance: The molecular docking method was validated when the docked ligand had the similar interactions with the residues of macromolecule as that was present in the downloaded crystallized macromolecule. The interactions

TABLE-1
GRID COORDINATES FOR HUMAN ACh ENZYME

Proteins	x-D	y-D	z-D	Spacing (Å)	x Center	y Center	z Center
5IKR	40	40	40	0.458	-17.575	-40.761	26.886

TABLE-2
MOLECULAR DOCKING RESULTS OF LIGAND MEFENAMIC ACID WITH THE HUMAN COX-2 RECEPTOR (5IKR)

Protein	Interacting residue	RMSD	Binding energy (kcal/mol)
5HF9	Tyr72, Tyr337, Tyr341, Phe295, Trp286, Val282 and Glu285	0.21	-7.02

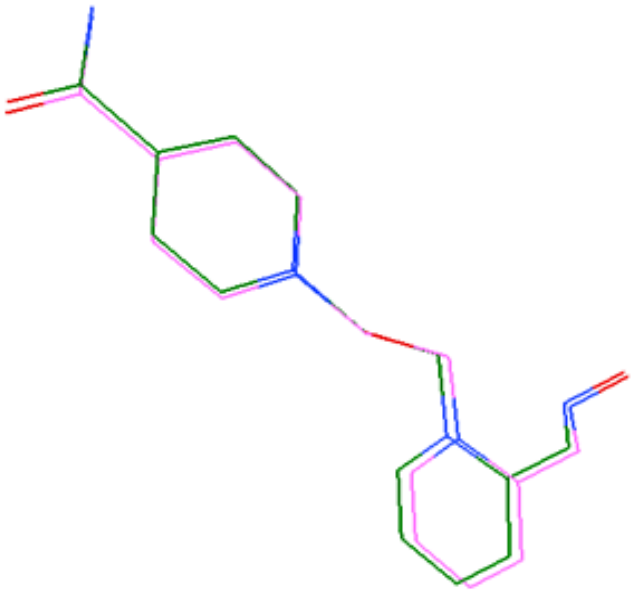


Fig. 3. Superimposition of the docked conformation of the ligand with reference to its bioactive conformation of the ligand was obtained from the crystal structure of the downloaded protein

present in crystal structure and the interactions present in the docked structure are shown in Fig. 4.

Docking studies of flavonoids: The binding affinity of all the ligand molecules were identified by analyzing the binding energy obtained for top ranking pose of each ligand and interactions of docked compound were visualized. The molecular docking results of all the 9 flavonoid molecules were obtained after performing AutoDock based molecular docking simulation against the human ACh enzyme are shown in Table-3.

The beverages such as green tea, black tea and red wine are considered as good source of flavonols, quercetin and kampferol and their glycosides. Onions and apples predominantly contain quercetin where as the flavanones largely found in citrus fruits [38,39]. There are more than 8000 well-known polyphenols found in foods including monomeric flavanols, flavanoness, flavones, anthocyanidins and flavonols. Flavonoids have diphenylpropane (C_6-C_3-C_6) basic skeleton and they are considered as primary class of polyphenols [40-43]. Individuals of same group and different groups have different biological activities which result from the variation in number and position of substitution with alkyl group, position and number of glycosylation and arrangement of hydroxyl groups. They are preferably glycosylated with the most usual sugar residue glucose but others include galactose, rhamnose, xylose [44]. In support to present study, it has been reported that flavonoid radicals have lower reduction potentials than others likes alkyl peroxyl radicals and superoxide radicals. This fact indicates that flavonoids may inactivate oxyl species and stop the deleterious consequences of these species and their reaction that is basis of free radical scavenging activity [45]. Number of hydroxyl groups determines the antioxidant potency of phenolic acids and their esters which is further enhanced by steric hindrance. If hydroxyl benzoates are substituted by electron withdrawing groups such as carboxylate group in bezoic acid, it negatively influences the H-donating abilities. The activity of all flavonoids usually enhanced if they have hydrophobic substitution such as nitrogen or oxygen containing heterocyclic moieties, alkyl chains, alkylamino chains and prenyl groups [46].

Conclusion

Nine reported flavonoids having anti-Alzheimer activity were evaluated for their binding affinity against human ACh enzyme for confirming their possible mechanism of action using AutoDock based molecular docking simulation technique. The flavonoid derivatives such as 3-hydroxy flavones, 5-hydroxy flavones, 6-hydroxy flavones, 7-hydroxy flavones, chrysin, baicalien, flavone, kaemferol and quercetin were evaluated for their possible mechanism of action in the present experimental

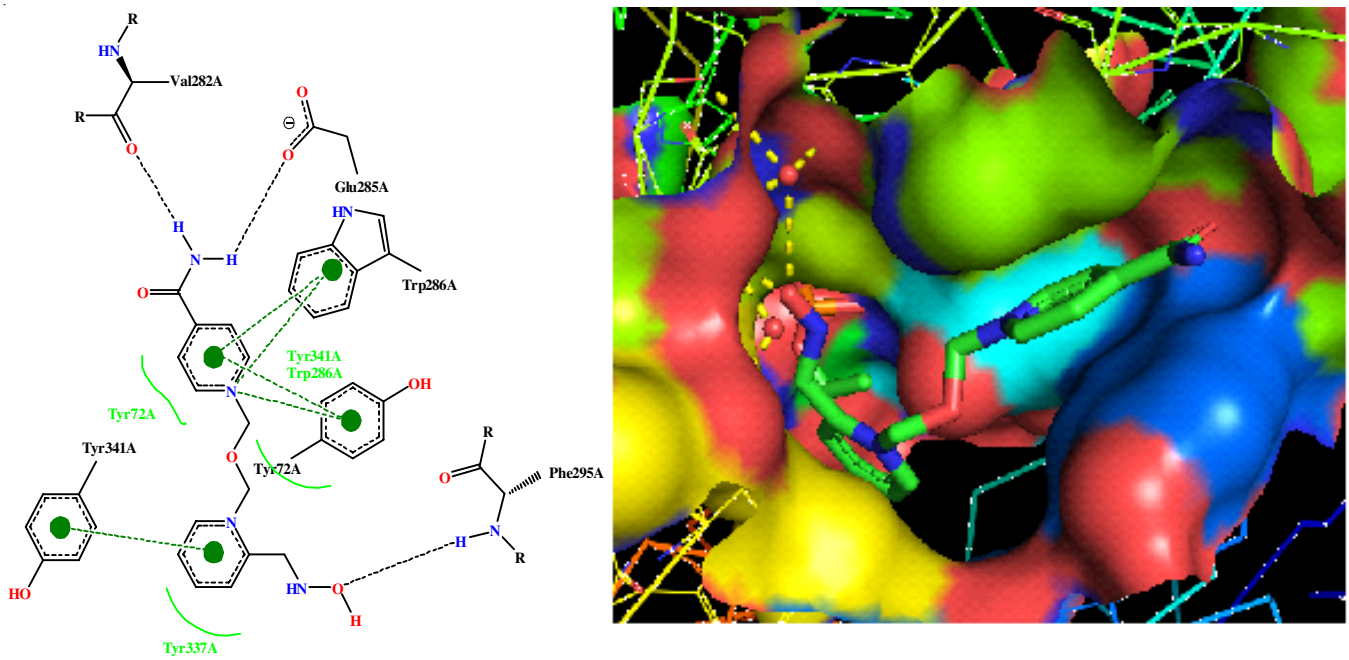
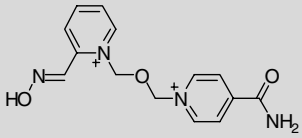
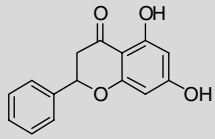
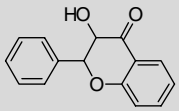
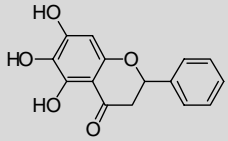
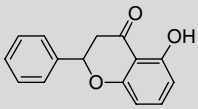
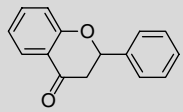
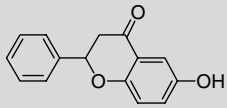
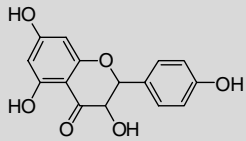
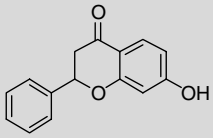
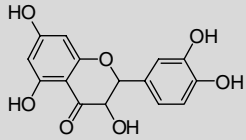


Fig. 4. Binding mode and chemical interactions of the bound ligand HI-6 within the active ligand binding site human ACh enzyme

TABLE 3
BINDING ENERGY OF ALL THE NINE SELECTED FLAVONOID LIGAND MOLECULES AND HI-6 AGAINST THE HUMAN ACh ENZYME

Name	Structure	Binding energy	Name	Structure	Binding energy
HI6 (Bound ligand)		-7.01	Chrysin		-8.56
3-Hydroxy flavones		-8.52	Baicalien		-8.60
5-Hydroxy flavones		-8.68	Flavone		-8.89
6-Hydroxy flavones		-8.79	Kaemferol		-8.41
7-Hydroxy flavones		-8.74	Quercetin		-8.57

study. All the 9 flavonoids exhibited strong binding affinity that promises potent inhibition of human ACh enzyme in *in silico*. Thus, these flavonoid compounds could be presumed to be potential anti-cholinesterase drugs. Further, these drug candidates could be considered as alternative options in the management of Alzheimer's disease.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. L.C. Dos Santos Picanco, P.F. Ozela, M. de Fatima de Brito-Brito, A.A. Pinheiro, E.C. Padilha, F.S. Braga, C.H.T. de Paula da Silva, C.B.R. Dos Santos, J.M.C. Rosa and L.I. da Silva Hage-Melim, *Curr. Med. Chem*., 25, 3141 (2018) https://doi.org/10.2174/0929867323666161213101126.
2. I.G. Onyango, J. Dennis and S.M. Khan, *Aging Dis.*, **7**, 201 (2016); https://doi.org/10.14336/AD.2015.1007.
3. H.Q. Sun, X. Zhang, W.J. Huang and W.W. Chen, *Eur. Rev. Med. Pharmacol. Sci.*, **20**, 1903 (2016).
4. H. Robert and M.D. Howland, *J. Psychosoc. Ment. Health Serv.*, **49**, 13 (2011).
5. M.R. Cesarone, G. Laurora, A. Ricci, G. Belcaco and P. Pomante, *J. Vas. Disease*, **21**, 76 (1992).
6. M. Lutz, E. Fuentes, F. Ávila, M. Alarcón and I. Palomo, *Molecules*, 24, 366 (2019); https://doi.org/10.3390/molecules24020366.
7. X. Han, T. Shen and H. Lou, *Int. J. Mol. Sci*., **8**, 950 (2007); https://doi.org/10.3390/i8090950.
8. C. Kandaswami and E. Middleton, *Adv. Exp. Med. Biol.*, **366**, 351 (1994); https://doi.org/10.1007/978-1-4615-1833-4_25.
9. D. Tungmunnithum, A. Thongboonyou, A. Pholboon and A. Yangsabai, *Medicines*, 5, 93 (2018); https://doi.org/10.3390/medicines5030093.
10. J. Duarte, F. Pérez-Vizcaíno, A. Zarzuelo, J. Jiménez and J. Tamargo, *Eur. J. Pharmacol.*, **239**, 1 (1993); https://doi.org/10.1016/0014-2999(93)90968-N.
11. C.A. Rice-Evans, N.J. Miller and G. Paganga, *Free Radic. Biol. Med.*, **20**, 933 (1996); https://doi.org/10.1016/0891-5849(95)02227-9.
12. A.J. Elliott, S.A. Scheiber, C. Thomas and R.S. Pardini, *Biochem. Pharmacol.*, **44**, 1603 (1992).
13. W.S. Chang, Y.J. Lee, F.J. Lu and H.C. Chiang, *Anticancer Res.*, **13**, 6A (1993).
14. Y. Hanasaki, S. Ogawa and S. Fukui, *Free Radic. Biol. Med.*, **16**, 6845 (1994); https://doi.org/10.1016/0891-5849(94)90202-X.
15. J.L. Cotelle Bernier, J.P. Henichart, J.P. Catteau, E. Gaydou and J.C. Wallet, *Free Radic. Biol. Med.*, **13**, 3 (1992).
16. G. Chen, M. Griffin, J. Lee Poyer, P.B. McCay and D.W.A. Bourne, *Free Radic. Biol. Med.*, **9**, 93 (1990); https://doi.org/10.1016/0891-5849(90)90110-5.
17. M. Erben-Russ, C. Michel, W. Bors and M. Saran, *J. Phys. Chem.*, **91**, 2362 (1987); https://doi.org/10.1021/j100293a033.
18. S.V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic and M.G. Simic, *J. Am. Chem. Soc.*, **116**, 4846 (1994); https://doi.org/10.1021/ja00090a032.
19. C.G. Frag, V.S. Martino, G.E. Ferraro, J.D. Coussio and A. Boveris, *Biochem. Pharmacol.*, **36**, 5 (1987).
20. A. Negre-Salvayre, Y. Alomar, M. Troly and R. Salvayre, *Biochim. Biophys. Acta*, **1096**, 291 (1991); https://doi.org/10.1016/0925-4439(91)90065-H.
21. J. Terao, M. Piskula and Q. Yao, *Arch. Biochem. Biophys.*, **308**, 278 (1994); https://doi.org/10.1006/abbi.1994.1039.
22. H. Mangiapane, J. Thomson, A. Salter, S. Brown, G.D. Bell and D.A. White, *Biochem. Pharmacol.*, **43**, 445 (1992); https://doi.org/10.1016/0006-2952(92)90562-W.
23. C.V. De Whalley, S.M. Rankin, J.R. Hoult, W. Jessup and D.S. Leake, *Biochem. Pharmacol.*, **39**, 1743 (1990); https://doi.org/10.1016/0006-2952(90)90120-A.

24. R.J. Nijveldt, E. van Nood, D.E. van Hoorn, P.G. Boelens, K. van Norren and P.A. van Leeuwen, *Am. J. Clin. Nutr.*, **74**, 418 (2001); https://doi.org/10.1093/ajcn/74.4.418.
25. T. Fotsis, M.S. Pepper, E. Aktas, S. Breit, S. Rasku, H. Adlercreutz, K. Wahala, R. Montesano and L. Schweigerer, *Cancer Res.*, **57**, 14 (1997).
26. D.H. Paper, *Planta Med.*, **64**, 686 (1998); https://doi.org/10.1055/s-2006-957559.
27. M.D. Losiewicz, B.A. Carlson, G. Kaur, E.A. Sausville and P.J. Worland, *Biochem. Biophys. Res. Commun.*, **201**, 589 (1994); https://doi.org/10.1006/bbrc.1994.1742.
28. C. Rice-Evans, N.J. Miller, G.P. Bolwell, P.M. Bramley and J.B. Pridham, *Free Radic. Res.*, **22**, 375 (1995); https://doi.org/10.3109/10715769509145649.
29. C. Castelluccio, G. Paganga, N. Melikian, G. Paul Bolwell, J. Pridham, J. Sampson and C. Rice-Evans, *FEBS Lett.*, **368**, 188 (1995); https://doi.org/10.1016/0014-5793(95)00639-Q.
30. B. Rosengarten, S. Paulsen, S. Molnar, R. Kaschel, B. Gallhofer and M. Kaps, *J. Neurol.*, **253**, 58 (2006); https://doi.org/10.1007/s00415-005-0926-5.
31. B. Matharu, G. Gibson, R. Parsons, T.N. Huckerby, S.A. Moore, L.J. Cooper, R. Millichamp, D. Allsop and B. Austen, *J. Neurol. Sci.*, **280**, 49 (2009); https://doi.org/10.1016/j.jns.2009.01.024.
32. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov and P.E. Bourne, *Nucleic Acids Res.*, **28**, 235 (2000); https://doi.org/10.1093/nar/28.1.235.
33. M.C. Franklin, M.J. Rudolph, C. Ginter, M.S. Cassidy and J. Cheung, *Proteins*, **84**, 1246 (2016); https://doi.org/10.1002/prot.25073.
34. D.S. Goodsell, G.M. Morris and A.J. Olson, *J. Mol. Recognit.*, **9**, 1 (1996); https://doi.org/10.1002/(SICI)1099-1352(199601)9:1<1::AID-JMR241>3.0.CO;2-6.
35. W.L. DeLano, CCP4 Newsletter On Protein Crystallography, **40**, 1 (2002).
36. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin, *J. Comput. Chem.*, **25**, 1605 (2004); https://doi.org/10.1002/jcc.20084.
37. S. Mujwar and K.R. Pardasani, *J. Med. Imaging Health Infor.*, **5**, 1 (2015); https://doi.org/10.1166/jmihi.2015.1357.
38. A.N. Panche, A.D. Diwan and S.R. Chandra, *J. Nutr. Sci.*, **5**, e47 (2016); https://doi.org/10.1017/jns.2016.41.
39. G. Gattuso, D. Barreca, C. Gargiulli, U. Leuzzi and C. Caristi, *Molecules*, **12**, 1641 (2007); https://doi.org/10.3390/12081641.
40. S. Bhattacharya, *Pharmacogn. Res.*, **3**, 147 (2011); https://doi.org/10.4103/0974-8490.81966.
41. J.C. Stoclet and V. Schini-Kerth, *Ann. Pharm. Fr.*, **69**, 78 (2011); https://doi.org/10.1016/j.pharma.2010.11.004.
42. K. Herrmann, *Int. J. Food Sci. Technol.*, **11**, 433 (1976); https://doi.org/10.1111/j.1365-2621.1976.tb00743.x.
43. J. Kuhnau, *World Rev. Nutr. Diet.*, **24**, 117 (1976); https://doi.org/10.1159/000399407.
44. K. Herrmann and C.W. Nagel, *Crit. Rev. Food Sci. Nutr.*, **28**, 315 (1989); https://doi.org/10.1080/10408398909527504.
45. S.V. Jovanovic, I. Jankovic and L. Josimovic, *J. Am. Chem. Soc.*, **114**, 9018 (1992); https://doi.org/10.1021/ja00049a037.
46. Y. Xie, W. Yang, F. Tang, X. Chen and L. Ren, *Curr. Med. Chem.*, **22**, 132 (2015); https://doi.org/10.2174/0929867321666140916113443.