

Antioxidant and Anti-inflammatory Related Activities of Selected Synthetic Chalcones: Structure–Activity Relationship Studies Using Computational Tools

Rajesh GACCHE,^{*,a} Mansi KHSIRSAGAR,^a Srikant KAMBLE,^b Babasaheb BANDGAR,^b Nagesh DHOLE,^a Kavita SHISODE,^a and Ajay CHAUDHARI^c

^aSchool of Life Sciences, Swami Ramanand Teerth Marathwada University; ^bSchool of Chemical Sciences, Swami Ramanand Teerth Marathwada University; and ^cSchool of Physical Sciences, Swami Ramanand Teerth Marathwada University; Nanded 431 606 (MS), India. Received December 15, 2007; accepted April 4, 2008

Synthetic derivatives of 1-(2-hydroxy-3-(2-hydroxy-cyclohexyl)-4,6-dimethoxy-phenyl)-methanone were evaluated *in-vitro* for their activities related to antioxidant and anti-inflammatory. The antioxidant potential was determined by calculating reducing potential, OH and DPPH (2,2-diphenyl-1-picryl hydrazine) radical scavenging activities. The *in-vitro* anti-inflammatory related activities of synthetic chalcones (SCs) were demonstrated by performing inhibition assays of trypsin, β -glucuronidase and diene conjugates. The results of the various parameters studied shows that the selected derivatives were found to be effective reducing agents and were reactive towards stabilizing the OH and DPPH radicals. The compounds have showed moderate to poor or no inhibition profile towards trypsin and β -glucuronidase, but were found to be effective inhibitor of diene conjugates (hydroperoxides). An attempt has been made to define structure activity relationship using BioMed CAChe 6.1.10: a computer-aided molecular modeling tool which applies equations from classical and quantum mechanics. The experimental and *in silico* results of the present investigation shows that the basic nucleus 1-(2-hydroxy-3-(2-hydroxy-cyclohexyl)-4,6-dimethoxy-phenyl)-methanone can be considered as a potential candidate for the design and development of lead antioxidant and anti-inflammatory agents.

Key words synthetic chalcone; antioxidant; anti-inflammatory; enzyme inhibition

A cursory look at the literature cited in relation to chalcones in recent years indicates that there is a growing interest in evaluating the pharmaceutically important biological activities of chalcones and its derivatives, presuming their role in the prevention of various degenerative diseases and other human ailments. Chalcones which are classified under polyphenolic compounds are basically flavonoids, ubiquitously present in plants especially more in fruits and vegetables.¹⁾ Scientific investigations on the bioavailability of chalcones from food sources are limited but variety of synthetic chalcones (SCs) has been reported to possess a wide range of pharmaceutically important biological activities. Plethora of literature has accumulated in the recent years suggesting the role of chalcones and its derivatives as an anti-inflammatory,²⁾ anticancer,³⁾ and antioxidant,⁴⁾ agents. Chalcones have also been reported as inhibitors of angiogenesis, because the process of angiogenesis (formation of new blood vessels) is proved to be crucial for the survival and proliferation of solid tumors. Arresting the angiogenesis process has been considered as a potential target for the development of anticancer drugs.⁵⁾ Hydroxy-methylated chalcone such as 2'-hydroxy-4'-methoxychalcone has been described as an effective anti-angiogenic and anti-tumor agent.⁶⁾ Nevertheless chalcones have also been attributed with wide range of antimicrobial activity like antimalarial,⁷⁾ antileishmanial,⁸⁾ and antibacterial⁹⁾ especially more effective against Gram-positive than Gram-negative bacteria. Recently we have reported series of derivatives of 1-(2-hydroxy-3-(2-hydroxy-cyclohexyl)-4,6-dimethoxy-phenyl)-methanone as an effective antioxidant agents.¹⁰⁾ Various derivatives of prenylated and nonprenyated SCs have been investigated *in vitro* for their antioxidant and prooxidant actions.¹¹⁾ Several SCs have been designed, synthesized and tested for inhibition of activation

of mast cells, neutrophils, macrophages and microglial cells which are important mediators in the initiation of inflammatory disorders.¹²⁾ It is this reputation of SCs in the main stream of pharmaceutical research, which has attracted researches in the recent years.

The research focus on free radicals and antioxidants in biology is producing medical revolution that promises an alternative approach for health and disease management. Using antioxidants efforts are being made to prevent oxidative reactions in foods, pharmaceuticals and cosmetics. Moreover the antioxidant therapy is coming up as an alternative, attractive and effective approach for the amelioration of several chronic degenerative diseases including cancer, inflammatory, cardiovascular, autoimmune, neurodegenerative (*e.g.* Alzheimer's disease, Parkinson's disease, multiple sclerosis, downs syndrome) and aging.¹³⁾ In recent years, it has been appreciated that antioxidants may also be involved in regulating signaling pathways and cellular responses.¹⁴⁾ Interestingly many of the anti-inflammatory agents are found to be effective in antioxidant activity.¹⁵⁾

In present investigations the antioxidant and anti-inflammatory related activities of several derivatives of 1-(2-hydroxy-3-(2-hydroxy-cyclohexyl)-4,6-dimethoxy-phenyl)-methanone are described with an effort to establish structure–activity relationship using computational tools.

Experimental

Materials The SCs to be tested were selected from the series of chalcones synthesized in the School of Chemical Sciences, S. R. T. M. University, Nanded (MS), India. The details of the synthetic methodology and characterization data of the selected SCs has been reported.¹⁶⁾ 2,2-Diphenyl-1-picrylhydrazine (DPPH), β -glucuronidase (EC 3.2.1.31, 25000 units, source: *E. coli*), were obtained from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.), *p*-nitrophenyl- β -D-glucopyranosiduric acid was procured from CALBIOCHEM (EMD Biosciences Inc. La Jolla, CA, U.S.A.), Trypsin was

* To whom correspondence should be addressed. e-mail: rngacche@rediffmail.com

obtained from SISCO Research Laboratories Pvt. Ltd. Mumbai. Glutathione (reduced) was obtained from s.d. Fine Chemicals Ltd. Mumbai. All other chemicals used were of AR grade and were obtained from commercial sources.

Reducing Activity Assay Reduction of Fe^{3+} of $\text{K}_3\text{Fe}(\text{CN})_6$ to Fe^{2+} by antioxidants is the underlying mechanism of the reducing activity assay.¹⁷⁾ The reducing potential of SCs was calculated by the fall in extinction of $\text{K}_3\text{Fe}(\text{CN})_6$ at 420 nm against appropriate blank. The reaction mixture contained 500 μl solution of individual SC (1 mM in 0.5% v/v dimethyl sulfoxide) in 3 ml of 1 mM potassium ferricyanide solution and the absorbance was recorded at 420 nm after 10 min reaction time. Glutathione (reduced, 1 mM) was used as a standard reducing agent.

OH Radical Scavenging Activity OH radicals were generated by using the Ferric ion (Fe^{3+})/ascorbic acid reaction system. The detection of OH radicals was carried out by measuring the amount of formaldehyde generated from the oxidation of dimethyl sulfoxide.¹⁸⁾ The reaction mixture contained 0.1 mM EDTA, 167 μM Fe^{3+} , 33 mM DMSO in phosphate buffer of 50 mM pH 7.4. 0.1 ml individual SC (1 mM) solution. Ascorbic acid (150 μl , 10 mM in phosphate buffer) was added finally to initiate the reaction. Trichloroacetic acid (17%, w/v) was used to terminate the reaction. The contents were observed spectrophotometrically at 412 nm for the detection of formaldehyde. Mannitol (1 mM) was used as a reference compound for comparative study.

Interaction with DPPH Radical¹⁹⁾ The ability of SCs towards DPPH radical scavenging SCs was carried out by mixing 1 mM solution of DPPH and individual SCs (in absolute ethanol). After 10 min reaction time the samples were observed spectrophotometrically at 517 nm. Quercetin (1 mM) was used as a standard compound.

Assay of Anti-proteolytic Activity²⁰⁾ One millimolar solution of individual SCs (0.1 ml) was incubated with trypsin (0.075 mg/ml) for 20 min followed by addition of bovine serum albumin (BSA, 6 g/100 ml, in 0.1 M phosphate buffer, pH 7.6). After 20 min incubation at 37 °C the reaction was terminated using trichloroacetic acid (3 ml, 5%). The resultant precipitates were separated by centrifugation at 5000 rpm. The acid soluble protein fractions were estimated using Lowry method.²¹⁾ Salicylic acid (1 mM) was used as a reference compound.

Inhibition of β -glucuronidase The β -glucuronidase inhibition assay was carried out as per the reported method²²⁾ in brief, 2.5 mM *p*-nitrophenyl- β -D-glucopyranosiduronic acid was incubated with 1 mM (0.1 ml) individual SCs in acetate buffer (0.1 M, pH 7.4) for 5 min followed by addition of 0.1 ml of β -glucuronidase solution. The contents were further retained for 30 min followed by addition of sodium hydroxide (2 ml, 0.5 N) for termination of the reaction. The amount of product formed was observed spectrophotometrically at 410 nm. Salicylic acid (1 mM) used as a reference drug for comparative study.

Inhibition of Hydroperoxide Formation The inhibition of formation of hydroperoxides (diene conjugates) was performed as per the reported method.²³⁾ The RBC membrane solution was prepared as per the procedure described elsewhere²⁴⁾ with slight modification.²⁵⁾ To the membrane solution (1.0 ml), 5 ml of chloroform:methanol (2:1) was mixed followed by centrifugation at 1000 g for 15 min for separation of the two phases. The chloroform layer was taken in a test tube and dried at 45 °C in water bath. The lipid residue leftover was dissolved in 1.5 ml of cyclohexane. The amounts of hydroperoxides generated were measured at 233 nm against a cyclohexane blank. Aspirin (acetyl salicylic acid, 1 mM) was used as a reference drug.

The results of the above studied parameters were calculated as % activity by using a formula.²⁶⁾

$$\% \text{ activity} = \left(1 - \frac{\text{absorbance of test sample}}{\text{absorbance of control sample}} \right) \times 100$$

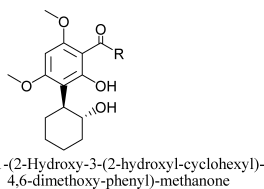
Computational Studies The log *P* value, molecular weights and quantum chemical descriptors like E_{HOMO} (energy of highest occupied molecular orbital), E_{LUMO} (energy of lowest unoccupied molecular orbital), ionization potential and electron affinity of the selected SCs were calculated using a BioMed CAChe 6.1.10 (Fujitsu Ltd.), a computer-aided molecular design modeling tool for Windows ME, 982000 and XP operating system. The structures of the selected SCs were built in the workplace and the sample files were subjected for energy minimizations using Augmented Molecular Mechanics (MM_3). The energy minimization is continued until the energy change is less than 0.001 kcal/mol, or until the molecule has been updated 300 times. All the quantum chemical descriptors were calculated in the Project Leader. The log *P* value is calculated using the atom typing scheme. E_{HOMO} and E_{LUMO} are determined after optimizing the molecular geometry

first using Augmented MM_2 and then using MOPAC with PM_3 parameters. The ionization potential and electron affinity are approximated by the energy of the HOMO and LUMO respectively, after optimizing the molecular geometry using Augmented MM_2 followed by MOPAC with PM_3 parameters. The selected quantum chemical descriptors are reported to have strong attributes with the free radical scavenging and biological activity of the compounds.²⁷⁾ The results of the studied computational parameters are summarized in Table 3.

Results and Discussion

Log *P* Values and Molecular Weights The molecular weights and theoretical log *P* values calculated *in silico* are summarized in Table 1. Log *P* is the logarithm of the partition coefficient between *n*-octanol and water. It is a physico-chemical property of a drug candidate widely used in medicinal chemistry as an index of lipophilicity and ability of drug molecule for crossing various biological membranes.²⁸⁾ According to the Lipinski's rule of five, the log *P* values more than 5 and molecular weight above 500 g/mol does not qualify as a drug candidate and such compounds might not be appropriate for *in vivo* administration.²⁹⁾ In the present studies, except compound **C1** (log *P* 6.758) all other SCs have log *P* values in a range of 3.069—4.571. The compounds **C1** (mol. wt. 592.771) and **C3** (mol. wt. 551.653) are having marginally higher molecular weights than the upper limit of 500 g/mol set by Lipinski's rule of five. All other SCs are having molecular weights within an acceptable range of 398.455—462.541 g/mol. The number of hydrogen bond donors and acceptors is also one of the important criteria set by Lipinski's rule of five, wherein it has been described that the number of hydrogen bond donors should be less than five whereas acceptors should be less than ten.²⁹⁾ All the test compounds under study are found to be within the Lipinski's range, in which the compounds **C3—C5** and **C7** possess three hydrogen bond donors, while **C1**, **C2**, **C6** and **C8** have two hydrogen bond donors. The compounds **C1—C3** and **C6—C8** possess five and four hydrogen bond acceptors respectively, while **C4** and **C5** have three hydrogen bond acceptors. In brief all the test samples fulfill the hydrogen bond donor and acceptor functions within the limit set by Lipinski's rule of five.

Reducing Ability of Selected SCs The results of the reducing ability of selected SCs (Table 2) shows that the compound **C6** (47.30%), **C8** (43.60%) followed by **C1** (36.10%) are found to be effective reducing agents, while all other SCs showed reduction potential in a range of 11.80—28.50% as compared to glutathione (58.90%). Reducing ability is a measure of the ability to donate electron. The donation of an electron has been strongly attributed with the E_{HOMO} and E_{LUMO} of the compounds. It has been also reported that the greater the value of E_{HOMO} , the greater the electron donating capability while the smaller the E_{LUMO} , the smaller the resistance to accept the electrons, in other words E_{LUMO} measures the electron accepting character of the compound.³⁰⁾ The results of the reducing ability of the selected SCs to a greater extent are in agreement with the *in silico* values of E_{HOMO} and E_{LUMO} wherein the E_{HOMO} of the **C6**>**C8**>**C1** and E_{LUMO} of **C1**>**C6**>**C8** (**C4** an exception) are higher as compared to all other SCs under study implying the electron donating nature rather than electron accepting. Nevertheless the compounds possessing considerable reducing potential can be further explored for the design of a novel anti-inflammatory

Table 1. Structures, Molecular Formulae, Molecular Weights, and Theoretical log *P* Values of the Selected SCs

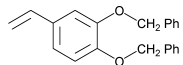
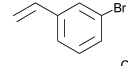
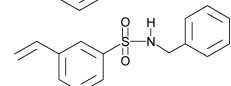
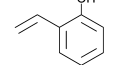
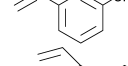
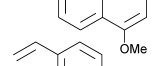
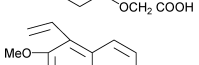
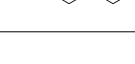
| Compound no. | Molecular formula | Molecular weight | Log <i>P</i> | R-group |
|--------------|---|------------------|--------------|---|
| C1 | C ₃₆ H ₄₈ O ₇ | 592.771 | 6.758 |  |
| C2 | C ₂₃ H ₂₅ O ₅ Br | 461.352 | 4.571 |  |
| C3 | C ₃₀ H ₃₃ NO ₇ S | 551.653 | 4.322 |  |
| C4 | C ₂₃ H ₂₆ O ₆ | 398.455 | 3.495 |  |
| C5 | C ₂₃ H ₂₆ O ₆ | 398.455 | 3.495 |  |
| C6 | C ₂₈ H ₃₀ O ₆ | 462.541 | 4.529 |  |
| C7 | C ₂₅ H ₂₈ O ₈ | 456.491 | 3.069 |  |
| C8 | C ₂₈ H ₃₀ O ₆ | 462.541 | 4.529 |  |

Table 2. Summary of the Results of % Reducing Ability (% RA), % OH and DPPH Radical Scavenging Activity of the Tested Synthetic Chalcones

| SCs ^{a)} | % RA | % OH | % DPPH |
|-------------------|---------------|---------------|---------------|
| C1 | 36.10 (±0.71) | 7.55 (±0.51) | 33.70 (±0.59) |
| C2 | 11.80 (±0.31) | NR | 13.60 (±0.62) |
| C3 | 28.80 (±0.28) | 7.12 (±0.97) | 27.80 (±1.3) |
| C4 | 21.62 (±1.21) | 6.10 (±0.48) | 26.20 (±0.85) |
| C5 | 14.70 (±0.89) | NR | 17.50 (±1.1) |
| C6 | 47.30 (±0.51) | 23.20 (±0.11) | 39.80 (±0.17) |
| C7 | 15.45 (±1.41) | 4.60 (±0.29) | 21.75 (±0.91) |
| C8 | 43.60 (±0.93) | 12.70 (±1.3) | 36.90 (±0.34) |
| GLU | 58.90 (±0.56) | ND | ND |
| MAN | ND | 30.10 (±0.77) | ND |
| QUE | ND | ND | 94.70 (±0.97) |

Percent activity shown here are the mean ± standard deviation of *n* = 3. GLU, glutathione (reduced); MAN, mannitol; QUE, quercetin; NR, no reaction under experimental conditions; ND, not determined. *a)* The effective concentration of SCs after dilution to 3 ml of reaction mixture was 0.5 mM.

agent.¹⁷⁾

OH and DPPH Radical Scavenging Activities The OH and DPPH radical scavenging activities of selected SCs are summarized in Table 2. Except **C2** and **C5**, all other SCs were found to be good to moderate scavengers of OH radicals. It is clear that the compound **C6** (23.20%) can be graded as an effective OH radical stabilizing agent, while rest of the SCs have shown OH radical scavenging activity in a range of 4.60–12.70% as compared to Mannitol (30.10%). In case of DPPH radical scavenging activity the order of re-

activity is **C6** (39.80%) > **C8** (36.90%) > **C1** (33.70%). Remaining SCs were found to possess the DPPH radical scavenging activity in a range of 13.60–27.80% as compared to Quercetin (94.70%).

The computational data obtained can be correlated with that of the OH and DPPH radical scavenging activity. The *in silico* results to a greater extent are in agreement with that of the experimental evaluation. Samples possessing higher E_{HOMO} were found to be effective agents for stabilizing the OH and DPPH radicals. Moreover it has also calculated that the molecules with smaller ionization potential are expected to be more reactive as nucleophiles.³¹⁾ The compounds **C6**, **C8** and **C1** are having higher HOMO as well as lower ionization potential as compared to all other SCs. The energy of HOMO is directly related to the ionization potential and characterizes the susceptibility of the molecule toward attack by electrophiles. While the energy of the LUMO has attributes with electron affinity and it implies the susceptibility of the molecule toward attack by nucleophiles^{32,33)} The smaller values of E_{LUMO} indicates the electron accepting character of the compounds.³⁰⁾ The compounds **C1**, **C6** and **C8** showing better reducing, DPPH and OH radical scavenging activities are having higher E_{LUMO} values implying the suitability of the compounds as an electron donors rather than electron acceptors. It was also observed that the *para*-substitution of methoxy group (**C6**) was found to be more susceptible for reducing and overall free radical scavenging activity as compared to *ortho*-substituted methoxy group on the *R*-naphtha-

Table 3. Profile of the Quantum Chemical Descriptors Studied *in Silico* for the Selected SCs

| SCs | E_{HOMO} (eV) | E_{LUMO} (eV) | I.P. (eV) | E.A. (eV) |
|-----------|------------------------|------------------------|-----------|-----------|
| C1 | -8.549 | -0.167 | 8.435 | 0.241 |
| C2 | -9.271 | -0.734 | 9.252 | 0.733 |
| C3 | -8.947 | -0.791 | 8.915 | 0.819 |
| C4 | -8.961 | -0.133 | 8.961 | 0.133 |
| C5 | -9.078 | -0.581 | 9.078 | 0.581 |
| C6 | -8.498 | -0.468 | 8.495 | 0.461 |
| C7 | -9.003 | -0.420 | 8.928 | 0.531 |
| C8 | -8.538 | -0.470 | 8.738 | 0.697 |
| ASA | -9.656 | -0.569 | 9.656 | 0.569 |
| QUE | -8.374 | -1.066 | 8.374 | 1.066 |

SCs: synthetic chalcones, HOMO: highest occupied molecular orbital, LUMO: lowest unoccupied molecular orbital, I.P.: ionization potential, E.A.: electron affinity, ASA: acetyl salicylic acid, QUE: quercetin.

lene moiety (**C8**); the same was observed with **C4** and **C5** in relation to substitution of hydroxyl group on the *R*-benzene moiety. Chalcones are basically phenolic compounds wherein a general structure–activity relationship in case of radical scavenging reactions has been described. The potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Similarly, high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging by specific functional groups.³⁴⁾

Assay of Anti-proteolytic Activity The results of the trypsin inhibition assay are summarized in Table 4. This assay was performed to realize the anti-proteolytic potentials of the selected SCs. All the SCs under study were found to be good to moderate inhibitors of the trypsin induced hydrolysis of BSA. The compounds **C2** (40.62%), **C4** (30.20%) and **C5** (27.30%) were found to effective inhibitors of trypsin. All other SCs have shown the inhibition in a range of 13.80–20.10%. Proteases, especially serine proteases (*e.g.* Trypsin) are reported to be key players in recruiting the initiation and progression of the inflammatory process.³⁵⁾ Moreover serine protease inhibition has been considered as one of the targets for designing the anti-inflammatory agents.³⁶⁾ The computational data shows that the compounds **C2** and **C5** are having low E_{LUMO} indicating the potential electron accepting candidature of the compounds but this can not be correlated to all the compounds showing trypsin inhibition.

Inhibition of β -glucuronidase The profile of the inhibition of β -glucuronidase by SCs is presented in Table 4. The tested SCs were found to be poor inhibitors of β -glucuronidase. The sample **C1** and **C6** were found to be no reactive with β -glucuronidase. The compounds **C3** (7.3%) and **C2** (6.8%) were observed to be a moderate enzyme inhibitors. All other SCs shown enzyme inhibition in a range of 1.8–4.8%. The enzyme β -glucuronidase is present in the lysosomes of neutrophils and has been implicated as one of the mediators in initiating the process of inflammation.^{37,38)} It is indeed difficult to discuss the structure–activity relationship in this regard, but one common observation is that, that the compounds showing moderate β -glucuronidase inhibition has a lower E_{LUMO} and higher electron affinity as compared to other SCs which indicates the electron withdrawing environment which possibly might be favoring the β -glucuronidase inhibition.

Table 4. Profile of % Inhibition Activity of Trypsin, β -Glucuronidase and Hydroperoxides by Selected SCs

| SCs (1 mM) | % Inhibition | | |
|---------------|---------------------|-------------------|---------------------|
| | Trypsin | β -GLU | OOH |
| C1 | 16.70 (\pm 0.92) | NR | 39.75 (\pm 1.3) |
| C2 | 40.62 (\pm 1.2) | 6.8 (\pm 0.51) | 16.45 (\pm 0.52) |
| C3 | 13.80 (\pm 1.4) | 7.3 (\pm 1.5) | 12.70 (\pm 1.31) |
| C4 | 30.20 (\pm 0.25) | 2.1 (\pm 0.5) | 34.15 (\pm 0.83) |
| C5 | 27.30 (\pm 1.2) | 4.8 (\pm 1.5) | 28.50 (\pm 0.45) |
| C6 | 18.90 (\pm 0.24) | NR | 18.31 (\pm 0.55) |
| C7 | 20.10 (\pm 1.4) | 1.8 (\pm 2.1) | 29.95 (\pm 1.3) |
| C8 | 16.40 (\pm 0.29) | 3.4 (\pm 0.5) | 62.80 (\pm 0.35) |
| ASA | 55.20 (\pm 0.82) | ND | 65.66 (\pm 1.34) |
| SA | ND | 1.3 (\pm 0.95) | ND |

Results summarized are the mean \pm standard deviation of $n=3$. β -GLU, β -glucuronidase; OOH, hydroperoxides; ASA, acetyl salicylic acid; SA, salicylic acid; NR, no reaction under experimental conditions; ND, not determined.

Inhibition of Hydroperoxide Formation All the test SCs were found to be excellent to good inhibitors of lipid peroxidation (Table 4). The compound **C8** (62.80%) was ranked as an excellent inhibitor of the formation of hydroperoxides. The other effective samples showed inhibition in a range of 12.70–29.95% as compared to aspirin (65.66%). Lipid peroxidation is a proinflammatory primary event produced by oxidative stress or as a consequence of tissue damage, which can exacerbate tissue injury, due to the potential cytotoxicity and genotoxicity of the end products of lipid peroxidation. Membrane lipids with double bonds are most susceptible to oxidation. Lipid peroxidation can reduce membrane fluidity, leading to increased rigidity throughout the hydrophobic space of membranes, decreased permeability, osmotic fragility and altered activity of certain membrane-bound enzymes and transport systems.³⁹⁾ Conjugated diene (hydroperoxides) formation is one of the intermediate step during lipid peroxidation which takes place as a result of the hydrogen capture from the unsaturated fatty acids.⁴⁰⁾ It has been reported that the donation of electron to the intermediate radicals is the mechanism of inhibition of lipid peroxidation by phenolic antioxidants. The same mechanism has also reported for the inhibition of lipid peroxidation by chalcones.¹¹⁾ The computational studies also supports that the compounds (**C8**, **C1**) having more electron donating potentials (higher E_{HOMO}) are better inhibitors of hydroperoxides but this is not a common property to be applied to all the SCs showing considerable inhibition of lipid peroxidation.

Considering the experimental and *in silico* results of the present investigations it can be concluded that the nucleus 1-(2-hydroxy-3-(2-hydroxy-cyclohexyl)-4,6-dimethoxyphenyl)-methanone can be further explored for the design and development of lead antioxidant and anti-inflammatory agents.

Acknowledgements The Authors are thankful to UGC India for financial assistance and Director, School of Life Sciences, Swami Ramanand Teerth Marathwada University Nanded (MS) for providing the necessary facilities during this work. The software facility provided by HOD, Department of Biotechnology & Bioinformatics, MGM College of Engineering & Technology, Nanded (MS) is duly acknowledged.

References

- 1) Calliste C. A., Le Bail J. C., Trouilas P., Pouget C., Habrioux G., Chulia A. J., Duroux J. L., *Anticancer Res.*, **21**, 3949–3956 (2001).

- 2) Jahng Y., Zhao L., Moon Y., Basnet A., Kim E., Chang H. W., Ju H. K., Jeong T. C., Lee E. S., *Bioorg. Med. Chem. Lett.*, **14**, 2559—2562 (2004).
- 3) Modzelewska A., Pettit C., Achanta G., Davidson N. E., Huang P., Khan S. R., *Bioorg. Med. Chem.*, **14**, 3491—3495 (2006).
- 4) Suksamrarn A., Poomsing P., Aroonrerk N., Punjanon T., Suksamrarn S., Kongkun S., *Arch. Pharm. Res.*, **26**, 816—820 (2003).
- 5) Nam N. H., Kim Y., You Y. J., Hong D. H., Kim H. M., Ahn B. Z., *J. Med. Chem.*, **38**, 179—187 (2003).
- 6) Lee Y. S., Lim S. S., Shin K. H., Kim Y. S., Ohuchi K., Jung S. H., *Biol. Pharm. Bull.*, **29**, 1028—1031 (2006).
- 7) Dominguez J. N., Leon C., Rodrigues J., Dominguez N. G. D., Gut J., Rosenthal P. J., *J. Med. Chem.*, **48**, 3654—3658 (2005).
- 8) Narender T., Khaliq T., Shweta, Nishi, Goyal N., Gupta S., *Bioorg. Med. Chem.*, **13**, 6543—6550 (2005).
- 9) Opletalova V., *Ceska Slov. Farm.*, **49**, 278—284 (2000).
- 10) Gacche R. N., Dhole N. A., Kamble S. G., Bandagar B. P., *J. Enzyme Inhib. Med. Chem.*, **iFirst**, 1—4 (2007).
- 11) Mirinda C. L., Stevens J. F., Ivanov V., McCall M., Frei B., Deinzer M. L., Buhler D. R., *J. Agric. Food Chem.*, **48**, 3876—3884 (2000).
- 12) Won S. J., Liu C. T., Tsao L. T., Weng J. R., Ko H. H., Wang J. P., Lin C. N., *Eur. J. Med. Chem.*, **40**, 103—112 (2005).
- 13) Okezie I. A., *Mutat. Res.*, **9**, 523—524 (2003).
- 14) Palmer H. J., Paulson K. E., *Nutr. Rev.*, **55**, 353—361 (1997).
- 15) Rajnarayana K., Reddy M. S., Chaluvadi M. R., Krishna D. R., *Indian J. Pharmacol.*, **33**, 2—16 (2001).
- 16) Kamble S. G., “Synthetic Manipulation of Biologically Active Molecules,” Ph. D. Thesis, Swami Ramanand Teerth Marathwada University, Nanded (MS) India, 2003.
- 17) Gacche R. N., Gond D. S., Dhole N. A., Dawane B. S., *J. Enzyme Inhib. Med. Chem.*, **21**, 157—161 (2006).
- 18) Nash T., *Biochem. J.*, **55**, 416—421 (1953).
- 19) Bartolome B., Nunez V., Monagas M., Gomez-Cordoves C., *Eur. Food Res. Technol.*, **218**, 173—177 (2004).
- 20) Tandon M., Tandon P., Barthwal J. P., Bhalla T. N., Bhargava K. P., *Drug Res.*, **32**, 1233—1235 (1982).
- 21) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265—275 (1951).
- 22) Gacche R. N., Dhole N. A., *Pharm. Biol.*, **44**, 389—395 (2006).
- 23) Dodge J. T., Mitchell C., Hanaban D. J., *Arch. Biochem. Biophys.*, **100**, 119—130 (1963).
- 24) Quist K. H., *Biochem. Biophys. Res. Commun.*, **92**, 631—637 (1980).
- 25) Buege J. A., Aust S. D., *Methods Enzymol.*, **52**, 302—305 (1978).
- 26) Gulgun A., Canan K., Tulay C., Benay C., Mumtaz I., *J. Enzyme Inhib. Med. Chem.*, **19**, 129—135 (2004).
- 27) Karelson M., Lobanov V. S., *Chem. Rev.*, **96**, 1027—1043 (1996).
- 28) Zhu H., Sedykh A., Chakravarti S. K., Klopman G., *Curr. Computer-Aided Drug Design*, **1**, 3—9 (2005).
- 29) Pop E., Oniciu D. C., Pape M. E., Cramer C. T., Dasseuxb J.-L. H., *Croatica Chem. Acta*, **77**, 301—306 (2004).
- 30) Honorio K. M., da Silva A. B. F., *Int. J. Quantum Chem.*, **95**, 126—129 (2003).
- 31) Nakayama A., Hagiwara K., Hashimoto S., Shimoda S., *Quant. Struct.-Act. Relat.*, **12**, 251—253 (1993).
- 32) Sklenar H., Jager J., *Int. J. Quantum Chem.*, **16**, 467—471 (1979).
- 33) Tuppurainen K., Lotjonen S., Laatikainen R., Vartiainen T., Maran U., Strandberg M., Tamm T., *Mutat. Res.*, **247**, 97—101 (1991).
- 34) Korycka-Dahl M., Richardson M., *J. Dairy Sci.*, **61**, 400—407 (1978).
- 35) Trapnell J. E., *Br. J. Surg.*, **61**, 177—182 (1974).
- 36) Bilfinger T. V., George B. S., *Curr. Pharm. Des.*, **8**, 125—133 (2002).
- 37) Ito K., *Drug Res.*, **32**, 117—122 (1982).
- 38) Savill J., Hasel C., *Semin. Cell Biol.*, **6**, 385—393 (1995).
- 39) Jayaprakasha G. K., Rao L. J., Sakariah K. K., *Bioorg. Med. Chem.*, **12**, 5141—5146 (2004).
- 40) Senevirathne M., Kim S.-H., Siriwardhana J. H. H., Lee K.-W., Jeon Y.-J., *Food Sci. Tech. Int.*, **12**, 27—38 (2006).