Bioorganic Chemistry 56 (2014) 1–7

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Combined experimental and in silico approaches for exploring antiperoxidative potential of structurally diverse classes of antioxidants on docetaxel-induced lipid peroxidation using 4-HNE as the model marker

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ARTICLE INFO

Article history:
Received 11 December 2013
Available online 14 May 2014

Keywords:
Antioxidant
Docetaxel
Lipid peroxidation
4-Hydroxy-2-nonenal
QSAR
Validation

ABSTRACT

The objective of the present work was tantamount to explain the antiperoxidative potential and structural requirements of twenty-eight structurally diverse classes of antioxidants on docetaxel-induced lipid peroxidation. Both experimental and computational approaches were taken to the work. The experiments were performed *in vitro* and goat liver was used as a source of lipid. 4-hydroxy-2-nonenal was used as model marker for estimation of docetaxel–lipid interaction. The computational portion of the work was limited to QSAR analysis of those antioxidants for better understanding of the structural requirements of antioxidants on docetaxel–lipid interaction. The study was done with freely online available 2D descriptors available on PaDEL (open source). Stepwise regression analysis was used as chemometric tool. The experimental study showed the lipid peroxidation induction capacity of docetaxel. It was also noted that all twenty-eight antioxidants had the ability to suppress the lipid peroxidation. But among them butylated hydroxyl toluene showed the highest potential (−20.5%) and flavone showing lowest potential (−0.8%) to suppress the docetaxel-induced lipid peroxidation. The computational study indicates the importance of topology of the whole molecules, topological distances among atoms within a molecule and specific fragment pattern present in a molecule required for inhibition of lipid peroxidation.

1. Introduction

In cancer chemotherapy, drugs are utilized to kill the cancerous cell with little damage to normal cell. But most of the cytotoxic drugs affect the rapidly dividing normal tissue, and produce to a greater or lesser extent toxic side effects such as bone marrow toxicity, impaired wound healing, loss of hair, damage to gastrointestinal epithelium, depression of growth in children, sterility, teratogenicity, kidney damage, reversible liver abnormalities, etc. [1]. Finally, virtually all cytotoxic drugs produce severe nausea and vomiting. These side effects are unwelcome as well as repulsive to patients. Patients in general suffer from fear psychosis. One of the causative factors of anticancer drug mediated damage to normal cells may be drug-induced lipid peroxidation.

Lipid peroxidation is a related free radical process that may occur in the biological system under enzymatic control or non-enzymatically [2–4]. The latter form is linked mostly with cellular damage as a result of oxidative stress [5]. Lipid peroxidation leads to generation of peroxides and hydroperoxides that can decompose to give a wide range of cytotoxic end products most of which are aldehydes as exemplified by molondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), etc. [6]. Free radicals are constantly being produced in the body through various mechanisms and also being removed by endogenous antioxidant defense mechanism that acts by scavenging free radicals, decomposing peroxides and/or binding with pro-oxidant metal ion. Free radical mediated oxidative stress results are usually from deficient natural antioxidant defense. In case of reduced or impaired defense mechanism and excess generation of free radicals that are not counter balanced by endogenous antioxidant defense exogenously administered antioxidants have been proven useful to overcome oxidative damage [7].

Docetaxel is a semi synthetic derivative of paclitaxel which is obtained from the rare Pacific yew tree *Taxus brevifolia* [8]. It is

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http://dx.doi.org/10.1016/j.bioorg.2014.05.002
0045-2068/

primarily used for the treatment of breast, ovarian and non-small cell lung cancer. As docetaxel is a cell cycle specific agent, it is cytotoxic to all dividing cells in the body [9] and produces several toxic side effects due to damage of normal cell like hair follicles, bone marrow and other germ cells. It was reported that docetaxel has the capability of inducing lipid oxidization and membrane damage in human hepatoma cells [10]. Another report shows the beneficial effect of nitroxide pirolin on oxidative stress generated by doxorubicin and docetaxel in blood plasma of rats bearing mammary tumor [11]. Lipid peroxidation induction capacity of drugs may be related to their toxic potential as exemplified by adriamycin-induced cardiotoxicity, which occur through free radical mediated process [12].

Considering the above findings the present work has been assessed in two parts. At first attempts have been made to find out the lipid peroxidation induction capacity of docetaxel on goat liver tissue and explore the beneficial role of twenty-eight antioxidants on docetaxel–lipid interaction. 4-hydroxy-2-nonenal is used as a marker of lipid peroxidation. Secondly QSAR analysis of those structurally diverse classes of antioxidants having lipid peroxidation inhibition potency has been performed to promote our understanding towards structural requirements of antioxidants on docetaxel-induced lipid peroxidation.

2. Materials and methods

2.1. Experimental works

Pure sample of docetaxel used in present study was provided by Fresenius Kabi, Kalyani, India. 2,4-dinitrophenylhydrazine (DNPH) and trichloroacetic acid (TCA) were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi; 2,6-di-tert-butyl-4-methyl phenol (butylated hydroxyl toluene, BHT) and butylated hydroxyl anisole (BHA), alpha tocopherol, ascorbic acid were from Merck, Mumbai; Morin, rutin, dextrose, uric acid were from CDH Pvt. Ltd., New Delhi; Naringin, flavone, flavanone, hesperidin, quercetin, curcumin, caffeic acid were from Himedia Bioscience, Mumbai; Apigenin, chrysin, kaempferol, fisetin, galangin, naringenin, taxifolin, vitexin were from Sigma–Aldrich, St. Louis, MO; Myricetin and *n*-propyl gallate were from SRL, Mumbai; Larycytrin and fustin were from Triveni Aromatics and Perfumery Pvt. Ltd., Vapi; Robinetin was from Clearsynth Labs (P) Ltd., Mumbai. The standard sample of 4-HNE was purchased from ICN Biomedicals Inc., Ohio. All other reagents were of analytical grade.

2.1.1. Preparation of tissue homogenate

The study was performed on goat (*Capra capra*) liver. 4-hydroxy-2-nonenal (4-HNE) content of the tissue sample was used as marker of lipid peroxidation. The goat liver was chosen because of its easy availability and close similarity to the human liver in its lipid profile [13]. Goat liver was collected from the Kolkata Municipal Corporation (KMC) approved outlet. Goat liver perfused with normal saline through the hepatic portal vein was harvested and its lobes were briefly dried between filter papers to remove excess blood and thin cut with a heavy-duty blade. The small pieces were subsequently transferred in a sterile vessel containing phosphate buffer (pH = 7.4) solution. After draining the buffer solution as completely as possible, the liver was immediately ground to make a tissue homogenate (1 g/ml) using freshly prepared phosphate buffer (pH = 7.4) solution [14].

2.1.2. Incubation of tissue homogenate with docetaxel and/or antioxidant

The tissue homogenate consisted of four equal parts. The first portion was kept as control (C), while the second portion was treated with docetaxel (D) at a concentration of 0.143 μM/g wet liver tissue homogenate. The third portion was treated both with docetaxel at a concentration of 0.143 μM/g wet liver tissue homogenate and antioxidant (Alpha-Tocopherol/Apigenin/Ascorbic acid/BHA/BHT/Caffeic acid/Chrysin/Curcumin/Dextrose/Fisetin/Flavanone/Flavone/Fustin/Galangin/Hesperidin/Kaempferol/Larycytrin/Morin/Myricetin/*n*-Propyl gallate/Naringenin/Naringin/Quercetin/Robinetin/Rutin/Taxifolin/Uric acid/Vitexin) at a concentration of 0.189 μM/g wet liver tissue homogenates (DA). The fourth one was treated only with the above mentioned antioxidant alone at a concentration of 0.189 μM/g wet liver tissue homogenate (A). After treatment with docetaxel and/or antioxidant, the different portions of liver homogenate were shaken for 5 h at ambient temperature and 4-HNE content of different proportions were estimated.

2.1.3. Estimation of 4-hydroxy-2-nonenal (4-HNE) level from tissue homogenate

The estimation was replicated in three animal sets. In each set three replicate samples of 2 ml of the incubation mixture were treated with 1.5 ml of 10% TCA solution and centrifuged at 3000 rpm for 30 min. Then 2 ml of the filtrate was treated with 1 ml of 2,4 dinitrophenyl hydrazine (DNPH) (100 mg/100 ml in 0.5 M HCl) and kept for 1 h at room temperature. After that the samples were extracted with hexane and the extract was evaporated to dryness under argon at 40 °C. After cooling to room temperature, 2 ml of methanol was added to each sample and the absorbance was measured at 350 nm against methanol as blank [15]. The values were established from the standard curve [16].

2.1.4. Statistical analysis

The results are expressed as mean of percent changes in various groups with respect to corresponding control along with the standard error. Interpretation of the result is corroborated by analysis of variance (ANOVA) and multiple comparison analysis [17–18].

2.2. Computational works

2.2.1. Data set

The percent changes in 4-HNE content of twenty-eight docetaxel-antioxidant treated groups were invoked as response variable (% DA) for subsequent QSAR analyses (Table 1).

2.2.2. Descriptors

Structures of twenty-eight compounds (Fig. 1) were sketched using ChemDraw Ultra version 6.0 [19] and saved in Mol Format which is one of the suitable input formats for PaDEL. Energies of structural configuration were minimized by AM-1 method using Chem 3D Ultra version 6.0 and used as input structure for descriptor calculations. Only 2D descriptors available on freely available PaDEL were taken into account in the present study [20]. Initially 1660 descriptors were calculated utilizing PaDEL software version 2.12. Then we deleted the descriptors with high intercorrelation (0.95), as well as zero and constant value descriptors. Finally pruned 232 descriptors were selected for QSAR analysis of selected data set. The categorical lists and values of the descriptors present in the developed QSAR model are listed in Supplementary materials.

2.2.3. Model development

For the development of model, the whole data set ($n = 28$) was divided into training ($n = 21$, 75% of the total number of compounds) and test ($n = 7$, 25% of the total number of compounds) sets by *k*-means clustering technique applied on standardized descriptor matrix. The QSAR model was developed using the training set compounds (optimized by Q^2), and then the developed

Table 1
Effects of antioxidant on docetaxel-induced lipid peroxidation: Changes in 4-HNE profile.

Sl. no.	Name of antioxidant	% Changes in 4-HNE content with respect to corresponding control (Average ± SE) (n = 3)			Analysis of variance and multiple comparison
		%D	%DA	%A	
1	Alpha-Tocopherol	18.38 ± 4.87	−8.51 ± 2.48	−13.75 ± 3.82	F1 = 18.35, F2 = 0.75, PV = 48.55, CD = 13.11, RM = (D) (DA, A)
2	Apigenin	11.96 ± 3.31	−7.93 ± 1.09	−9.05 ± 1.51	F1 = 35.08, F2 = 1.63, PV = 11.93, CD = 6.50, RM = (D) (DA, A)
3	Ascorbic acid	10.52 ± 3.07	−15.50 ± 0.26	−10.32 ± 2.11	F1 = 27.70, F2 = 0.04, PV = 20.56, CD = 8.53, RM = (D) (DA, A)
4	BHA	12.28 ± 3.73	−9.94 ± 2.54	−7.35 ± 0.76	F1 = 14.39, F2 = 0.045, PV = 30.77, CD = 10.44, RM = (D) (DA, A)
5	BHT	11.67 ± 3.17	−20.50 ± 0.63	−9.09 ± 1.67	F1 = 162.64, F2 = 6.09, PV = 4.91, CD = 4.17, RM = (D) (DA) (A)
6	Caffeic acid	21.15 ± 4.07	−1.20 ± 0.24	−15.80 ± 1.18	F1 = 47.26, F2 = 0.46, PV = 21.98, CD = 8.83, RM = (D) (DA) (A)
7	Chrysin	21.92 ± 4.23	−17.94 ± 6.8	−18.79 ± 4.5	F1 = 16.06, F2 = 0.51, PV = 101.11, CD = 18.93, RM = (D) (DA, A)
8	Curcumin	13.16 ± 2.19	−7.26 ± 1.84	−12.29 ± 2.61	F1 = 27.18, F2 = 0.253, PV = 20.04, CD = 8.43, RM = (D) (DA, A)
9	Dextrose	10.77 ± 1.34	−15.2 ± 0.11	−5.85 ± 1.47	F1 = 103.05, F2 = 0.38, PV = 5.03, CD = 4.22, RM = (D) (DA) (A)
10	Fisetin	15.01 ± 1.49	−4.56 ± 3.52	−11.19 ± 0.67	F1 = 163.01, F2 = 0.45, PV = 3.41, CD = 3.47, RM = (D) (DA) (A)
11	Flavanone	8.85 ± 1.98	−3.16 ± 0.44	−7.44 ± 0.81	F1 = 39.08, F2 = 0.63, PV = 5.47, CD = 4.4, RM = (D) (DA, A)
12	Flavone	8.71 ± 2.46	−0.80 ± 0.16	−4.69 ± 0.74	F1 = 17.95, F2 = 0.5, PV = 7.96, CD = 5.31, RM = (D) (DA, A)
13	Fustin	18.21 ± 2.8	−10.65 ± 1.1	−13.29 ± 2.96	F1 = 49.74, F2 = 0.91, PV = 18.41, CD = 8.08, RM = (D) (DA, A)
14	Galangin	15.81 ± 1.17	−10.05 ± 1.19	−12.03 ± 2.65	F1 = 60.35, F2 = 0.45, PV = 11.98, CD = 6.51, RM = (D) (DA, A)
15	Hesperidin	7.40 ± 0.61	−4.67 ± 0.77	−7.26 ± 1.08	F1 = 107.87, F2 = 1.75, PV = 1.70, CD = 2.45, RM = (D) (DA) (A)
16	Kaempferol	9.55 ± 1.8	−5.82 ± 1.65	−8.89 ± 2.62	F1 = 18.1, F2 = 0.38, PV = 16.16, CD = 7.57, RM = (D) (DA, A)
17	Larycytrin	17.20 ± 0.89	−6.85 ± 0.89	−7.63 ± 2.08	F1 = 71.64, F2 = 0.14, PV = 8.34, CD = 5.54, RM = (D) (DA, A)
18	Morin	26.71 ± 3.68	−1.80 ± 0.12	−11.63 ± 1.38	F1 = 65.67, F2 = 0.57, PV = 18.11, CD = 8.01, RM = (D) (DA) (A)
19	Myricetin	11.32 ± 3.53	−5.93 ± 2.04	−10.86 ± 2.87	F1 = 11.43, F2 = 0.10, PV = 35.58, CD = 11.23, RM = (D) (DA, A)
20	*n*-Propyl gallate	18.45 ± 1.95	−5.59 ± 1.01	−10.14 ± 1.67	F1 = 92.16, F2 = 0.96, PV = 7.67, CD = 5.21, RM = (D) (DA, A)
21	Naringenin	9.42 ± 2.11	−4.11 ± 0.61	−10.70 ± 1.07	F1 = 51.76, F2 = 0.94, PV = 6.09, CD = 4.65, RM = (D) (DA) (A)
22	Naringin	14.77 ± 3.12	−3.21 ± 1.41	−8.78 ± 2.52	F1 = 17.21, F2 = 0.06, PV = 26.39, CD = 9.67, RM = (D) (DA, A)
23	Quercetin	15.82 ± 2.42	−6.73 ± 0.38	−8.86 ± 0.27	F1 = 109.93, F2 = 1.58, PV = 5.10, CD = 4.25, RM = (D) (DA, A)
24	Robinetin	12.23 ± 2.58	−3.20 ± 0.11	−10.59 ± 2.85	F1 = 18.79, F2 = 0.05, PV = 2.37, CD = 2.89, RM = (D) (DA) (A)
25	Rutin	12.40 ± 1.5	−1.25 ± 0.04	−8.54 ± 1.35	F1 = 143.06, F2 = 3.16, PV = 21.64, CD = 8.76, RM = (D) (DA, A)
26	Taxifolin	25.43 ± 5.44	−7.89 ± 2.92	−16.82 ± 5.32	F1 = 18.83, F2 = 0.52, PV = 79.01, CD = 16.73, RM = (D) (DA, A)
27	Uric acid	11.32 ± 2.21	−1.52 ± 0.16	−9.60 ± 0.27	F1 = 70.43, F2 = 1.14, PV = 4.74, CD = 4.10, RM = (D) (DA) (A)
28	Vitexin	7.96 ± 2.04	−7.20 ± 0.4	−7.24 ± 1.14	F1 = 39.37, F2 = 0.88, PV = 5.85, CD = 4.55, RM = (D) (DA, A)

Averages of three sets; SE = Standard Error (n = 3); Theoretical values of F: p = 0.1 level F1 = 4.32 [df = (2,4)], F2 = 4.32 [df = (2,4)]; p = 0.05 level F1 = 6.94 [df = (2,4)], F2 = 6.94 [df = (2,4)], F1 and F2 corresponding to variance ratio between groups and within groups respectively; D, DA, A indicate docetaxel-treated, docetaxel and antioxidant-treated and only antioxidant-treated respectively; PV = Pooled variance, CD = Critical difference according to least significant procedure (p = 0.05), RM = Ranked means (two means not included within same parenthesis are statistically significantly different at p = 0.05 level).

model was validated (externally) using the test set compounds. Stepwise regression was used as chemometric tool [21]. The stepping criterion was based on F value (F = 4.0 for inclusion; F = 3.9 for exclusion). MINITAB version 14 software [22] was used for the stepwise regression method. K-means clustering, standardization of the variables was conducted in SPSS version 9.0 software [23]. STAISTICA version 7 software [24] was used only for the determination of the leave-one-out (LOO) values of the training set compounds.

2.2.4. Model validation

The statistical qualities of developed equation were judged by calculating several metrics namely determination coefficient (R^2) as a measure of the total variance of the response explained by the regression models (fitting), explained variance ($R_a{}^2$) and variance ratio (F) at specified degrees of freedom (df) [17].

Both internal and external validations are performed to assess to reliability and the predictive potential of the developed model. To determine the predictive quality of the models, models are required to be further validated using different validation techniques: (a) internal validation or cross-validation using the training set compounds, (b) external validation using the test set compounds

2.2.4.1. Internal validation. Internal validation of generated model was performed by the leave-one-out (LOO) procedure (Q^2_{int}) [25]. It can be expressed as follows:

$$Q^2_{int} = 1 - \frac{\sum (Y_{pred} - Y_{obs})^2}{\sum (Y_{obs} - \overline{Y}_{training})^2} \quad \text{(i)}$$

where Y_{pred} and Y_{obs} indicate LOO predicted and observed activity of training set compounds. $\overline{Y}_{training}$ indicates mean of activity of training set respectively.

2.2.4.2. External validation. The external validation of the QSAR model was judged by parameter like $Q^2_{ext(F1)}$ [26]. It is expressed as follows:

$$Q^2_{ext(F1)} = 1 - \frac{\sum (Y_{pred(test)} - Y_{obs(test)})^2}{\sum (Y_{obs(test)} - \overline{Y}_{training})^2} \quad \text{(ii)}$$

where $Y_{obs(test)}$ and $Y_{pred(test)}$ indicate observed and predicted activity of test set compounds. $\overline{Y}_{training}$ and $\overline{Y}_{test}$ indicate mean of activity of training and test set respectively. $n_{training}$ and n_{test} are the number of compounds in training and test set respectively.

2.2.4.3. Further test on external validation. As external validation is the optimum tool for establishing the predictive QSAR models, so besides the above parameters two more external validation parameters were also employed to check the predictive ability of the developed models.

The parameters $\overline{r^2_m}$ and Δr^2_m are utilized to indicate better both the internal and external predictive capacities of a model and to ascertain the proximity to the values of the predicted and observed response data [27–28]. They are calculated as follows:

$$\overline{r^2_m} = (r^2_m + r'^2_m)/2 \quad \text{(i)}$$

$$\Delta r^2_m = |(r^2_m - r'^2_m)| \quad \text{(ii)}$$

where $r^2_m = r^2 * \left(1 - \sqrt{r^2 - r^2_0}\right)$ and $r'^2_m = r^2 * \left(1 - \sqrt{r^2 - r'^2_0}\right)$.

Squared correlation coefficient values between the observed and predicted values of the test set compounds (leave-one out predicted values for training set compounds) with intercept (r^2) and without intercept (r^2_0) were calculated for determination of r^2_m

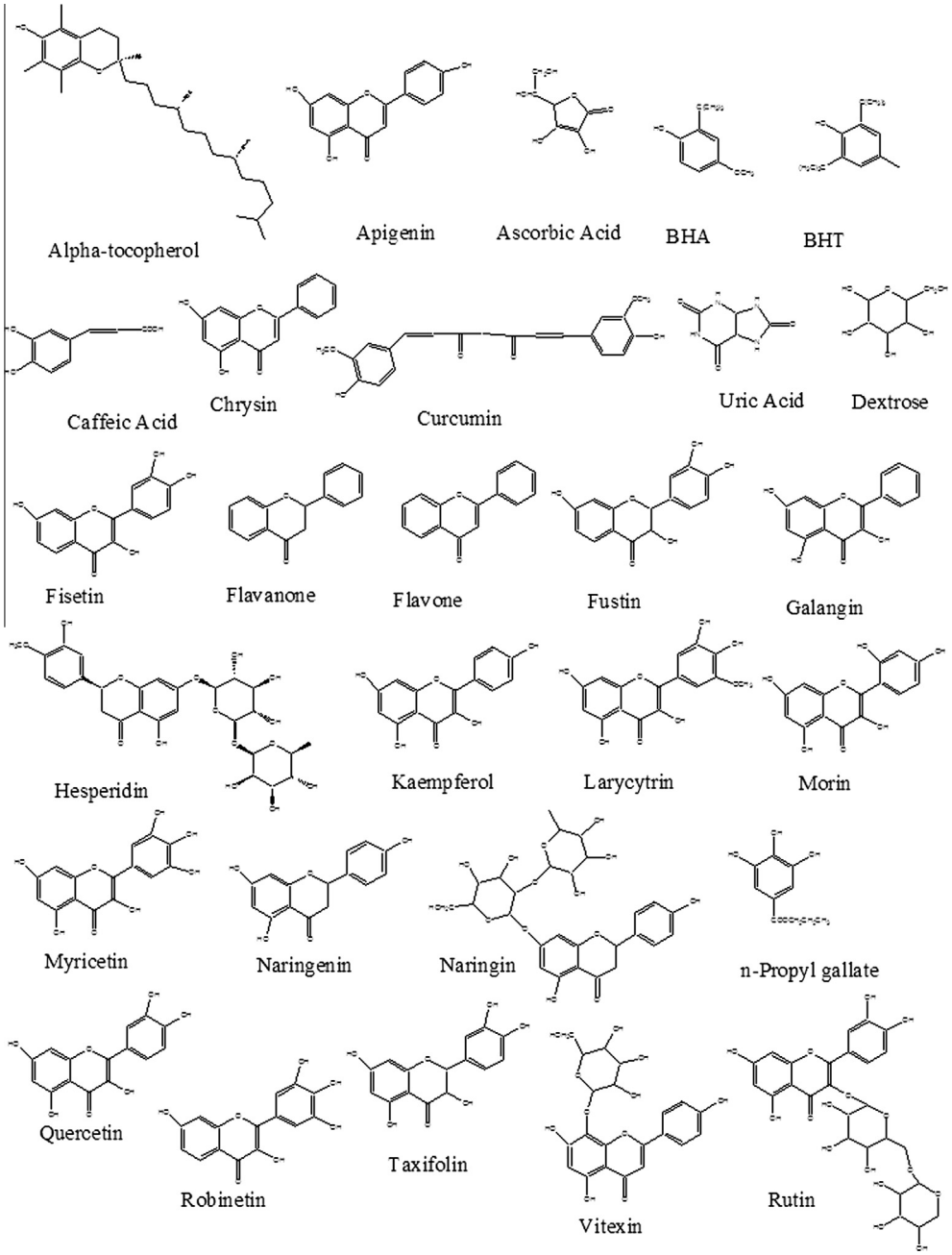

Fig. 1. Structural features of antioxidants.

change of the axes gives the value of r'^2_0 and the r'^2_m metric is calculated based on the value of r'^2_0. The $\overline{r^2_m}$ and Δr^2_m matrices are applied for internal validation of training set compounds ($\overline{r^2_{m(LOO)}}$) as well as $\Delta r^2_{m(LOO)}$, external validation of test set compounds ($\overline{r^2_{m(test)}}$ as well as $\Delta r^2_{m(test)}$) and overall validation for all compounds ($r^2_{m(overall)}$, $\Delta r^2_{m(overall)}$). QSAR models bearing acceptable values for all the traditional parameters can be finally evaluated in terms on the $r_m{}^2$ metrics. Those with $\overline{r^2_m}$ value above the threshold of 0.5 and with a Δr^2_m value less than 0.2 are considered to be predictive and reliable ones.

Again the developed equations are validated applying the parameters proposed by Golbraikh and Tropsha [29]. For a high predictive ability of a developed model the correlation coefficient between actual and calculated activity must be close to one. So the regression of actual activity against calculated activity or calculated activity against actual activity through the origin can be characterized by the slope (k/k'). The slope should be close to one. They can be calculated as follows:

$$k = \frac{\sum y_a y_c}{\sum (y_c)^2} \quad \text{(iii)}$$

$$k' = \frac{\sum y_a y_c}{\sum (y_a)^2} \quad \text{(iv)}$$

where y_a, y_c represent actual and calculated activity respectively.

The criteria for a statistically validated models according to Golbraikh and Tropsha are (i) $Q^2_{int} > 0.5$, (ii) $r^2 > 0.6$, (iii) r^2_0 or r'^2_0 is close to r^2, such that $[(r^2 - r^2_0)/r^2]$ or $[(r^2 - r'^2_0)/r^2] < 0.1$ and $0.85 \leqslant k \leqslant 1.15$ or $0.85 \leqslant k' \leqslant 1.15$).

Additionally, another parameter for model randomization ${}_cR_p{}^2$ was also calculated to ensure that the model is not by chance for 100 scrambling iterations. The parameter ${}_cR_p^2$ which should be more than 0.5 for passing the test is calculated by using the formula

$${}_cR_p^2 = R \times \sqrt{R^2 - R_r^2}$$

where ${R_r}^2$ is mean squared correlation coefficients of the randomized models and R^2 is squared correlation coefficients of non-randomized model [30,31].

3. Results and discussion

3.1. Experimental works

The percent changes in 4-HNE content of different samples at 5 h of incubation were calculated with respect to the control of the corresponding time of incubation and was considered as an indicator of the extent of lipid peroxidation. The results of the studies on docetaxel-induced lipid peroxidation and its inhibition with Alpha-Tocopherol/Apigenin/Ascorbic acid/BHA/BHT/Caffeic acid/Chrysin/Curcumin/Dextrose/Fisetin/Flavanone/Flavone/Fustin/Galangin/Hesperidin/Kaempferol/Larycytrin/Morin/Myricetin/*n*-Propyl gallate/Naringenin/Naringin/Quercetin/Robinetin/Rutin/Taxifolin/Uric acid/Vitexin was shown in Table 1.

It was also evident from Table 1 that tissue homogenates treated with docetaxel showed an increase in 4-HNE (7.4% to 26.71%) content in samples with respect to control to a significant extent. The observations suggest that docetaxel could significantly induce the lipid peroxidation process. 4-HNE is formed due to lipid peroxidation occurring during episodes of oxidant stress, readily forms adducts with cellular proteins; these adducts can be assessed as a marker of oxidant stress in the form of lipid peroxidation [32]. Lipid peroxidation leads to the generation of a variety of cytotoxic products. Moreover it causes disruption of membrane structure and change in fluidity [33]. But the 4-HNE content was significantly reduced (−0.8% to −20.5%) in comparison to control and docetaxel-treated group when tissue homogenates were treated with docetaxel in combination with above mentioned antioxidant. 4-HNE as well as related aldehydes display strong cytotoxicity [34–35] and their effective removal could play an important role in a broad defense system of the liver *in vivo* against damaging effects of lipid peroxidation [36–38]. Again the tissue homogenates was treated only with the above mentioned antioxidant then the 4-HNE level was reduced (−4.69% to −18.79%) in comparison to the control and the docetaxel treated group. This decrease may be explained by the free radical scavenging property of the antioxidant.

It is observed from Table 1 that there are significant differences among various groups (F1) such as docetaxel-treated, docetaxel and antioxidant-treated and only antioxidant-treated group. However within a particular group, differences (F2) are insignificant which shows that there are no statistical differences in goat liver tissue homogenate in a particular group. If *F*-test is significant and more than two treatments are incorporated into the experiment it may not be obvious immediately which treatments are different. To solve the problem multiple comparison analysis is suggested. We are using least significant different procedure [17–18] on the percent changes data of various groups such as docetaxel-treated (D), docetaxel and antioxidant (DA) and only antioxidant-treated (A) with respect to control group of corresponding time. It was observed that the level of 4-HNE in docetaxel-treated group, docetaxel and BHT/caffeic acid/dextrose/fisetin/hesperidin/morin/naringenin/robinetin and uric acid-treated group and only BHT/caffeic acid/dextrose/fisetin/hesperidin/morin/naringenin/robinetin and uric acid-treated groups are statistically significantly different from each other. But docetaxel treated samples (containing 4-HNE) are significantly different from docetaxel and other antioxidants containing samples namely alpha-tocopherol/apigenin/ascorbic acid/BHA/chrysin/curcumin/flavanone/flavone/fustin/galangin/kaempferol/larycytrin/myricetin/*n*-propyl gallate/naringin/quercetin/rutin/taxifolin and vitexin. The docetaxel-treated group is also significantly different from only alpha-tocopherol/apigenin/ascorbic acid/BHA/chrysin/curcumin/flavanone/flavone/fustin/galangin/kaempferol/larycytrin/myricetin/*n*-propyl gallate/naringin/quercetin/rutin/taxifolin and vitexin-treated group. But there is no statistically significantly different among the docetaxel and antioxidant-treated group and antioxidant-treated group.

3.2. Computational works

Membership of compounds in discrete clusters generated using *k*-means clustering technique is given in Supplementary materials. The PCA score plot of first three principal components of the standardized descriptor matrix suggesting that test set compounds lies in the near vicinity of some training set molecules (Fig. 2). In the developed model, difference between R^2 and Q^2 values is not very high (less than 0.3) [39].

$$\%\mathrm{DA} = -20,507 + 1279\mathrm{BCUTw} - 1\,\mathrm{h} - 72\mathrm{VCH} - 6 + 80\mathrm{Petitjean\ Number} - 5.4\mathrm{PubchemFP662} + 12.1\mathrm{ATSc4}$$

$$R^2 = 0.8802, R_a^2 = 0.8403, Q_{int}^2 = 0.7877, F = 22.04(df5, 15), s = 2.17, n_{training} = 21, Q_{ext(F1)}^2 = 0.5167, n_{test} = 7$$

The relative order of importance of the descriptors is this: BCUTw-1h > Petitjean Number > VCH-6 > PubchemFP662 > ATSc4. The equation could explain and predict respectively 84.03 and 78.77% of variance. When this equation was applied for prediction of test set compounds, the predictive R^2 value for the test set was found to be 0.5167.

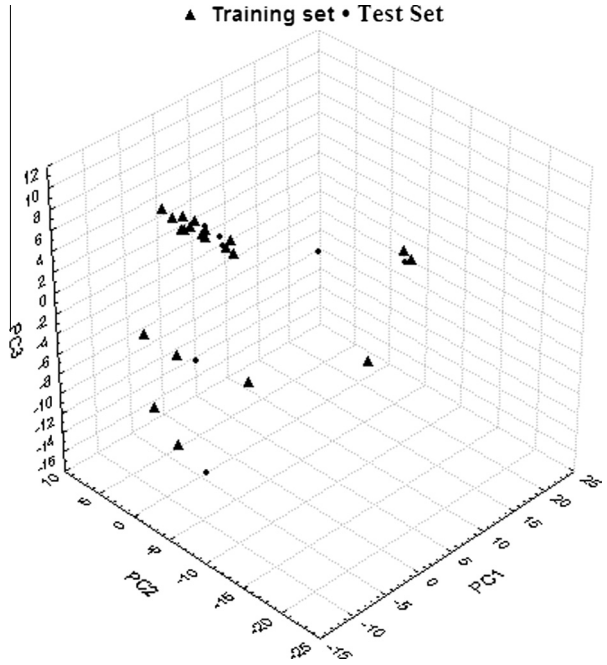

Fig. 2. The PCA score plot of first three principal components of the standardized descriptor matrix.

The positive coefficient of BCUT-1h indicates that activity increases with increase in number of the lowest eigenvalue in highest atom weighed compounds. This descriptor considers both the connectivity and atomic properties of a molecule. The lowest eigenvalue will belong to the totally symmetric representation of the molecular point graph and the eigenvectors will have coefficients belonging to every atom, except in highly symmetric cases. This indicates that the lowest eigenvalues contain contributions from all atoms and so reflect the topology of the whole molecule. Compounds like BHT, chrysin, BHA, alpha-tocopherol, galangin contain heavy atoms and possesses the lowest eigenvalue shows comparatively better activity.

Petitjean Number has a positive contribution towards activity. It is a charge index and expressed as (diameter − radius)/diameter for a particular molecular graph. According to Petitjean, the eccentricity of a vertex is corresponding to the distance from that vertex to the most remote vertex in the graph. The distance is available from the distance matrix as the count of edges between the two vertices. If r_i is the largest matrix entry in row i of the distance matrix D, then the radius is defined as the smallest of the r_i. The graph diameter D is defined as the largest vertex eccentricity in the graph. Compounds like BHT and dextrose having a lowest value of Petitjean Number possesses comparatively higher activity. It shows that in these two molecules eccentricity of vertices is close to each other. So the difference between the diameters to the radius is small, which in turn indicates that the distance between atoms is small.

The parameter VCH-6 signifies the importance of sixth order valence molecular connectivity of chain type and is negatively contributed towards activity. BHT has the lowest value showing the highest activity. Compound like flavone have higher value showing the lowest activity.

The descriptor PubchemFP662 signifying the importance of fragment pattern O–C–C–O–C and has negative contribution towards activity. Molecules like BHT and chrysin do not possess this fragment showing better activity than compounds like ascorbic acid and dextrose where this fragment is present.

The autocorrelation of a topological structure of lag 4 (ATSc4) has a positive coefficient towards activity. The values are calculated considering weight equal to charges. The 2D-autocorrelation descriptors in general explain how the values of certain functions, at intervals equal to the lag d, are correlated. In the case of the descriptors used, lag is the topological distance, and the atomic properties (weight or charge) are the functions correlated. The descriptors can be obtained by summing up the products of definite properties of the two atoms located at a given topological distance or spatial lag. It can be expressed as follows:

$$ATS_d = \sum_{i=1}^{A}\sum_{j=1}^{a}\delta_{ij}.(w_i - w_j)_d$$

where w is any atomic property, A is the atom number, d is the considered topological distance (i.e. the lag in autocorrelation terms), δ_{ij} is the Kronecker delta ($\delta_{ij} = 1$ if $\delta_{ij} = d$, zero otherwise). Compounds like dextrose, ascorbic acid, fustin possesses comparatively higher value showing better lipid peroxidation inhibition potency. In all three molecules the topological distance between two hydroxyl groups which are attached to vicinal carbon atom is four.

4. Overview and conclusions

In this present work both experimental and computational approaches were adopted to explain the antiperoxidative potential and structural requirements of antioxidants on docetaxel-induced lipid peroxidation. The experimental part shows that BHT has the highest potential (−20.5%) and flavone has lowest potential (−0.8%) to suppress the docetaxel-induced lipid peroxidation among twenty-eight structurally diverse classes of antioxidants. The computational study indicates the importance of topology of the whole molecules, topological distances among atoms within a molecule, specific fragment pattern present in a molecule required for the lipid peroxidation inhibition activity. Fig. 3 shows a scatter plot of observed vs calculated/predicted values of the training and test set compounds respectively of the developed model. The model satisfy (Table 2) all the criteria for external validation except the slope (k) value for a test set where it is just near to the threshold value. The intercorrelation among the parameters used in the equation is shown in Table 3 and utmost care was exercised to avoid collinearities among the variables. Finally the multicollinearity for stepwise models was also checked (Table 4). In all cases, variable inflation factor was found to be less than 10 and tolerance value more than 0.1, suggesting absence of multicollinearity. Finally the ${}_cR_p{}^2$ value of 0.730 for model randomization

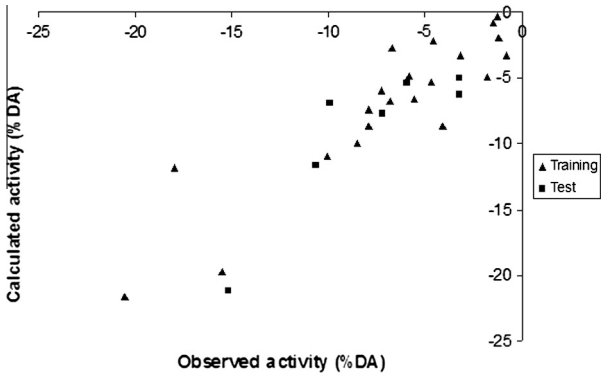

Fig. 3. Scatter plot of observed vs calculated/predicted values of the training and test set compounds respectively of the developed model.

Table 2
Further test on external validation of the QSAR model.

Set	$(r^2 - r_0^2)/r^2$	$(r^2 - r'^2_0)/r^2$	k	k'	$\overline{r_m^2}$	Δr_m^2
Test	0.063	0.000005	0.813	1.15	0.687	0.169
Training	0.0077	0.00859	0.936	0.987	0.738	0.005
Overall	0.015	0.0067	0.897	1.03	0.72	0.0285

Table 3
Intercorrelation among descriptors used in the model from stepwise analysis.

	BCUTw-1h	VCH-6	Petitjean Number	PubchemFP662	ATSc4
BCUTw-1h	1.000	0.562	0.305	0.415	−0.133
VCH-6	0.562	1.000	0.301	0.517	−0.016
Petitjean Number	0.305	0.301	1.000	0.302	−0.190
PubchemFP662	0.415	0.517	0.302	1.000	0.315
ATSc4	−0.133	−0.016	−0.190	0.315	1.000

Table 4
Test for multicollinearity for stepwise regression analysis.

Descriptors	Tolerance	VIF
BCUTw-1h	0.621	1.610
VCH-6	0.578	1.730
Petitjean Number	0.802	1.248
PubchemFP662	0.550	1.818
ATSc4	0.752	1.330

indicates that the model is not by chance. From the total study it was observed that compounds like BHT, chrysin, ascorbic acid, dextrose, fustin, galangin showed promising inhibitory activity against docetaxel-induced lipid peroxidation.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.05.002.

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