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# Design and QSAR study of analogs of α-tocopherol with enhanced antiproliferative activity against human breast adenocarcinoma cells

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**Abstract**

Quantitative structure–activity relationships (QSAR) have been established for two sets of the antitumor drugs, α-tocopherol derivatives. Constitutional, geometrical, physico-chemical and electronic descriptors (using the B3LYP/6-31G (d, p) basis set) were computed and analyzed. The most relevant of these descriptors were grouped and multiple linear regressions have been carried out. QSAR model with four variables, $R^2 = 0.98$ and cross-validation parameter $q^2_{pre} = 0.91$, was selected. Analogs of α-tocopherol (compounds D-1 and D-2) have been designed and their antiproliferative activities were evaluated using the proposed regression model.

Calculated antiproliferative activities of the designed lysine/α-tocopherol/cholesterol conjugates, $IC_{50}$ (D-1) = 2.25 μM and $IC_{50}$ (D-2) = 3.42 μM, were significantly stronger than activities of the other analyzed compounds $IC_{50} > 4$ μM.


*Keywords:* QSAR; α-Tocopherol; Cholesterol; Lysine; Human breast cancer

## 1. Introduction

The biomembranes of lysosomes and mitochondria are important sites for triggering apoptosis of human breast cancer cells [1,2]. Previous studies measured significant activity of α-tocopheryl-lysine derivatives against human breast cancer MCF-7 cell lines (Fig. 1) [3]. The lipophilic cations are selectively accumulated in lysosomes and mitochondria of the breast cancer cells [3–5].

Also, α-tocopheryl-succinate (4, Fig. 2) induced human breast cancer cells to undergo apoptosis *via* destabilization of lysosomal and mitochondrial membranes [6], involvement of transforming growth factor-ß (TGF-ß), Fas (CD95/APO-1) apoptosis *via* mitogen-activated protein kinase (MAPK) and block of anti-apoptotic bcl-2 protein activation [2,7–10]. The charged succinyl moiety together with the long alkyl chain of tocopherol behaves as a typical anionic detergent and interacts with the lysosomal and mitochondrial membranes in cancer cells, causing an increase in the membrane permeability and destabilization of the lysosomes [2,4]. The released lysosomal content causes a drop in the mitochondrial membrane potential and induction of apoptosis [1,2,4,11–13]. Furthermore, α-tocopheryloxy butyric acid (3, Fig. 2), cholesteryl-succinate (7, Fig. 2) and cholesteryloxy butyric acid (5, Fig. 2), have shown significant antiproliferative activity on MCF-7 human breast cancer cells [14].

Since the α-tocopheryl-lysine, α-tocopheryl-succinate and cholesteryl-succinate derivatives (1–10, Figs. 1 and 2) show activity against MCF-7 cells *via* similar pathways, destabilization of lysosomal and mitochondrial membranes, QSAR analysis was performed on these compounds [15]. Constitutional, geometrical, physico-chemical and electronic descriptors (using the B3LYP/6-31G (d, p) basis set) were computed and analyzed. The most relevant of these descriptors were grouped and multiple linear regressions have been carried out.

Analogs of α-tocopherol (compounds D-1 and D-2) have been designed and their antiproliferative activities were evaluated using the proposed regression model.

## 2. Experimental

$IC_{50}$ activity data of the anticancer compounds were collected from literature [3,14], as they were tested using identical 3-(4-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium

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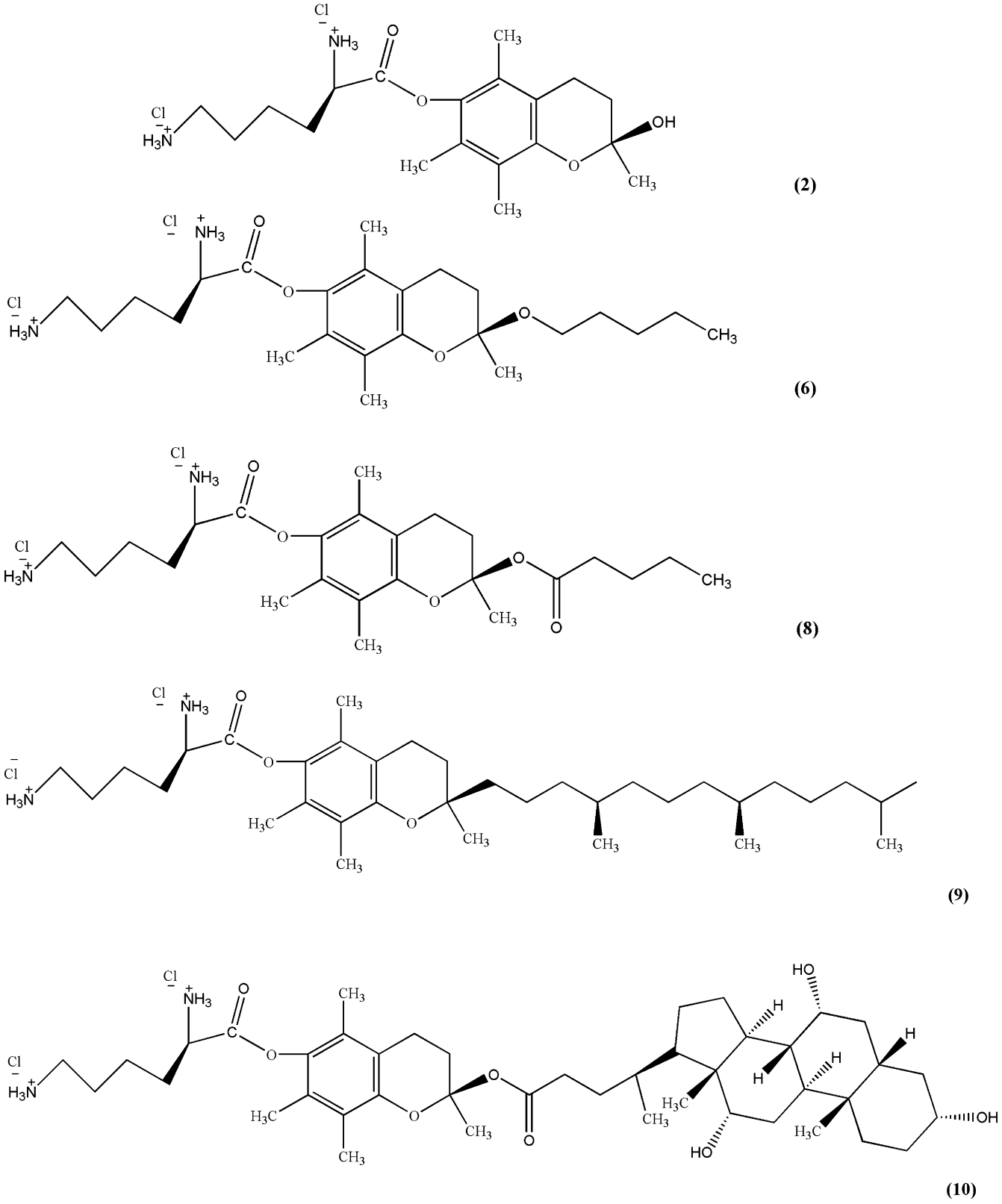


Fig. 1. Chemical structures of lysine-α-tocopheryl compounds.

bromide (MTT) cytotoxicity assay against the MCF-7 cell line. The compounds were selected with an intention of covering a wide range of activity and the logarithm of their $IC_{50}$ values ($\log(IC_{50})$) were calculated.

Minimum energy conformations of the compounds were obtained by using the MOPAC/$PM_3$ method [16,17]. The CS Gaussian 98 program [18] by density functional theory (DFT) using the B3LYP hybrid functional including 6-31G (d, p) basis

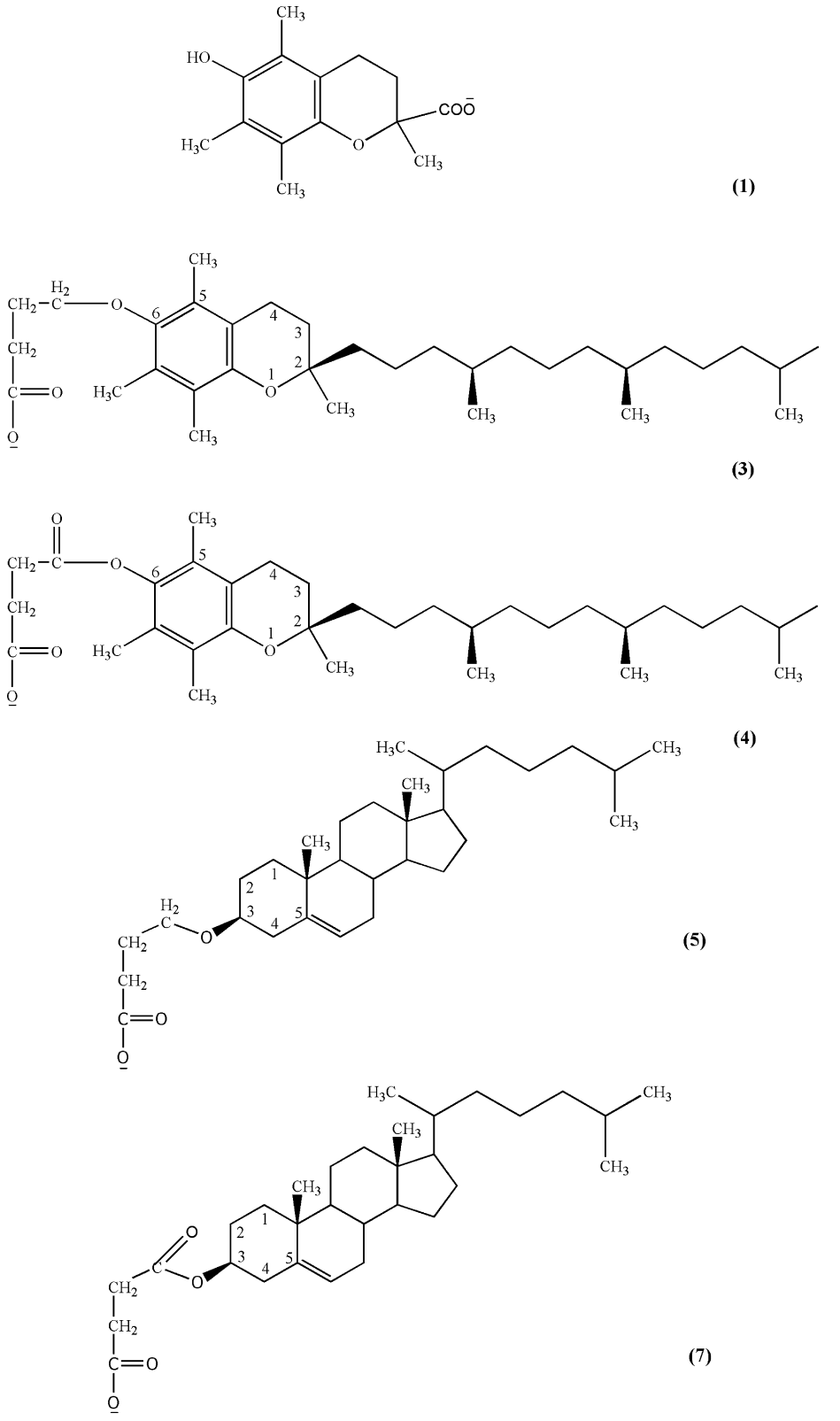


Fig. 2. Chemical structures of trolox anion (1), α-tocopheryloxy-butyric acid anion (3), α-tocopheryl-succinate anion (4), Cholesteryloxy butyric acid anion (5), and Cholesteryl-succinate anion (7).

[19,20] with the Mulliken population analysis and natural population analysis (NPA), was applied for partial atomic charge computations of the optimized models. Their partition coefficient octanol/water ($C \log P$), $pK_a$, and distribution coefficient ($\log D$) were determined by using the ChemPro, Marvin 4.0.5 ChemAxon and MM2 programs, respectively.

The selected Gaussian methods for examination of the models have proven to be a very good choice to predict

the molecular parameters of related aromatic compounds [21–28].

The correlation coefficients for all calculated molecular parameters with inhibition activities ($\log(IC_{50})$) were determined. The molecular properties with the highest correlation coefficients were selected for multiple linear regression study. Single and multivariable linear regression models were developed for the data set by use of the Microsoft-Excel 2000/Regression Data Analysis and Multi-precision Floating Point Computation for Excel (XNUMBERS.XLA-Ver.4.7-2006).

The quality of the regression fits was estimated using parameters such as the regression factor ($r$), square of regression factor ($R^2$), adjusted square of regression factor ($R^2_{adj}$), $q^2_{pre}$ (validation $R^2$), $F$ ratio, and $P$ values [29].

One measure of performance of a model is its ability to make predictions. In this context, withhold-1 cross-validation of the created models was carried out. In this setting, Allen defined predicted sum of squares (PRESS) as:

$$\text{PRESS} = \sum_{i=1}^{n} e^2_{(i)} \quad (1)$$

$$q^2_{pre} = 1 - \frac{\text{PRESS}}{\text{SST}_o} \quad (2)$$

Models with $q^2_{pre} \geq 0.6$ can be considered to have good predictive capability [29].

## 3. Results and discussion

Since the examined molecules very easily undergo ionization at physiological pH (5.0–7.0) [15], was decided to use the ionic forms of the anticancer compounds as models in the QSAR study.

Relatively high correlation factors between $IC_{50}$ values [14] and calculated partial charges (B3LYP/6-31G(d, p)$^{water}$) of carboxyl-O ($r$: 0.982), and carboxyl-C ($r$: 0.819) in succinic/butyric acid derivatives (Table 1) selected the free carboxyl group as the active group in the acidic moiety derivatives of the compounds. Also, good correlations between $IC_{50}$ values [3] and calculated partial charges (B3LYP/6-31G(d, p)$^{water}$) of ammonium-N ($r$: −0.907) and ammonium-H ($r$: −0.813) in lysine derivatives (Table 1) indicated on ammonium group as the active group in the lysine moiety of the examined structures.

Distances between the positively charged ammonium group and methyl groups in the side chain of the lysine conjugates were similar with distances between negatively charged carboxyl group and methyl groups in the aliphatic chain of the succinic/butyric acid derivatives (R.S.D. < 7%) [15].

Therefore, it was supposed that the succinic/butyric acid and lysine compounds (Figs. 1 and 2) could express anticancer activity *via* similar pathway, as ionic detergents that selectively destabilizing lysosomal and mitochondrial membranes of the cancer cells.

The succinic/butyric acid and lysine compounds, with at least three methyl groups in the side chain expressed high positive correlation between their solubility in water at physiological pH 5.0 ($r$: 0.979) [15] and anticancer activity on MCF-7 breast cancer cells [3,14].

Also, O-6 partial charges (B3LYP/6-31G (d, p) $^{gas}$, Mulliken population analysis) of tocopherol derivatives (2, 3, 4, 6, 8, 9, and 10) expressed good agreement with the $\log(IC_{50})$ values ($r$: −0.702).

A QSAR model with four variables was created, number of methyl groups in the side chain, liposolubility ($\log D_{pH\ 5.0}$), partial O-6/O-3 charges and dipole moment attempted to fit the data with the corresponding regression parameters $r$, $R^2$ (0.98), $R^2_{adj}$, $q^2_{pre}$, $F$ ratio, and $P$ values. The multivariable model has succeeded the performed cross-validation with a $q^2_{pre}$ value of 0.91 (Table 2).

Thus was recommended the regression equation,

$$\text{Log}(IC_{50}) = -4.180 - 0.186(N_{CH_3}) + 0.021(\log D_{pH\ 5.0}) - 13.081(\text{O-charge}) - 0.047(\text{dipole})$$

as optimal model for anticancer activities evaluation of novel compounds.

Since increased flow of cholesterol into mitochondria is associated with breast cancer progression [30], it was supposed that incorporation of a cholesterol moiety into the structures of

Table 1
Correlation analysis between the calculated partial atomic charges (B3LYP/6-31G(d, p)$^{water}$ Onsager solvation model, Mulliken population analysis) and the antiproliferative activities of the succinic/butyric acid and lysine derivatives, obtained by MTT assay on MCF-7 cells [3,14]

| Succinic/butyric acid derivatives | Carboxyl-O-part charge | Carboxyl-C4-part charge | Carbonyl-O-part charge | $IC_{50}$ [μM] [14] |
|---|---|---|---|---|
| 3 | −0.55219 | 0.52576 | −0.57140 | 45 ± 15 |
| 4 | −0.56767 | 0.52593 | −0.54760 | 30 ± 10 |
| 5 | −0.58053 | 0.51425 | −0.56711 | 25 ± 7 |
| 7 | −0.58304 | 0.50884 | −0.56978 | 20 ± 5 |
| Correlation factor, $r$ | 0.982 | 0.819 | −0.105 | |

| Lysine derivatives | Amino-N-part charge | Amino-H-part charge | $IC_{50}$ [μM] [3] |
|---|---|---|---|
| 2 | −0.62228 | 0.25170 | 194 ± 62 |
| 6 | −0.51671 | 0.38027 | 22 ± 6 |
| 8 | −0.51001 | 0.37713 | 15 ± 1 |
| 9 | −0.55817 | 0.30152 | 12 ± 2 |
| 10 | −0.51893 | 0.38029 | 4 ± 1 |
| Correlation factor, $r$ | −0.907 | −0.813 | |

Table 2
Regression analysis relating the four variables, number of methyl groups in the side chain, liposolubility (log $D_{pH\ 5.0}$), partial charges on the (O-6 or O-3) oxygens (B3LYP/6-31G(d, p)$^{gas}$, Mulliken population analysis) and dipole moment (B3LYP/6-31G(d, p)$^{gas}$, NPA), with anticancer activity

| Compound | $IC_{50}$[μM] [3,14] | Log(IC50) | No. $CH_3$ sites | Log *D* at pH 5.0 | O-6/O-3 charge (Mulliken) | Dipole (NPA) |
|---|---|---|---|---|---|---|
| 1 | 1461 | 3.165 | 0 | 1.97 | −0.57057 | 4.574 |
| 2 | 194 | 2.288 | 1 | −3.00 | −0.55241 | 12.803 |
| 3 | 22 | 1.653 | 4 | 8.80 | −0.56522 | 21.790 |
| 4 | 15 | 1.477 | 4 | 8.13 | −0.56315 | 21.284 |
| 5 | 20 | 1.398 | 3 | 6.83 | −0.51333 | 16.451 |
| 6 | 25 | 1.342 | 2 | −1.12 | −0.53626 | 19.995 |
| 7 | 45 | 1.301 | 3 | 6.11 | −0.49398 | 12.058 |
| 8 | 30 | 1.176 | 2 | −1.43 | −0.53463 | 23.583 |
| 9 | 12 | 1.079 | 4 | 3.00 | −0.53630 | 23.890 |
| 10 | 4 | 0.602 | 4 | 0.65 | −0.54573 | 37.354 |
| Regression equation | | Log(IC50) = −4.180 − 0.186 (No.$CH_3$) + 0.021 (log D at pH 5.0) − 13.081 (O-charge) − 0.047 (dipole) | | | | |
| $R^2$ | | | | | | 0.980 |
| $R^2_{adj}$ | | | | | | 0.963 |
| $q^2_{pre}$ | | | | | | 0.912 |
| *F*-ratio | | | | | | 59.890 |
| *P*-value | | | | | | 0.0002 |

| Designed compounds | Evaluated $IC_{50}$[μM] | Evaluated log(IC50) | No. $CH_3$ sites | Log *D* at pH 5.0 | O-6/O-3 charge (Mulliken) | Dipole (NPA) |
|---|---|---|---|---|---|---|
| D-1 | 2.249 | 0.352 | 4 | 2.12 | −0.54602 | 40.657 |
| D-2 | 3.420 | 0.534 | 4 | 1.28 | −0.55635 | 39.289 |

$IC_{50}$ concentration, which inhibits the proliferation of MCF-7 cells in culture by 50%, obtained by MTT assay [3,14].

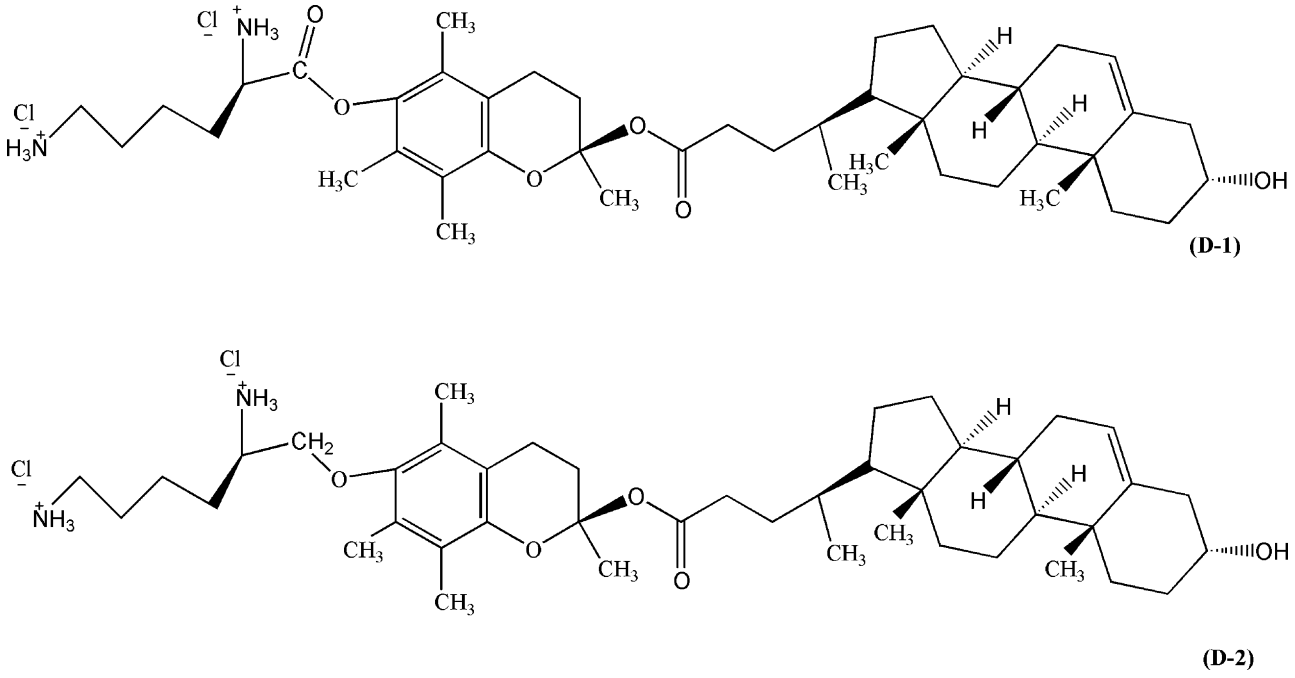


Fig. 3. Chemical structures of designed α-tocopherol analogs.

α-tocopheryl-lysine compounds could facilitate their interaction with mitochondrial membranes and enhance their activities. Based on this prediction, novel lysine/α-tocopherol/cholesterol conjugates (D-1 and D-2, Fig. 3) have been designed and their antiproliferative activities were calculated using the proposed regression model.

Evaluated antiproliferative activities of the designed lysine/α-tocopherol/cholesterol conjugates, $IC_{50}$ (D-1) = 2.25 μM and $IC_{50}$ (D-2) = 3.42 μM, were significantly stronger than activities of the other analyzed compounds with $IC_{50} > 4$ μM (1–10, Figs. 1 and 2).

## 4. Conclusion

The charged succinyl or lysine compounds with lipophilic α-tocopherol/cholesterol moieties behave as typical anionic or

cationic detergents able to selectively interact with the lysosomal or mitochondrial membranes of the human breast cancer cells and cause destabilization of the organelles and apoptosis. Performed quantitative structure-activity relationship study of the α-tocopherol/cholesterol derivatives selected number of methyl groups in the side chain, liposolubility ($\log D_{\mathrm{pH\ 5.0}}$), partial charges on the (O-6 or O-3) oxygens and dipole moment, as important factors for their efficient anticancer activity.

As incorporation of cholesterol moiety into the structures of α-tocopheryl-lysine compounds could facilitate interaction with mitochondrial membranes and enhance their activity, novel lysine/α-tocopherol/cholesterol conjugates (D-1 and D-2) were designed. The developed regression model has predicted stronger antiproliferative activities of the designed α-tocopherol analogs, $IC_{50} < 3.42\ \mu M$.

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