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Design and QSAR study of analogs of γ-tocotrienol with enhanced antiproliferative activity against human breast cancer cells

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ABSTRACT

Quantitative structure-activity relationships (QSAR) study has been performed for two sets of the antitumor drugs against human breast cancer MCF-7 cell lines, α-tocopherol and cholesterol derivatives. Constitutional, geometrical, physico-chemical and electronic descriptors (using the density functional theory, 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. Optimal QSAR models with three and four variables, $R^2 > 0.95$ and cross-validation parameter $q^2_{pre} > 0.88$, were selected. Based on the QSAR study, novel vitamin-E derivatives (compounds **D-1** and **D-2**) were designed and their antiproliferative activities were evaluated using the proposed regression models.

Calculated antiproliferative activities of the designed compounds, IC_{50} (**D-1**): 3.09 μM and IC_{50} (**D-2**): 3.54 μM, were significantly stronger than anticancer effect of the other analyzed compounds IC_{50}: 4–1461 μM.

1. Introduction

The biomembranes of lysosomes and mitochondria are important sites for triggering apoptosis of human breast cancer cells [1,2]. Previous studies were measured significant activity of α-tocopheryl-lysine derivatives against human breast cancer MCF-7 cell lines (**2**, **6**, **8**–**10**, 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) inducing human breast cancer cells to undergo apoptosis *via* destabilization of lysosomal and mitochondrial membranes [6], involvement of transforming growth factor-β (TGF-β), 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 long alkyl chain of tocopherol behaves as typical anionic detergents and interacts with the lysosomal and mitochondrial membranes in cancer cells, causing increase in the membrane permeability and destabilization of the lysosomes [2,4]. The released lysosomal content cases drop in the mitochondrial membrane potential and induction of apoptosis [1,2,4,11–13]. Furthermore, α-tocopheryloxy butyric acid, cholesteryl-succinate and cholesteryloxy butyric acid, have expressed significant antiproliferative activity on MCF-7 human breast cancer cells [14].

Previous cytological study of human breast cancer cells indicated on acidification of extracellular medium of cancer tissue (pH 5.0) [15]. Thus was calculated pK_a, microspecies distribution in function of pH, percentage of ionic form in function of pH (from 5.0 to 7.0), and distribution coefficient (log *D* from pH 5.0 to 7.0) of the analyzed compounds.

Since the α-tocopheryl-lysine, α-tocopheryl-succinate and cholesteryl-succinate derivatives (**1–10**) are expressing activity against MCF-7 cells *via* similar pathways, destabilization of lysosomal and mitochondrial membranes, was previously developed QSAR models for these compounds [16]. Based on the QSAR study [16], novel vitamin-E derivatives (compounds **D-1** and **D-2**) were designed and their antiproliferative activities were evaluated using the proposed regression models.

2. Experimental

Anticancer activity data (IC_{50}) for the α-tocopherol and cholesterol derivatives were collected from literature [3,14], as they were tested using identical 3-(4-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium 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}, i.e. ($\log(IC_{50})$) values were calculated.

The pK_a calculation and selection of dominant molecules/cations at physiological pH 5.0–7.4, was performed for all examined compounds (**1–10**, **D-1** and **D-2**) using the Marvin 4.0.5 ChemAxon program [17]. The computer program Marvin

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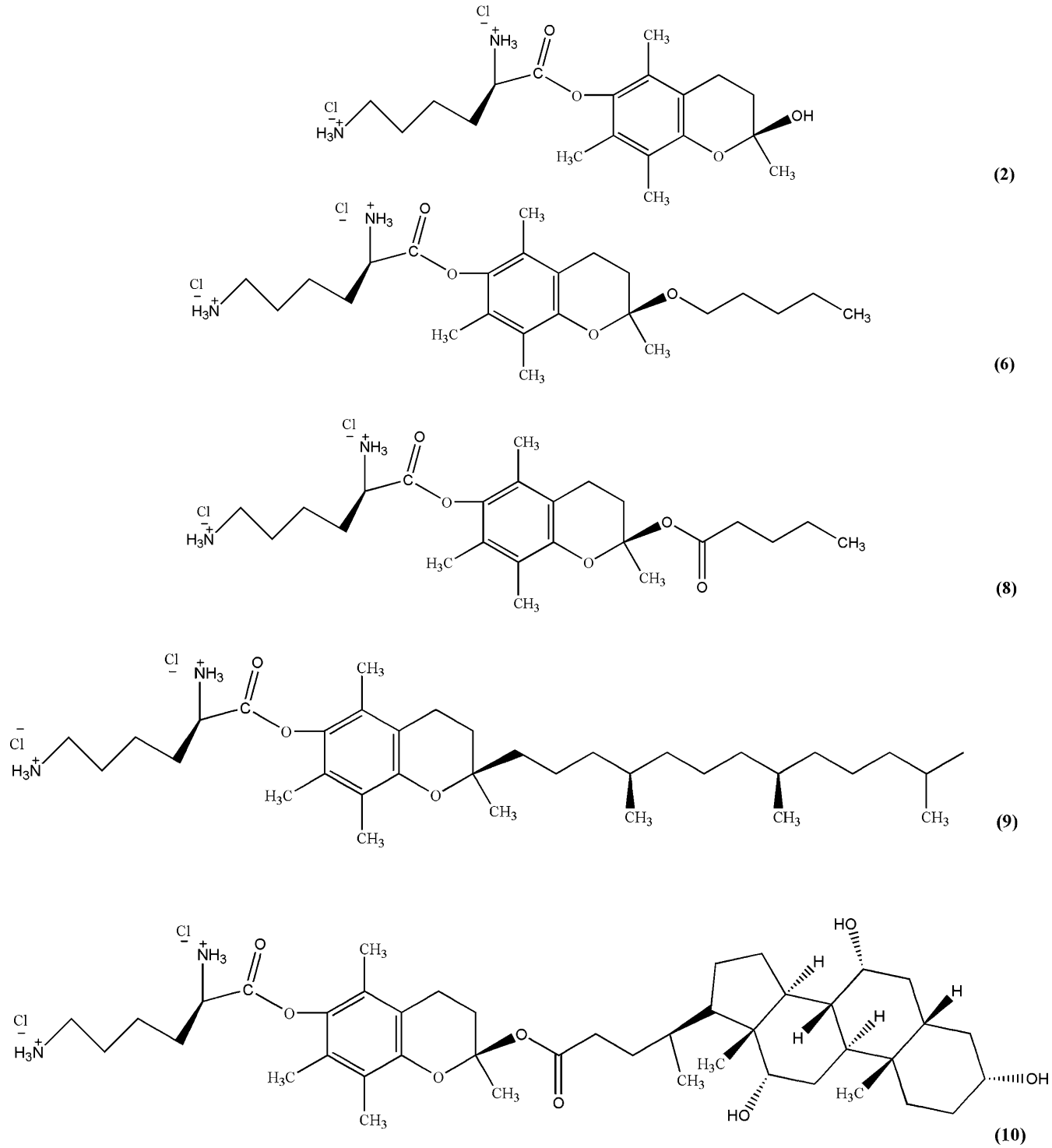

Fig. 1. Chemical structures of lysine-α-tocopheryl compounds (major microspecies at physiological pH).

4.0.5 uses computational algorithms based on the fundamental chemical structure theory to estimate a variety of chemical reactivity parameters.

Minimum energy conformations of all analyzed compounds (**1**–**10**, **D-1** and **D-2**) were obtained by using the MOPAC/PM_3 method [18,19]. The CS Gaussian 98 program [20] by density functional theory (DFT) using the B3LYP hybrid functional including 6-31G (d,p) basis [21,22] 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 [23] and Marvin 4.0.5 ChemAxon programs [17].

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 [24–31].

Furthermore, the partition coefficient octanol/water ($\log P$), pK_a, distribution coefficient ($\log D$), isoelectric point, total charge, and other electronic, steric and thermodynamic properties were

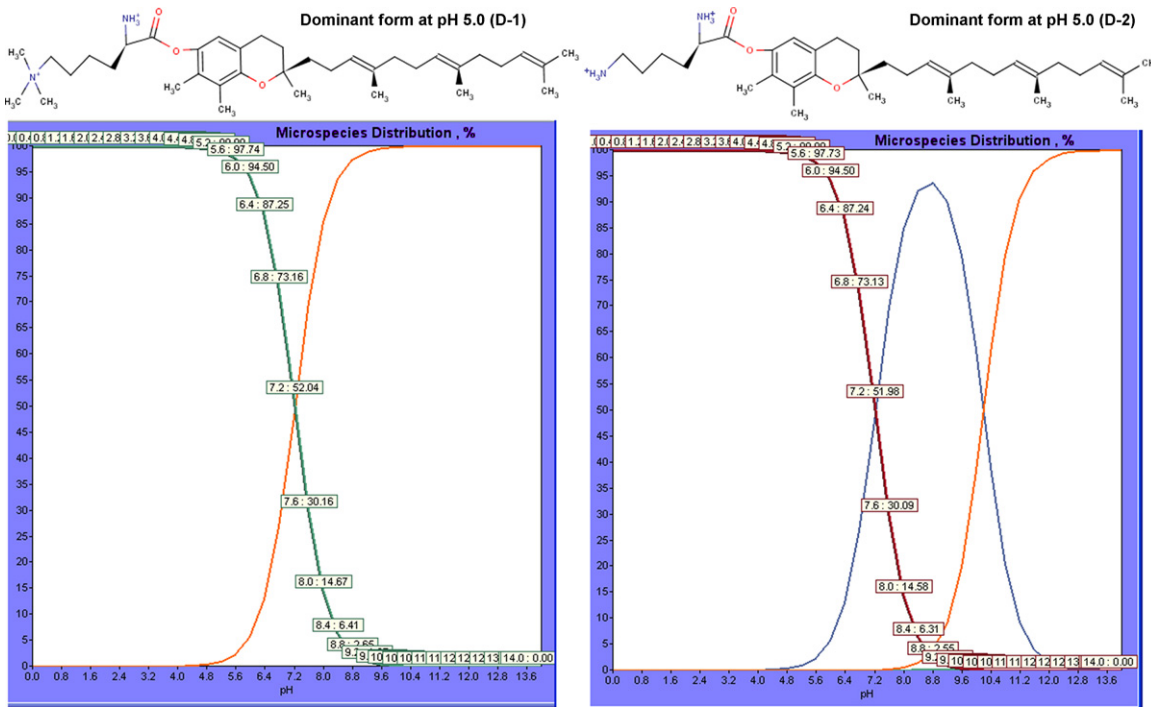

Fig. 2. Microspecies distribution in function of pH (calculated by use of the Marvin 4.0.5 ChemAxon program [17]).

determined by use of the ChemAxon Marvin 4.0.5 [17] and ChemProp [23] programs.

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) [32].

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}), root mean square error of estimation (RMSEE), root mean square error of prediction (RMSEP), q^2_{pre} (validation R^2), F ratio, and P-values [33].

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 PRESS (Predicted Sum of Squares), RMSEP and q^2_{pre} as

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

$$\mathrm{RMSEP} = \sqrt{\frac{\mathrm{PRESS}}{n}} \quad (2)$$

$$q^2_{pre} = 1 - \frac{\mathrm{PRESS}}{\mathrm{SSTo}} \quad (3)$$

Models with $q^2_{pre} \geq 0.6$ can be considered to have good predictive capability [33]. Student t-test was performed for each descriptor in the developed regression models in order to test any significant relationship between y and x.

$$t = \frac{b\sqrt{S_{xx}}}{s_{yx}} \quad (4)$$

$$S_{xx} = \sum_{i=1}^{n} (x_i - \bar{x})^2$$

b, coefficient for the variable in the regression equation.

The value of t to be used is t for $\alpha/2 = 0.025$, for a given number of degrees of freedom, $n - 2$. The calculated P-value in the t-test, for each descriptor in the regression model, is actually a probability to reject the null hypothesis Ho: b (or β) = 0 (*there is no any significant relationship between the variable and y in the regression model*). If the P-value in the t-test for the descriptor in the regression model is above the critical value $\alpha/2 = 0.025$, the null hypothesis Ho:b (or β) = 0 is accepted and the variable is considered as unimportant for the regression.

PLS-regression (PLSR), recently developed generalization of multiple linear regression (MLR) [34], has been used for calculation of variable importance in the projection (VIP).

In multilinear modeling, a summary of the importance of every variable (x_k) for both Y and X matrices is given by VIP_k parameter. The x-variables with VIP value larger than 1 are the most relevant for explaining the regression model, the x-variables with $1.0 > \mathrm{VIP} > 0.5$ are moderately influential, while x-variables with VIP value smaller than 0.5 are not relevant for the model [34].

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 active site in acidic moiety of the compounds. Also, good correlation between IC_{50} values [3] and the 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 active group in lysine moiety of the examined structures.

Distances between positively charged ammonium group and methyl groups in side chain of the lysine conjugates were similar with distances between negatively charged carboxyl group and methyl groups in aliphatic chain of the succinic/butyric acid derivatives (RSD <7%) [16].

Table 1
Correlation analysis between the calculated partial atomic charges (B3LYP/6-31G (d,p)^{water}, Onsager salvation 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 charge	Carboxyl-C4 charge	Carbonyl-O charge	IC_{50} [μM] [14]
α-Tocopheryloxy-butiric acid anion (**3**)	−0.55219	0.52576	−0.57140	45 ± 15
α-Tocopheryl-succinate anion (**4**)	−0.56767	0.52593	−0.54760	30 ± 10
Cholesteryloxy-butiric acid anion (**5**)	−0.58053	0.51425	−0.56711	25 ± 7
Cholesteryl-succinate anion (**7**)	−0.58304	0.50884	−0.56978	20 ± 5
Correlation factor-*r*	0.982	0.819	−0.105	

Lysine/α-Tocopherol derivatives	Ammonium N_ε^+ 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	

Therefore was supposed that the succinic/butyric acid and lysine compounds (**1**–**10**) 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 side chain expressed high positive correlation between their solubility in water at physiological pH 5.0 (*r*: 0.979) [16] 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).

For all selected variables were performed calculation of VIP parameter. The number of methyl groups (no. of CH_3, VIP: 1.2238) and dipole moment (VIP: 1.2944) have expressed the strongest influence on the multilinear regression model (VIP > 1), while partial O6/O3 atomic charge (VIP: 0.7864) and log $D_{pH\ 5.0}$ (VIP: 0.5035) are moderately influential on the model. Therefore, we decided to create QSAR models with different combination of these four variable and compare obtained statistical and cross-validation parameters. Three QSAR models with the best regression and predictive capacity were selected.

The QSAR model-1 with four variables, number of methyl groups in side chain (no. of CH_3), liposolubility (log $D_{pH\ 5.0}$), partial charges on the O-6 (tocopherol's derivatives) or O-3 (cholesterol derivatives) oxygens and dipole moment, attempted to fit the data with the corresponding regression parameters R^2 (0.98), R^2_{adj} (0.96), RMSEE (0.10), *F* ratio (59.89), and *P*-values (0.0002). The QSAR model-1 has succeeded the performed cross-validation with q^2_{pre} values 0.91 (Table 2).

Furthermore, QSAR model-2 with three independent variables, number of methyl groups in side chain (no. of CH_3), liposolubility (log $D_{pH\ 5.0}$) and partial O-6/O-3 charges, was developed with corresponding regression parameters R^2 (0.94), R^2_{adj} (0.91), RMSEE

Table 2
Multilinear regression analysis relating the four variables, number of methyl groups in side chain, liposolubility (log $D_{pH\ 5.0}$), partial charges on the (O-6 or O-3) oxygen (B3LYP/6-31G (d,p)^{gas}, Mulliken population analysis) and dipole moment (B3LYP/6-31G(d,p)^{gas}, NPA), with anticancer activity, log(IC_{50}) [3,14].

Compound	log(IC_{50})[a]	No. of CH_3 sites	Log *D*, pH 5.0	O6/O3 Charge (Mulliken)	Dipole (NPA)
1	3.165	0	1.97	−0.57057	4.574
2	2.288	1	−3.00	−0.55241	12,803
3	1.653	4	8.80	−0.56522	21,790
4	1.477	4	8.13	−0.56315	21,284
5	1.398	3	6.83	−0.51333	16,451
6	1.342	2	−1.12	−0.53626	19,995
7	1.301	3	6.11	−0.49398	12,058
8	1.176	2	−1.43	−0.53463	23,583
9	1.079	4	3.00	−0.53630	23,890
10	0.602	4	0.65	−0.54573	37,354
Variable Importance for the Projection (VIP)		1.2238	0.5035	0.7864	1.2944

Regression equation, QSAR model-1: log(IC_{50}) = −4,180 − 0.186 (no. of CH_3 sites) + 0.021 (log $D_{pH\ 5.0}$) − 13,081 (O6/O3-charge) − 0.047 (dipole)
Regression Equation, QSAR model-2: log(IC_{50}) = −2.673 − 0.530 (NCH_3) + 0.093 (log $D_{pH\ 5.0}$) − 9.930 (O6/O3-charge)
Regression Equation, QSAR model-3: log(IC_{50}) = −4.539 − 0.102 (NCH_3) − 13.805 (O6/O3-charge) − 0.0572 (dipole)

Statistical and leave-one out validation parameters for QSAR models	QSAR model-1	QSAR model-2	QSAR model-3
R^2	0.980	0.940	0.977
R^2_{adj}	0.963	0.911	0.966
RMSEE	0.096	0.165	0.102
q^2_{pre}	0.912	0.879	0.910
F-ratio	59.890	31.522	85.214
P-value	0.0002	0.0004	2.615E−05

Designed compounds	Evaluated log(IC_{50}), QSAR model-2	No. of CH_3 sites	Log *D*, pH 5.0	O6/O3 Charge (Mulliken)	Dipole (NPA), out of QSAR model-1 range	Eval-IC_{50} [μM], QSAR model-2	Ammonium N_ε^+ charge
N,N,N-Trimethyl-Lys-gamma-tocotr (**D-1**)	0.490568	4	0.99	−0.52117	82.556	3.094	−0.40486
Lys-gamma-tocotr (2+) (**D-2**)	0.548487	4	1.36	−0.52302	87.200	3.536	−0.51753

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

Table 3
Student *t*-test results for each variable in the QSAR models **1**–**3** and confidence intervals for the coefficients values in the regression equations (QSAR models **1**–**3**).

	Coefficients	Standard Error	*t*-Stat	*P*-value	Lower coefficient value (95%)	Upper coefficient value (95%)
Statistical parameters for regression equation (QSAR model-1)						
Intercept	−4.180674	1.172093	−3.566843	0.016097	−7.193631	−1.167716
No. of CH_3 sites	−0.186108	0.118113	−1.575672	0.175922	−0.489727	0.117512
O6/O3 charge (Mulliken)	−13.080712	2.182575	−5.993248	0.001855	−18.691190	−7.470234
Log *D*, pH 5.0	0.020869	0.026759	0.779877	0.470750	−0.047917	0.089655
Dipole (NPA)	−0.046955	0.015162	−3.096957	0.026946	−0.085929	−0.007981
R^2	0.980	R^2_{adj}	0.963	q^2_{pre}	0.912	
Statistical parameters for regression equation (QSAR model-2)						
Intercept	−2.673111	1.662719	−1.607675	0.159031	−6.741640	1.395418
No. of CH_3 sites	−0.529849	0.062984	−8.412490	0.000154	−0.683964	−0.375733
O6/O3 charge (Mulliken)	−9.930300	3.011303	−3.297676	0.016455	−17.298699	−2.561902
Log *D*, pH 5.0	0.092850	0.020678	4.490257	0.004147	0.042252	0.143447
R^2	0.940	R^2_{adj}	0.911	q^2_{pre}	0.879	
Statistical parameters for regression equation (QSAR model-3)						
Intercept	−4.539300	1.042325	−4.354974	0.004797	−7.089780	−1.988820
No. of CH_3 sites	−0.101699	0.045720	−2.224398	0.067786	−0.213572	0.010173
O6/O3 charge (Mulliken)	−13.805500	1.909256	−7.230826	0.000355	−18.477285	−9.133715
Dipole (NPA)	−0.057225	0.007264	−7.878325	0.000222	−0.074998	−0.039452
R^2	0.977	R^2_{adj}	0.966	q^2_{pre}	0.915	

(0.16), *F* ratio (31.52) and *P*-value (0.0004). The QSAR model-2 has passed the performed cross-validation with q^2_{pre} values 0.88 (Table 2).

Finally, QSAR model-3 with three independent variables, number of methyl groups in side chain (no. of CH_3), partial O-6/O-3 charges and dipole moment was obtained with corresponding regression parameters R^2 (0.98), R^2_{adj} (0.97), RMSEE (0.10), *F* ratio (85.21) and *P*-value (0.00003). The QSAR model-3 has passed the performed cross-validation with q^2_{pre} values 0.91 (Table 2).

In order to test any significant relationship between *y* and *x*, the student *t*-test was performed for each descriptor in the developed regression models.

If the *P*-value in the *t*-test for the descriptor in the regression models (QSAR model 1–3) is bellow the critical value $\alpha/2 = 0.025$, the null hypothesis Ho:b (or β) = 0 can be rejected and the variable is considered as important for the regression (Table 3).

The student *t*-test of the QSAR models selected the QSAR model-2, as regression equation with *P*-value for all descriptors ((no. of CH_3, $\log D_{pH\ 5.0}$ and O-6/O-3 charges) bellow critical value $\alpha/2 = 0.025$ (Table 3).

Finally, the $\log IC_{50}$ = f (no. of CH_3, $\log D_{pH\ 5.0}$ and O-6/O-3 charges) linear regression model (QSAR model-2) created, was used for evaluation of test molecules (**T1**–**T4**, Fig. 3) with experimentally determined IC_{50} [35,36] (Table 4).

Novel phenyl-seleno-succinyl-α-tocopherol consists of two isomers, the phenyl-seleno-succinyl-1-α-tocopherol (PSS-1-AT (**T1**), Fig. 3) and the phenyl-seleno-succinyl-4-α-tocopherol (PSS-4-AT (**T2**), Fig. 3) in 7 to 3 ratio, respectively, as evident by ^{77}Se and ^{1}H NMR spectra [35]. The mixture was used in cell viability assays on the estrogen-receptor positive MCF-7 breast cancer cells [35]. Determined antiproliferative activity of the PSS-1-AT was IC_{50}: 26.2 μM [35], while the QSAR model-2 has predicted IC_{50}: 18.9 μM value (Table 4).

Furthermore, amamistatin B fragment analogs (**T3** and **T4**, Fig. 3) were examined as test compounds for the external validation of the QSAR model-2. The amamistatin B fragment analogs contain the lysine moiety and lipophilic aromatic structure, similar as the set compounds of the QSAR model. Differences in anticancer activity between enantiomers of the **T3** and **T4** molecules were measured (Table 4) [36]. The *S*-enantiomers of both test molecules have expressed stronger activity against MCF-7 cells than *R*-enantiomer. Since the descriptors of the QSAR model-2 are descriptors related to electronic effects (partial O-6/O-3 charges) and hydrofobicity/lipofilicity ((no. of CH_3, $\log D_{pH\ 5.0}$), was decided to use average of the experimental IC_{50} (*R* enantiomer) and IC_{50} (*S* enantiomer) values for **T3** and **T4** as reference. The optimized **T3** and **T4** conformations were selected for the molecular parameters calculations and QSAR modeling. The amino-Nε group of lysine moiety in the amamistatin B fragment analogs was modified in N-formyl hydroxylamine group, while the amino-Nα group of the lysine moiety has created imid. Since the Nα-atom in the imid group is at similar distance from heterocyclic O-atom (4.38 Å) as O-6 atom in tocopherol derivatives (5.53 Å), was decided to use partial atomic charge of the N-imid atom as O-charge in the QSAR model-2 prediction of the IC_{50} activity. Furthermore, phenyl moiety of the amamistatin B fragment analogs was selected as the lipophilic part with equivalent of no. of CH_3 = 3.

Table 4
Test molecules (**T1**–**T4**) with evaluated anticancer activities (IC_{50} [μM] against MCF-7 cells) by QSAR model-2.

Test compound	Experimental IC_{50} on MCF-7 [μM] [35]	Evaluated IC_{50} [μM] by QSAR model-2	Evaluated log(IC_{50}) by QSAR model-2	No. of CH_3 sites	O6/O3 charge (Mulliken)	Log *D*, pH 5.0
PSS-1-AT (**T1**)		16.75	1.224	4	−0.515555	9.66
PSS-4-AT (**T2**)		24.03	1.381	4	−0.533020	9.48
T1/T2 mixture, 7:3	26.18	18.93	1.271			
Test compound	Experimental IC_{50} on MCF-7 [μM] [36]	Evaluated IC_{50} [μM] by QSAR model-2	Evaluated log(IC_{50}) by QSAR model-2	No. of CH_3 sites	N-imid charge (Mulliken)	Log *D*, pH 5.0
(**T3**), R/S enantiomers	34.01	34.20	1.534	3	−0.58103	0.29
(**T4**), R/S enantiomers	35.47	36.02	1.556	3	−0.58058	0.58

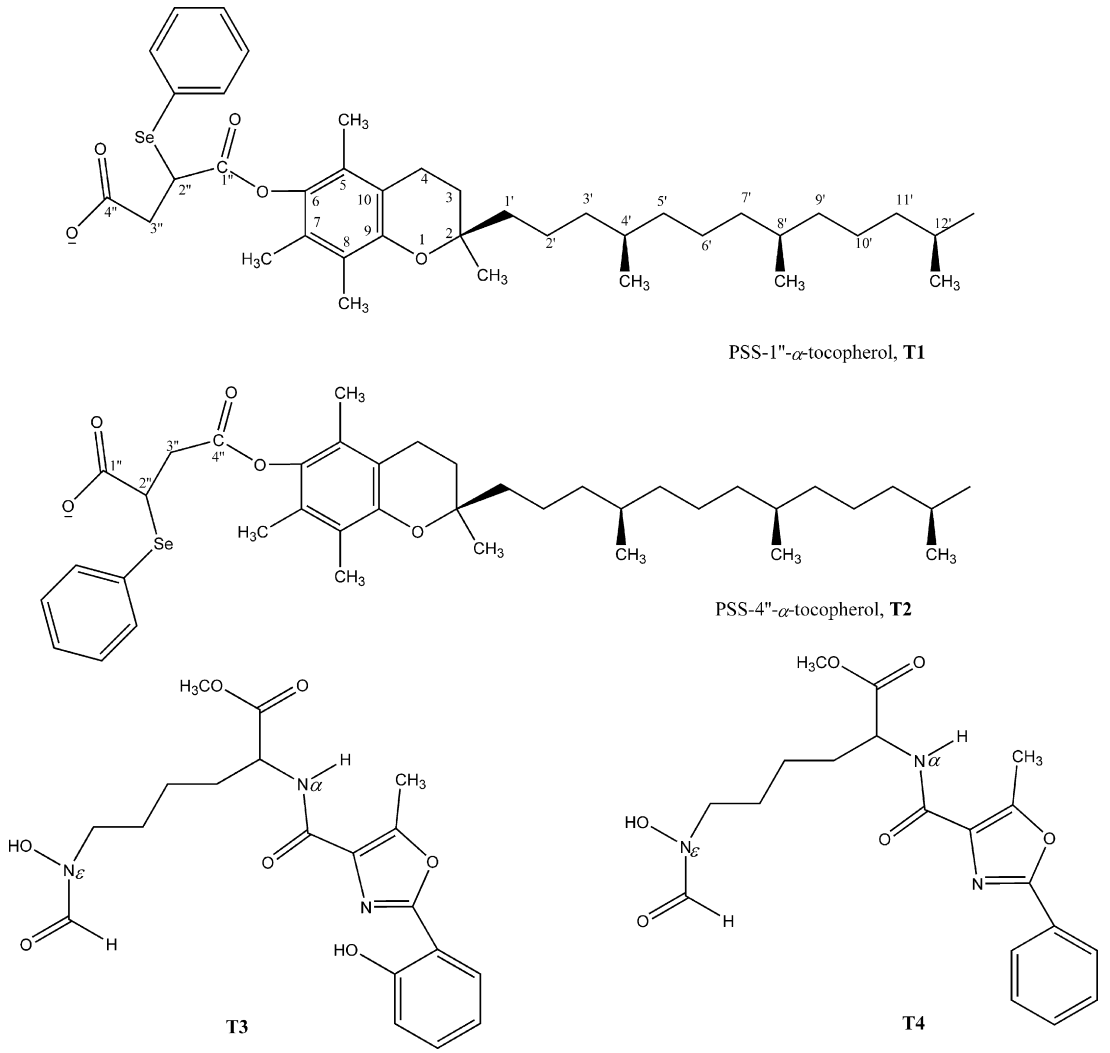

Fig. 3. Test molecules (major microspecies at physiological pH) with determined antiproliferative activity against MCF-7 breast cancer cells [35,36].

Further 3D-QSAR and molecular docking studies on specific active site should be performed for the **T3** and **T4** structures in order to explain the difference in anticancer activity of the *R* and *S* enantiomers on MCF-7 cells.

The average of the experimentally determined MCF-7 antiproliferative activities for **T3** and **T4** enantiomers were IC_{50}: 34.0 and 35.5 μM, respectively, while the QSAR model-2 predicted IC_{50} (**T3** and **T4**): 34.2 and 36.0 μM (Table 4).

The small differences between the observed and evaluated IC_{50} [μM] indicated the high prognostic capability of the $\log(IC_{50}) = f(NCH_3, \log D_{pH\ 5.0}, O6/O3\text{-charge})$ linear regression model. Therefore the QSAR model-2 was applied in IC_{50} prediction of novel proposed compounds.

Summary, the high number of methyl groups in side chain, low liposolubility (log *D* at pH 5.0) and higher O-6/O-3 partial atomic charges values are the most important factors for stronger anticancer activity of the α-tocopherol/cholesterol derivatives against MCF-7 human breast cancer cells.

Better antiproliferative and pro-apoptotic properties of tocotrienols than tocopherols [35,37] inspire us to design and examine novel tocotrienol derivatives. Tocotrienols have been shown to induce apoptosis by activating caspase-8 and caspase-3 in neoplastic mammary epithelial cells. Recent studies have shown that treatment of highly malignant +SA mouse mammary epithelial cells with 20 μM γ-tocotrienol decreased +SA cell viability by inducing apoptosis [38]. These findings demonstrate that γ-tocotrienol-induced caspase-8 activation and apoptosis in malignant +SA mammary epithelial cells is associated with suppression in PI3K/PDK-1/Akt mitogenic signaling and subsequent reduction in intracellular FLIP levels in the malignant mammary cells [38]. These results demonstrate that γ-tocotrienol-induced caspase-8 activation and apoptosis is not associated with death receptor signaling in malignant +SA mouse mammary epithelial cells [38]. Since the resistance to death receptor-induced apoptosis is an important contributor for multidrug resistance in a variety of cancer cell types [39–41], it is possible that γ-tocotrienols may provide additional therapeutic benefits when combined with traditional chemotherapeutic agents in the breast cancer treatment.

Based on the pharmacological findings [37,38] and the QSAR study results [16], was designed novel lysine-γ-tocotrienol ester (**D-1** and **D-2**, Fig. 4) and its antiproliferative activity was evaluated using the developed QSAR models.

Evaluated antiproliferative activities of the designed lysine-γ-tocotrienol esters by use of the QSAR model-2, IC_{50} (**D-1** and **D-2**): 3.09–3.54 μM, were significantly stronger than activities of the other analyzed compounds with IC_{50}: 4–1461 μM (**1**–**10**).

Relatively high ammonium-Nε charge/IC_{50} correlation (*r*: −0.907) in group of lysine derivatives (Table 1) indicated that higher Nε charge in ammonium group of the lysine moiety has further influence on enhanced anticancer effect. Thus, significantly lower ammonium-Nε charge of the proposed **D-1** (Nε: −0.4049)

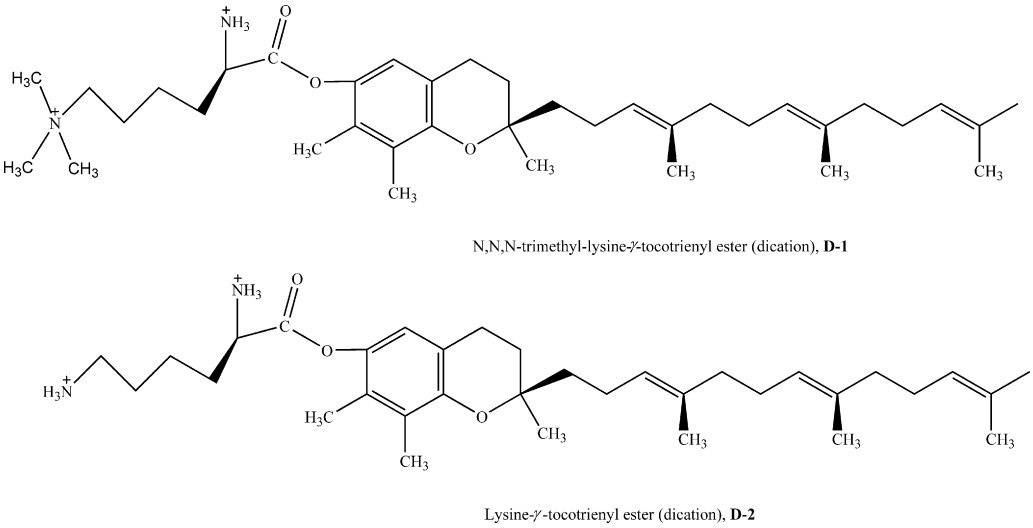

Fig. 4. Designed lysine ester with pro-apoptotic γ-tocotrienol molecule (major microspecies at physiological pH).

and **D-2** (Nε: −0.5175) compounds that the training set structures (Nε: −0.6222 to −0.5100) is additional indicator of stronger anticancer potential of the designed **D-1** and **D-2** molecules.

4. Conclusion

Understanding the molecular mechanisms involved in tocotrienol-induced apoptosis may provide critical leads for the development of novel therapies to treat breast cancer cases that have acquired resistance to death receptor signaling. Based on the pharmacological findings [37,38] and the QSAR study results [16], was supposed that esterification of the lysine with γ-tocotrienol could result in formation of novel compounds with enhanced anticancer activity.

Designed lysine ester with pro-apoptotic γ-tocotrienol molecule (**D-1** and **D-2**) has been examined by the developed QSAR models [16]. The QSAR study has proposed that both novel structures, the N,N,N-trimethyl-lysine-γ-tocotrienyl ester (**D-1**), with IC_{50}: 3.09 μM and Nε: −0.4049, and the lysine-γ-tocotrienyl ester (**D-2**), with IC_{50}: 3.54 μM and Nε: −0.5175, are compounds with potentially enhanced anticancer activity.

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