Quantitative Structure-Activity Relationship Study and Directed Synthesis of Thieno[2,3-*d*]pyrimidine-2,4-diones as Monocarboxylate Transporter 1 Inhibitors

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ABSTRACT

The aim of any quantitative structure-activity relationship (QSAR) study is not only to reveal relationships between structure of molecules and their biological activity, but also to explain it within the bounds of theoretical conceptions and to use the obtained model for prediction of properties of new compounds. That provides possibility to execute directed synthesis of new compounds with required biological activities. Monocarboxylate transporter 1 (MCT1) is one of the targets in a search for new immune-response modulating and antitumor agents. In the present

study, QSAR model for MCT1 binding affinity is developed. Decisive influence of relative negative partial charge, solvation energy and radius of gyration on MCT1 inhibition has been detected. Theoretical explanation of obtained model is given and biological activity prediction for a series of *N*-vinyl derivatives of thieno[2,3-*d*]pyrimidine-2,4-dione is made. Directed synthesis of three leading compounds has been executed according to prediction results.

Keywords: QSAR; Thieno[2,3-*d*]pyrimidine-2,4-dione; Monocarboxylate Transporter 1 Inhibitor; *N*-vinyl derivatives; *in silico* screening

1. Introduction

Activation of T-cells following antigen challenge is an important component of the immune response. However, excessive activation of T-cells can lead to plenty of autoimmune diseases and a reaction of transplant rejection. Immunosuppressive agents, which are used to prevent the rejection of transplanted organs or tissues and to treat autoimmune diseases or diseases that are most likely of autoimmune origin (e.g., rheumatoid arthritis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, Crohn's disease, pemphigus, ulcerative colitis, etc.), have numerous side-effects and risks (Berchtold et al., 1996; Niethammer et al., 1999; Rifai et al., 2006). Therefore, the search for new compounds with immunosuppressive activity is important and actual.

The monocarboxylate transporters are a family of proteins which transport lactate and other small monocarboxylates. It was proved that monocarboxylate transporter 1 (MCT1) expression grows rapidly after T-lymphocyte activation in order to eliminate lactate from the cell, the quantity of which grows as a result of increased glycolytic rate. Inhibition of lactate efflux by potent blockade of lactate transport results in an accumulation of lactate within the cell and feedback inhibition of glycolysis. This suppression of cellular metabolism, without being cytotoxic, results in an inability of T-lymphocytes to sustain the rapid rate of cell division, that takes place during the early immune response to antigen appearance. Thus, blockade of MCT1 is a new mechanism of immunosuppression distinct from current therapies (Murray et al., 2005).

In addition, it has been reported that the blocking of lactate transport to tumor cells is one of the reliable methods to fight tumor growth. Experimental studies promise MCT1 inhibition to be a new, efficient anticancer treatment both by itself and combined with radiotherapy (Sonveaux et al., 2008).

Thieno[2,3-*d*]pyrimidine-2,4-dione derivatives are characterized by several biological activities. Alpha-1 adrenoreceptor antagonists (Russell et al., 1988), luteinizing hormone-releasing hormone receptor antagonists (Sasaki et al., 2003), gonadotropin-releasing hormone receptor antagonists (Betz et al., 2008), melanocortin receptor agonists or antagonists (Sharma et al., 2005) and also MCT1 inhibitors (Guile et al., 2006) have been discovered in this class of compounds.

The purpose of current study is to reveal the dependence of MCT1 inhibiting rate on molecular structure and to execute directed synthesis of new thieno[2,3-*d*]pyrimidine-2,4-dione derivatives with high MCT1 inhibiting activity.

2. Results and discussion

2.1. Data sets

The comparativeness and homogeneity of experimental data is an important precondition for perfect QSAR model development because the values of the same activity for same compounds vary due to experimental conditions and methods. Thus, MCT1 inhibition coefficients (K_i) used for QSAR modeling were obtained during careful data analysis from literature (Guile et al., 2006). The analysis shows that out of 37 compounds with obtained K_i there were only 22 compounds evaluated in the same assay (filter binding assay). So only these compounds and their experimental data were used for model development and validation. The complete set of compounds was divided into corresponding training and test sets based on MCT1 binding affinity. Since the K_i varied by orders of magnitude, to guarantee the linear distribution of the dependent variable, the K_i values were transformed to logarithmic values ($pK_i = \log 1/K_i$), listed in Table 1. Prior to descriptors calculations, molecular geometries were pre-optimized by molecular mechanics calculations using the MMFF94x force field as implemented in MOE 2007.09 (Chemical Computing Group Inc).

2.2. QSAR model building

About 250 descriptors used as independent variables in QSAR modeling were calculated with MOE software. Then, all data processing was performed in MATLAB 7.7 (The MathWorks, Inc). The whole data was divided into a training (16 compounds) and a test set (6 compounds). This proportion was selected as the compromise between 1/3 of data as recommended (Gramatica, 2007) and the apprehensiveness to get too small training set. In an ideal case, all compounds have to be uniformly split into two samples both in the descriptors' space and by their activity. In the present study it was decided to split data set using pK_i values. For this purpose compounds were sorted by the activity in the ascending order and every fourth compound (starting from the first) was classified as test one. The uniformity of test and training sets in the descriptors' space was checked using principal component analysis (PCA), particularly by the projection of data into first two principal components plane. The result (Fig. 1) shows satisfactory compounds distribution by their descriptor values.

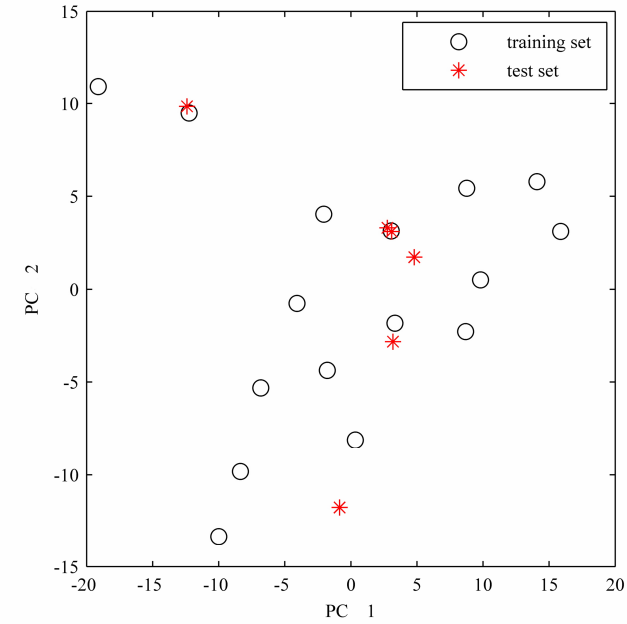
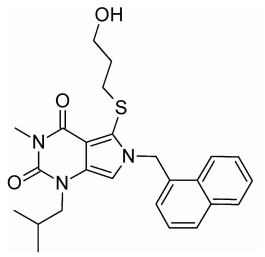
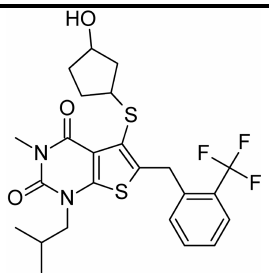
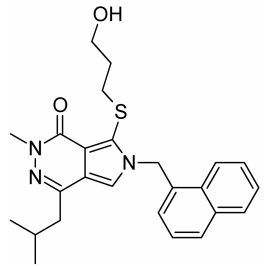
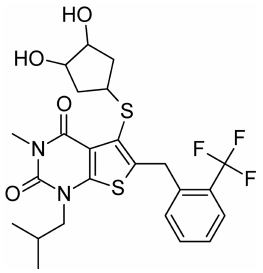
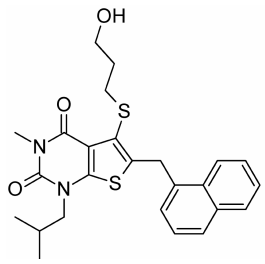
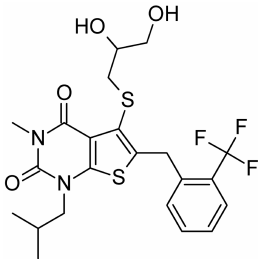
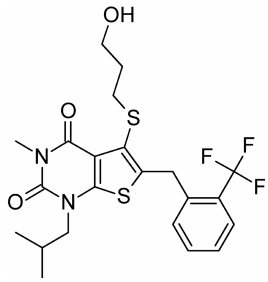
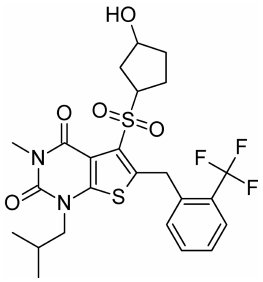
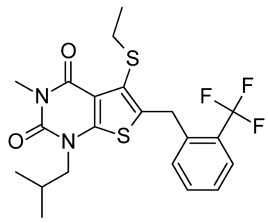
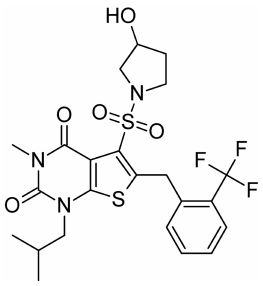
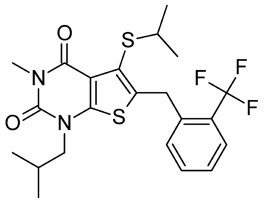
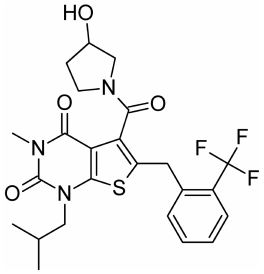
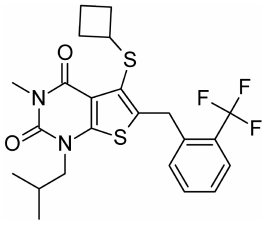
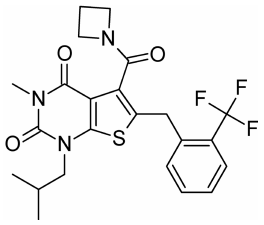
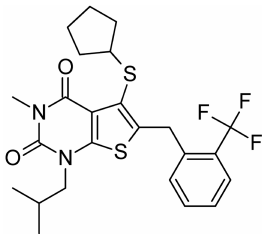
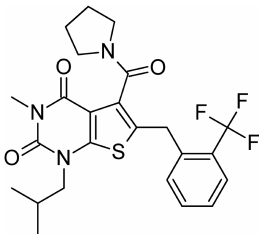
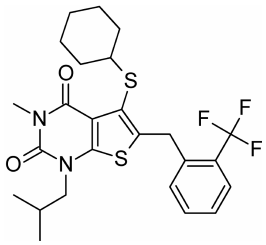
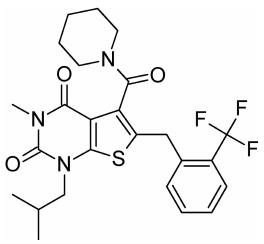
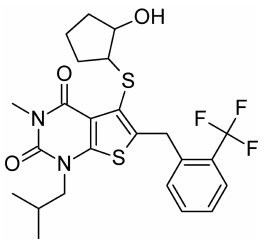
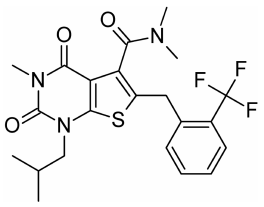
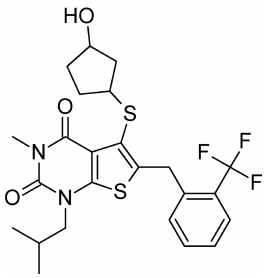
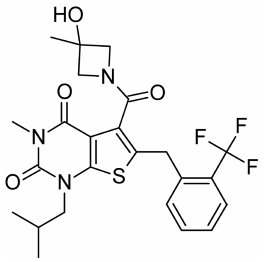


Fig.1. Projection of compounds into first two principal components plane (PC 1 and PC 2 describe 34.2% and 19.7% of total variance respectively).

Table 1.

Molecular structures with corresponding experimental and predicted MCT1 inhibition activity

№	Molecular Structure	pK_i observed	pK_i calculated	№	Molecular Structure	pK_i observed	pK_i calculated
1		9.48	9.63	**12***		9.38	9.16
2		10.00	9.95	**13**		9.17	9.21
3*		9.55	10.08	**14**		8.19	8.28
4		9.46	9.59	**15**		7.92	8.30
5		8.22	8.18	**16**		8.96	8.56

6		8.66	8.37	**17**		8.31	8.55
7		7.89	8.19	**18**		8.04	7.82
8		8.49	8.54	**19***		8.26	8.00
9		8.22	8.29	**20***		8.06	8.03
10*		8.57	8.94	**21***		7.43	7.80
11		9.54	9.15	**22**		8.46	8.39

Compounds marked with "*" are the test set; other compounds are the training set.

A stepwise regression technique was used in order to construct the model of MCT1 binding affinity. Stepwise regression is a systematic method for adding and removing terms from a multilinear model based on their statistical significance in a regression (Draper et al., 1998). Using

default regression parameters (maximum *p*-value for a term to be added = 0.05, minimum *p*-value for a term to be removed = 0.10), only three descriptors were selected:

- *PEOE_RPC-* – relative negative partial charge;

- *E_sol* – solvation energy;

- *rgyr* – radius of gyration.

The calculated values for selected descriptors are listed in Table 2.

Table 2.

Calculated values for descriptors of chemical structures

Compound	*PEOE_RPC-*	*E_sol*	*rgyr*
1	0.1563	3.3014	4.3210
2	0.1835	-16.4083	4.2096
3*	0.1696	-6.2025	4.4744
4	0.1508	8.1924	4.3448
5	0.1336	1.8911	3.8918
6	0.1312	6.9452	4.0009
7	0.1297	2.6764	3.9850
8	0.1268	9.3244	4.1868
9	0.1241	-7.7311	4.3740
10*	0.1446	-8.9567	4.3396
11	0.1467	-7.5846	4.4109
12*	0.1467	-7.2084	4.4112
13	0.1296	21.6715	4.3766
14	0.1316	-7.5933	4.1793
15	0.1364	-10.9105	4.1285
16	0.1282	1.8018	4.2967
17	0.1266	-0.9526	4.3732

18	0.1251	-4.7219	3.9626
19*	0.1229	-11.4607	4.2640
20*	0.1206	-10.8194	4.3337
21*	0.1279	-2.6092	3.8486
22	0.1244	3.1249	4.2520

Compounds marked with "*" are the test set; other compounds are the training set.

In order to reveal intercorrelations of independent variables and to discover the role of each descriptor in the resulting model, a pair-wise correlation analysis was performed and correlation matrix of selected descriptors and pK_i was calculated (Table 3). Multi-collinearity between the above mentioned three descriptors was tested by calculating their variance inflation factors (*VIF*) (Studenmund, 2006). If *VIF* equals to 1.0 - no intercorrelation exists for each variable, and if it is larger than 5.0 –that means that the related model is unstable and a recheck is necessary. The corresponding *VIF* values of selected descriptors are shown in Table 4. As can be seen from this table, all variables have *VIF* values less than 2.0. Both results show the absence of significant intercorrelations between selected descriptors, indicating that obtained model's coefficients have obvious statistic significance (Allen, 1997).

Table 3.

Correlation matrix for experimental pK_i values and molecular descriptors selected for MCT1 binding affinity model development

	pK_i observed	*PEOE_RPC-*	*E_sol*	*rgyr*
pK_i observed	1.000	0.782	0.057	0.537
PEOE_RPC-		1.000	-0.373	0.172
E_sol			1.000	0.079
rgyr				1.000

Table 4 shows obtained correlation model.

Table 4.

Descriptors, coefficients, normalized coefficients, standard errors, *t*-values and variance inflation factors for the linear model

Physico-chemical meaning	Descriptors	Coefficient	Normalized coefficient	Standard error	*t* value	*VIF*
Intercept	*Constant*	-2.1411	-3.3483	1.6909	-1.266	
Relative negative partial charge	*PEOE_RPC-*	35.3745	0.8479	4.6509	7.6059	1.2193
Solvation energy	*E_sol*	0.0245	0.3448	0.0078	3.1294	1.1908
Radius of gyration	*rgyr*	1.425	0.3637	0.4066	3.5047	1.0563

Statistical parameters of QSAR model are the following:

N =16; R^2 = 0.878; $p < 10^{-5}$; F = 28.71; Q^2_{LOO} = 0.8105; Q^2_{ext} = 0.7946, $RMSE$ = 0.2236; $RMSEP_{LOO}$ = 0.2789; $RMSEP_{ext}$ = 0.3342,

where N is the number of compounds used, R^2 is the squared correlation coefficient, p is the significance level of the model, F is the Fisher ratio, Q^2_{LOO} and Q^2_{ext} are the squared correlation coefficients for Leave-One-Out and external validations respectively, $RMSE$ is the Root Mean Squared Error of the model (also known as standard deviation of regression, S), $RMSEP_{LOO}$ and $RMSEP_{ext}$ are Root Mean Squared Errors of Prediction in Leave-One-Out and external validation procedures respectively.

The obtained statistical parameters and model validation results indicate its high quality and predictive power. A plot of predicted pK_i versus experimental pK_i values for both training and test sets is shown on Fig. 2.

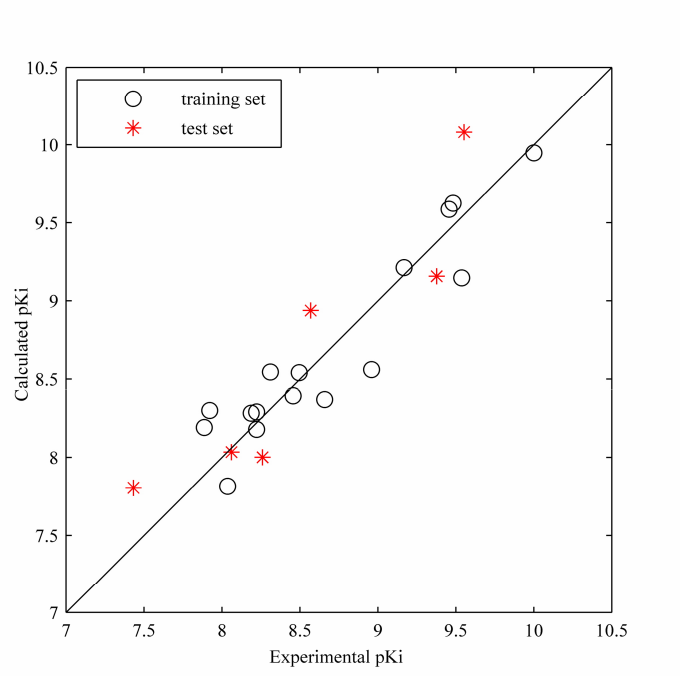


Fig. 2. Calculated *versus* experimental pK_i values for both training and test sets. The diagonal in the plot is the y = x line.

The agreement observed between the predicted and experimental values confirmed the efficiency of this QSAR model. In order to find outliers the plot of the residuals (predicted pK_i – experimental pK_i) versus experimental pK_i (Fig. 3) was studied. Considering the 3 standard deviation limit line (3*S*) for spotting outliers, all data were retained in the model.

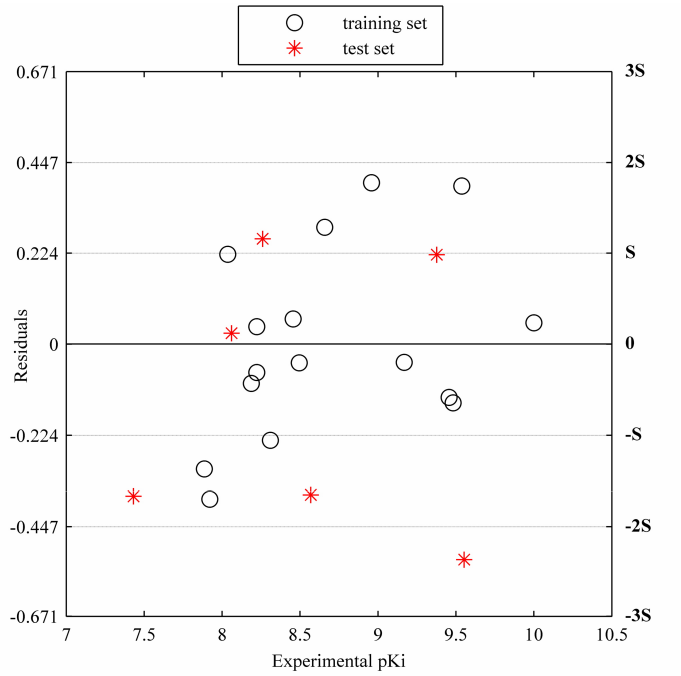


Fig. 3. Residuals *versus* experimental pK_i values for both training and test sets.

Stepwise regression method gives the ability to find a minimum number of variables necessary to build an adequate model, but there is no guarantee that the obtained model is globally optimal. Thus, the genetic algorithm as more powerful global search heuristics was used as implemented in QSAR_BENCH software (Konovalov et al., 2007). Particularly, the variable selection procedure was carried out by *RMSEP* minimization in Monte-Carlo cross-validation with genetic algorithm (GA-MCVS) with iterations number $N = 100\,000$, validation set size $n\_v = 7$ and number of variables $n = 3$ (Konovalov et al., 2008). The data filtering was executed and constant, repeated and intercorrelated (with $r > 0.9$) descriptors were discarded preliminarily. The results have shown that the minimum root mean squared error of prediction (*RMSEP* = 0.3607) is represented by the previously obtained descriptor set. It means that the obtained model has a great probability to be globally optimal.

2.3. QSAR model interpretation

As it is indicated by model normalized coefficients shown in Table 4 and correlation coefficients between descriptors and pK_i (Table 3), the highest significance for MCT1 inhibition activity belongs to relative negative partial charge (*PEOE_RPC-*). It is calculated as the smallest negative partial charge q_i divided by the sum of the negative partial charges q_i. And atomic partial charges in a molecule are obtained using Partial Equalization of Orbital Electronegativities (PEOE) method (Gasteiger et al., 1980). It means an important contribution of electrostatic interaction to MCT1 low molecular weight ligand binding. The highest *PEOE_RPC-* values are represented by compounds that contain an atom with considerable negative partial charge on it. This opin ion is perfectly harmonized by the fact that natural MCT1 ligands are monocarboxylates – anions of short-chain organic acids (such as lactate, pyruvate, acetate, acetoacetate, β-hydroxybutyrate) (Halestrap et al., 1999). Monocarboxylates contain negative charge delocalized over two carboxylate oxygen atoms.

As a result, MCT1 blockers have to be found among organic acids and other compounds that can form anions. Nevertheless it is known that ions with a localized charge exist in the solvated

state in polar solvents (such as water and biological fluids). Therefore, solvent molecules coordinated to ligand become a barrier for ligand binding. Thus, the solvation energy barrier overcoming is a necessary precondition for the interaction. Just that very case is considered by the second descriptor – solvation energy *E_sol*. Its positive coefficient value means that the pK_i increases when solvent coordination is more difficult and solvation sphere removing is easier. Since there is no even a minimal correlation between *E_sol* and pK_i (Table 3), this descriptor does not define MCT1 affinity itself, but it is an important restrictive factor for *PEOE_RPC-*. This is confirmed by the negative value of correlation coefficient between *PEOE_RPC-* and *E_sol*. Thereby the obtained in this study QSAR model once more confirms the impossibility to develop perfect models using approaches that discard so-called insignificant (from the standpoint of a single-parameter correlation) descriptors (e.g. heuristic descriptor pre-selection procedure implemented in CODESSA PRO (University of Florida, 2005).

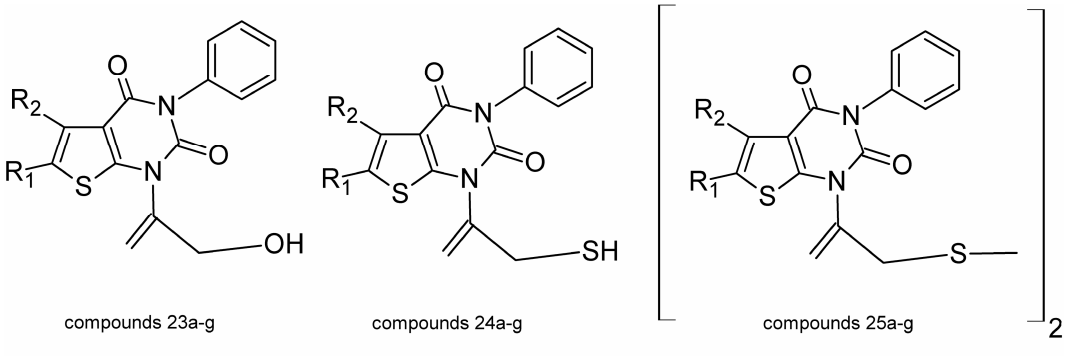
The third descriptor included in the model is radius of gyration *rgyr*. Its positive coefficient value means that spatially branched molecules will show higher affinity to MCT1. According to the authors' opinion, it can be the result of additional weak hydrogen and van der Waals bonds formation. This formation plays a key role in ligand emplacement near the active site of protein molecule in the moment of proton accepting by anion because of equilibrium proton exchange between anionic ligand and positively charged amino acid.

2.4. QSAR model application

In order to reveal leading compounds among potent MCT1 blockers *in silico* screening of *N*-vinyl derivatives of thieno[2,3-*d*]pyrimidine-2,4-dione was carried out. The whole set of evaluated compounds is presented in Table 5.

Table 5.

Selected descriptors and predicted pK_i values for analyzed compounds



compounds 23a-g compounds 24a-g compounds 25a-g

Compound	R_1	R_2	*PEOE_RPC-*	*E_sol*	*rgyr*	pK_i (predicted)
23a	CH_3	H	0.2005	-14.8547	3.4041	9.44
23b	CH_3	CH_3	0.1979	-13.2489	3.4415	9.44
23c	CH_3	C_2H_5	0.1931	-13.7838	3.5130	9.36
23d	CH_3	*n*-C_3H_7	0.1882	-9.7605	3.5775	9.37
23e	CH_3	*iso*-C_3H_7	0.1890	-12.4710	3.5961	9.36
23f		$(CH_2)_3$	0.1965	-12.7736	3.5247	9.51
23g		$(CH_2)_4$	0.1913	-12.5710	3.6372	9.50
24a	CH_3	H	0.1595	-12.8694	3.4455	8.09
24b	CH_3	CH_3	0.1572	-12.1241	3.4873	8.09
24c	CH_3	C_2H_5	0.1529	-11.8150	3.5528	8.04
24d	CH_3	*n*-C_3H_7	0.1485	-11.4918	3.6061	7.97
24e	CH_3	*iso*-C_3H_7	0.1493	-12.5344	3.6415	8.02
24f		$(CH_2)_3$	0.1559	-12.7663	3.5669	8.14
24g		$(CH_2)_4$	0.1513	-12.6884	3.6773	8.14
25a	CH_3	H	0.0839	-15.4518	5.2819	7.97
25b	CH_3	CH_3	0.0826	0.0983	5.2233	8.23
25c	CH_3	C_2H_5	0.0803	-6.9993	5.8252	8.83
25d	CH_3	*n*-C_3H_7	0.0779	-17.4794	5.9173	8.62
25e	CH_3	*iso*-C_3H_7	0.0782	-15.2369	5.6233	8.27

25f	$(CH_2)_3$	0.0819	6.0621	5.6502	8.96
25g	$(CH_2)_4$	0.0794	-16.3663	5.8241	8.57

Deliberately reducing the molecular weight and complexity (compounds **23a-g** and **24a-g** in comparison with compounds **1-22** used for model building and validation) an increase of *PEOE_RPC-* values was achieved, but *rgyr* and *E_sol* values decreased (Table 2 *vs* Table 5). As a result of the model application high pK_i values for a number of derivatives containing alcoholic hydroxyl (compounds **23a-g**, Table 5) were predicted. The predicted pK_i values for thiol derivatives (compounds **24a-g**) were lower. And for both series of molecules larger pK_i were observed for compounds with condensed cyclopentene or cyclohexene rings (**23f,g** and **24f,g**). Several dimers with considerably decreased *PEOE_RPC-* simultaneously with the increased *rgyr* and *E_sol* values (compounds **25a-g**) were also included to the set of screening compounds. This dimers have shown higher predicted MCT1 affinities than the corresponding monomers (**24a-g**). But the anion formation from compounds **25a-g**, that may be essential for MCT1 binding, is possible only after former hydrolysis. So these compounds can be regarded only as prodrugs.

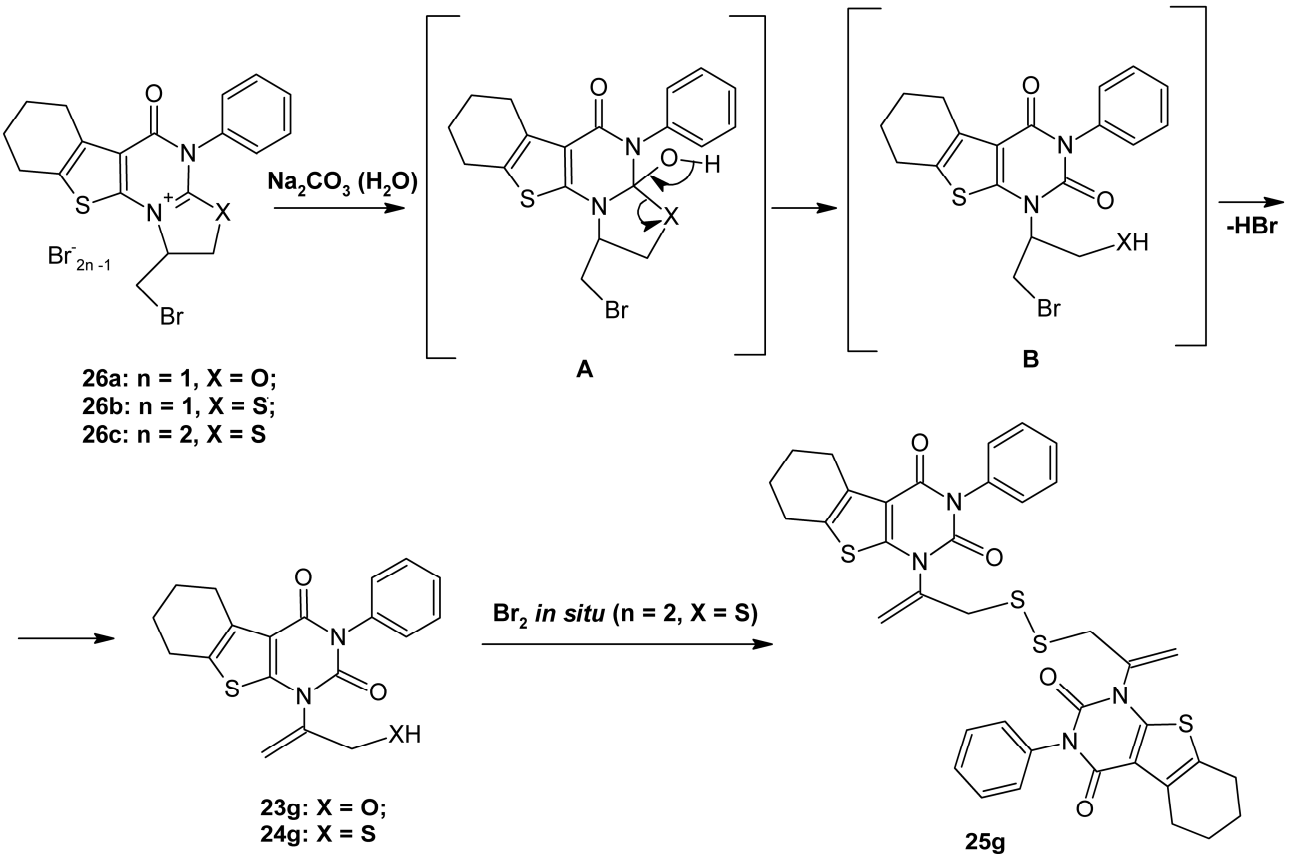
In modern drug discovery programs compliance of molecular structures with drug likeness criteria has a high importance. Therefore, Lipinski's drug-like test (Lipinski et al., 1997) and Oprea's lead-like test (Oprea, 2000) (both implemented in MOE) were conducted for studied compounds. Results showed that molecular structures **23a-g** and **24a-g** meet all criteria. Molecular structures **25a-g** failed both drug-like and lead-like tests.

Since the compounds **23f** and **23g**, and **24f** and **24g** have the very close predicted pK_i values, in order to select leading compounds to synthesize, the following analysis was made. From the drug structure database *DrugBank* (http://www.drugbank.ca) two samples of molecules containing respectively cyclopentene or cyclohexene ring as a substructure were received. So 115 structures with cyclohexene ring and only 10 structures with cyclopentene ring were found. Since there is more than a 10-fold difference in the sizes of the received samples, it was decided to synthesize cyclohexene derivatives, specifically:

- Compound **23g** – as a leading compound with the highest predicted pK_i value;
- Compounds **24g** and **25g** – as model representatives of the series **24a-g** and **25a-g** respectively.

2.5. Chemistry

A convenient way to get vinyl functional derivatives of heterocycles by splitting oxazoline or thiazoline rings was chosen (Slivka et al., 2008). Oxazolino(thiazolino-)thienopyrimidine salts **26a-c** were chosen as starting compounds. An active electrophilic center located at the "nodal" Carbone was used as the reaction center. The preparative methods of splitting oxazolino(thiazolino-)thienopyrimidinium bromides **26a-c** were developed by varying synthesis conditions and the nature of nucleophilic reagent (Scheme 1.).



Scheme 1.

Nucleophilic cleavage was carried out by the action of sodium carbonate. In the first phase the attack of hydroxyl group on the "nodal" carbon of pyrimidine cycle is implemented with the nex addition and pseudo-base (**A**) formation. Then the destruction of oxazolidine (thiazolidine) ring takes place with intermediate (**B**) formation and under the influence of strong nucleophile the

elimination of hydrogen bromide follows. This leads to the formation of *N*-vinyl derivatives of thieno[2,3-*d*]pyrimidine-2,4-dione **23g** and **24g**. In the case of formation of *N*-thiovinyl derivatives from corresponding tribromides compound **25g** is received. This can be explained by the presence of free bromine molecules in the reaction atmosphere, acting as oxidants of previously formed aliphatic mercapto groups. The overall reaction is shown in Scheme 1.

Known methods of synthesis from the corresponding allyl(thio-)ethers were used for the preparation of starting oxazolino(thiazolino-)thienopyrimidinium bromides **26a-c** (Khripak et al., 2004).

3. Conclusions

The study of quantitative relationship between molecular structure and monocarboxylate transporter 1 inhibition is carried out and QSAR model that can predict pK_i is obtained in present work. It is shown that the inhibition constant increases with increasing relative negative partial charge, solvation energy and radius of gyration of molecules. Using obtained QSAR model the activities of *N*-vinyl derivatives of thieno[2,3-*d*]pyrimidin-2,4-dione are predicted and the leading compound is identified. The synthesis of the leading compound and two model representatives of the studied molecules is executed and preparative methods of current synthesis are developed. Further investigations of the synthesized compounds *in vitro* and *in vivo* are necessary for QSAR model optimization and for optimization of the leading compound structure.

4. Experimental section

4.1. Statistical methods and model validation

The goodness of fit of the model was evaluated using the following statistical parameters: the squared correlation coefficient (R^2), the Root Mean Squared Error of the model (*RMSE*, also known as the standard deviation of regression *S*), the significance of the model (*p*) and the Fisher ratio value (*F*).

The predictive stability and robustness of the model depends not only on model building approaches, but also on the quality of initial data. Therefore, in order to detect multicollinearity

between the selected descriptors the variance inflation factors (*VIF*) were calculated as follows: $VIF = 1/(1-R^2)$, where R^2 is the correlation coefficient of multiple regression between one variable and the others in the model.

Validation procedure is the most important step in the development of reliable QSAR models. Obtained QSAR model was first verified by internal cross-validation by calculating the following parameters: Q^2_{LOO} (the squared correlation coefficient for Leave-One-Out cross-validation) and $RMSEP_{LOO}$ (the Root Mean Squared Error of Prediction in Leave-One-Out validation). Using test set, the model was further checked by external validation by calculating parameters: Q^2_{ext} (the squared correlation coefficient for external validation) and $RMSEP_{ext}$ (the Root Mean Squared Error of Prediction in external validation procedure).

4.2. Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of compounds was confirmed by the thin-layer chromatography (TLC) performed on Sorbfil plates (Russia) at 27°C (silicagel, ethanol/diethyl ether/hexane 1:3:1). Spots were detected by their absorption under UV light and by visualization with 0.05 mol I_2 in 10% HCl. IR spectra were recorded on an UR-20 spectrometer as KBr pellets and wave numbers were given in cm^{-1}.

^1H NMR spectra were obtained in a Varian VXR-300 spectrometer (300 MHz) in DMSO-d6. Chemical shifts are reported in δ values (ppm) relative to TMS δ = 0 (^1H), as internal standard. The microanalyses were performed on Perkine-Elmer 240C elemental analyzer.

The starting oxazolino(thiazolino-)thienopyrimidinium bromides 26a-c were prepared according to literature procedure (Khripak et al., 2004).

*4.2.1. 1-(3-hydroxyprop-1-en-2-yl)-3-phenyl-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine-2,4(1H, 3H)-dione (**23g**)*

Monobromide 26a (0.50 g, 1 mmol) was dissolved when heated in 20 ml DMSO. To the stirred resulting mixture a solution of sodium carbonate (0.53 g, 5 mmol) in 5 ml of water was

added. The reaction mixture was stirred under heating at 70-80°C for 4 hours and then the hydrolysis product was precipitated by adding 100 ml of cooled water. Precipitate was filtered and washed by 50 ml of warm water acidified by 1 ml of acetate acid and then was dissolved in ethanol heated to boiling. The mixture was cooled and formed during cooling precipitate was separated. The reaction product was participated from the filtrate by adding water and was crystallized from 50% ethanol-water solution. Yield: 69%. Colorless crystals; mp: 148-149°C; TLC: R_f = 0.78; ^{1}H NMR (300 MHz, DMSO-d6) δ: 1.75 (m, 4H, 2CH_2), 2.69 (m, 2H, CH_2), 2.76 (m, 2H, CH_2), 4.81-4.83 (m, 2H, CH_2), 5.78 (s, 1H, =CH_2), 6.14 (s, 1H, =CH_2), 7.25-7.51 (m, 5H, C_6H_5) ppm; IR (KBr), ν: 3400-3600 (O-H), 1680 (C=O) cm^{-1}. Anal. Calcd. for $C_{19}H_{18}N_2O_3S$ (354.423), %: C, 64.41; H, 5.08; N, 7.91; S, 9.04. Found, %: C, 64.11; H, 5.11; N, 7.97; S, 9.09.

*4.2.2 1-(3-mercaptoprop-1-en-2-yl)-3-phenyl-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine-2,4(1H, 3H)-dione (**24g**)*

Monobromide 26b (1.03 g, 2 mmol) was dissolved in 20 ml of heated DMSO. To the stirring and cooling resulting mixture a solution of sodium carbonate (0.53 g, 5 mmol) in 5 ml of water was added dropwise. The reaction mixture was stirred at room temperature for 10 minutes and then the hydrolysis product was precipitated by adding 100 ml of cooled water. Precipitate was filtered and washed by 50 ml of warm water acidified by 1 ml of acetate acid. The reaction product was crystallized from methanol. Yield: 54%. Light yellow crystals; mp: 123-125°C; TLC: R_f = 0.86; ^{1}H NMR (300 MHz, DMSO-d6) δ: 1.76 (m, 4H, 2CH_2), 2.72 (m, 2H, CH_2), 2.78 (m, 2H, CH_2), 4.12 (s, 2H, CH_2), 5.66 (s, 1H, =CH_2), 5.92 (s, 1H, =CH_2), 7.20-7.50 (m, 5H, C_6H_5) ppm; IR (KBr), ν: 2480 (S-H), 1640 (C=O) cm^{-1}. Anal. Calcd. for $C_{19}H_{18}N_2O_2S_2$ (370.488), %: C, 61.62; H, 4.86; N, 7.57; S, 17.30. Found, %: C, 61.51; H, 4.81; N, 7.62; S, 17.39.

*4.2.3 1,1'-(disulfanediyldiprop-1-ene-3,2-diyl)bis(3-phenyl-5,6,7,8-tetrahydrobenzo[b]thieno[2,3-d]pyrimidine-2,4(1H, 3H)-dione) (**25g**)*

Tribromide 26c (0.68 g, 1 mmol) was dissolved in 20 ml of heated DMSO. To the stirring and cooling resulting mixture a solution of sodium carbonate (0.53 g, 5 mmol) in 5 ml of water was added dropwise. The reaction mixture was stirred at room temperature for 10 minutes and then the hydrolysis product was precipitated by adding 100 ml of cooled water. Precipitate was filtered and washed by 50 ml of warm water acidified by 1 ml of acetate acid. The reaction product was crystallized from dioxane. Yield: 72%. Light yellow crystals; mp: 227-228°C; TLC: R_f = 0.64; ^{1}H NMR (300 MHz, DMSO-d6) δ: 1.74 (m, 4H, 2CH_2), 2.74 (m, 2H, CH_2), 2.79 (m, 2H, CH_2), 4.06 (s, 2H, CH_2), 5.64 (s, 1H, =CH_2), 5.90 (s, 1H, =CH_2), 7.22-7.48 (m, 5H, C_6H_5) ppm; IR (KBr), ν: 1640 (C=O) cm^{-1}. Anal. Calcd. for $C_{38}H_{34}O_4N_4S_4$ (738.961), %: C, 61.79; H, 4.61; N, 7.59; S, 17.34. Found, %: C, 61.62; H, 4.51; N, 7.62; S, 17.42.

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