Journal of Computer-Aided Molecular Design **17:** 291–297, 2003.

MCASE study of the multidrug resistance reversal activity of propafenone analogs

Gilles Klopman[1,*], Hao Zhu[1], Gerhard Ecker[2] & Peter Chiba[3]
[1]*Department of Chemistry, Case Western Reserve University, 10900 Euclid Ave, Cleveland, Ohio 44106, USA;* [2]*Institute of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Wien, Austria;* [3]*Institute of Medical Chemistry, University of Vienna, Währingerstrasse 10, A-1090 Wien, Austria*

Key words: BAIA, MCASE, multidrug resistance, propafenone, QSAR

Summary

A database containing 130 propafenone type chemicals which have been tested for their multidrug resistance (MDR) reversal activity was compiled. Using the Multiple Computer-Automated Structure Evaluation (MCASE) program to analyze this database, an underlying relationship between MDR reversal activity and octanol/water partition coefficient was found. An MDR reversal model was created based on this database by the baseline activity identification algorithm (BAIA) of the MCASE program. The main phamacophores relevant to MDR reversal activity were identified.

Introduction

Chemotherapy is one of the most common approaches to the treatment of human cancers. However, multidrug resistance (MDR) represents a major obstacle to a successful chemotherapeutic treatment. MDR is a term used to describe the phenomenon characterized by the ability of tumors to develop resistance to a number of 'structurally and functionally unrelated chemotherapeutic agents' [1]. A large variety of human cell lines displaying such an MDR phenotype have been identified [2].

One of the major factors leading to MDR is believed to be due to an overexperssion of P-glycoprotein (PGP) in tumor cells. Human P-glycoprotein is a 1280 amino acid surface ATP-binding cassette transport protein. Most transport proteins transport specific chemical substrates. P-glycoprotein, however, is unusual in that it transports chemicals with varying molecular structures out of the cell. In MDR cell lines, drugs entering the cells through passive diffusion bind to PGP and are actively pumped out of the cells. This active efflux directly results in the decrease of accumulation of anticancer drugs in tumor cells. In the past decade, great efforts have been made to discover effective and nontoxic chemicals that are able to reverse MDR by modulating the activity of PGP activity. As a result, a number of inhibitors of PGP-mediated drug efflux have been identified [2].

Propafenone (Figure 1) has been used clinically as an antiarrhythmic agent due to its ability to block cardiac sodium channels [3]. It was shown to be an inhibitor of PGP-mediated drug resistance [4]. Ecker and Chiba have performed a considerable amount of work on propafenone analogs and their MDR reversal activity [4–21]. In their recent reports [16, 19–21], the octanol/water partition coefficient (logP) was shown to correlate with the MDR reversal activity of relevant chemicals. Hence, the overall lipophilicity was suggested to be an important global predictive parameter for the activity of propafenone type MDR reversal agents. However, some large deviation between LogP values and MDR reversal activities exist in a number of cases [16, 19–21]. In this paper, a new algorithm recently introduced in the Multiple Computer-Automated Structure Evaluation (MCASE) program [22] was used to develop a quantitative structure-

*To whom correspondence should be addressed. E-mail: gxk6@po.cwru.edu

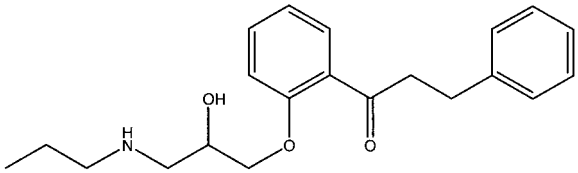


Figure 1. Molecular structure of propafenone

activity model of a large set of MDR reversal agents.

Materials and methods

MDR reversal agent database

The MDR reversal agent database contained 130 propafenone type chemicals. The activity data of 105 of these compounds had been reported in previous publications [4–8, 9–11, 13–15, 17–18, 20] and those of the remaining 25 chemicals are from more recent experimental results**. The daunomycin efflux assay was used to measure the MDR reversal activity of the chemicals in the database. The experimental protocol was described in details in previous publications [16]. Briefly, expressing CCRF_CEM vcr1000 cells were loaded with daunomycin, and the time-dependent decrease in mean cellular fluorescence was determined in the presence of various concentrations of chemicals. The first order rate constants (Vmax/Km) were obtained by nonlinear regression analysis. A correction for simple diffusion was achieved by substraction of the efflux rates observed in parental CCRF-CEM cells. The potential MDR reversal activity of each molecule was expressed as the EC50 value calculated from dose response curves of Vmax/Km vs. chemical concentration. The experimental EC50 values of these chemicals range from 0.01 μM to 1585 μM. The structures of the molecules were entered into a database as Simplified Molecular Input Line Entry System (SMILES) code [23]. When submitted to the MCASE program, the breakpoint of the training set was selected automatically by the MCASE program as 1.5 μM. Therefore, 60 of the molecules were designated as active (EC50 $<$ 0.5 μM), 46 as marginally active (0.5 $\leq$ EC50 $<$ 6.5 μM) and 24 as inactive (EC50 $\geq$ 6.5 μM).

Ecker G. and Chiba P. unpublished observations.

LogP calculation

The octanol-water partition coefficients were calculated by an extended group contribution model implemented in the MCASE program [24].

Modeling methodology

First, we simply tried to correlate the MDR reversal activities of the molecules in the database with their LogP values (Model 1). The statistics were poor (Equation 1). Although there is a correlation between the lipophilicity and the activity, this relationship is neither clear nor reliable.

$$\begin{aligned} &\mathrm{Log}(1/\mathrm{EC50}) = -1.6381 + 0.3792\mathrm{LogP} \\ &R^2 = 0.35, \quad S = 0.73, \quad F = 70.3, \quad N = 130 \end{aligned} \tag{1}$$

The algorithm used in the MCASE program was previously described by Klopman [22]. This program is able to identify the structural descriptors, named biophores, that may be responsible for the biological activity. Once the chemicals containing a particular biophore are identified, MCASE then identifies potential modulators of activity for this series of chemicals (i.e. descriptors that will enhance or decrease the activity of the biophore). These modulators may be structural fragments, physical properties (e.g., log P, water solubility) or quantum chemical parameter such as HOMO and LUMO energies that change the activity of the chemicals containing the biophore. The biophore and relevant modulators are then used to derive a quantitative structure activity relationship (QSAR) equation for the subset of chemicals containing this biophore. After that, the program will remove from consideration the molecules already explained by this biophore and will search for the next biophore and associated modulators. The process is repeated until all the active molecules in the learning set have been explained or no further biophore can be identified.

We then attempted to use the MCASE program to generate a MDR reversal agent model (Model 2). The results were better compared to those obtained with Model 1. But the MCASE model still gives a relative low correlation ($R^2 = 0.64$) and shows large deviations ($S = 0.54$).

In a recently released version of the MCASE program, a new algorithm, named Baseline Activity Identification Algorithm (BAIA), was introduced to identify the potential of existence of baseline activity due to a specific physical attribute (e.g., log P) of the molecules of the data set. A review of this algorithm

and its applications to models of fish toxicity has been published by Klopman et al. [25, 26].

The procedure we currently use, now called BAIAplus, starts off with the creation of a classical QSAR with logP and calculates a first approximation to the baseline by the classic 'Robust QSAR' technique [27]. Then the routine enters into an iterative algorithm, aiming at identifying and correcting significant errors, the major criteria being the distribution of errors around the baseline.

The baseline will slide down incrementally, based on the calculated error distributions until the significant errors are decreased or even completely eliminated. As soon as these major adjustments are completed, the remaining errors are iteratively reevaluated and eliminated by a series of rotate-slide adjustments of the baseline. The final statistical parameters are then recalculated using all the points in the proximity of the newly found baseline and the results are returned unless the resulting correlation does not fulfill specific requirements

When the current database was submitted to the new MCASE program, a good baseline linear correlation was found by the BAIAplus algorithm between the MDR reversal activity and lipophilicity (Equation 2). Thus the weak MDR reversal activity of 37 molecules (28.5% of the total) can simply be explained by their lipophilic property (Figure 2). The difference between the observed activities of the other molecules and that predicted for them by the baseline was then submitted to the MCASE program to identify the specific features responsible for this extra activity (Model 3). The results were substantially better than those obtained from the previous models.

$$\text{Log}(1/\text{EC50}) = -2.8022 + 0.3865\text{LogP}$$
$$R^2 = 0.86, \quad S = 0.30, \quad F = 214.8, \quad N = 37 \qquad (2)$$

Six outliers, located below the baseline, are seen to exist in Figure 2. They are lower than expected activity may be due to an incorrect estimation of their LogP values, to unspecific membrane effects rather than interaction with PGP or other unknown reasons. Since there is no biophores relevant to these chemicals, they are defined as 'inactives' by MCASE program in the learning set.

A comparison between the three models is made in Table 1. The results of model 1 indicate that lipophilicity is definitely not the only factor that determines the MDR reversal activity of the molecules in the database. However, compared to model 2 which was created by using structural descriptors only (biophores), model 3 shows significantly improved correlation statistics (Table 1). Therefore, model 3 which was created using the BAIAplus utility of the MCASE program was selected as the best one, in spite of its inability to account for the unexpected low activity (or incorrect calculated logP) of a few of the compounds.

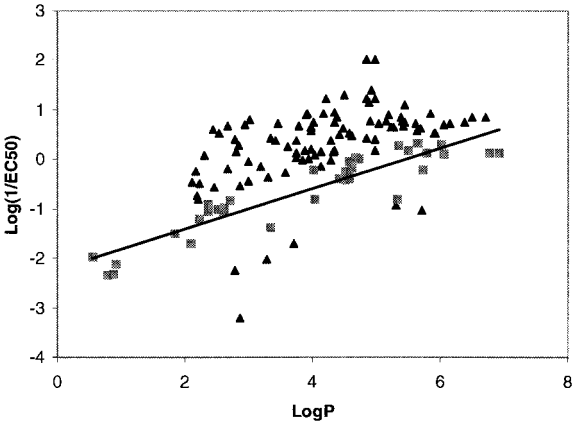


Figure 2. Baseline determined by the Baseline Activity Identification Algorithm (BAIA) utility in the MCASE program: (■) Data selected to create the baseline; (▲) all remaining data.

Table 1. Comparisons between the results obtained from three MDR reversal agent models based on the same training set

	No. of parameters	R^2	S	F
Model 1	1	0.35	0.73	70.3
Model 2	7	0.64	0.54	32.0
Model 3	11	0.80	0.40	43.3

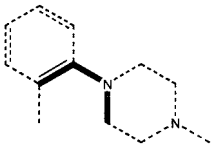
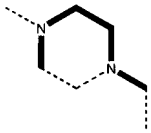
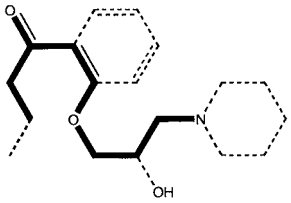
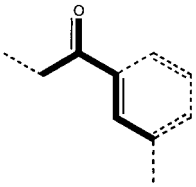
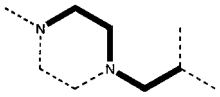
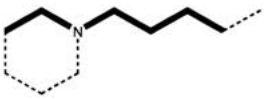
R^2: square of correlation coefficient; S: standard deviation; F: F-test value.

Results and discussion

Biophores

After implementation of BAIAplus, the MCASE program identified biophores for those compounds whose activity cannot be completely explained by their lipophilic property. A total of 10 biophores were identified by MCASE. Each biophore was then used as a template to create specific QSARs for the molecules that contain this biophore. Of the 10 biophores, 6 gave statistical significant QSAR. The nature of these 6 biophores and the statistical parameters relevant to the corresponding QSARs are shown in the following table (Table 2).

Table 2. The biophores and relevant parameters of local QSAR.

Nm.	Biophore	I/M/A	Avg. Act.	R^2	S	F	N. of mod.
B1		0/4/25	38.0	0.76	0.3	8	8
B2		0/8/28	37.0	0.72	0.32	9	8
B3		0/13/25	36.0	0.73	0.37	11	7
B4		0/5/11	33.0	0.79	0.34	8	6
B5		2/11/31	35.0	0.78	0.34	16	8
B6		0/2/5	33.0	0.97	0.10	41	3

Nm: biophore identification number; I,M,A: number of inactive, marginally active and active molecules containing the biophore; Avg. Act.: average activity; R^2 : square of correlation coefficient; S: standard deviation; F: F-test value; N of mod.: number of modulator relevant to the biophore.

Due to the obscure mechanisms of MDR and P-glycoprotein functionality, it is difficult to explain each of the biophores. Nevertheless, certain combinations of some biophores and modulators are recognized as leading to MDR reversal, probably by efficiently binding to P-glycoprotein.

The importance of a nitrogen atom attached to saturated carbons as a potential 'binding center' with the P-glycoprotein in MDR reversal agents has been noticed for several years [20, 28]. In our study, biophore 1, 2, 3, 5 and 6 (B1, B2, B3, B5 and B6) clearly show that the presence of a nitrogen atom in such an environment is relevant to MDR reversal activity. Similar results have been reported in previous publications. For examples, several 1st and 2nd generation MDR reversal agents like flupentixol [29], trifluorperazine [30] and MS209 [31] all contain a piperazine substructure. Our results, however, may indicate that while

Table 3. Modulators relevant to biophore 1 (B1)

Modulator	Activating(+)/ Deactivating(−)	Parameter or Fragment
M1	−	Log (nr.Bio/MW)
M2	+	cH =cH -cH =c >-c <=
M3	+	cH =cH –c =cH - <3-N>
M4	−	CH3-CO -c =cH –cH =c <-
M5	+	CH3-c =cH -c″ –CO -CH2-
M6	−	CH2-CH -CH2-N -CH2-CH2-N -c=c -cH = <9-O>
M7	−	CH3-CO -c =c <-cH =c -
M8	−	c <=cH –c =c <-

c <: aromatic carbon attached to an electron donor; c > : aromatic carbon attached to an electron acceptor; <9-O> indicates an oxygen substituent attached to the ninth atom of the modulator.

Table 4. Statistical evaluation of the MDR reversal agent model through cross-validation

Nr.	Molecules in the learning set (I/MA)	Molecules in the test set (I/MA)	Conc. (%)	Sens. (%)	Spec. (%)	R^2	S
Retrofit experiment	130(24/46/60)	130(24/46/60)	100	100	100	0.80	0.40
1	116 (22/41/53)	14 (2/5/7)	100	100	100	0.71	0.54
2	116 (22/40/54)	14 (2/6/6)	100	100	100	0.78	0.37
3	116 (22/41/53)	14 (2/5/7)	88.9	85.7	100	0.72	0.56
Average	116 (22/41/53)	14 (2/5/7)	96.3	95.2	100	0.74	0.48

I,M,A: number of inactive, marginally active and active molecules containing the biophore; Con.: concordance; Sens.: sensitivity (percentage of correctly predicted actives); Spec.: specificity (percentage of correctly predicted inactives); R^2: square of correlation coefficient; S: standard deviation.

the nitrogen atom in such environment interacts with the P-glycoprotein, the ring structure is conducive to a more favorable interaction. 25 active chemicals and 4 marginally active chemicals were found to contain the top biophore which consists of a nitrogen atom in a substituted aliphatic ring connected to an ortho substituted aromatic ring. The local QSAR performed with the chemicals containing this biophore showed that 8 modulators were also relevant to this biophore (Table 3). It is shown that the presence of an additional nitrogen (M3) attached to the aromatic ring will enhance the activity of the chemicals containing this biophore. But if there is an acetyl group attached to the ortho or the para position of the aromatic ring (M4, M7), the activity decreases.

Biophore 4 (B4) shows a substructure that consists of a carbonyl group attached to a meta substituted aromatic ring. The carbonyl group is a potential electron donor and can therefore act as a hydrogen bonding acceptor. Compared to modulators 4 and 7 (M4, M7) (Table 3), the difference between the substituted positions of the aromatic ring shows that a substituent in meta of the carbonyl group is needed for it to unfurl MDR reversal functionality. However, an ortho or para electron donor substituent will decrease the MDR reversal activity.

Testing the model

We used the model created by the BAIA utility of the MCASE program (model 3) to calculate the MDR reversal activity of the 130 molecules of the database (retrofit experiment). We obtained a linear relationship between the experimental and calculated values with the following statistical characteristics: $\boldsymbol{R}^2 = 0.80$, $\boldsymbol{F} = 43.3$, $\boldsymbol{s} = 0.40$, $\boldsymbol{N} = 130$. Because the MCASE program ignores 'marginal activity' when se-

Table 5. Comparison between the experimental and predicted values of 11 'unknown' chemicals

No.	Smiles Code of the Chemical	Exp. Act. [Log(1/EC50)]	Cal. Act. [Log(1/EC50)]
1	OC2C(C)(C)OC1=CC=C(C#N)C=C1C2NCC3=CC=CC=C3	−1.77	−0.96
2	OC2C(C)(C)OC1=CC=C(C#N)C=C1C2N3CCN(CC4=CC=CC=C4)CC3	0.19	0.12
3	OC2C(C)(C)OC1=CC=C(C#N)C=C1C2NC	−3.00	−1.73
4	OC2C(C)(C)OC1=CC=C(C#N)C=C1C2NCC(OC(C)(C)C)=O	−1.67	−1.31
5	OC2C(C)(C)OC1=CC=C(C#N)C=C1C2N(CC3=CC=CC=C3)CC(OC(C)(C)C)=O	−0.39	−0.80
6	CC2N(C(C3=CC=C(C)C=C3)=O)C1=CC=CC=C1C(NCC4=CC=CC=C4)C2O	−0.51	−0.40
7	CC3N(C2=CC=CC=C2C(N4CCN(CC5=CC=CC=C5)CC4)C3O)C(C1=CC=C(C=C1)C)=O	0.37	1.00
8	CC3N(C2=CC=CC=C2C(N4CCCC4)C3O)C(C1=CC=C(C=C1)C)=O	−0.90	−0.96
9	CC2N(C(C3=CC=C(C)C=C3)=O)C1=CC=CC=C1C(NC(C)CCCC(CC)CC)C2O	−0.29	−0.06
10	O=C(C4=CC=C(C)C=C4)OC2C(C)NC1=CC=CC=C1C2N3CCCCC3	−0.75	−0.80
11	O=C(C3=CC=C(C)C=C3)OC2C(C)NC1=CC=CC=C1C2NC(CCCN(CC)CC)C	0.47	−0.17

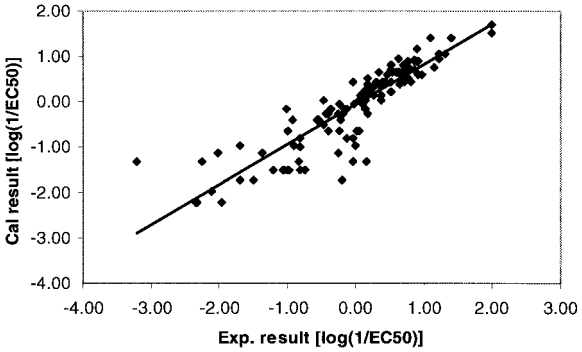


Figure 3. Correlation of predicted activity Vs experimental activity values for 130 chemicals

lecting biophores, most of the outliers were found for chemicals with 'marginally active' experimental values (Figure 3).

In a study like this, it is essential to evaluate the statistical relevance of the model. To this effect, the reliability and prediction potential of the model were evaluated through cross-validation experiments. 14 compounds were randomly removed from the original database and the remaining 116 compounds were used to create a new learning set. The model created from the reduced learning set was then used to predict the MDR reversal activities of the 14 compounds that were not included in the reduced learning sets. This procedure was repeated three times. The results of these validation tests are presented in Table 4. On the average, the R^2 and standard error of the predictions are 0.74 and 0.48 respectively. It can be seen that the reduced models were pretty reliable. The average concordance is over 90%, indicating that most of the current biophores and modulators were also identified in the reduced models.

Prediction of unknown chemicals

After our study was finished, an external test was performed using 11 'unknown' chemicals which were tested by the same experimental protocol in a previous report [19]. These chemicals, however, were not derivatives of propafenone and were not included in our database either. Nevertheless, using our model to test this 'unknown' dataset, the overall correlation R^2 and standard error S were found to be 0.75 and 0.41 respectively. These correlation results are thus similar to what we obtained from our propafenone database so, apparently, our model may also be useful to predict the MDR reversal activity of other types of chemic-

als. The experimental results and relevant calculation values are shown in Table 5.

Conclusion

Propafenone type MDR reversal agents show a potential dependence on lipophilicity. This relationship was obtained with the help of a new released version of the MCASE program. Several structural descriptors were used to account for the remaining activity after excluding the baseline activity. Using the current model to predict the potential MDR reversal activity of 11 'unknown' chemicals, their MDR reversal activity were predicted with excellent accuracy.

References

1. Krishna, R., Mayer, D.L., Eur. J. Pharm. Sci., 11 (2000) 265.
2. Wiese, M., Pajeva, K.I., Cur. Med. Chem., 8 (2001) 685.
3. Jazwinska-Tarnawska, E., Orzechowska-Juzwenko, K., Niewinski, P., Rzemislawska, Z., Loboz-Grudzien, K., Dmochowska-Perz, M., Slawin, J., Int. J. Clinic. Pharm. Therap., 39 (2001) 288.
4. Chiba, P., Burghofer, S., Richter, E., Tell, B., Moser, A., Ecker, G., J. Med. Chem., 38 (1995) 2789.
5. Albrecht, G., Ecker, G., Tumova, I., Sci. Pharm., 61 (1993) 161.
6. Ecker, G., Fleischhacker, W., Helml, T., Noe, C.R., Scasny, S., Lemmens-Gruber, R., Studenik, C., Marei, H., Heistracher, P., Chirality, 6 (1994) 329.
7. Ecker, G., Fleischhacker, W., Noe, C.R., Arch. Pharm., 327 (1994) 691.
8. Ecker, G., Fleischhacker, W., Helml, T., Noe, C.R., Studenik, C., Schade, B., Heistracher, P., Arch. Pharm., 328 (1995) 343.
9. Chiba, P., Ecker, G., Schmid, D., Drach, J., Tell, B., Goldenberg, S., Gekeler, V., Mol. Pharmacol.,, 49 (1996) 1122.
10. Spatzenegger, M., Ecker, G., Jäger, W., Biomed. Chromatogr., 10 (1996) 127.
11. Ecker, G., Chiba, P., Hitzler, M., Schmid, D., Visser, K., Cordes, H.P., Csollei, J., Seydel, J.K., Schaper, K.J., J. Med. Chem., 39 (1996) 4767.
12. Ecker, G., Chiba, P., Schaper, K.J., J. Pharm. Pharmcol., 49 (1997) 305.
13. Chiba, P., Hitzler, M., Richter, E., Huber, M., Tmej, C., Giovagnoni, E., Ecker, G., Quant. Struct.-Act. Relat. 16 (1997) 361.
14. Chiba, P., Tell, B., Jäger, W., Spatzenegger, M., Richter, E., Hitzler, M., Ecker, G., Arch. Pharm. Pharm. Med. Chem., 330 (1997) 343.
15. Chiba, P., Annibali, D., Hitzler, M., Richter, E., Ecker, G., Il Farmaco, 53 (1998) 357.
16. Chiba, P., Holzer, W., Landau, M., Bechmann, G., Lorenz, K., Plagens, B., Hitzler, M., Richter, E., Ecker, G., J. Med. Chem., 41 (1998) 4001.
17. Tmej, C., Chiba, P., Huber, M., Richter, E., Hitzler, M., Schaper, K.J., Ecker, G., Arch. Pharm. Pharm. Med. Chem., 331 (1998) 233.
18. Salem, M., Richter, E., Hitzler, M., Chiba, P., Ecker, G., Sci. Pharm., 66 (1998) 147.
19. Hiessbock, R., Wolf, C., Richter, E., Hitzler, M., Chiba, P., Kratzel, M., Ecker, G., J. Med. Chem., 42 (1999) 1921.
20. Ecker, G., Huber, M., Schmid, D., Chiba, P., Mol. Pharmcol., 56 (1999) 791.
21. Schmid, D., Ecker, G., Kopp, S., Hitzler, M., Chiba, P., Biochem. Pharmcol., 58 (1999) 1447.
22. Klopman, G., Quant. Struct.-Act. Relat., 11 (1992) 176.
23. Weininger, D., J. Chem. Inf. Comput. Sci., 28 (1988) 31.
24. Klopman, G., Li. J., Wang, S., Dimayuga, M., J. chem. Inf. Comput. Sci., 34 (1993) 752.
25. Klopman, G., J. chem. Inf. Comput. Sci., 38 (1998) 78.
26. Klopman, G., Saiakhov, R., Rosenkranz H.S., Hermens, J.L., Environ. Toxicol. Chem., 18 (1999) 2497.
27. Press, W.H., Flannery, B.P., Teukolsky, S.A., Vetterling, W.T., Numerical Recipes in Fortran. 2nd ed. Cambridge: University Press, 1992.
28. Zamora, J.M., Pearce, L.H., Beck, T.W., Mol. Pharmacol., 33 (1988) 454.
29. Pajeva, I.K., Wiese, M., Cordes, H.P., Seydel, J.K., J. Cancer Res. Clin. Oncol., 122 (1996) 27.
30. Ford, J.M., Prozialeck, W.C., Hait, W.N., Mol. Pharmacol., 35 (1989) 105.
31. Takeshita, A., Shigeno, K., Shinjo, K., Naito, K., Ohnishi, K., Hayashi, H., Tanimoto, M., Ohno, R., Leukemia Lymphoma, 42 (2001) 739.