International Journal of Drug Design and Discovery

Volume **2** • *Issue* **1** • *January – March* **2011. 425-433**

Quantitative Structure Activity Relationship Analysis of Benzimidazole Derivatives as Aldose Reductase Inhibitors

P.K. Singour[1]*and D. V. Kohli[2]

[1]Department of Pharmaceutical Chemistry, VNS Institute of Pharmacy, Neelbud, Bhopal (M.P.)

[2]Department of Pharmaceutical Sciences, Dr. H.S. Gour Vishwavidyalaya, Sagar(M.P.)

ABSTRACT: Hyperglycemia is involved in the pathogenesis of diabetic neuropathy, retinopathy, nephropathy, and macro-vascular disease via multiple mechanisms, of which increased aldose reductase activity. Aldose reductase inhibitors, when administered from the onset of hyperglycemia, prevent the progression of polyol accumulation–linked complication. In this work, we report quantitative structure activity relationship analysis performed on the Triazino[4,3-α]benzimidazole acetic acid derivatives as aldose reductase inhibitors. The molecular modeling study was performed by using the software BioMed CAChe 6.0 and the regression analysis by SYSTAT 10.2. Various physicochemical parameters belonging to different classes viz. hydrophobic, steric and electronic etc. were calculated. QSAR models were generated using 17 compounds. The model showed a good correlative and predictive ability having convention coefficient (r^2) of 0.846 and cross-validated correlation coefficient (q^2) of 0.6866 by LOO external validation method. Based on the developed QSAR model, it may be concluded that aldose reductase inhibition activity of benzimidazole acetic acid derivatives is strongly influenced by the thermodynamic and electronic nature of the substituents. QSAR model shows that LOGP, LUMO energy, and steric energy are negatively affecting the biological activity. For a molecule to have higher aldose reductase activity, compound should be less lipophilic and should have more electronegative groups or atoms than electropositive groups or atoms present in the compound.

KEY WORDS: Polyol pathway; Aldose Reductase; Triazino[4,3-α]benzimidazole acetic acid; Quantitative Structure Activity Relationship (QSAR)

Introduction

Diabetes mellitus (DM) is a common chronic metabolic disease characterized by hyperglycaemia and various metabolic imbalances; its prevalence is about 6 % worldwide and the number of cases, presently estimated at more than 150 million, is predicted to double by 2025.[1] The diabetic individual is prone to late onset complications that are largely responsible for the morbidity and mortality observed in the patients.[2]

Through various clinical studies, including the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), the development and progression of these complications in type I and type II patients has been clearly linked to elevated blood glucose levels. During euglycemic conditions glucose is preferentially metabolized through the glycolytic pathway where it is phosphorylated with ATP by hexokinase to form glucose-6-phosphate.[3] Diabetic hyperglycemi a causes a marked increase in glucose metabolism through the polyol pathway in those tissues in which glucose transport is insulin-independent. The correlation existing between enhanced polyol pathway glucose metabolism and the onset and progression of long-term diabetic complications been well documented.[4] Hyperglycemia is involved in the pathogenesis of diabetic neuropathy, retinopathy, nephropathy, and macrovascular disease via multiple mechanisms, of which increased aldose reductase activity[5-7], nonenzymatic glycation and glycooxidation[8,9], activation of protein kinase C (PKC)[10,11], and oxidative-nitrosative stress[12,13] are the best studied. According to several studies performed in the diabetic lens[14,15], nerve[16], retina[17], and high-glucose–exposed endothelial cells[18], increased aldose reductase activity leads to oxidative stress, cellular swelling, and increased membrane permeability and perturbation of membrane transport processes. These changes can initiate cellular lesions associated with late-onset diabetes complication such as neuropathy, nephropathy, keretopathy, angiopathy, and cataract.[19]

* *For correspondence:* Vivek K. Vyas,

Tel.: +91-755-2696748/46 Fax: +91-755-2696748

E-mail: psingour@rediffmail.com

Experimental studies indicate that aldose reductase inhibitors (ARIs), which block the flux of the glucose through polyol pathway and prevent the intracellular accumulation of sorbitol[20], can retard, or reverse these complication of chronic diabetes.[21] These observations have spurred great interest in the development of ARIs, which can be divided into two general groups, those containing rigid spirohydantoins or related ring system, such as Sorbinil, and those containing a carboxylic acid moiety, like Alrestatin, Tolrestat, and Zopolrestat.[22]

Several spirohydantoins, including the most important member of this class, Sorbinil, show an excellent activity in both in vitro and in vivo models of diabetes complications. However, due to a certain incident of phenytoin-like hypersensitivity side effect in clinical trials, the present search for new ARIs is mainly directed towards the family of carboxylic acids.[23]

Nevertheless, relatively few carboxylic acids show any *in vivo* activity, which underlines the difficulty of obtaining a good conversion of in vitro activity to in vivo activity, particularly among compound of this class. All this seems to be due to the higher polarity of carboxylic acids, which are almost completely ionized at physiological pH, and could thus encounter greater difficulty than the hydantoins in crossing a biological membrane.[24]Currently no universally potent inhibitor exits.

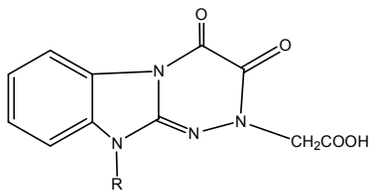
Taking the above-mentioned details into account, the present study describes the quantitative structure-activity relationship (QSAR) analysis of benzimidazole acetic acid derivatives. The relevance of the best QSAR model obtained for the design of novel derivatives should be assessed not only in terms of predictivity, either internal or external, but also in terms of their ability to provide a chemical and structural explanation of their binding interaction. These results could serve as a guideline in the design of more potent aldose reductase inhibitors.

Experimental

Data set for analysis

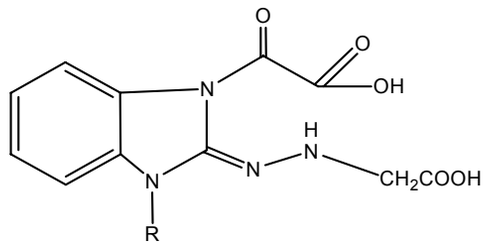
The first step in developing QSAR equations is to compile a list of compounds for which the experimentally determined inhibitory activity is known. The aldose reductase inhibitory activity data of Triazino[4,3-α]benzimidazole acetic acid derivatives were taken from the reported work of Da Settimo *et al.*[25] (Table 1). The biological activity data (IC_{50} in μM) was converted to negative logarithmic dose (pIC50) for QSAR analysis. The dataset of Aldose reductase inhibitors consisted of 25 compounds. For the external validation of QSAR models, the molecules were rationally divided into a training and test set on the basis of structural diversity and cover the complete range of variations in inhibitory activity as the guidelines for dividing into training and test sets by Oprea *et al.*[26]. The size of the final training set therefore became 17 compounds and in the test set are 8 compounds. (Table 1)

Table 1 Structures and inhibitory activities of benzimidazole derivatives against aldose reductase 1 (ALR1) enzyme

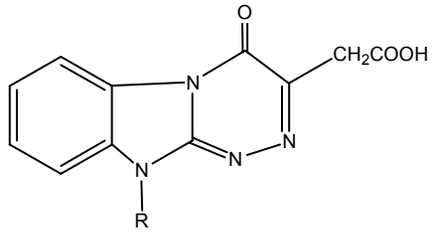


Compound	R	IC_{50} (μM)	-logIC_{50}
1	CH_3	24.8	-1.394
2	$CH_2CH_2CH_3$	37.2	-1.57
3	$CH_2C_6H_5$	0.36	0.443
4	$CH_2C_6H_4$-4-CH_3	13.3	-1.124
5	$CH_2C_6H_4$-4-OCH_3	42.6	-1.629
6	$CH_2C_6H_4$-4-Cl	4.15	-0.618
7*	$CH_2C_6H_4$-4-F	4.58	-0.66
8	$CH_2C_6H_4$-4-CF_3	23.9	-1.38
9*	$CH_2C_6H_4$-3,4-F_2	4.42	-0.645
10	$CH_2C_6H_4$-2-F-4-Br	4.47	-0.65
11*	CH_2COOH	13.5	-1.13

Table 1 *Contd…*



Compound	R	IC_{50} (µM)	-logIC_{50}
12	CH_3	108.6	-2.036
13*	$CH_2CH_2CH_3$	46.50	-1.667
14	$CH_2C_6H_5$	4.50	-0.653
15	$CH_2C_6H_4$-4-CH_3	45.9	-1.661
16*	$CH_2C_6H_4$-4-OCH_3	44.5	-1.648
17*	$CH_2C_6H_4$-4-Cl	10.0	-1
18	$CH_2C_6H_4$-4-F	14.8	-1.17
19*	$CH_2C_6H_4$-4-CF_3	2.63	-0.42
20	$CH_2C_6H_4$-3,4-F_2	9.72	-0.987
21	$CH_2C_6H_4$-2-F-4-Br	12.5	-1.097
22	CH_2COOH	236.0	-2.373



Compound	R	IC_{50} (µM)	-logIC_{50}
23*	H	35.9	-1.555
24	CH_3	17.0	-1.23
25	$CH_2C_6H_5$	5.44	-0.735

*Molecules in test set (8 compounds), remaining in training set (17 compounds);
IC_{50} = Dose in µM required to produce 50% inhibition.

Chemical Structure Construction and optimization

The molecular modeling study was performed by using the BioMed CAChe 6.0 software[27]. The structure of the molecules was drawn and saved as mol2 file. The energy minimization was carried out by MOPAC using Austin model-1 (AM1) method after performing geometry optimization in Mechanics using augmented MM3 force field in BioMed CAChe 6.0 software. To avoid the local stable conformations of the compounds, geometry optimization was run many times with different starting points for each molecule, and conformation with the lowest energy was considered for calculation of the molecular descriptors.

Descriptors Calculation

Various physicochemical parameters belonging to different classes viz. hydrophobic, steric and electronic etc. were calculated using "PROJECT LEADER" program in BioMed CAChe software. The abbreviations used for physicochemical properties are given in table 2 and values of the calculated parameters given in table 3.

Table 2 Selected Descriptors used

S.No.	Abbreviation	Full name	Description
1.	BA	Biological activity	Biological activity (Observed or Predicted)
2.	CI_1	Connectivity Index (First order)	First-order (bond) molecular connectivity index Chi1 for the chemical sample.
3.	SE	Steric Energy	The steric energy of a molecule is the sum of the molecular mechanics potential energies calculated for the bonds, bond angles, dihedral angles, nonbonded atoms and so forth. It is specific to Mechanics and depends upon the force-field used.
4.	LUMO	LUMO Energy	The energy gained when an electron is added to the lowest unoccupied molecular orbital (LUMO).
5.	LOGP	Log p	The octanol-water partition coefficient.
6.	MR	Molecular Refractivity	The molar refractivity of the chemical sample.

Table 3 Value of calculated descriptors

COMPOUND	CI_1	SE	LUMO	LOGP	MR
1.	9.503	27.224	-0.785	0.291	67.603
2.	13.363	8.489	-0.825	2.998	100.06
3.	10.897	17.56	-0.885	-0.168	73.688
4.	9.93	11.405	-0.578	0.177	68.181
5.	10.968	13.08	-0.584	0.988	77.453
6.	12.986	5.632	-0.793	1.954	92.793
7.	13.38	7.986	-1.004	2.421	97.835
8.	13.918	18.781	-0.719	1.701	99.257
9.	13.38	7.959	-0.76	2.472	97.598
10.	13.38	5.85	-0.973	2.093	93.01
11.	14.591	12.712	-1.06	2.837	98.767
12.	10.541	28.11	-0.767	1.102	76.875
13.	13.791	10.882	-0.853	2.233	93.226
14.	13.791	-1.236	-0.811	2.885	100.63
15.	11.324	23.947	-0.641	-0.281	74.265
16.	8.665	54.069	-1.41	0.505	63.455
17.	9.092	49.184	-1.349	0.751	68.351
18.	12.148	37.078	-1.295	2.528	92.964
19.	12.559	13.696	-0.972	2.067	92.216
20.	12.952	17.437	-0.931	2.534	97.257
21.	13.49	17.427	-0.77	1.814	98.679
22.	12.952	29.979	-1.013	2.585	97.02
23.	12.952	24.855	-1.012	2.207	92.432
24.	14.164	25.942	-1.13	2.95	98.189
25.	13.363	27.374	-1.086	2.346	92.648

Statistical analysis

The relationship between structural parameters and biological activities was quantified by the multiple linear regressions implemented in SYSTAT 10.2 software[28]. The cross-validation analysis was performed using the leave - one-out (LOO) method where one compound is removed from the dataset and its activity is calculated using the model derived from the rest of the dataset.

The correlation matrix of calculated descriptors with each other's and with the activity data was determined. The most significant for ALR inhibiting activity were chosen on the basis of their inter correlation (≤ 0.6) (table 4). In order to select the predominant descriptors affecting the activity, the correlation analysis was performed. Multiple regression analysis was performed for aldose reductase inhibiting activity of Triazino[4,3-α]benzimidazole acetic acid derivatives, *i.e.*-log IC considered as dependent variable and the different descriptors considered as the independent variables.

Model Development and Validation

The QSAR models were developed by stepwise multiple linear regression (MLR) analysis. The stepwise selection of variables, a combination of forward selection and backward elimination procedure, was used to select the most relevant subset of descriptors. Regression analyses were performed by SYSTAT 10.2 software.

Internal and external validation was performed to validate the QSAR model. In this approach, the activity of each compound in test set is computed. With the help of observed activity and calculated activity cross-validation coefficient q^2 was calculated. Cross-validation coefficient q^2 can be considered as an indicator of the predictive performance and stability of a model. For a reliable model, the square of cross-validation coefficient $q2$ should be≥0.5.

Results and Discussion

Multiple linear regression and other statistical analysis were carried out on all the compounds of training set. Descriptors were selected for the model based on their correlation coefficient and those descriptors having intercorrelation coefficient below 0.6 were considered. Various models were obtained after performing multiple linear regression (MLR) analysis. Model predictive power was judged based on various statistical parameters like Multiple correlation coefficient (r), Explained variance (Squared multiple r), Variance ratio at specific degree of freedom (df) (Fischer's F-test for significance) (F), Standard error of estimate (s). All these statistical parameters were computed as defined in the SYSTAT 10.2.

The r^2 statistic is a measure of the extent to which the total variation of the dependent variable is explained by the regression. The QSAR model having higher r^2 and F-ratio among the several models was tested by cross-validated (leave-one-out) procedure. The output of the cross validation is q^2 (Cross validated r^2). The best QSAR model was externally validated by test set. Predicted activities of training set were calculated by using this model. Residuals of prediction of training set were calculated by subtracting predicted activities from actual activities of training set.

The results suggest that the model-III is best models among other significant models and are good enough to rank molecular activities for further drug discovery.

Model I

$-\log (IC_{50}) = -0.181(\pm0.122)$ CI_1 $-2.434(\pm1.029)$ LUMO $-0.053(\pm0.018)$ SE $- 0.047(\pm1.276)$

N= 17, r =0.645, r^2 =0.417, F-ratio =3.094, s =0.543, P = 0.064

Model II

$-\log (IC_{50}) = - 0.031(\pm0.014)$ MR $- 2.887(\pm0.958)$ LUMO $- 0.058(\pm0.016)$ SE $+0.192(\pm1.004)$

N= 17, r =0.716, r^2 =0.513, F-ratio =4.556, s =0.496, P = 0.022

Model III

$-\log (IC_{50}) = -0.565(\pm0.121)$ LOGP $- 4.187(\pm0.783)$ LUMO $-0.069(\pm0.012)$ SE$-2.548(\pm0.442)$

N= 17, r =0.864, r^2 =0.846, s =0.358, Adjusted r^2 = 0.687, q^2 = 0.6866, PRESS = 3.21, S_{PRESS} = 0.496, $F_{observed}$ 3, 13 = 12.729 ($F_{\alpha\ 0.05\ 3,\ 13}$ = 3.41), P = 0.000,

Table 4 Pearson correlation matrix for terms in QSAR models

	BA	LOGP	LUMO	SE	CI1	MR
BA	1.000					
LOGP	-0.107	1.000				
LUMO	-0.150	-0.585	1.000			
SE	-0.270	-0.087	-0.478	1.000		
CI1	0.102	0.823	-0.438	-0.232	1.000	
MR	-0.005	0.912	-0.486	-0.174	0.958	1.000

The best QSAR model-III was selected on the basis of statistical parameters discussed earlier. This model explains 84.6 % of aldose reductase inhibitory activity for the training set. The internal predictive power of the model was confirmed by LOO cross validation (q^2) and a q^2 value of 0.6866 indicates robust model. The F-ratio of 12.729 shows that this model is at least 95% significant ($F_{\alpha\ 0.05\ 3,13}$ = 3.41). Tolerance is the percentage of the variance in given predictor that cannot be explained by the other predictors. When the tolerance is close to zero, there is high multicollinearity and the standard error of regression coefficients will be inflated. In collinearity statistics, the high tolerance showed that variance in given predictor cannot be explained by the other predictors, which means there is low multicollinearity. Variance inflation factor (VIF) is a measure of multicollinearity. Data in the model of the series shows that there is absence of any serious multicollinearity problem among the descriptors. (VIF<10)

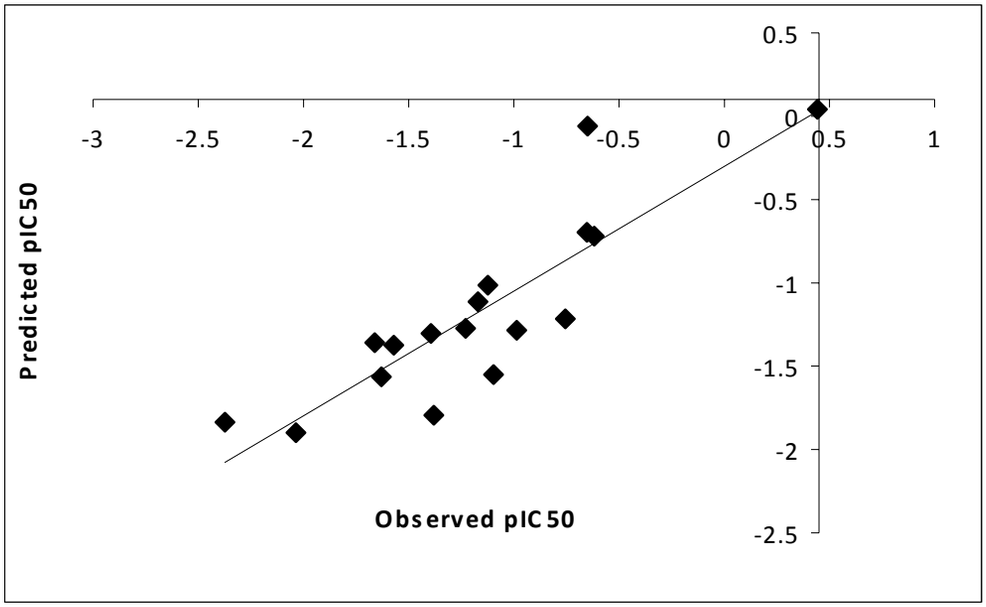


Fig. 1 The graph between actual and predicted activities of training set by using model-III

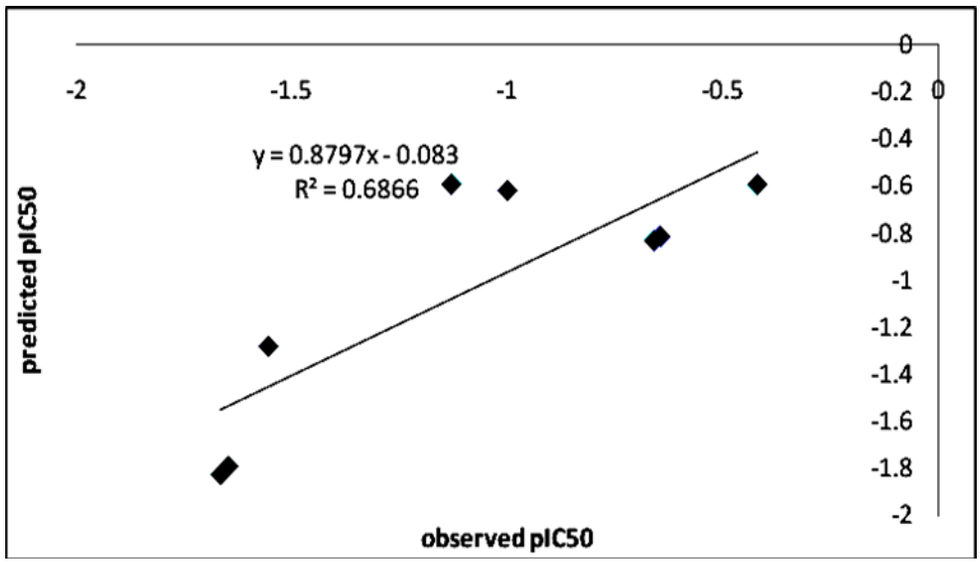


Fig. 2 The graph between actual and predicted activities of test set by using model-III

The model III is internally and externally validated with the training and test set respectively and judge by LOO method. The graph (figure 2 and 3) between actual and predicted values showed that their distribution is acceptable and not grouped. This model is used for the internal and external predictivity of the training sets and test sets respectively. (Table 5 and 6)

Table 5 LOO predicted value for aldose reductase inhibition for training set from model-III

S.No.	Compound	Actual pIC_{50}	Predicted pIC_{50}
1	1	-1.394	-1.304
2	2	-1.57	-1.373
3	3	0.443	0.041
4	4	-1.124	-1.015
5	5	-1.629	-1.564
6	6	-0.618	-0.720
7	8	-1.38	-1.795
8	10	-0.65	-0.060
9	12	-2.036	-1.899
10	14	-0.653	-0.697
11	15	-1.661	-1.358
12	18	-1.17	-1.113
13	20	-0.987	-1.285
14	21	-1.097	-1.551
15	22	-2.373	-1.836
16	24	-1.23	-1.273
17	25	-0.755	-1.215

Table 6 LOO predicted value for aldose reductase inhibition for test set from model-III

S. No.	Compound	Actual pIC_{50}	Predicted pIC_{50}
1	7	-0.66	-0.83
2	9	-0.645	-0.812
3	11	-1.13	-0.59
4	13	-1.667	-1.826
5	16	-1.648	-1.791
6	17	-1	-0.618
7	19	-0.42	-0.591
8	23	-1.555	-1.281
Predicted r^2		**0.6866**	

This model-III shows that LOGP, LUMO energy, and steric energy are negatively affecting the biological activity. LOGP is a partition coefficient in octanol-water system. Partition coefficient plays an important role in transportation of molecules through lipid membranes. In this model, LOGP is negatively affecting which means compounds should have less lipophilic groups for higher biological activity. LUMO is lowest unoccupied molecular orbital energy which means the lowest energy level in the molecule that contains no electrons. This characteristic is important in governing molecular reactivity and properties. When a molecule acts as a Lewis acid (an electron pair acceptor) in bond formation, incoming electron pairs are received in its LUMO. Molecules with low-lying LUMOs are more able to accept electrons than those with high-energy LUMOs; thus descriptor LUMO should measure the electrophilicity of a molecule. The negative correlation of LUMO with biological activity indicates that the

electron withdrawing groups are favorable for the activity. The steric energy of a molecule is the sum of the molecular mechanics potential energies calculated for the bonds, bond angles, dihedral angles, non bonded atoms and so forth. The model shows compounds should have low potential energy for better biological activity. Considering all the above indications it can be said that for a molecule to have higher aldose reductase activity, it should be less lipophilic and should have more electronegative groups or atoms than electropositive groups or atoms.

Conclusion

We have examined the ability of a large, diverse, and consistently tested training set to provide predictive QSAR models of aldose reductase enzyme inhibition. QSAR studies on Triazino[4,3-α]benzimidazole acetic acid derivatives have been done by using the different type of physicochemical parameters such as electronic steric and thermodynamic. The developed QSAR model, concluded that aldose reductase inhibitory activity by the Triazino[4,3-α]benzimidazole acetic acid derivatives is strongly influenced by the thermodynamic and electronic nature of the substitutuents. This QSAR model can be utilized for the further development of new molecules to exhibit good enzyme inhibitory activity. Based on the developed QSAR model, it may be concluded that LOMO, LOGP and Stretch energy are the properties that are to be consider apart from enzyme inhibition activity, while designing newer compounds, for their potential aldose inhibitory activity.

Acknowledgements

Authors are thankful to Head, department of Pharma. Science, Dr. H. S. Gour V.V. Sagar for providing necessary facilities.

Reference

[1] Maccari, R.; Ottana, R.; Ciurleo, R.; Vigorita, M.G.; Rakowitz, D; Steindl, T.; Langer, T. Bioorg. Med. Chem. 2007, 17, 3886-3893.

[2] Alexiou, P.; Nicolaou, I.; Stefek, M.; Kristl, A.; Demopoulos, V.J. Bioorg. Med. Chem. 2008, 16, 3926-3932.

[3] Van-Zandt, M.C.; Sibley E.O.; McCann, E.E.; Combs, K.J.; Flam, B.; Sawicki, D.R.; Sabetta, A.; Carrington, A.; Sredy, J.; Howard, E.; Mitschler, A.; Podjarny, A.D. Bioorg. Med. Chem. 2004, 12, 5661-5675.

[4] Da Settimo, F.; Primofiore, G.; La Motta, C.; Salerno, S.; Novellino, E.; Greco, G.; Lavecchia, A.; Laneri, S,; Boldrini, E. Bioorg. Med. Chem. 2005, 13, 491-499.

[5] Oates, P.J.; Mylari, B.L. Expert Opin. Investig. Drugs 1999, 8, 2095–2119.

[6] Obrosova, I.G.; Van Huysen, C.; Fathallah, L.; Cao, X.C.; Greene, D.A.; Stevens, M.J. FASEB J. 2002, 16, 123–125.

[7] Kato, N.; Yashima, S.; Suzuki, T.; Nakayama, Y.; Jomori, T. J. Diab. Compl. 2003, 17, 374 –379.

[8] Thornalley, P.J. Int. Rev. Neurobiol. 2002, 50, 37–57.

[9] Vlassara, H.; Palace, M.R. Mt. Sinai J. Med. 2003,70, 232–241.

[10] Nakamura, J.; Kato, K.; Hamada, Y.; Nakayama, M.; Chaya, S.; Nakashima, E.; Naruse, K.; Kasuy, Y.; Mizubayashi, R.; Miwa, K.; Yasuda, Y.; Kamiya, H.; Ienaga, K.; Sakakibara, F.; Koh, N.; Hotta, N. Diabetes 1999, 48, 2090-2095.

[11] Way, K.J.; Katai, N.; King, G.L. Diabet. Med. 2001,18, 945-959.

[12] Obrosova, I.G.; Int. Rev. Neurobiol. 2002, 50, 3–35.

[13] Pennathur, S.; Heinecke, J.W. Front Biosci. 2004, 9, 565–574.

[14] Lou, M.F.; Dickerson, J.E.; Jr Garadi, R.; York, B.M. Exp Eye Res. 1998,46,517–530.

[15] Obrosova, I.G.; Fathallah, L. Diabetologia 2000,43,1048 –1055.

[16] Lowitt, S.; Malone, J.I.; Salem, A.F.; Korthals, J.; Benford, S. Metabolism 1995, 44, 677– 680.

[17] Obrosova, I.G.; Minchenko, A.G.; Vasupuram, R.; White, L.; Abatan, O.I.; Kumagai, A.K.; Frank, R.N.; Stevens, M.J. Diabetes 2003,52,864–871.

[18] El-Remessy, A.B.; Abou-Mohamed, G.; Caldwell, R.W.; Caldwell, R.B. Invest Ophthalmol. Vis. Sci. 2003,44,3135–3143.

[19] Inoue, J.; Cui,Y. S.; Sakai, O.; Nakamura, Y.; Kogiso, H.; Peter F.; Kador, P. F. Bioorg. Med. Chem. 2000, 8, 2167-2173.

[20] Donkora, I. O.; Ghanyb, Y. S. A.; Kadorc, P. F.; Mizoguchic, T.; Malikc, A. B.; Millera, D. D. Eur. J. Med. Chem. 1998,33, 15-22.

[21] Costantino, L.; Corso, A. D.; Rastelli, G.; Petrash, J. M.; Mura, U. Eur. J. Med. Chem. 2001, 36, 697-703.

[22] Rakowitz, D.; Maccari, R.; Ottana, R.; Vigorita, M. G. Bioorg. Med. Chem. 2006,14, 567-574.

[23] Settimo, A. D.; Primofiore, G.; Settimo, F. D.; Simorini, F.; Motta, C. L.; Martinelli, A.; Boldrini, E. Eur. J. Med. Chem. 1996, 31, 49-58.

[24] Maccari, R.; Ottana, R.; Ciurleo, R.; Vigorita, M. V.; Rakowitz, D.; Steindl, T.; Langer, T. Bioorg. Med. Chem.Lett. 2007,17, 3886-3893.

[25] Da Settimo, F.; Primofiore, G.; Da Settimo, A.; La Motta, C.; Taliani, S.; Simorini, F.; Novellino, E.; Greco, G.; Lavecchia, A.; Boldrini, E. J. Med. Chem. 2001, 44, 4359-4369.

[26] Oprea, T. J.; Waller, G. L.; Marshall, G. R. J. Med. Chem. 1994, 37, 2206-2215.

[27] www.cache.fujitsu.com/biomedcache/index.shtml.

[28] SYSTAT Software 10.2 for Windows. SYSTAT Software, Inc.2002, Richmond, California.