Quantitative Structure−Activity Relationship Studies of [(Biphenyloxy)propyl]isoxazole Derivatives. Inhibitors of Human Rhinovirus 2 Replication

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The 50% cytotoxic concentration (CC_{50}) in HeLa cells, the 50% inhibitory concentration (IC_{50}) against human rhinovirus 2 (HRV-2), and the selectivity index ($SI = CC_{50}/IC_{50}$) of [(biphenyloxy)propyl]isoxazole derivatives were used to develop quantitative structure−activity relationships (QSAR) based on simplex representation of molecular structure. Statistic characteristics for partial least-squares models are quite satisfactory ($R^2 = 0.838 - 0.918$; $Q^2 = 0.695 - 0.87$) for prediction of CC_{50}, IC_{50}, and SI values and permit the virtual screening and molecular design of new compounds with strong anti-HRV-2 activity. The quality of prognosis for designed compounds was additionally estimated by analysis of domain applicability for each QSAR model. A hypothesis to the effect that terminal benzene substituents must have negative electrostatic potential and definite length (approximately 5.5−5.6 Å) to possess strong antiviral activity has been suggested. The quality of developed analysis, i.e., high level of antiviral action of three new designed compounds, has been confirmed experimentally.

Introduction

Human rhinoviruses (HRV[a]) are estimated to cause approximately one-third of all upper respiratory tract viral infections. The characteristic clinical syndrome found after rhinovirus infection is the common cold.[1] But human rhinovirus infections have also been associated with the development of otitis media, pneumonia, and sinusitis in children and with more serious diseases in both the upper and lower respiratory tracts. These infections can be more serious in patients suffering from chronic respiratory diseases, triggering acute exacerbations of asthma, emphysema, and chronic obstructive pulmonary disease. HRV infections may also be associated with substantial morbidity in the elderly. The most important route of transmission is through hand to hand contact.[2] The optimum environment for replication of the majority of HRV in cell culture is 33−35 °C. This may explain why HRV replicates well in the nasal passages and upper respiratory tract but less well in the lower respiratory tract. However, recent studies have established that rhinoviruses can also replicate in the lower respiratory tract and induce fever, pneumonia, and gastrointestinal symptoms.[3−5] Because of their high incidence, HRV infections are associated with an enormous economic burden both in lost productivity and in expenditure for treatment. In addition, billions of dollars per year are spent in the U.S. on inappropriate antibiotic prescription and over-the-counter medicines associated with treatments for the common cold.[6] Thus, prevention or treatment of HRV infections to avoid these complications would have an enormous socioeconomic impact with regard to both morbidity and economic cost.

Because more than 100 different rhinovirus serotypes exist, vaccine development for prevention of rhinovirus infections is considered to be impracticable. The present treatment options for HRV infections are unsatisfactory.[7−9] But there are ongoing attempts to develop antiviral drugs.[10−16] Capsid-binding agents like pleconaril are among the best studied compounds and seem to be most promising at present. Pleconaril, an antiviral with good activity against a broad spectrum of entero- and rhinoviruses in cell culture,[16−18] has modest effects in clinical studies when given orally.[19,20] Moreover, it was found to induce cytochrome P-450 3A enzymes[21] and resistance during the course of treatment.[20,22] Clinical benefit correlates strongly with the pleconaril susceptibility of baseline isolates.[18] Now, Schering-Plough, under license from ViroPharma, is developing an intranasal formulation of pleconaril for the potential treatment of the common cold in high-risk populations.[8]

In the search for antiviral agents, a new class of perspective compounds, derivatives of [(biphenyloxy)propyl]isoxazole, has been investigated.[23] Because of their good tolerability and potent antiviral activity, [(biphenyloxy)propyl]isoxazole derivatives are of considerable interest in antiviral therapy as high-quality lead compounds for further synthesis. Therefore, a computational approach that can distinguish highly active inhibitors from less useful compounds and predict more potent compounds should be established and applied in the present study. For many years, QSARs have been used for the analysis of toxicity and antiviral activity.[24−29] This technique quantitatively relates variations in biological activity to changes in molecular properties.

The aims of the present study were (i) to determine structural elements of [(biphenyloxy)propyl]isoxazole derivatives enhancing the antiviral activity as well as selectivity and decreasing cytotoxicity, (ii) to understand chemical−biological factors governing mechanisms of their antiviral action, and (iii) to predict and design novel compounds with improved antiviral activity.

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[a] Abbreviations: CC_{50}, 50% cytotoxic concentration; IC_{50}, 50% inhibitory concentration; SI, selectivity index, HRV, human rhinoviruses; CPE, cytopathic effect; pleconaril, 5-[3-[2,6-dimethyl-4-[5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl]phenoxy]propyl]-3-methylisoxazole; SiRMS, simplex representation of molecular structure; PLS, partial least squares or projections to latent structures; DA, domain applicability.

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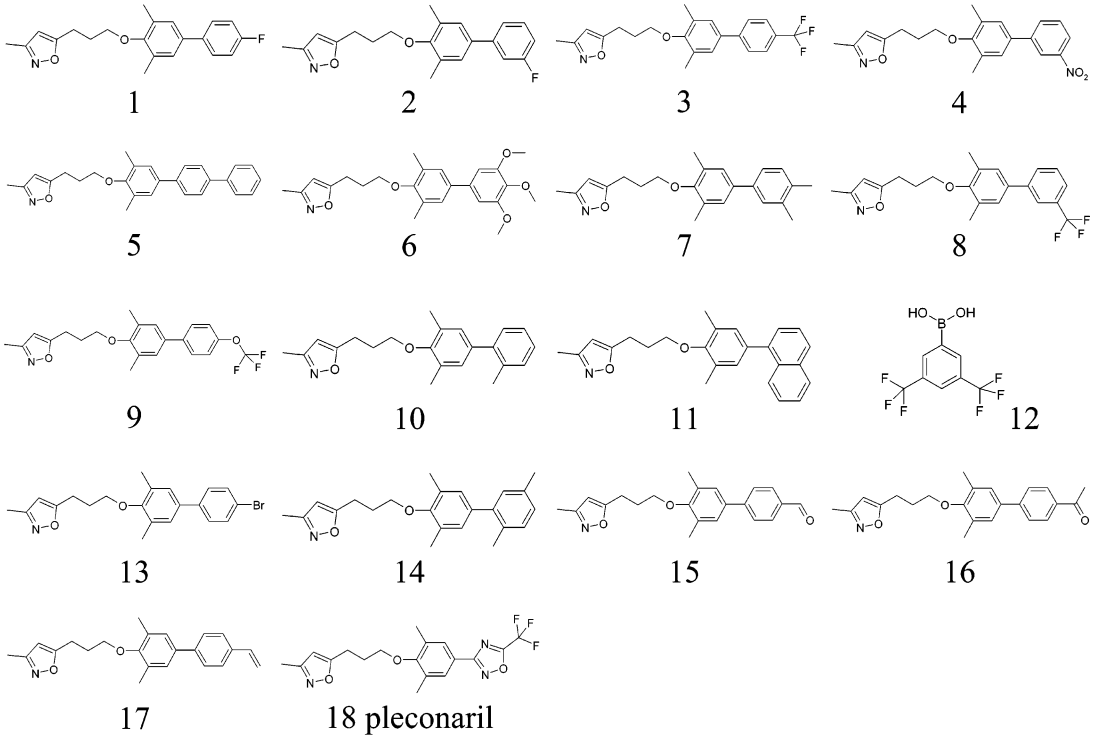


Figure 1. Structure of training set compounds.

Results and Discussion

The work set consists of 18 compounds (Figure 1). They have not been divided into training and test sets because of their small amount (i.e., structural information containing in each molecule in our case is unique and useful). Approximately 8000 simplex descriptors have been calculated during the initial stage of work. Differentiation of atoms in simplexes has been developed on the base of following characteristics: (1) atom type (used as mark in the simplex vertexes), (2) partial charge, (3) lipophilicity, (4) refraction, and (5) a mark that characterizes the atom as a possible donor or acceptor of an H-bond. The atoms weighted by properties have been divided into seven groups corresponding to their (i) partial charges, $A \le -0.1 < B \le -0.05 < C \le -0.01 < D \le 0.01 < E \le 0.05 < F \le 0.1 < G$, (ii) lipophilicity, $A \le -1 < B \le -0.5 < C \le -0.1 < D \le 0.1 < E \le 0.5 < F \le 1 < G$, and (iii) refraction, $A \le 2 < B \le 3 < C \le 4 < D \le 6 < E \le 9 < F \le 12 < G$.

After the data mining procedure, PLS QSAR models were established (see Experimental Section) using 190 simplex descriptors from the initial set. Their determination coefficient values are shown in Table 2 ($R^2 = 0.838 - 0.918$) and confirm that for the majority of compounds the observed values are in excellent correlation with predicted values of cytotoxicity, antiviral activity, and selectivity (Table 2) (observed vs predicted diagrams for all compounds are in Supporting Information). Because of the absence of a test set, the model prediction power will be determined through Q^2, the determination coefficient calculated in cross-validation terms. Its quality ($Q^2 = 0.695 - 0.870$) is quite satisfactory. This indicates that the variables employed provide a model with good predictive ability for cytotoxic and antiviral activity as well as selectivity index of the investigated compounds. Additional model validation by comparison of observed/predicted values of investigated properties for new designed compounds predicted and tested with these QSAR models will be discussed later.

By use of the established QSAR models, the influence of selected physicochemical properties of investigated compounds on cytotoxicity, antiviral activity, and selectivity was studied (Figure 2). The results indicate a high influence of the atom's individuality on all investigated properties (~40%). The impact of electrostatic factors increases during the passage from antiviral activity (~20%) to selectivity (~50%), where these

Table 1. Observed and Predicted Values of Investigated Properties of [(Biphenyloxy)propyl]isoxazole Derivatives

	property					
	anti-HRV-2 activity (log IC_{50})		selectivity index (log SI)		cytotoxicity (log CC_{50})	
structure	obsd	predicted	obsd	predicted	obsd	predicted
1	−2.05	−1.81	3.74	3.59	1.70	1.68
2	−1.40	−1.27	2.36	2.34	0.96	0.94
3	−0.97	−1.08	2.05	2.24	1.08	1.21
4	−1.47	−1.28	2.25	2.12	0.79	0.73
5	1.70	1.77	0	−0.14	1.70	1.72
6	−0.54	−0.61	1.53	1.61	1.00	1.01
7	−1.31	−1.01	1.97	1.39	0.66	0.66
8	−1.24	−0.98	2.32	2.24	1.08	1.21
9	0.05	−0.38	1.00	1.14	1.03	0.94
10	−1.09	−0.33	1.76	1.33	0.66	1.06
11	−0.20	0.10	1.16	1.25	0.96	1.03
12	1.70	1.94	0	−0.10	1.70	1.68
13	−0.15	−0.69	0.85	1.12	0.68	0.69
14	1.06	−0.25	0.6	1.40	1.70	1.12
15	−1.09	−0.89	2.09	2.31	1.00	1.05
16	−0.63	−0.81	2.33	2.29	1.70	1.69
17	−0.90	−1.01	3.20	3.26	2.30	2.29
18	−1.70	−1.63	2.80	2.62	1.10	1.09

Table 2. Statistical Characteristics of Obtained QSAR Models[a]

activity	R^2	Q^2	*S*(ws)	*S*(cv)	*A*	*N*	*M*	outliers
HRV-2	0.838	0.767	0.441	0.586	2	10	18	no
SI	0.918	0.870	0.294	0.407	2	15	18	no
cytotoxicity	0.851	0.695	0.181	0.27	3	8	18	no

[a] R^2: correlation coefficient. Q^2: cross-validation correlation coefficient. *S*(ws): standard error of a prediction for work set. *S*(cv): standard error of prediction for work set in cross-validation terms. *A*: number of PLS latent variables. *N*: number of descriptors. *M*: number of molecules in work set.

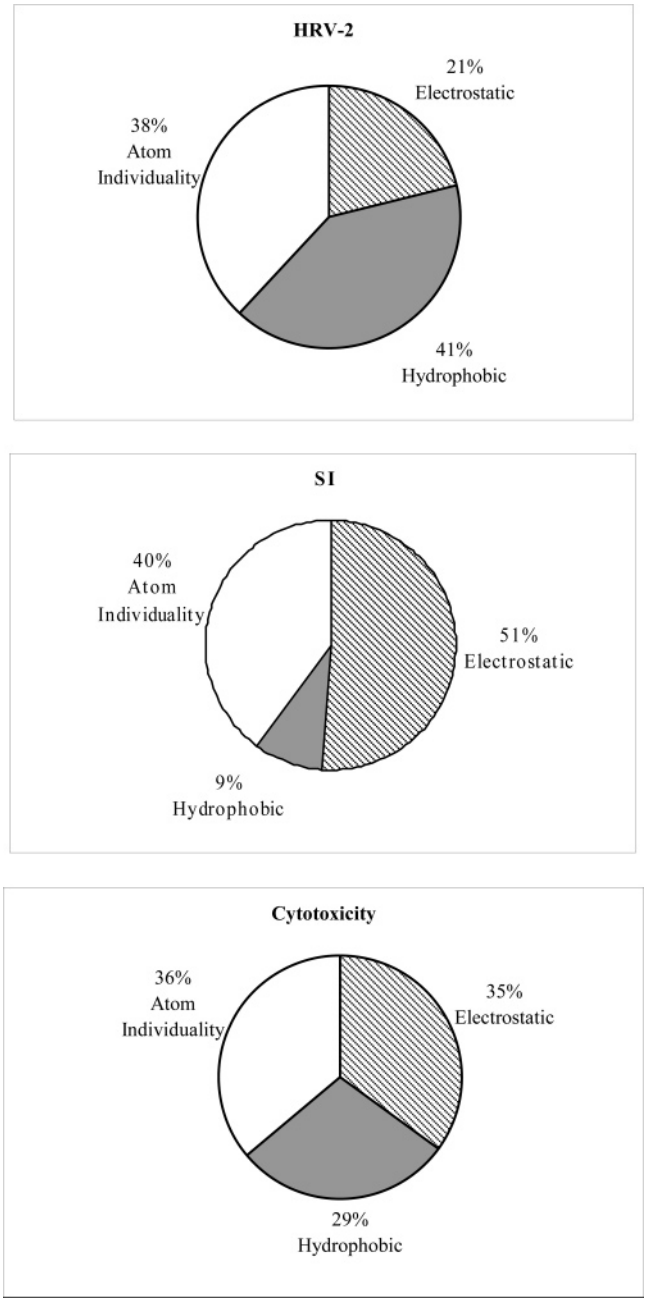


Figure 2. Relative influence of some physicochemical factors on variation of cytotoxicity, anti-HRV-2 activity, and selectivity index estimated on the basis of QSAR models.

factors along with atom individuality play the determining role. The situation for hydrophobic interactions is diametrically opposite. They do not play an important role for selectivity (~10%) but are quite important for antiviral activity (~40%). It is necessary to note that all of the mentioned factors have nearly the same importance for antiviral activity and cytotoxicity.

It is well-known[30] that the PLS equation can be represented as

$$Y = b_0 + \sum_{i=1}^{N} b_i x_i$$

where Y is appropriate activity, b_i is PLS regression coefficients, x_i is the ith descriptor value (the number of simplexes of ith type in the SiRMS), and N is the total number of descriptors. By use of this equation, it is not difficult to make the reverse analysis (interpretation of QSAR models) by using the SiRMS approach. The contribution of each j atom (C_j) in the molecule can be defined as ratio of the sum of PLS regression coefficients (b_i) of all simplexes this atom contains (M), to the number of atoms in the simplex: $C_j = (1/4)\sum_{i=1}^{M} b_i$.[31] Thus, the atoms having a positive or negative influence on the studied biological activity of compounds can be determined easily. For example the structure of compound **5** (Table 1) is shown in Figure 3. Atoms and structural fragments necessary for antiviral activity and selctivity are colored in light-gray. Dark-gray was used to visualize atoms and structural fragments reducing antiviral activity.

By use of this information, molecular fragments promoting or interfering with investigated activities were determined (Table 3). Thus, the presence of fragments 1−4 and 17 (Table 3) in the molecule leads to strong enhancement of its useful properties, i.e., increase of activity toward HRV-2 as well as selectivity and decrease of cytotoxicity. Fragment 1 is the best for selectivity increase. Both fragments 1 and 17 exhibited the strongest influence on antiviral activity, along with fragment 16 which decreases the cytotoxicity more than others. In turn, fragment 16 increases selectivity but has quite a weak influence on antiviral activity. An additional aromatic ring, naphthalene (R11) or phenyl (R5), strongly decreases activity toward HRV-2 and to a lesser degree SI. The presence of R9 and R10 is also undesirable. Insertion of bromine slightly increases cytotoxicity and decreases activity toward HRV-2 and SI. The influence of other fragments reflected in the Table 3 does not have prominent character.

By use of the results from the established QSAR on strength and direction of the influence of distinct structural fragments, new compounds **19**−**26** (Table 4) were designed. The values of the investigated properties for these compounds have been predicted by the obtained PLS models (Table 4). As is obvious from Table 4, designed compounds **19**, **21**, and **23** are more active than pleconaril, compounds **19**−**23** are more selective, and compounds **20**−**23** are less toxic, where the difference in toxicity between pleconaril and compounds **22** and **23** is more than 1 logarithmic unit.

It was interesting to try to estimate the reliability of prognosis by the DA procedure. DA ellipsoids and rectangles (see Experimental Section) for all obtained models are shown in Figure 4. Values h_i from approach 1 have been represented in this article only for compounds with $h_i > h_{cr}$ ($h_{cr} = 0.5$ for HRV-2 and SI models; $h_{cr} = 0.67$ for cytotoxicity model) (h_i values for all compounds are in Supporting Information). As is obvious from Figure 5, in the case of the HRV-2 model, compounds **1** and **19** were unsatisfactory for both DA ($h_1 = 0.59$ and $h_{19} = 0.67$). According to the obtained SI model, only compound **22** has a less reliable prognosis by all three DA procedures ($h_{22} = 0.8$). Although compounds **1**, **20**, and **23** fall into DA rectangle, they are located out of the DA ellipsoid and have h_i values higher than h_{cr} ($h_1 = 0.68$, $h_{20} = 0.7$, $h_{23} = 0.74$). In the cytotoxicity model, compound **12** does not correspond to all DA ($h_{12} = 0.92$) and compounds **22** and **23** do not conform to leverage criterion ($h_{22} = 0.89$, $h_{20} = 0.96$). In summary, all mentioned DA estimation procedures give nearly the same results whereas leverage and ellipsoid DA are in better agreement with each other than with rectangle DA. If a work set molecule (**1** and **12** in our case) does not correspond to DA criteria, it is called "influential", i.e. has unique (for a given work set) structural features that distinguish it from other compounds. If designed molecules are situated outside the DA, their prediction is less reliable.

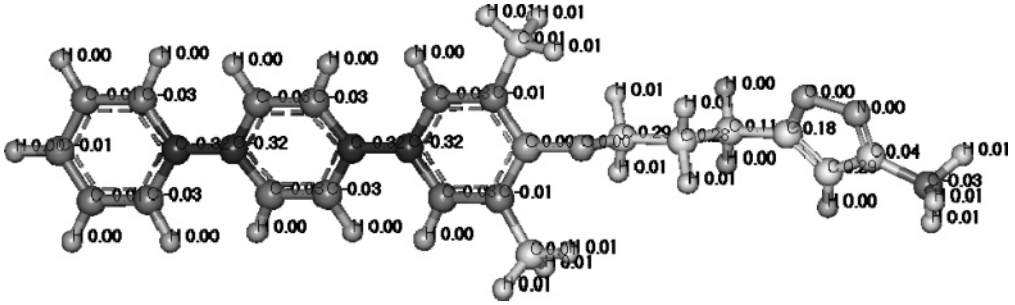


Figure 3. Example of color-coded structure of compound **5**. Atoms and structural fragments reducing antiviral activity and selectivity are colored in dark-gray, and those enhancing antiviral activity and selectivity are in light-gray and white.

Compounds **19**−**21** have been synthesized and tested for cytotoxicity and anti-HRV2 activity. Observed values of investigated properties are presented in Table 4. As is obvious from Table 4, the predicted values for these compounds are in excellent correlation with the experimental ones with only one exception: the cytotoxicity value for structure **20**. These results confirm once again the high predictive power of the obtained models and show that their real competence region is wider than the DA. As is obvious from Figure 5, compound **20** corresponds to DA for the cytotoxicity model. It has been poorly predicted and is situated outside DA for the SI model but has been well-predicted like compound **19** in the HRV-2 model. These results reflect the relative character of any DA procedure and are a reminder that DA is only a probable recommendation.

Predicted SI values can be easily compared with that calculated by HRV-2 and cytotoxicity models: $\log SI_{calculated} = \log CC_{50} - \log IC_{50}$ (represented in parentheses in the Table 4). The $SI_{observed}$ values are in good correlation with both $SI_{predicted}$ and $SI_{calculated}$ for compounds **19** and **21** and bad correlation with $SI_{calculated}$ of structure **20**. But this discrepancy is caused by an incorrect prediction of the cytotoxicity value. By application of this procedure to compounds **22** and **23** with unreliable DA prediction of SI, a great difference (more than 1 logarithmic unit) between $SI_{predicted}$ and $SI_{calculated}$ becomes apparent. On the basis of these data, it can be suggested with great probability that real SI values are lower than predicted ones.

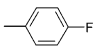
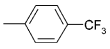
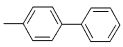
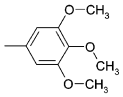
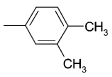
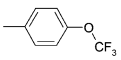
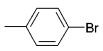
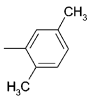
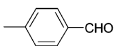
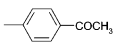
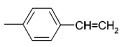
Taking into account the quite satisfactory quality (by statistic characteristics) of the obtained QSAR models as well as the good results of their external validation, the development and virtual screening of new compounds **27**−**37** were considered to be possible (Table 4). With the aim to understand their mechanism of influence, substituents in the aromatic ring of the variable part of investigated compounds were exchanged. Even simple visual analysis of molecules from the work set shows that there is no unambiguous influence of substituents on antiviral activity in relation to their donor or acceptor effects. Thus, for example, compound **6** that contains typical electron-donating $-OCH_3$ groups and compound **16** with an aldehyde group (typical acceptor) have close activity values. More detailed analysis with the usage of polar Hammett σ substituents constants shows the absence of correlation with activity ($R = 0.35$). Nevertheless, we draw our attention to the following: a relationship with activity is possible for substituents that create negative electrostatic potential, i.e., contain a heteroatom with partial negative charge and/or π-electron fragments, when the length of these substituents has been used as a structural descriptor. Quite satisfactory dependence ($R = 0.88$) between anti-HRV-2 activity and their L value (L is a substituent steric descriptor that reflects it length;[32] L values for these compounds are in Supporting Information) authenticates this hypothesis. It is possible to propose an approximate scheme of interaction of these substituents with the fragment of biological target (Figure 5A). Most probably the fluorine atom in the para position of aromatic ring B (compound **1** ($L = 5.59$ A) is quite complementary to cavity C for such interaction. It is necessary to note that the molecule of pleconaril ($L = 5.54$ Å) completely satisfies the indicated criteria. Insertion of additional fluorine atoms at the meta positions of the benzene ring (compounds **20**, **32**, **33**) most likely leads to "extrusion" of the para substituent from cavity C (Figure 5B), which decreases activity (see Table 5). For nitrosubstituted compounds the other situation has been observed. The nitro group at the para position does not join into cavity C because of steric reasons. But enough strong electrostatic interactions "hold" this group in the cavity area. Thus, the accumulation of nitro groups in the region of cavity C will lead to strengthening of electrostatic interactions and an increase in activity (Figure 5C). Despite some speculation of this analysis, we suggest this hypothesis is "viable". However, we are aware that additional synthesis and screening experiments are necessary for its confirmation.

Conclusion

The SiRMS based QSAR approach allows us to analyze successfully the antiviral action of [(biphenyloxy)propyl]isoxazole derivatives on a 2D level of molecular structure representation. Our results indicate a nearly constant high influence of the atom's individuality on alteration of all investigated properties (~40%). The molecular fragments promoting or interfering investigated activities were determined. A fluorine atom at the para position of the benzene ring strongly promotes activity. The virtual screening and molecular design of new well-tolerated compounds with strong anti-HRV-2 activity have been carried out on the base of QSAR analysis. Three different DA approaches give near the same results for each QSAR model and allow us to estimate additionally the quality of prognosis for all of the designed compounds. A hypothesis to the effect that the external benzene substituent must have negative electrostatic potential and definite length (approximately 5.5−5.6 Å) to possess strong antiviral activity has been suggested. In the case of nitroaromatics the accumulation of nitro groups in the region of cavity C will lead to strengthening of electrostatic interactions and an increase of activity.

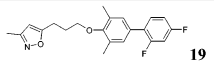
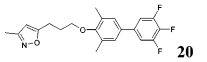
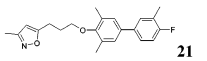
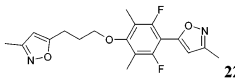
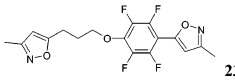
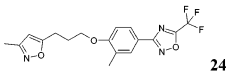
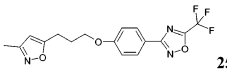
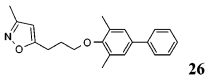
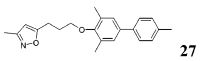
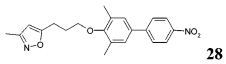
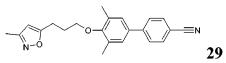
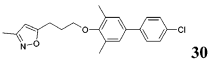
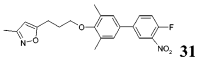
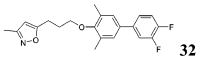
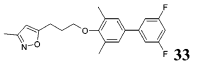
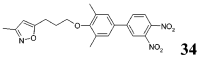
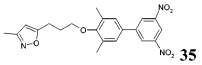
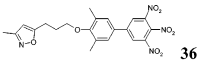
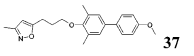
Eighteen new compounds have been computationally designed and predicted as high active. Three of them were synthesized and experimentally tested. A strong coincidence between experimental and predicted anti-HRV-2 activity and SI has been observed. In the case of cytotoxicity only for one compound, a quite big error of prediction occurred when two structures were predicted well.

Table 3. Examples of Molecular Fragments and Their Relative Influence[a] on the Investigated Properties

Code	Fragment	Anti-HRV-2 activity (ΔLogIC$_{50}$)	SI (Δlog SI)	Cytotoxicity (Δlog CC$_{50}$)
1		-0.91	1.79	0.98
2		-0.45	0.64	0.24
3		-0.18	0.34	0.38
4		-0.18	0.37	0.12
5		1.67	-1.16	0.79
6		0.12	0.04	0.29
7		-0.45	-0.08	-0.04
8		-0.10	0.06	0.38
9		0.17	-0.39	0.18
10		0.29	-0.11	0.30
11		0.50	-0.15	0.24
12		0.10	-0.22	0.07
13		0.05	-0.08	0.00
14		-0.07	0.37	0.34
15		-0.05	0.31	0.68
16		-0.10	0.69	0.96
17		-0.91	0.14	0.38

[a] In every concrete case, the fragment's contributions are dependent on their position in the molecule. Notes: "0", without influence; "+", SI increase, antiherpetic activity as well as cytotoxicity decrease; "−", SI decrease, antiherpetic activity as well as cytotoxicity increase.

Table 4. Perspective Compounds: Molecular Design Results[a]

Structure	Property					
	Anti-HRV-2 activity (log IC$_{50}$)		Selectivity Index (log SI)		Cytotoxicity (log CC$_{50}$)	
	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
 **19**	-2.30	-2.28	3.20	3.27 (3.05)	0.90	0.75
 **20**	-1.46	-1.53	3.34	3.52 (2.65)	1.88	1.11
 **21**	-1.82	-1.83	3.10	3.03 (3.05)	1.28	1.25
 **22**		-1.40		5.15 (3.57)		2.17
 **23**		-1.81		5.13 (4.01)		2.10
 **24**		-1.10		2.50 (2.00)		0.90
 **25**		-0.58		2.37 (1.3)		0.72
 **26**		-0.41		1.24 (1.42)		1.01
 **27**		-0.33		1.32 (1.39)		1.06
 **28**		-1.39		2.13 (2.12)		0.73
 **29**		-0.61		1.73 (1.68)		1.05
 **30**		-0.69		1.14 (1.38)		0.69
 **31**		-2.14		3.57 (3.46)		1.32
 **32**		-1.80**		3.58** (3.16)		1.36
 **33**		-1.24		2.75 (2.29)		1.05
 **34**		-2.16		2.73 (2.15)		0.99
 **35**		-2.16		2.72 (2.61)		0.45
 **36**		-2.94**		3.47 (3.91)		0.97
 **37**		-0.61		1.37 (1.35)		0.74

[a] (**) This value is less reliable than others (out of DA).

Experimental Section

QSAR. The Work Set. The work set consists of 17 structurally characterized derivatives of [(biphenyloxy)propyl]isoxazole (Figure 1) that were examined for cytotoxic as well as antiviral activity under in vitro conditions. The synthesis procedures, chemical structures, cytotoxicity, and activity against HRV-2 of these compounds were determined and published by Makarov et al. in 2005.[23] Additionally, pleconaril **18** was included in these studies.

Good tolerability and strong antiviral activity are both important for the development of antiviral drugs. Therefore, a thorough investigation of the relationship between structure and (i) cytotoxicity in Hela cells, (ii) antirhinoviral activity, and (iii) the selectivity index (SI) of [(biphenyloxy)propyl]isoxazole derivatives has been carried out in the present study. The cytotoxicity is expressed in terms of 50% cytotoxic concentration (CC$_{50}$, μg/mL) in HeLa cells. The antirhinoviral activity is expressed in terms of 50% inhibitory concentration (IC$_{50}$, μg/mL) determined against HRV-2 in HeLa cells. The SI represents the ratio of CC$_{50}$ to IC$_{50}$. All original data

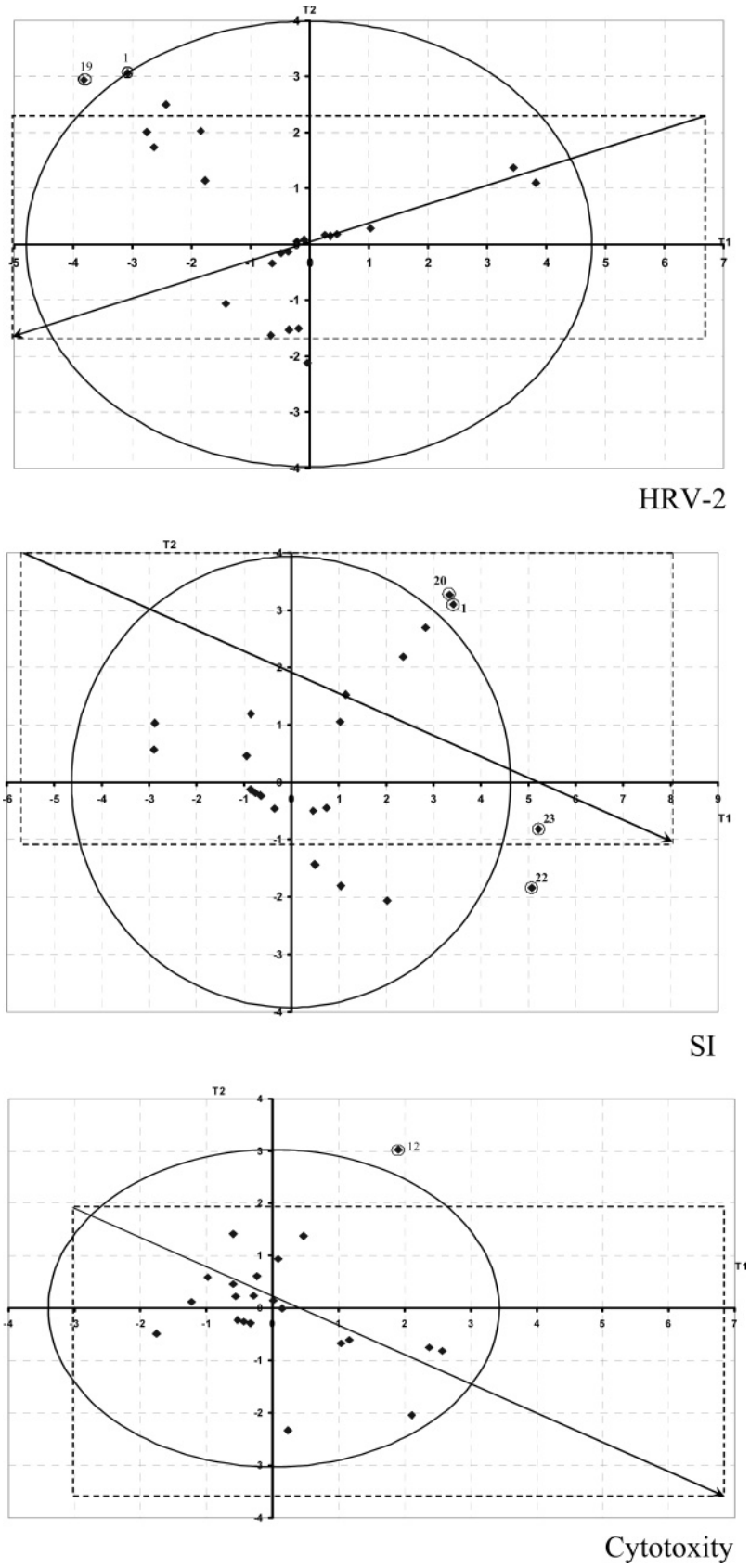


Figure 4. QSAR models domain applicability.

have been converted to $\log_{10}$ response variables ($\log CC_{50}$, $\log IC_{50}$, log SI). The corresponding values (observed) are presented in Table 1.

Simplex Representation of Molecular Structure (SiRMS) Method. SiRMS[27,33−35] has been used as the main tool for QSAR investigations. This method showed good results in previous studies for solving different "structure−activity" problems.[35,36] The simplex descriptors (SD = number of four-atom fixed structure fragments) were used to describe the molecular structure. This approach accounts not only for the atom type but also for other atom

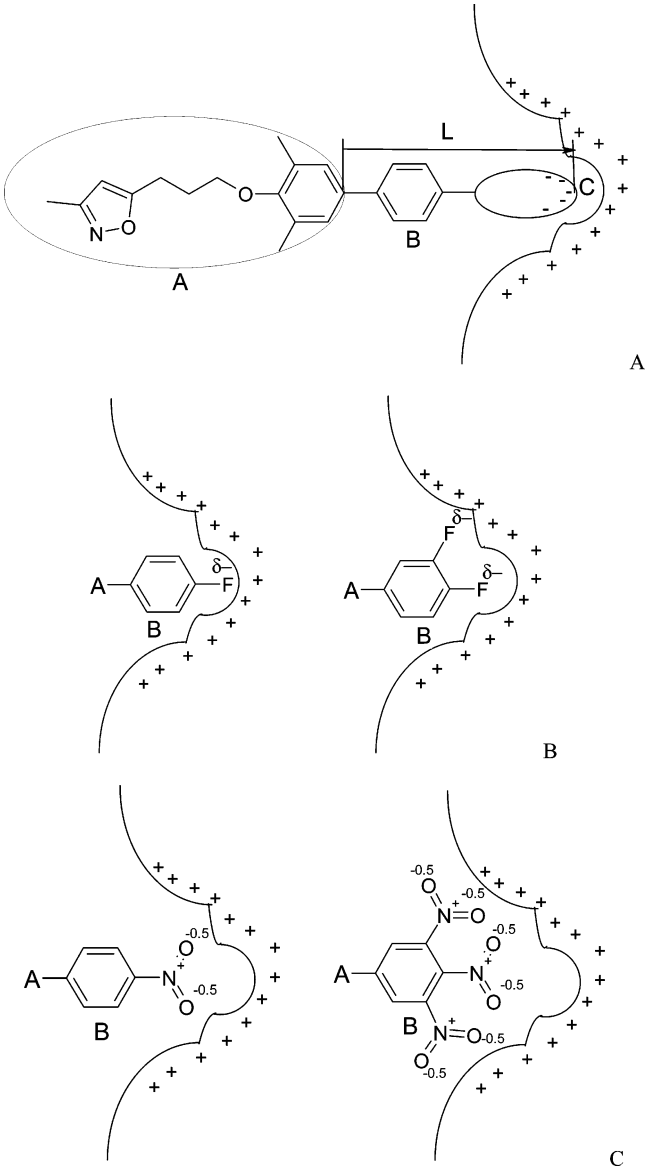


Figure 5. Scheme of interaction of selected substituents with the fragment of biological target.

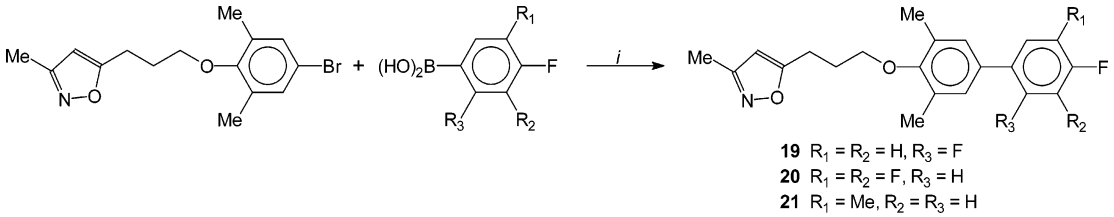
characteristics important for possessing biological activity of compounds, e.g., partial charge,[37] lipophilicity,[38] refraction,[39] and atom ability for being a donor/acceptor in hydrogen-bond formation (H-bond). For atom characteristics, which have real values (charge, lipophilicity, refraction) at the preliminary stage, division of the value range into discrete groups is carried out. The number of groups (G) is a tuning parameter and can be varied (as a rule $G = 3-7$). For atom H-bond characteristics, the atoms has been divided into three groups: A (acceptor of hydrogen in H-bond), D (donor of hydrogen in H-bond), I (indifferent atom). The use of diverse variants of differentiation of simplex vertexes (atoms) represents the principal feature of the offered approach. The main advantages of SiRMS are the opportunity of analysis of molecules with noticeable structural differences within a training set of compounds as well as the possibility to reveal individual molecular fragments (simplex combinations) promoting or interfering biological activities, e.g., cytotoxicity, toxicity, and antiviral activity. The large number of simplex descriptors has been generated on the base of SiRMS approach. The PLS method[30,40,41] proved to be efficient with a great number of variables. The removal of highly correlated and constant descriptors, genetic algorithm (GA),[42] trend-vector method,[43−45] and automatic variable selection (AVS) strategy based on interactive[41] and evolutionary[40] variables selection have been used for selection of descriptors in PLS.

QSAR investigations can be used to make predictions for compounds with unknown activity values (sometimes called "virtual screening"). It is evident that any QSAR model cannot be used for activity prediction of compounds with random structure. Each QSAR has it own "domain applicability" (DA)[46] in the space of structural features, where compounds relatively similar by structure are located. Activity prediction for new compounds that have strong differences in structure in comparison with training set ones is not very reliable. Thus, the determination of DA, i.e., that region of space of structural features where we can trust the results of QSAR prognoses, is an important stage of QSAR research. There are several approaches for DA estimation.[46] In the given work, three of them have been used. The first one is based on the estimation of leverage value h_i.[47] The molecule is situated out of DA if $h_i > h_{cr} = 3(A + 1)/M$, where A is the number of PLS latent variables and M is the number of molecules in a work set. Developed by us, the DA procedure has been used as the second approach. Its essence consists of the following: the distribution of molecules from training set in a space of latent variables T_1-T_A (axes of coordinates) can be obtained from PLS. For each coordinate axis (T_1 and T_2 in our case) the average values A_{T1} and A_{T2} and their corresponding root-mean-squared deviations S_{T1} and S_{T2} have been determined. DA represents an ellipsoid that is built from molecules of the training set distribution center with the S semiaxes lengths being $3S_{T1}$ and $3S_{T2}$, respectively. Thus, if a new molecule from the prediction set is situated out of DA (square inside ellipsoid), its prognosis by the corresponding QSAR model is not very reliable. And naturally, the prognoses for molecules approximated to the center of DA are the most reliable. The third approach is based on the SiRMS. Two extreme points in a space of structural features are determined in it. The first one contains maximum number of simplexes (work set data) promoting activity and minimum interference. This point corresponds to the hypothetical molecule and is peculiar activity etalon. The second point, analogically, is inactivity etalon, i.e. contains a maximal amount of simplexes interfering activity and minimal promoting. The vector that unites these points (directed from inactive to active one) depicts the tendency for toxicity change in the variables space. This vector is a diagonal for rectangle that determines DA.

Chemistry. Reagents and Instrumentation. All reagents and solvents were purchased from commercial suppliers and used without further purification. Melting points were determined on an Electothermal 9001 instrument and are uncorrected. ^{1}H and ^{13}C NMR were measured at 400 MHz on a Varian Unity +400 spectrometer. Shifts for NMR are reported in ppm downfield from TMS (σ). A Waters Micromass ZQ detector was used in ESI MS for identification of various products. Elemental analyses were carried out on a Carlo-Erba 5500 elemental analyzer for C, H, F, N, and the results are within ±0.3% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC. Column chromatography was performed on Merck silica gel 60 (70−230 mesh).

5-{3-[(2′,4′-Difluoro-3,5-dimethylbiphenyl-4-yl)oxy]propyl}-3-methylisoxazole (19). A suspension of 5-[3-(4-bromo-2,6-dimethylphenoxy)propyl]-3-methylisoxazole (0.60 g, 1.93 mmol), 2,4-difluorophenylboronic acid (0.33 g, 2.10 mmol), and tetrakis-(triphenylphosphine)palladium(0) (0.09 g, 0.08 mmol) in isopropyl alcohol (25 mL) was treated by a water solution (10 mL) of $NaHCO_3$ (0.78 g, 9.28 mmol) and heated at 80 °C for 1 h (Scheme 1), at which time TLC (hexane/acetone, 2:1) indicated the reaction had gone to completion.[23] The reaction mixture was cooled and diluted by 60 mL of water and 30 mL of of ethyl acetate. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The combined organic phases were washed twice by a 1 N solution of NaOH and twice by water, dried over Na_2SO_4, treated with activated carbon, filtered off, and concentrated by rotary evaporation. The residue was separated by column chromatography on silica gel (hexane/acetone, 10:1) to give **19** (200 mg, 31%) as a white solid, mp 71−73 °C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18 (m, 2H), 2.26 (s, 3H), 2.29 (s, 6H), 2.99 (t, J = 129.5 Hz, 2H), 3.84 (t, J = 144 Hz, 2H), 5.87 (s,

Scheme 1. Synthesis of 5-{3-[(3,5-Dimethylbiphenyl-4-yl)oxy]propyl}-3-methylisoxazoles **19**−**21**[a]



[a] (i) Tetrakis(triphenylphosphine)palladium(0), $NaHCO_3$, ^{i}PrOH, 80 °C, 1 h.

1H), 6.82−6.92 (m, 2H), 7.12 (s, 2H), 7.32 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.2, 16.2, 23.3, 28.2, 70.5, 101.6, 104.0 (t, J = 26 Hz), 111.2 (q, J = 21.4, 3.8 Hz), 124.9 (q, J = 13.7, 3.8 Hz), 129.2, 130.3, 130.8, 131.2 (q, J = 9.1, 5.0 Hz), 155.3, 159.4 (q, J = 249, 11.4 Hz), 161.8 (q, J = 249.1, 12.2 Hz), 159.6, 172.3. MS (ESI) *m/z*: 358.39 [M + H$^+$].

5-{3-[(3′,4′,5′-Trifluoro-3,5-dimethylbiphenyl-4-yl)oxy]propyl}-3-methylisoxazole (20) was synthesized in a 61% yield from 3,4,5-trifluorophenylboronic acid using the procedure described above, mp 82−83 °C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.19 (m, 2H), 2.26 (s, 3H), 2.29 (s, 6H), 2.99 (t, J = 129 Hz, 2H), 3.82 (t, J = 143 Hz, 2H), 5.87 (s, 1H), 7.06−7.14 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.3, 16.3, 23.3, 28.2, 70.6, 101.6, 110.6, 127.2, 131.5, 133.6, 137.0 (q, J = 20 Hz), 138.5 (dt, J = 250, 15 Hz), 151.2 (dq, J = 248.7, 9.9, 4.5 Hz), 156.0, 159.7, 172.2. MS (ESI) *m/z*: 376.40 [M + H$^+$].

5-{3-[(4′-Fluoro-3′-methyl-3,5-dimethylbiphenyl-4-yl)oxy]propyl}-3-methylisoxazole (21) was synthesized in a 46% yield from 4-fluoro-3-methylphenylboronic acid using the procedure described above, mp 77−79 °C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18 (m, 2H), 2.27 (s, 3H), 2.30 (s, 6H), 2.31 (d, J = 2.4 Hz, 3H), 3.00 (t, J = 129.4 Hz, 2H), 3.83 (t, J = 144 Hz, 2H), 5.87 (s, 1H), 7.00 (q, J=17.9 Hz, 1H), 7.15 (s, H), 7.28 (m, 1H), 7.32 (m, J = 7.7 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.3, 16.3, 23.4, 28.2, 70.6, 101.6, 115.0 (d, J = 22 Hz), 124.7 (d, J = 16.8), 125.6 (d, J = 8.4 Hz), 127.3, 129.9 (d, J = 4.6 Hz), 130.9, 135.9, 136.6 (d, J = 3.8 Hz), 155.0, 159.7, 160.7 (d, J = 244 Hz), 172.4. MS (ESI) *m/z*: 354.43 [M + H$^+$].

Biological Evaluation. Virus and Cells. Virus stock of human rhinovirus 2 (HRV-2, kindly provided by Dr. J. Seipelt, Greenhills Biotechnology, Ltd., Vienna, Austria) was prepared in HeLa cells, aliquoted, and stored at −80 °C until use. HeLa cells were grown in Eagle's minimal essential medium (MEM/E, Cambrex) supplemented with 10% neonatal calf serum (NCS, HeLa Ohio, Greiner no. 758010, Germany), 100 U/mL PEN, and 100 μg/mL STR. The test medium contains only 2% of NCS.

Determination of Cytotoxicity. To determine the 50% cytotoxic concentration (CC_{50}), 2-day-old confluent HeLa cell monolayers grown in 96-well plates were incubated with serial 2-fold dilutions of compounds for 72 h (37 °C, 5% CO_2). Then the cells were fixed and stained with a crystal violet formalin solution as described previously.[48] After dye extraction, the optical density of individual wells was quantified spectrophotometrically at 550/630 nm with a microplate reader. Cell viability of individual compound-treated wells was evaluated as the percentage of the mean value of optical density resulting from six mock-treated cell controls, which were set to 100%. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration reducing the viability of untreated cell cultures by 50%.

Cytopathic Effect (CPE) Inhibitory Assay. CPE inhibitory assays have been performed as described previously.[23] Briefly, the tests were carried out in 1-day-old confluent HeLa cell monolayers growing in 96-well flat-bottomed microtiter plates (Falcon 3075). After removal of the culture medium, 50 μL of drug solution and a constant amount of virus in a volume of 50 μL (multiplicity of infection of 0.01) were added to the cells. Six wells of uninfected and six wells of infected cells without the test compound served as cell and virus control, respectively, on each plate. Pleconaril was used as the reference compound. By use of the crystal violet uptake assay described for cytotoxic investigations, the inhibition of the virus-induced CPE was scored 72 h after infection when untreated infected control cells showed maximum cytopathic effects and the positive control compound-treated wells a 50% protection at a pleconaril concentration of 0.02 μg/mL. The IC_{50} values of antiviral active compounds were determined from the mean dose response curves of at least three separate experiments.

Acknowledgment. This work was partially supported by a grant from the President of Ukraine (President of Ukraine Grant for Young Investigators GP/F11/0115) and the Science & Technology Center in Ukraine (STCU Project 3147)

Supporting Information Available: Descriptors, elemental analysis results of final compounds, and observed vs predicted diagrams for obtained QSAR models. This material is available free of charge via the Internet at http://pubs.acs.org.

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