Virtual Combinatorial Syntheses and Computational Screening of New Potential Anti-Herpes Compounds[1]

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Received December 14, 1998

The activity of new anti-HSV-1 chemical structures, designed by virtual combinatorial chemical synthesis and selected by a computational screening, is determined by an in vitro assay. A virtual library of phenol esters and anilides was formed from two databases of building blocks: one with carbonyl fragments and the other containing both substituted phenoxy and phenylamino fragments. The library of virtually assembled compounds was computationally screened, and those compounds which were selected by our mathematical model as active ones were finally synthesized and tested. Our antiviral activity model is a "tandem" of four linear functions of topological graph−theoretical descriptors. A given chemical structure was selected as active if it satisfies every discriminant equation in that model. The final result was that five new structures were selected, synthesized, and tested: all of them demonstrated activity, and three showed appreciable anti-HSV-1 activity, with IC_{50} values of 0.9 μM. The same model, applied to a database of known compounds, has identified the anti-herpes activity of the following compounds: 3,5-dimethyl-4-nitroisoxazole, nitrofurantoin, 1-(pyrrolidinocarbonylmethyl)piperazine, nebularine, cordycepin, adipic acid, thymidine, α-thymidine, inosine, 2,4-diamino-6-(hydroxymethyl)pteridine, 7-(carboxymethoxy)-4-methylcoumarin, 5-methylcytidine, and others that showed less activity.

Introduction

New trends in the field of drug design comprise the combinatorial chemical syntheses of large libraries of compounds and further high-throughput screening using quick and cost-effective assays. In particular, very large libraries of peptides and peptide-like molecules have been synthesized, leading to the identification of novel, active compounds.[2,3] Often these molecules display limited bioavailability, so they are not good drugs. Thus, there was considerable interest in using other different synthons from amino acids to extend combinatorial libraries to small organic nonoligomeric structures.[4−13]

The concepts of virtual combinatorial chemical synthesis and virtual library design have also been developed and applied as much as peptoids[14−16] as other nonoligomeric[17] virtual libraries. Such concepts involve, as a first step, to select on a determined mathematical model of the chemical structure, such as a topological[14−16] or 3D-based[17] one. Virtual combinatorial synthesis is the computational simulation of the generation of new chemical structures, which constitute a virtual library, within the aforementioned model. Virtual library design is the selection of a manageable subset of molecules from a larger library of possible compounds.[18,19] It is also called computational or virtual screening, and it requires an additional model that evaluates the possibility of pharmacological activity of the compounds represented in a database or in the virtual library. The computational screening will result in a filtered set of chemical structures which has to be assayed by high-throughput or traditional screening, depending on the size of such a set. In brief, virtual combinatorial synthesis−virtual screening is an attempt to merge two powerful and contradictory strategies: combinatorial chemistry and rational drug design.

It is generally assumed that virtual combinatorial chemical synthesis was born as a tool in virtual library design, that is, as a derived concept of combinatorial chemistry to overcome the informational explosion associated to all combinatorial problems by controlling the generation of molecular diversity and, so, to guide the chemical synthesis steps in an efficient way. But, as a matter of fact, the concept of inverse QSAR, the computational generation of candidate chemical structures, is chronologically previous to the idea of combinatorial chemistry. We can allude, as an example, the works of our research group from the second half of the 1980s. We generally use a "scaffold", which is denominated by a base structure, and a "library", constituted by carbon atoms and functional groups. One or several attachment sites are established in the base structure, and the elements of the library are computationally assembled to these sites. Every new added element was able to attach other elements of the library, depending on its own nature and the parameters inputed to control the program. This process generates high molecular diversity. Every new "synthesized" molecule was "tested" as potentially active, according to a previously established QSAR model.[20]

In our research group also, we have developed the concept of computational screening, in structurally heterogeneous[21−24] databases of known compounds, to the level that allows us to focus the in vitro screening in sets with very low number of compounds and high probabilities of success. We obtained new diverse active

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10.1021/jm981132u CCC: $18.00
Published on Web 08/03/1999

Table 1. New Biological Activities Discovered through Virtual Screenings[a]

activity found	main authors and reference	selected drugs
antibacterial	R. Soler, C. de Gregorio, R. García-Domenech, and J. Gálvez[30]	1-chloro-2,4-dinitrobenzene, 3-chloro-5-nitroindazole, 1-phenyl-3-methyl-2-pyrazolin-5-one, neohesperidin, amaranth, mordant brown 24
antifungal	L. Pastor and J. Gálvez [31]	neotetrazolium chloride, benzotropine mesilate, 3-(2-bromoethyl)indole, 1-chloro-2,4-dinitrobenzene
hypoglycaemic	G. M. Antón, F. Pérez, and R. García-Domenech[32]	3-hydroxybutyl acetate, 4-(3-methyl-5-oxo-2-pyrazolin-1-yl)benzoic acid, 1-(mesitylene-2-sulfonyl)-1*H*-1,2,3-triazole
antineoplastic	M. J. Gómez-Lechón and J. Gálvez[33]	carminic acid, tetracycline, piromidic acid, doxycycline
antimalarial	R. Gozalbes and R. García-Domenech[34]	hexetidine, hydroxyzine
antitoxoplasma	R. Gozalbes and R. García-Domenech[35]	cefamandole nafate, prazosin, andrographolide, dibenzothiophene sulfone, 2-acetamido-4-methyl-5-thiazolesulfonyl chloride
antihystaminic	E. Casabán, G. M. Antón, and R. García-Domenech[36]	benzydamine, 4-(1-butylpentyl)pyridine, *N*-(3-bromopropyl)phthalimide, *N*-(3-chloropropyl)phthalimide, *N*-(3-chloropropyl)piperidine hydrochloride, 5-bromoindole
bronchodilator	I. Ríos, R. García-Domenech, and J. Gálvez[37]	griseofulvin, anthrarobin, 9,10-dihydro-2-methyl-4*H*-benzo[5,6]cyclohept-[1,2-*d*]oxazol-4-ol, 2-aminothiazole, maltol

[a] Details on assays and protocols can be found in the cited references.

compounds and even more new unexpected activities of known biologically active compounds, as shown in Table 1.

Human herpesviruses are distributed worldwide and are among the most frequent causes of viral infections in both immunocompetent and immunosuppressed patients. Herpes simplex virus type 1 (HSV-1) is generally associated with primary and recurrent mucocutaneous facial or ophthalmic lesions. Two categories of antiviral drugs are clinically active against herpesvirus infections: nucleoside analogue inhibitors of viral DNA synthesis such as acyclovir (ACV) and ganciclovir (GCV), and direct inhibitors of viral DNA polymerase such as phosphonoformate (PFA; foscarnet) and phosphonoacetate. However, the emergence of resistant herpes simplex virus strains, particularly noted in immunosuppressed patients (AIDS and recipients of organ transplants), demands the development and evaluation of newer antiviral drugs.

The aim of this study is to determine the anti-HSV-1 activity of new chemical structures. They are first designed by virtual combinatorial chemical synthesis and eventually are selected by a computational screening. We tried, for this purpose, one of the simplest couplings in preparative chemistry. A virtual library of phenol esters and anilides was formed from two databases of building blocks: one containing carbonyl fragments that foreshadow cyclic acid anhydrides in a virtual chemical reaction and the other with substituted phenoxy and phenylamino fragments that symbolize phenols and anilines, respectively. The virtually assembled compounds were computationally screened, and those selected by our mathematical model as active compounds were finally synthesized and tested. We have assumed a topological graph−theoretical description of the molecular structure, to obtain the utmost speed in the calculations. Our anti-herpes activity model is a "tandem" of four linear functions of connectivity Randic′−Kier−Hall type indices.[25,26] A given chemical structure is selected as active if it satisfies every discriminant equation in that model.

Methods and Results

There are two basic stages in our plan: (a) development of the discriminant model (Figure 1) and (b) design and synthesis of the new active compounds (Figure 2). Each one of these stages comprises both computational and experimental steps, as will be revealed.

Figure 1 represents the steps involved in the development of the theoretical model. The first step is the

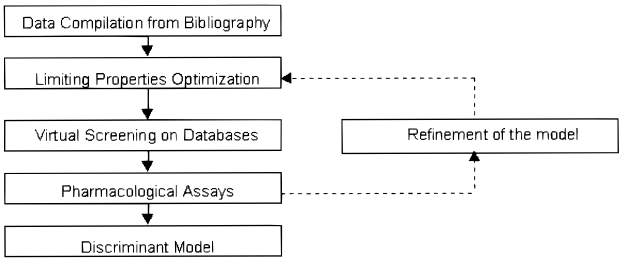


Figure 1. Steps involved in the development of the theoretical discriminant model.

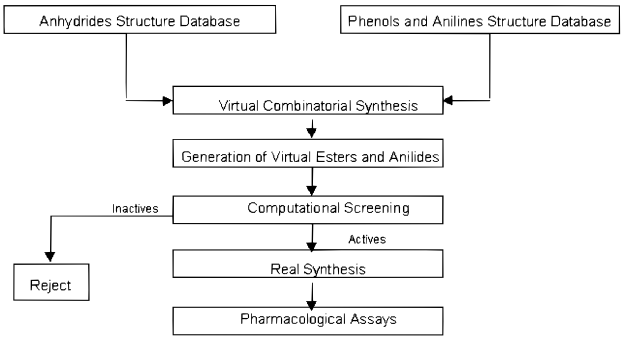


Figure 2. Steps involved in obtaining new active compounds.

compilation of data from the bibliography. These data comprise the structural formula of all the active compounds and the data of biological activity as many as possible to collect for every active compound. The structural formulas are used to calculate a set of descriptors. The properties are correlated with subsets of descriptors to obtain several connectivity functions. We use the Furnival−Wilson algorithm to obtain subsets of descriptors, and we initially select equations with the least Mallow Cp. Other connectivity functions can be obtained through stepwise linear discriminant analysis (LDA). We call limiting property to any property whose prediction equation is able to cluster active compounds in a different way from inactive ones. This clustering must work out of the training group used to obtain that equation. This topic was widely treated in a previous paper in which plots called pharmacological distribution diagrams (PDDs) are defined.[27] The limiting properties are selected among the aforementioned connectivity functions. This constitutes the second step shown in Figure 1. PDDs are histograms of calculated values of the connectivity function in which expectancies appear in the ordinate axis. For an arbitrary interval of values of a given function, we can define an expect-

ancy of activity as

$$Ea = a/(i + 1)$$

where a is the number of active compounds in the interval/total number of active compounds and i is the number of nonactive compounds in the interval/total number of nonactive compounds. The expectancy of nonactivity is defined in a symmetrical way:

$$Ei = i/(a + 1)$$

These plots are useful to select and visualize limiting properties from a set of connectivity functions and to fix thresholds, that is, to find intervals of the selected limiting properties in which the expectancy of activity is high. PDDs are built using two test groups, sets of molecules that have not been used in the correlations or discriminations: one group containing active compounds and the other with inactive ones. This test set of nonactives is composed of other drugs, which show other activities different from the studied one. The procedure followed makes unnecessary a cross-validation to evaluate the fitting of the data because the important fact for the model is the clustering of the test groups. The limiting properties constitute a preliminary model, in which "property" equations become "discriminant" equations by means of the assignment of the interval of predicted activity. One compound is discriminated as potentially active if all its calculated limiting properties are within assigned intervals. The discriminant ability is tested in databases of known compounds, revealing several of them as new active compounds by means of in vitro pharmacological assays. False positive compounds, predicted as active ones and not confirmed by assays, are used in the refinement of the model through a new optimization of the limiting properties. As displayed in Figure 1, this process is repeated until one obtains a good discriminant model. If, eventually, the set of descriptors used does not reflect the main structural features involved in the activity that one tries to modelize, it will not be possible to achieve a good model. In this case, it will be necessary to introduce new descriptor sets which give an account of these features. This is the best way to enhance the discriminant model. Also, the model could be improved using nonlinear fittings and other approaches such as artificial neural networks (ANN), genetic algorithms (GA), or classification and regression trees (CART).

We see, in Figure 2, a graphic representation of the concepts involved in the design of the new active compounds by virtual combinatorial synthesis−computational screening. Two fragment databases are combined to generate a virtual library of esters and amides. A virtual screening allows, using the theoretical discriminant model previously developed, to discard most of the members of the library as potential actives. The potentially active compounds selected from this virtual library are chemically synthesized and tested in vitro for the predicted biological activity.

The application of this method to other design problems would follow the same steps described for antiviral compounds and reflected in Figures 1 and 2. Also, other synthetic reactions, with different building-block databases, can be considered for the virtual combinatorial process.

Discriminant Model. A number of structurally heterogeneous antiviral drugs were selected, irrespective of their mode of action. This set was used in a previous work.[21] For every molecular structure was calculated a set of 16 descriptors. These were subgraph connectivity indices[25] ($^0\chi$,$^1\chi$,$^2\chi$,$^3\chi_p$,$^3\chi_c$,$^4\chi_p$,$^4\chi_c$,$^4\chi_{pc}$) in which multiple bonds contribute to δ_i values and their corresponding valence indices. The first eight are valence Kier−Hall connectivity indices in which all heteroatoms were taken as carbons, while the last indices were identical to the valence indices previously defined by Kier and Hall.[26]

We included in the bibliographic research three kinds of biological properties for these antiviral drugs: pharmacokinetic (maximum plasma concentration, percentage of unchanged drug in urine, and clearance half-life), pharmacodynamic (LD_{50} oral in mice and LD_{50} intraperitoneal in mice), and microbiologic (IC_{50} in HSV-1, ID_{50}, and maximum subtoxic concentration for HSV-1). All these properties were linearly correlated with the aforementioned descriptors. These equations and several classification functions obtained by linear discriminant analysis, using an additional training group of nonantiviral drugs,[21] were candidates to become limiting properties. The finally selected limiting properties, among the initially studied ones, were

IC_{50} (μM):

$$IC_{50} = -17.36\ ^4\chi_p + 41.39\ ^4\chi_{pc}{}^v + 21.71$$

$$N = 18,\ r = 0.914,\ \mathrm{Cp} = 6.0,\ \sigma = 0.59$$

interval of predicted activity = between −10 and 20

ID_{50} (μM):

$$\log(ID_{50}) = -1.42\ ^0\chi + 4.81\ ^0\chi^v - 11.41\ ^3\chi_p{}^v + 1.32\ ^3\chi_c{}^v + 4.17\ ^4\chi_{pc} - 8.42$$

$$N = 25,\ r = 0.9287,\ \mathrm{Cp} = 5.04,\ \sigma = 3.31$$

interval of predicted activity = between −5 and 3

Percentage of unchanged drug found in urine:

$$\log(UDU) = -4.67\ ^1\chi^v + 8.70\ ^2\chi - 3.64\ ^3\chi_p + 3.15\ ^3\chi_p{}^v - 8.05\ ^3\chi_c - 9.23$$

$$N = 25,\ r = 0.9568,\ \mathrm{Cp} = 3.03,\ \sigma = 12.41$$

interval of predicted activity = between −4 and 4

Discriminant function of antiviral activity:

$$D = -1.17\ {}^{0}\chi^{v} + 2.11\ {}^{3}\chi_{p} + 2.79$$

$$N = 81,\ F = 23.4,\ \lambda = 0.28$$

interval of predicted activity = between −1 and 5

Figures 3−6 show the PDDs for the respective equations applied to test groups of active and nonactive compounds. Expectancy[27] values are represented in the ordinate axis. A new molecule will be selected as active if its calculated values for every limiting properties are within the assigned intervals. These thresholds were selected leaving out the zones of limiting property in which the assignment of activity could be doubtful, to minimize the number of false actives selected.

It is noteworthy that the selected properties are not related with specific anti-HSV-1 activity. Although we assay anti-HSV-1 activity to confirm our predictions, the structures that fit the model probably have a broader antiviral spectrum. The preliminary virtual screening, using these four equations, on a homemade database formed by compounds from the Merck Index[28] and from the Aldrich catalog[29] resulted in the identification of 22 new potentially active compounds. All of them showed calculated values within the established intervals of the limiting properties. Its anti-herpes potencies were determined using the assay methods described in the Experimental Section. These results, and the cytotoxic evaluation, are shown in Table 2. It can be seen that 16 out of 22 compounds showed activity. Thus, we concluded that the discriminant model was good enough to try the following step.

Virtual Combinatorial Synthesis−Computational Screening. Figure 2 displays the scheme of virtual synthesis employed. The composition of the "anhydride" and the "nucleophile" databases is shown in Figures 7 and 8, respectively. These compounds were chosen among the commercially available ones,[29] paying care to avoid the presence of substituents that could provoke lateral products in the real synthesis. The virtual combinatorial synthesis process formed the new phenol esters and anilides from anhydride and nucleophile fragments taken from each database.

Not all the possible binary combinations of fragments were obtained. Previous to the generation of the virtual library, the contributions of every fragment of both databases to the values of the limiting properties were estimated. When the sum of the fragments that contribute to a limiting property for a given virtual compound was far enough from the limits of the prefixed intervals, this combination was skipped. The virtual compounds that passed this first filter constituted the virtual combinatorial library of potential antivirals and contained about 600 virtual structures. The computational screening process selected those virtual compounds that showed all four limiting properties within their established intervals.

The finally selected compounds (Figure 9) were synthesized and tested. The results of the pharmacological assay are shown in Table 3. The five compounds predicted to be active were active. They have potencies comparable to the known antiviral agent foscarnet.

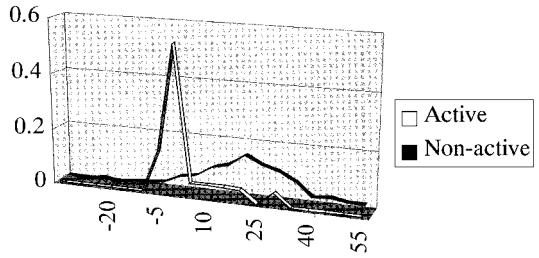


Figure 3. PDD for $IC_{50} = -17.36\ {}^{4}\chi_{p} + 41.39\ {}^{4}\chi_{pc}{}^{v} + 21.71$. Abscises: IC_{50}, μM. Ordinates: expectancy of activity in white, expectancy of nonactivity in black. Interval: between −10 and 20.

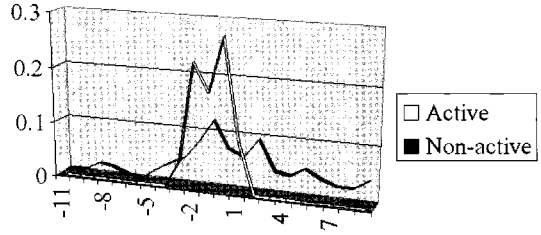


Figure 4. PDD for $\log(ID_{50}) = -1.42\ {}^{0}\chi + 4.81\ {}^{0}\chi^{v} - 11.41\ {}^{3}\chi_{p}{}^{v} + 1.32\ {}^{3}\chi_{c}{}^{v} + 4.17\ {}^{4}\chi_{pc} - 8.42$. Abscises: $\log(ID_{50})$, ID_{50} expressed in μM. Ordinates: expectancy of activity in white, expectancy of nonactivity in black. Interval: between −5 and 3.

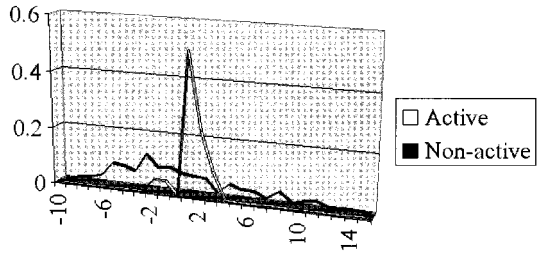


Figure 5. PDD for $\log(UDU) = -4.67\ {}^{1}\chi^{v} + 8.70\ {}^{2}\chi - 3.64\ {}^{3}\chi_{p} + 3.15\ {}^{3}\chi_{p}{}^{v} - 8.05\ {}^{3}\chi_{c} - 9.23$. Abscises: logarithm of percent of unchanged drug in urine. Ordinates: expectancy of activity in white, expectancy of nonactivity in black. Interval: between −4 and 4.

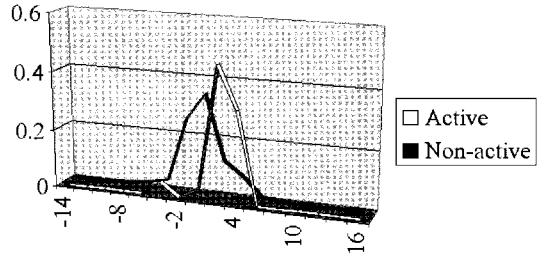


Figure 6. PDD for $D = -1.17\ {}^{0}\chi^{v} + 2.11\ {}^{3}\chi_{p} + 2.79$. Abscises: classification function obtained by linear discriminant analysis. Ordinates: expectancy of activity in white, expectancy of nonactivity in black. Interval: between −1 and 5.

Discussion

In relation to the initial computational screening, 16 out of 22 compounds selected from the database of known substances exhibited in vitro activity. Among these we can find 7 nucleoside analogues, 7 diverse heterocycles that could be considered as 7 new lead drugs, and 2 very simple diacids. The pharmacological assays confirmed the discriminant ability of the proposed model in structurally heterogeneous groups of compounds. The prediction found is accurate enough to

Table 2. Compounds Selected through Virtual Screening on Databases, with Their Experimental IC_{50} on HSV-1 and Semiquantitative Cytotoxicity Results

compound	IC_{50}, μM	cytotoxicity[a]
1,2,3-triazole-4,5-dicarboxylic acid	5	medium
2′-deoxyuridine	2.2	medium
5-nitrobarbituric acid trihydrate	1.7	high
succinic acid	1.3	no
3,5-dimethyl-4-nitroisoxazole	0.9	medium
nitrofurantoin	0.9	medium
1-(pyrrolidinocarbonylmethyl)piperazine	0.6	medium
nebularine	0.6	medium
cordycepin	0.5	low
adipic acid	0.4	no
thymidine	0.4	no
α-thymidine	0.3	low
inosine	0.23	no
2,4-diamino-6-(hydroxymethyl)pteridine	0.16	medium
7-(carboxymethoxy)-4-methylcoumarin	0.07	no
5-methylcytidine	0.06	low
4,4-dihydroxyazobenzene	NA	
2,4-dihydroxy-5-nitropyrimidine	NA	
formaldoxime	NA	
7-hydroxycoumarin	NA	
2,4,6-trihydroxypyrimidine	NA	
tropine	NA	

[a] Effect on cell growth of noninfected cellular monolayers, at the corresponding IC_{50}. NA, no IC_{50} reached without damage to the cell monolayer.

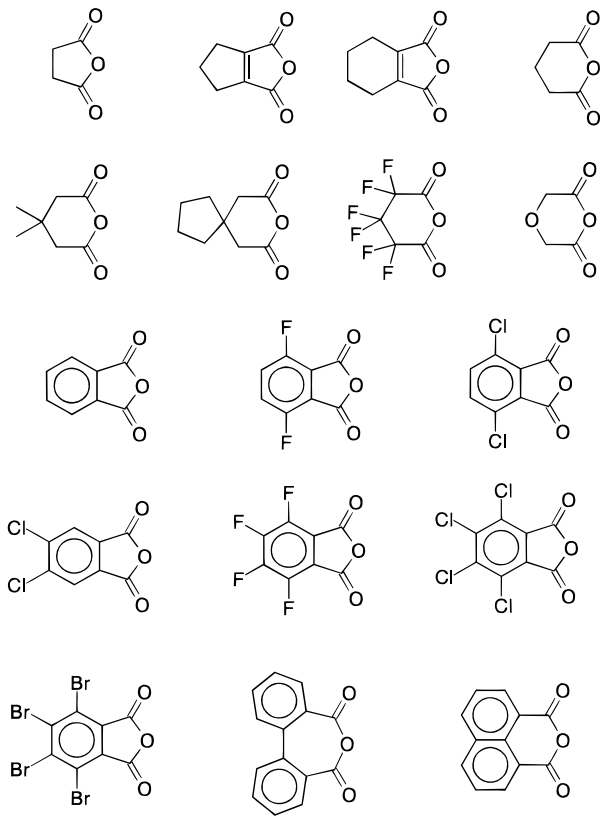
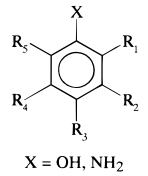


Figure 7. Symmetrical cyclic anhydrides represented in the carbonyl fragments database.

make unnecessary the introduction of additional descriptors, which could afford more information, or the use of more sophisticated or nonlinear discriminant analyses.

With respect to the virtual combinatorial synthesis, only cyclopentene-1,2-dicarboxylic derivatives were selected. Thus, only one of the 17 fragments of the carbonyl database appears in the designed structures. In the same way, only di- or trifluorophenyl derivatives



X = OH, NH_2

R_1, R_2, R_3, R_4, R_5 = H, Me, Et, Pr, iPr, F, Cl, Br, OMe, OEt, Ph

Figure 8. Phenols and anilines represented in the phenoxy and phenylamino fragments database (not all the possible combinations, only commercially available ones).

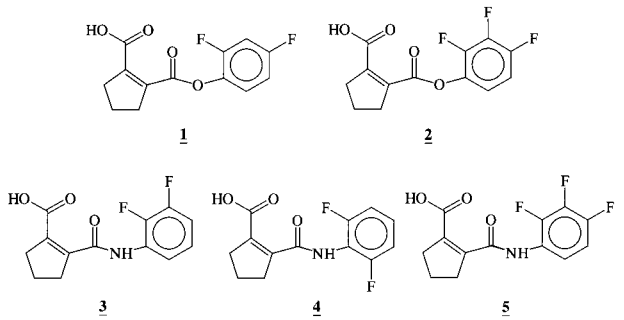


Figure 9. Compounds designed by virtual combinatorial chemical syntheses and selected by computational screening.

with F in position 2 and H in position 5 were finally kept in the resulting compounds, among all the examined substitutions. Both structural features are not present in actual antiviral drugs. Other difluoro or trifluoro compounds did not pass all the discriminant equations, or the corresponding nucleophilic precursor was not available. The designed amides were more active than the esters. Only mechanistic studies will enlighten the reasons for these observations. We cannot ensure that other compounds of this screened space could not be active. Maybe with wider limiting conditions we could find some others, but the actual level of restriction has prevented the obtaining of false positives.

Even though a computational screening will always require experimental confirmation, when such a reliability level is reached, there is no essential difference between a screening based on in vitro assays and a virtual screening made in silicio. An in silicio assay is an entirely abstract model, while in an in vitro assay we are using an analogical model of the pharmacological activity, but it is only a model of the real effect in humans. Both approaches allow us to restrict the number of compounds that will be assayed in further steps.

Conclusions

Combinatorial chemical synthesis and its associated techniques have largely demonstrated their capabilities in the search of new bioactive compounds. If it were possible to devise something better, that would be, without any doubt, the computational simulation of all these processes. But this will only be a real alternative if a trustworthy virtual screening method exists. In this work, we demonstrated that it is more than a possibility in the concrete field of antiviral drugs.

The mathematical model employed in this work retains the main structural features that condition the anti-herpes activity and embody the different mechanisms of action. That pattern is not linked to an easily recognized organic structure or functional group. Thus,

Table 3. Compounds Designed by Virtual Combinatorial Chemical Syntheses and Selected by Computational Screening, with Their Experimental IC_{50} on HSV-1 and Cytotoxicity Results

no.	compound	IC_{50}, μM	cytotoxicity[a]
1	2-(2,4-difluorophenoxycarbonyl)-1-cyclopentene-1-carboxylic acid	1.4	low
2	2-(2,3,4-trifluorophenoxycarbonyl)-1-cyclopentene-1-carboxylic acid	1.8	medium
3	2-(2,3-difluorophenylcarbamoyl)-1-cyclopentene-1-carboxylic acid	0.9	no
4	2-(2,6-difluorophenylcarbamoyl)-1-cyclopentene-1-carboxylic acid	0.9	no
5	2-(2,3,4-trifluorophenylcarbamoyl)-1-cyclopentene-1-carboxylic acid	0.9	no

[a] Effect on cell growth of noninfected cellular monolayers, at the corresponding IC_{50}.

it seems that there is no common chemical or geometrical features between the designed structures and the molecules of the training group, but there must be a subtler topological common pattern of these structures. These new molecules constitute in fact new lead drugs in the field of anti-HSV-1 therapy. There remain many open questions, for example, the mechanisms of action involved, but the results disclose the potential of the method of design developed. Thus, there is no a priori indication that there could exist any active substance within the space of chemical structures studied.

Experimental Section

Chemistry. General. 1-Cyclopentene-1,2-dicarboxylic anhydride, 96%; 2,4-difluorophenol, 99%; 2,3,4-trifluorophenol, 97%; 2,3-difluoroaniline, 98%; 2,6-difluoroaniline, 97%; and 2,3,4-trifluoroaniline were acquired from Aldrich and used directly. Infrared absorption spectra were obtained on a Perkin-Elmer model 843 spectrometer (Norwalk, CT), with samples as KBr pellets. NMR spectra were recorded on a Bruker AC 250-MHz spectrometer (Fällanden, Switzerland) using methanol-d_4 as solvent, and chemical shifts are reported in ppm downfield from internal TMS. Low-resolution mass spectra were obtained with a VG Autoespec-Q (Fisons) with ionization energy of 70 eV. Elemental analyses were performed at the Centre d'Investigació i Desenvolupament del CSIC, Barcelona, Spain. Melting points were determined in a Cambridge Instruments apparatus and are uncorrected.

2-(2,4-Difluorophenoxycarbonyl)-1-cyclopentene-1-carboxylic Acid (1). To a solution of NaOH (0.11 g, 2.8 mmol) in bidistilled water (5.6 mL) was added 2,4-difluorophenol (0.182 g, 1.4 mmol), and the reaction was stirred at room temperature. When the phenol was dissolved it was added to 1-cyclopentene-1,2-dicarboxylic anhydride (0.193 g, 1.4 mmol). The reaction was stirred for 10 min and then neutralized with concentrated HCl (ca. 0.12 mL). The precipitate was filtered, dried in vacuo, and recrystallized in a hexane−dichloromethane mixture, to give **1**: 150 mg, 40%; mp 98 °C; IR 3409 (OH), 1764 (ester), 1689 (acid) cm^{-1}; ^1H NMR δ 2.0 (m, 2 H), 2.8 (m, 4H), 7.1 (m, 3 H); ^{13}C NMR δ 23.8, 35.8, 36.5, 105.9, 106.7, 112.6, 113.4, 126.1, 126.3, 138.1, 144.3, 154.1, 157.9, 160.1, 164.5, 169.2; MS 268 (M^+). Anal. ($C_{13}H_{10}O_4F_2$) H; C: calcd, 58.22; found, 57.70.

2-(2,3,4-Trifluorophenoxycarbonyl)-1-cyclopentene-1-carboxylic Acid (2). Following the procedure described for the synthesis of **1**: 160 mg, 40%; mp 100 °C; IR 3414 (OH), 1762 (ester), 1681 (acid) cm^{-1}; ^1H NMR δ 2.0 (m, 2 H), 2.8 (m, 4H), 7.0 (m, 2 H); ^{13}C NMR δ 23.8, 35.8, 36.5, 105.9, 106.7, 112.8, 113.4, 119.6, 119.8, 135.8, 137.8, 140.1, 140.8, 144.1, 146.6, 147.8, 148.8, 148.9, 152.2, 152.9, 164.2, 169.2; MS 286 (M^+). Anal. ($C_{13}H_9O_4F_3$) H; C: calcd, 54.56; found, 54.12.

2-(2,3-Difluorophenylcarbamoyl)-1-cyclopentene-1-carboxylic Acid (3). 1-Cyclopentene-1,2-dicarboxylic anhydride (0.4 g, 2.8 mmol) was dissolved in toluene at 60 °C. Then 2,3-difluoroaniline (0.362 g, 2.8 mmol) was added with stirring. When it dissolved, the solution was left to cool at room temperature. The precipitate was filtered, washed with concentrated HCl, dried in vacuo, and recrystallized in a hexane−dichloromethane mixture, to give **3**: 598 mg, 80%; mp 180 °C; IR 3410 (OH), 1691 (acid), 1621 (amide I), 1573 (amide II) cm^{-1}; ^1H NMR δ 1.6 (m, 2 H), 2.5 (m, 4H), 6.5−7.5 (m, 3 H); ^{13}C NMR δ 21.4, 34.5, 36.7, 112.1, 113.0, 119.5, 126.5, 128.1, 128.2, 135.2, 140.1, 144.4, 147.3, 148.3, 148.4, 152.2, 152.5, 165.2, 166.5; MS 267 (M^+). Anal. ($C_{13}H_{11}NO_3F_2$) C, H, N.

2-(2,6-Difluorophenylcarbamoyl)-1-cyclopentene-1-carboxylic Acid (4). Following the procedure described for the synthesis of **3**: 464 mg, 62%; mp 218 °C; IR 3406 (OH), 1688 (acid), 1626 (amide I), 1607 (amide II) cm^{-1}; ^1H NMR δ 1.9 (m, 2 H), 2.8 (m, 4H), 7.0−7.3 (m, 3 H); ^{13}C NMR δ 22.4, 36.2, 37.6, 112.5, 112.9, 129.5, 129.6, 129.8, 131.7, 145.6, 157.5, 161.5, 167.6, 167.9; MS 267 (M^+). Anal. ($C_{13}H_{11}NO_3F_2$) C, H, N.

2-(2,3,4-Trifluorophenylcarbamoyl)-1-cyclopentene-1-carboxylic Acid (5). Following the procedure described for the synthesis of **3**: 510 mg, 64%; mp 192 °C; IR 3408 (OH), 1695 (acid), 1627 (amide I), 1577 (amide II) cm^{-1}; ^1H NMR δ 1.9 (m, 2 H), 2.7 (m, 4H), 7.0−7.5 (m, 2 H); ^{13}C NMR δ 21.3, 34.1, 36.4, 111.7, 112.0, 119.6, 123.6, 123.7, 141.3, 141.5, 145.2, 146.8, 147.9 165.2, 166.0; MS 285 (M^+). Anal. ($C_{13}H_{10}NO_3F_3$) C, H, N.

Biology. Cells and Viruses. The cell line used for the antiviral activity assays was Vero. The cells were grown in Eagle's minimun essential medium (MEM) supplemented with 10% inactivated fetal bovine serum, 1% L-glutamine, and 0.3% sodium bicarbonate. Titrated viral suspension of HSV-1 McIntyre strain was employed.

Antiviral Assays. Antiviral activity was measured in a plaque inhibition assay. Confluent Vero cell monolayer in 6-well multidishes were infected with 100 PFU in 1 mL of MEM medium. After adsorption at 37 °C for 2 h, residual inoculum was replaced with 1 mL of MEM medium containing 0.2% γ-globulin and 1 mL of a dilution of varying concentrations of the test compounds. Virus-infected wells without compounds were used as cytopathogenicity controls. Viral cytopathogenicity (CPE) was completed 1−2 days after viral infection. Antiviral activity is expressed as the IC_{50} (50% inhibitory concentration).

Cytotoxicity. Cytotoxicity of the compounds for the host cells was evaluated in parallel with their antiviral effects, based on the inhibition of cell growth.

Acknowledgment. We express our gratefulness to Dr. Rosa Soler and Mr. Javier Llompart for the development of some of the programs used and to Prof. Lãcrãmioara Popa (Dept of Physical Chemistry, "Carol Davila" University, Bucharest) and Prof. Ovidiu Ivanciuc (Dept of Organic Chemistry, Polytechnic University, Bucharest) for careful reading of the manuscript and useful suggestions. This study has been supported by CICYT, SAF96-0158-C02-02 (Ministerio Español de Educación y Cultura).

Note Added in Proof: We have recently known the interesting works of Dr. Roger Lahana's group, related to the method presented here; see, for example: Lahana, R. Virtual Combinatorial Chemistry. *Sci. Am. (French Ed.)* **1997**, *241*, 56−58. Also: Grassy, G.; Calas, B.; Abdelaziz, Y.; Lahana, R.; Woo, J.; Jyer, S.; Kaczorek, M.; Floc'h, R.; Buelow, R. Computer-assisted rational design of immunosuppressive compounds. *Nature Biotech.* **1998**, *16*, 748−752.

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JM981132U