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Topological Virtual Screening: A Way to Find New Anticonvulsant Drugs from Chemical Diversity

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Abstract—A topological virtual screening (*tvs*) test is presented, which is capable of identifying new drug leaders with anticonvulsant activity. Molecular structures of both anticonvulsant-active and non active compounds, extracted from the Merck Index database, were represented using topological indexes. By means of the application of a linear discriminant analysis to both sets of structures, a topological anticonvulsant model (*tam*) was obtained, which defines a connectivity function. On the basis of this model, 41 new structures with anticonvulsant activity have been identified by a topological virtual screening.

Epilepsy, one of the most frequent neurological disorders, is a major public health issue, affecting about 4% of individuals over their lifetime.[1] Despite the increasing understanding of the pathogenesis of seizures and epilepsy,[2] the cellular basis of human epilepsy remains a mystery. In the absence of a specific etiological understanding, approaches to drug therapy for epilepsy must necessarily be directed toward the control of symptoms, that is, the suppression of seizures by chronic administration of anticonvulsant drugs (AEDs). However, seizures remain uncontrolled in at least 30% of all epilepsies, even when adequate AED therapy is administered. During recent years, a large number of new AEDs have been marketed worldwide, but the proportion of patients failing to respond to drug treatment has not been changed in a significant extent. Furthermore, none of the old or new AEDs appears to represent a 'cure' for epilepsy or an efficient way to prevent epilepsy or its progression.[3] Thus, new concepts and original ideas for developing AEDs are urgently needed.

Virtual screening, or in silico screening, is a new approach attracting increasing levels of interest in the pharmaceutical industry as a productive and cost-effective technology in the search for novel lead compounds.[4–7] The principles involve the computational analysis of chemical databases, in order to identify those compounds that are most likely to have a given biological activity. These ideas are not new, but have been pursued for several years in drug design groups. However, the availability of inexpensive high-performance computing platforms has transformed these processes, in a way that, at present, increasingly complex and more accurate analyses can be performed on very large data sets.[8] The topologic virtual screening is based on the analysis of a chemical diversity of molecules with known structures. They are not classified according to their biological activity, but described by their topological indexes. After a computational study of the structures of the set, only those complying with a topological model are kept. The model results from a discriminant linear analysis involving two sets of structures. One of them has a well defined pharmacological activity. The other one is built from structures having other biological activity different from the one for which the model is mend to be created. The resulting model, associated with the desired pharmacological activity, generates a set of topological descriptors capable of differentiate potentially active compounds from those devoiced of activity. This method represents a very detailed and relevant artifice to search for leaders, prioritizing the selection of compounds that are advisable to be tested in a biological

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assay. It offers a new option, a new methodology that shows itself powerful to face the hunting for the new gene, the new target, the new lead compound, the new drug candidate, that finally allows hunting for the new drug.

Computational chemistry (computer-aided drug design, molecular modeling, etc.) was being hailed as the newest, safest and fastest method to put new chemical entities on the drug market in the late 1980s. This time came for combinatorial chemistry (applied molecular evolution, multiple parallel synthesis, etc.), combined with highthroughput screening (HTS), in the mid-1990s. These technologies have partially delivered their promise—but not in the manner initially described by enthusiastic supporters.[9] This paper discusses the broader scope of topological virtual screening focusing, as a relevant example, on the description of our recent research. This research deals with the search for new leaders using a database of compounds with anticonvulsant activity. We are aimed to highlight the technical feasibility of performing large scale virtual screening as a route to identify novel drug leaders, useful in the therapeutic (or for the treatment) of any disease or condition for which a database exists or can be built.

We have developed a new model capable of searching for new anticonvulsant lead structures. We have also demonstrated its validity when succeeding in the prediction of a new set of compounds sharing the desired activity. In order to comply with this goal we have considered two large sets, one of them comprising substances with demonstrated anticonvulsant activity, and the other comprising compounds that are devoid of it. The latter are, however, associated with other therapeutic applications, as anabolic, analgesic, antibacterial, diuretic and so on. The complete data set consists of 128 compounds. 57 of these are active, anticonvulsant drugs, whereas the other 71 do not have anticonvulsant activity. They have all been extracted from the Merck Index.[10] The assignment of the activity has been done, in each case, following the Merck Index nomenclature. Every compound within the inactive set was carefully checked in the literature in order to avoid erroneous inputs. Of course, the absence of report on activity does not mean necessarily lack of activity, but, as long as the number of data is significant, this risk becomes very low. The mechanism of action of the 57 active compounds has not been considered at the time they were selected to be used to create the topological model. No information based on 3D geometries has been either taken into account. It should be remarked that the topological method applied to the anticonvulsant activity binding data is used here for the first time to analyze chemical diversity. Moreover, the method is significantly less time-consuming and less expensive to apply than other formalisms such as those based on molecular mechanics,[11] quantum chemical descriptors,[12] similarity/dissimilarity approaches,[13] 3D-QSAR.[14]

The compounds of the set, chosen among those included in the Merck index, are shown in Tables 1 and 2. Both sets are characterized by a wide structural diversity. We have quantified the molecular diversity through the Tanimoto coefficient, TC,[15] using the topological indexes as structural descriptors for each compound. TC can be calculated easily by the expression:

$$TC_{ij} = \frac{\Sigma X_i X_j}{\Sigma X_i^2 + \Sigma X_j^2 - \Sigma X_i X_j}$$

where X_i and X_j represent the topological indexes of the compounds i and j whose molecular similarity we want to evaluate. Table 3 shows the values of $TC_{\max}$, $TC_{\min}$

Table 1. Results obtained by the LDA on anticonvulsant activity: training active group

Active group		Class
Compd	Prob (activ.)	
3-Methyl-5-phenylhydantoin	0.791	A
4-Amino-3-hydroxybutiric acid	0.990	A
5-Methy-5-(3-phenanthryl)hydantoin	0.496	I
Acetylpheneturide	0.798	A
Albutoin	0.659	A
Aloxidone	0.748	A
Aminogluthimide	0.913	A
Aminopentamide	0.806	A
Atrolactamide	0.773	A
Beclamide	0.549	NC
Carbamazepine	0.508	NC
Cinromide	0.303	I
Clobazan	0.586	NC
Clomethiazole	0.557	NC
Clonazepan	0.394	I
Decimemide	0.318	I
Dimethadione	0.935	A
Doxenitoin	0.818	A
Eterobarb	0.974	A
Ethadiona	0.859	A
Ethosuximide	0.984	A
Ethotoin	0.704	A
Felbamate	0.660	A
Fluoresone	0.725	A
Gabapentin	0.743	A
Glutamic acid	0.969	A
Lamotrigine	0.060	I
Mephenytoin	0.923	A
Mephobarbital	0.973	A
Metharbital	0.978	A
Methetoin	0.892	A
Methsuximide	0.965	A
Narcobarbital	0.415	NC
Nimetazepan	0.447	NC
Nitrazepan	0.649	A
Paramethadione	0.967	A
Phenacemide	0.471	NC
Phenetharbital	0.986	A
Phenobarbital	0.986	A
Phensuximide	0.914	A
Phenturide	0.901	A
Phenylmethylbarbituric acid	0.977	A
Phenytoin	0.882	A
Phethenylate sodium	0.717	A
Primidone	0.978	A
Progabide	0.398	I
Suclofenide	0.412	NC
Sulthiame	0.931	A
Tetrantoin	0.915	A
Tiagabine	0.533	NC
Topiramate	0.950	A
Trimethadione	0.976	A
Vigabatrin	0.938	A
Valproic acid	0.899	A
Valpromide	0.871	A
Zonisamde	0.987	A

Table 2. Results obtained by the LDA on anticonvulsant activity: training inactive group

Inactive group		
Compd	Prob (activ.)	Class
Abikoviromycin	0.718	A
Acecainide	0.12	I
Aceclofenac	0.087	I
Acefylline	0.326	I
Acetanilide	0.125	I
Acetiaminosalol	0.081	I
Acetamidoeugenol	0.533	NC
Acetanophen	0.234	I
Acetiamine	0.077	I
Acifran	0.798	A
Adenosine	0.076	I
Adrafinil	0.521	NC
Agroclavine	0.199	I
Alacepril	0.352	I
Alazopeptin	0.322	I
Alclofenac	0.179	I
Albendazole	0.474	NC
alfachor	0.001	I
Alizarine Blue	0.768	A
Amlexanox	0.259	I
Amocarzine	0.015	I
Amolanone	0.886	A
Amoscanate	0.042	I
amoxici	0.009	I
Amphotalide	0.639	A
Ampicillin	0.024	I
Androisoxazole	0.019	I
Arbutin	0.006	I
Arecoline	0.249	I
Aristolochic acid	0.094	I
Arotinolol	0.012	I
Atenolol	0.029	I
Aureothin	0.149	I
Azimilide	0.02	I
Benomyl	0.615	A
Benzocaine	0.398	I
Bupivacaine	0.679	A
Camphor	0.507	NC
Carbachol	0.124	I
Carticaine	0.47	NC
Cyclopentamine	0.394	I
Deoxicorticosterone acetate	0.007	I
Deoxiepinephrine	0.072	I
D-Lactic acid	0.071	I
Fenpiprane	0.702	A
Fluacizine	0.054	I
Furazolidone	0.029	I
Gemeprost	0.046	I
Glutethimide	0.96	A
Glybuthiazole	0.357	I
Heptenophos	0.326	I
Hexamethylolmelamine	0.016	I
Hydroprene	0.386	I
Hygrine	0.226	I
Tevenel	0.124	I
Ifosfamide	0.757	A
Imidacloprid	0.109	I
Improsulfan	0.402	NC
Iproclozide	0.021	I
Iproniazid	0.172	I
Lidamidine	0.174	I
Lotrifen	0.467	NC
Metaraminol	0.082	I
Moclobemide	0.086	I
N-Hydroxymethyl)nicotinamide	0.796	A
Nidroxyzone	0.016	I
Nimidane	0.065	I
Physostigmine	0.293	I
Prednisolone	0.144	I
Ritonavir	0.022	I

and TC_{mean} for each group. As may be observed, the structural diversity is elevated in all them (a home-made version to calculate *TC* is at reader's disposal under e-mail request).

In the present research, we have used 62 molecular structure descriptors: 20 topological charge indices,[16] 16 molecular connectivity indices up to the fourth order,[17] 10 ad-hoc indices such as number of atoms and ramifications (N and R), Wiener index, W, quaternary ramifications, PR0, and other 16 indices such as differences and quotients between valence and non-valence connectivity ($\Delta\chi_i$ and $Q\chi_i$).These topological descriptors have been used to develop models for many activities and properties.[18–21]

The calculated topological indexes, used to describe the molecules of both sets, have been input in the BMDP 7M program package.[22] The linear discriminant analysis included in this program is based on a mathematical algorithm capable of distinguishing among two or more categories of objects, and of finding, in this way, a linear discriminant function (anticonvulsant topological model, atm). This function allows to discern between active and inactive compounds with high accuracy. Discriminant ability is assessed in terms of the percentage of correct classifications attained for each set.

The topological anticonvulsant model, *tam*, was selected on the basis of F-Snedecor's value, and the classification criterion used was the shortest Mahalanobis distance (distance between each particular case and the mean of all cases used in the regression equation). The BMDP 7M program chooses the variables used in computing the linear classification functions in a stepwise manner: in each step, the variable adding the most to the separation of the groups is entered into (or the variable adding the least is removed from) the discriminant function. The quality of the discriminant function is evaluated by the Wilk's lambda parameter (λ) or U-statistic, which is obtained by a multivariate analysis of variance statistic that tests the equality of group means for the variable(s) in the discriminant function.

The *tam* function selected was:

$$Tam = -28.88 - 1.94\,{}^4\chi^v_{pc} - 0.21G^y_1 + 4.64G_5 + 20.11J^y_3 - 45.87J_4 - 3.42\Delta^0\chi + 40.65Q^0\chi - 10.47Q^3\chi_p + 2.79\Delta^4\chi_p + 1.32*PR0$$

$N = 128 \qquad \lambda = 0.54 \qquad F = 10$

N: number of total of compounds analyzed;
λ, Wilk's lambda parameter; F, Fischer index;
${}^4\chi^v_{pc}$ is the fourth order valence path-cluster connectivity index, whilst G^y_1, G_5, J^y_3 and J_4 are charge indices of the orders 1, 5, 3 and 4, respectively. Those labeled as 'v' are valence indices, in which the calculation is performed from the topological matrix, introducing in the corresponding entry the value of the Pauling' electronegativity of the heteroatom, taking oxygen = 2 as the reference value.[15]

The indices labeled as Δ and Q are differences and quotients between valence and non-valence indices, respectively. Thus, $\Delta^0\chi$ stands for the difference $= {}^0\chi - {}^0\chi^v$,

Table 3. Coefficients Tanimoto maxim, minim and mean obtained with each set of compounds used in this work

Set	TC_{min}	TC_{max}	TC_{mean}
Active	0.0314 Decimemide-4-amino-butiric	0.9999 Valpromide-valproic acid	0.5903
Inactive	0.0089 D-Lactic acid-azimilide	0.9999 Acetaminosalol-acetiamine	0.5682
Complete	0.0089	0.9999	0.5650

whereas, for instance Q $^3\chi_p$ is the quotient $= {}^3\chi_p / {}^3\chi_p^v$. Finally PR0 stands for the number of sp3 carbons bound in four substituents (quaternary ramifications). Though not outlined here, the model was checked as for its stability and randomness.

Tables 1 and 2 summarize the classification results obtained with *tam* function for each group. A compound will be classified as anticonvulsant (A) if their probability assigned is 0.600 or superior and non-anticonvulsant (I) if probability is below 0.4. The remaining intermediate values do not allow an appropriate classification, and the compounds are referred to as non-classified NC (see Tables 1 and 2, second and third columns).

From the analysis of the data, it becomes evident that, in the active group, six false inactive compounds have been predicted, whereas in the inactive group eleven false active structures have developed. The percentage error (10.5 and 15.5%, respectively) is, nevertheless, perfectly acceptable as far as the high structural heterogeneity of the training set is considered.

Our topological anticonvulsant model is, therefore, highly predictive of the anticonvulsant activity (ACA). A broad range of properties or structural features are implicitly included in this model. This features becomes relevant at different stages of the drug optimization process (solubility and lipophilia can be mentioned, among others). Selection of the *tam* function is particularly important when compounds are sourced from suppliers' catalogs, from a combinatorial library date base or any other database able to be found in the related literature, as those basis usually provide information about the pharmacological profiles. The pharmaceutical industry is presently headed in knowing the pharmacokinetic and toxicological profiles since the earlier stages of the drug discovery processes. Therefore, it is useful to bring some of these concepts into the topological virtual screening stage, while accepting that the calculable properties currently available are at best only vague indicators of metabolic fate or toxicity. This possibility is afforded by the use of topological indexes. The properties can be calculated quickly and can be easily applied to filtering a large database. Likewise, filters can be applied in the selection of an specific biological activity or chemical property

The anticonvulsant model obtained as described above, has been further used to predict the ACA of other compounds, that have not been considered in its derivation. We have used the data-base of the Merck Index for this purpose. Using this model, the *tvs* differentiates 108 compounds as potential anticonvulsants with probability above 98%. For this subset of compounds, a bibliographic search has been performed, looking for previously reported data on their pharmacological profile. We found that in 41 cases the AC activity of the structures had been previously reported as positive (Table 4). This result shows the accuracy of the model, which has been capable of finding out 41 new potential leaders from structures known to show a different pharmacological profile.

Furthermore, the results also demonstrate that our model is not just a pure mathematical approach but it

Table 4. Results of prediction of anticonvulsant activity obtained when applying the *tam* proposed to Merck Index base

Compd	Merck Index therapeutics category [10]	Prob[a]. (ACA)
Aconitine	Neuralgia	1.000(23)
Adinazolan	Antidepressant	0.931(24)
α-Methylenebutyrolactone	N/D	0.943(25)
Amido-G-Acid	N/D	0.990(26)
Amlodipine	Antianginal; antihipert	0.956(27)
Cannabidiol	N/D	0.978(28)
Cannabinol	N/D	0.907(29)
Caprolactam	N/D	0.982(30)
Cycloheptanone	N/D	0.976(31)
Cycloleucine	N/D	0.995(32)
Cyclopentanone	N/D	0.991(31)
Cychlothiazide	Diuretic; antihipert	0.960(33)
Diethadione	Analeptic	0.978(34)
Ectylurea	Sedative; hypnotic	0.933(35)
Felodipine	Antihipert; antianginal	0.939(36)
Guvacine	N/D	0.968(37)
Linoleic acid	Nutrient	0.920(38)
L-Pyroglutamic acid	N/D	0.977(39)
Nifedipine	Antianginal; antihipert	0.961(40)
Flunarizine	Vasodilator	0.815(40)
Diltiazem	Antianginal. antiarrhyth.	0.540(40)
Nicardipine	Antianginal; antihipert	0.610(40)
Nisoldipine	Antianginal; antihipert	0.974(40)
Nitrendipine	Antihipert	0.600(40)
Nimodipine	Vasodilator cerebral	0.584(40)
Verapamil	Antianginal; antihipert	0.741(40)
Prenylamine	Vasodilator coronary	0.862(40)
Nipecotic acid	N/D	0.984(41)
Phencyclidine	Analgesic, anesthetic	0.911(42)
Phthalimide	N/D	0.917(43)
Pipecolic acid	N/D	0.961(41)
Proline	N/D	0.932(44)
Riluzole	Neuroprotective	0.916(45)
Biperiden	Anticholinergic, antiparkins	0.719(46)
Scopolamine	Anticholinergic	0.760(47)
Trihexyphenidyl	Anticholinergic, antiparkins	0.871(47)
Benactyzine	Antidepres, anticholinerg	0.643(46)
Benztropine	Anticholinergic	0.685(47)
Sulfanilamide	Antimibacterial	0.921(48)
Caramiphen	Anticholinergic, antitussive	0.690(49)
Carbetapentane	Antitussive	0.571(50)

[a]Probability of anticonvulsant activity by the *tam* function and the reference where is described.

encodes ADME information closely related to the in vivo activity and it has been obtained without an explicit knowledge about the mechanism of action and without any sort of quantitative measurement of activity.

Virtual screening is increasingly gaining acceptance in the pharmaceutical industry as a cost-effective and timely strategy for analyzing very large chemical data sets. This procedure is computationally intensive for analyzing a large database but provides the most detailed basis for determining which compounds are likely to be potent leaders. Our results demonstrate that the *tvs* can accurately reproduce an already known pharmacological activity, and it is capable of discriminating between reference drugs and random compounds, either on the basis of a predicted *tam*, or any other topological model associated with a biological activity.

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