Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1684–1690

# Discovery of anticonvulsant activity of abietic acid through application of linear discriminant analysis

Alan Talevi,[a,b] Mariana Sella Cravero,[b] Eduardo A. Castro[a] and Luis E. Bruno-Blanch[b,*]

[a]*Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Department of Chemistry, Faculty of Exact Sciences, Universidad Nacional de La Plata, B1900AVV, La Plata, Buenos Aires, Argentina*
[b]*Medicinal Chemistry, Department of Biological Sciences, Faculty of Exact Sciences, Universidad Nacional de La Plata, B1900AVV, La Plata, Buenos Aires, Argentina*

Received 26 September 2006; revised 21 December 2006; accepted 22 December 2006
Available online 8 January 2007

**Abstract**—Linear discriminant analysis was performed to derive discriminant functions based on 2D descriptors and capable of classifying anticonvulsant from non-anticonvulsant compounds. Through application in virtual screening of the discriminant function which performed best in the validation steps, abietic acid was identified as a potential new anticonvulsant agent. The anticonvulsant activity of abietic acid at 30 and 100 mg/kg was confirmed in the Maximal Electroshock Test, both orally and intraperitoneally.


Epilepsy is the most common among brain disorders that disturbs human condition, affecting around 50 million people worldwide. Current chemotherapy remains ineffective in up to 30% of the patients. In non-developed countries up to 80% of epilepsy sufferers receive no treatment.[1] Moreover, even the new generation of anticonvulsant agents present important side effects such as headache, drowsiness, diplopia, ataxia, dizziness, nausea, allergies, blood dyscrasias, and hepatotoxicity.[2] Thus, new medications for the treatment of epilepsy are urgently needed.

Linear discriminant analysis (LDA) is a statistical technique that aims to find the linear combination of features which best separates two or more classes of objects or events. This linear combination of features (the Discriminant Function – DF) has the general formula:

$$\text{Class} = a_0 + a_1X1 + a_2X_2 + \cdots + a_nX_n$$

where $X_1, X_2, \ldots, X_n$ represent the chosen descriptors and $a_1, \ldots, a_n$ represent the coefficients of the classification function determined by the least-squares method.

LDA is closely related to regression analysis, which also attempts to express a dependent variable as a linear combination of other features or measurements. In regression analysis, however, the dependent variable is a continuous quantity while for LDA is a categorical variable (the class label).

LDA has been applied extensively in drug discovery, mainly by the Las Villas and the Valencia groups.[3–7] Our group has previously applied this methodology in the identification of new antichagasic agents with good results.[8] For the application of LDA to drug discovery, the DF should be capable of distinguishing between compounds with and without a biological activity of interest. To this purpose, a training set is generated from compounds with the activity of interest and compounds with other bioactivities. It is assumed that these latter do not possesx the activity being searched. After the DF is derived from the training set and it is properly validated, it is applied to the classification of other chemical structures not included in the training set, selecting those with highest probability of being active at biological tests.

In this letter, we report the generation and validation of a DF to identify new anticonvulsants with activity in the Maximal Electroshock (MES) test.[9] The DF has been successfully applied in the identification of a novel anticonvulsant compound active in mice at 30 and 100 mg/kg, both through ip and oral administration.

*Keywords*: Anticonvulsant; Epilepsy; Linear discriminant analysis; Abietic acid.
* Corresponding author. Tel.: +54 02214235333; e-mail: lbb@biol.unlp.edu.ar

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.098

The DF was derived from a training set composed by 21 anticonvulsant compounds with proven activity in the MES test and 27 compounds with other biological activities. The 21 anticonvulsants chosen for the model generation are either market drugs or in clinical stage of development; they possess $ED_{50}$'s in the MES test below 200 mg/kg (mice, ip) and were defined as the ACTIVE category. The 27 compounds with other bioactivities compose the INACTIVE category (among them: antiulceratives, antiinflammatories, antibacterials, antivirals, antiparasitaries, antihypertensives, bronchodilators, antitussives, and others). Both categories were codified through a dummy variable (Active compound = 1; inactive compound = −1). Figure 1 presents the structures of the 21 molecules with anticonvulsant activity in the training set. Figure 2 shows the structures of the 27 non-anticonvulsant compounds in the training set. Note the structural diversity of the 48 compounds, which assures the generality of the derived models.

Dragon computer software was used for the computation of 877 molecular descriptors classified, according to their dimensionality, in 0D, 1D and 2D.[10] LDA was applied to determine which subset of these descriptors contributes the most to the separation of the ACTIVE and the INACTIVE categories. Several DFs were generated and the best DF was selected according to the following criteria:

- We search for a DF with low value of the statistical Wilk's $U$ ($U = 0$ means perfect discrimination between the classes considered; $U = 1$ means no discriminating ability at all).
- We looked for a DF with high percentage of good classifications both in the training and the test sets. We paid special attention to the percentage of good classifications in the inactive category, because a misclassification in this group (a false positive) means that an inactive compound will be sent to bioassays, with consequent loss of time and resources.
- Between two DFs with similar performance we preferred that with fewer descriptors, following the principle of parsimony.

The following DF was selected:

$$\begin{aligned} \mathrm{DF} &= 8.110 - 2.206 \times HVcpx - 4.277 \times BIC2 \\ &\quad + 0.443 \times GATS7e + 1.089 \times GATS8p \\ &\text{Wilks' } U: 0.32530 \quad F(4,43) = 22.297 \quad p < .0000 \quad n = 48 \end{aligned}$$

HVcpx represents the graph vertex complexity index,[11] BIC2 symbolizes the Bond Information Content (neighborhood symmetry of second order),[12] GATS7e denotes Geary autocorrelation – lag 7, weighted by atomic Sanderson electronegativities, and GATS8p stands for Geary autocorrelation – lag 8, weighted by atomic polarizabilities.[13]

Table 1 presents the values of the DF and the posterior probabilities of belonging to the active category for the 48 compounds which compose the training set. 93.75% of the compounds from the training set are correctly classified. The Pharmacological Distribution Diagram (PDD),[14] a representation of the expectancies to find an active and a non-active compound for each interval of values of the DF, confirms the low superposition of the DF values for the two considered categories (Fig. 3).

Validation of the DF was carried out through internal and external validation methodologies.[15] We used Leave-group-out (LGO) cross-validation to assure the model robustness (randomly removing 6 compounds through 8 LGO runs) and 48 randomization tests.

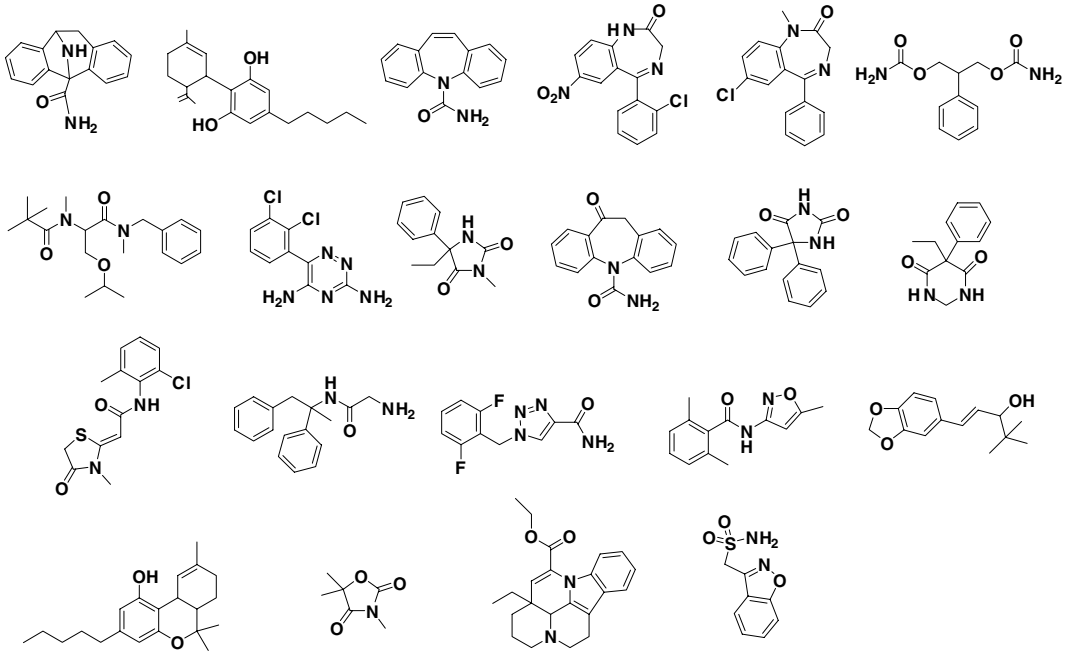

**Figure 1.** Structures of the 21 anticonvulsants that compose the ACTIVE category of the training set.

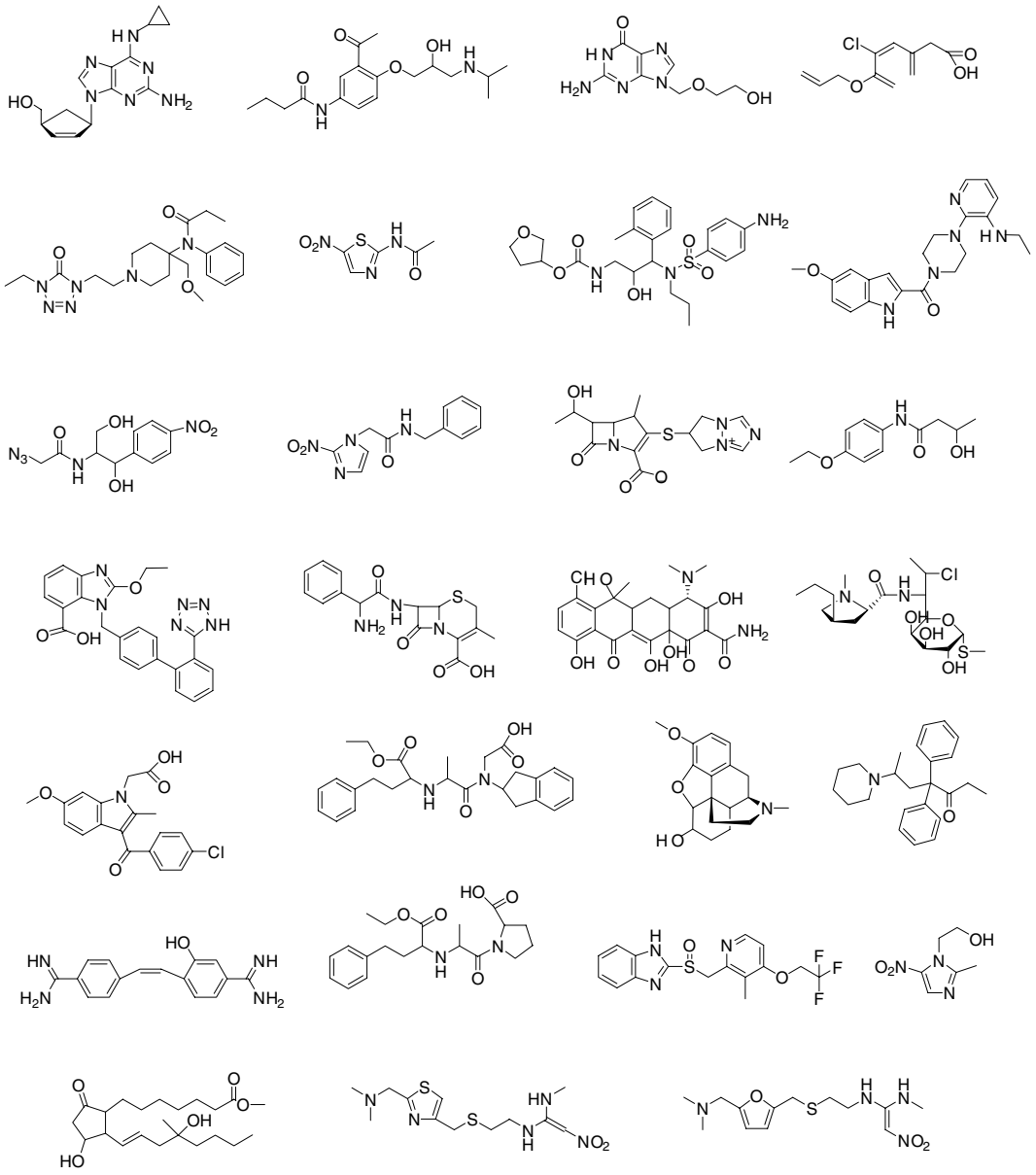

**Figure 2.** Structures of the 27 non-anticonvulsants that compose the INACTIVE category of the training set.

Table 2 presents the results of the LGO cross-validation, as well as the names of the compounds that were removed in each run. Figure 4 shows the results of the randomization test. As can be appreciated, the original DF always performed much better than the ones obtained through randomization of the dependent, dummy variable (in the randomized models, the Wilk's *U* statistical assumes values similar to 1, meaning non-discriminating power, and both the *F* statistical and the percentage of hits decrease considerably). This minimizes the risk of chance correlation between the set of descriptors of the DF and the dependent variable.

External validation was performed on a test set of 48 structurally diverse compounds (20 active compounds with activity in the MES test; the remaining 28 belonging to the inactive category and having other bioactivities). The active compounds are in preclinical stage of development and were extracted from different literature sources.[16–23] The results of the external validation are shown in Table 3.

The DF was applied in virtual screening of 10,250 compounds from Merck Index 13th (with exclusion of inorganic compounds).[24] Table 4 presents a list of forty of the drugs (20 hits and 20 non-hits) tested through virtual screening with their DF values and their therapeutic categories and uses according to Merck Index. Abietic acid (Fig. 5), a natural terpenoid present in pine resin and with reported antiinflammatory and antithrombotic activities,[25,26] was selected for evaluation in the MES test on the basis of its DF value (0.37) and its structure, which is very different from the known anticonvulsant compounds (see Fig. 1 and 5). Abietic acid is also used,

**Table 1.** DF values for the 48 compounds that compose the training set

| Compound | DF value | Prob (Act) |
|---|---|---|
| ADCI | 0.80 | 99.2 |
| Cannabidiol | 0.33 | 88.0 |
| Carbamazepine | 1.21 | 99.9 |
| Clonazepam | 0.16 | 72.5 |
| Diazepam | 1.10 | 99.9 |
| Felbamate | 0.96 | 99.7 |
| Lacosamide | 0.99 | 99.7 |
| Lamotrigine | 0.96 | 99.7 |
| Mephenytoin | 0.51 | 95.5 |
| Oxcarbacepine | 0.75 | 98.9 |
| Phenytoin | 1.39 | 100.0 |
| Primidone | 0.41 | 92.1 |
| Ralitoline | 0.07 | 60.1 |
| Remacemide | 0.65 | 98.0 |
| Rufinamide | 0.01 | 52.0 |
| Soretolide | 0.68 | 98.3 |
| Stiripentol | 0.17 | 73.9 |
| THC | 0.30 | 85.8 |
| Trimetadione | 0.79 | 99.1 |
| Vinpocetine | 0.74 | 98.8 |
| Zonisamide | 0.32 | 87.2 |
| *Abacavir* | −0.47 | 5.7 |
| *Acebutolol* | −1.22 | 0.1 |
| *Acyclovir** | 0.21 | 77.6 |
| *Alclofenac* | −0.83 | 0.7 |
| *Alfentanil* | −1.01 | 0.2 |
| *Aminitrozole* | −0.75 | 1.1 |
| *Amprenavir* | −1.27 | 0.1 |
| *Atevirdine* | −1.54 | 0.0 |
| *Azidamphenicol* | −1.44 | 0.0 |
| *Benznidazole* | −0.62 | 2.3 |
| *Biapenem* | −0.92 | 0.4 |
| *Bucetin* | −0.71 | 0.01 |
| *Candesartan* | −0.97 | 0.00 |
| *Cephalexin* | −0.82 | 0.01 |
| *Clortetraciclin* | −0.48 | 0.05 |
| *Clindamicine* | −1.05 | 0.00 |
| *Clometacine* | −0.70 | 0.01 |
| *Delapril* | −1.04 | 0.00 |
| *Dihydrocodeine** | 0.50 | 0.95 |
| *Dipipanone** | 0.65 | 0.98 |
| *Enalapril* | −0.83 | 0.01 |
| *Hidroxystilbamidine* | −0.16 | 0.28 |
| *Lansoprazol* | −0.99 | 0.00 |
| *Metronidazol* | −0.17 | 0.27 |
| *Misoprostol* | −0.94 | 0.00 |
| *Nizatidine* | −1.00 | 0.00 |
| *Ranitidine* | −0.77 | 0.01 |

Compounds from the INACTIVE category are written in italic. An asterisk indicates misclassifications.

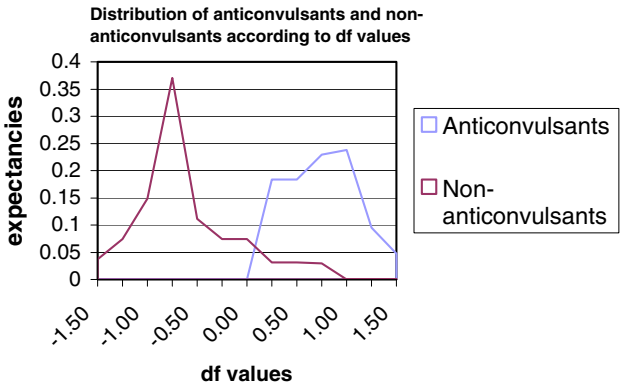


**Figure 3.** Pharmacological distribution diagram for the two considered categories.

according to Merck Index 13th,[24] in the manufacture of ester gums and metal resinates, and to assist growth of lactic and butyric acid bacteria.

The pharmacological tests were performed according to standard procedures provided by the Antiepileptic Drug Development (ADD) Program of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS).[27]

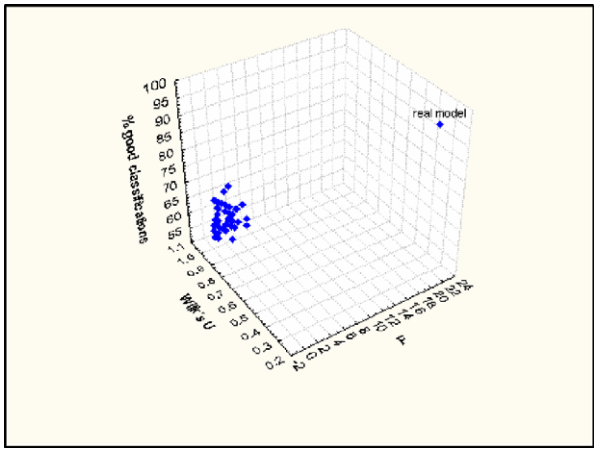


**Figure 4.** Results from the randomization study. The real DF clearly outperforms those obtained by randomization of the dependent, dummy variable.

**Table 2.** Results of the LGO cross-validation

| Removed compounds | *N* | Wilk's *U* | % hits training set | % hits test set |
|---|---|---|---|---|
| None (original DF) | 48 | 0.33 | 94 | 75 |
| Clonazepam, lacosamide, oxcarbacepine, biapenem, candesartan, delapril | 42 | 0.36 | 90 | 72 |
| Rufinamide, THC, Vinpocetine, Benzmidazole, Bucetina, Clometacine | 42 | 0.33 | 93 | 72 |
| ADCI, Mephenytoin, Primidone, Alfentanil, Atevirdine, Enalapril | 42 | 0.35 | 90 | 74 |
| Cannabidiol, Diazepam, Phenytoin, Aminitrozole, Amprenavir, Nizatidine | 42 | 0.35 | 93 | 74 |
| Carbamazepine, Felbamate, Lamotrigine, Acebutolol, Cephalexin, Dipipanone | 42 | 0.3 | 93 | 72 |
| Ralitoline, Soretolide, Stiripentol, Azidamphenicol, Lansoprazol, Ranitidine | 42 | 0.32 | 90 | 72 |
| Remacemide, Trimetadione, Zonisamide, Alclofenac, Clortetracicline, Dihydrocodeine | 42 | 0.29 | 95 | 74 |
| Clonazepam, Lamotrigine, Amprenavir, Clindamicine, Clometacine, Misoprostol | 42 | 0.35 | 90 | 80 |

The DFs obtained after random removal of six compounds in each LGO run perform almost identically to the original DF.

**Table 3.** Results of the external validation

| Compound | DF value |
|---|---|
| 534U87 | 0.26 |
| *N*-benzyl-2-ethylamino-3-methoxypropionamide* | −0.18 |
| *N*-benzyl-2-(1-oxo-ethylamino)-2-phenylacetamide | 0.05 |
| *N*-(2-fluorobenzyl)-2-azaspiro[4.4]nonane-1,3-dione | 0.73 |
| *N*-(3-fluorophenyl)-2-azaspiro[4.5]decano-1,3-dione | 0.30 |
| *N*-(3-trifluoromethyl phenyl)-2-azaspiro [4.4]nonane-1,3-dione | 0.27 |
| CFM11 | 0.29 |
| CFM2* | −0.13 |
| CFM2S | 0.20 |
| 1-(4-Methylpiperazin-1-yl)-3-(3-chlorophenyl)-pyrrolidine-2,5-dione [78]* | −0.13 |
| 1-(4-Methylpiperazin-1-yl)-3-(4-chlorophenyl)-pyrrolidine-2,5-dione [78] | 0.05 |
| GYKI 53655 | 0.00 |
| NBQX* | −0.43 |
| 4-Amino-1,3,5-trimethylpyrazole | 0.68 |
| *N-Cylohexyl-N_-(3,5-dimethylpyrazole-4-yl)thiourea* | 0.20 |
| *N-(4-Methoxyphenyl)-N_-(3,5-dimethylpyrazole-4-yl)thiourea** | −0.54 |
| *N-Phenyl-N_-(3,5-dimethylpyrazole-4-yl)urea* | 0.55 |
| 3,3,3-Trifluoro-2-hydroxy-2-phenyl-propionamide | 0.48 |
| 3,3,3-Trifluoro-2-hydroxy-*N*-methyl-2-phenylpropionamide | 0.96 |
| THIQ-10c* | −0.07 |
| *Acetoxolone* | −0.30 |
| *Alacepril* | −1.37 |
| *Albuterol* | −0.02 |
| *Amoxicillin* | −1.02 |
| *Aranidipine* | −0.03 |
| *Atenolol* | −1.09 |
| *Atorvastatin* | −1.01 |
| *Balofloxacin* | −0.48 |
| *Bambuterol* | −0.02 |
| *Benorylate* | −0.80 |
| *Bromfenac* | −0.44 |
| *Capravirine* | −0.56 |
| *Caromoxirole* | −1.52 |
| *Ceftezole* | −1.58 |
| *Chlorobutanol** | 1.62 |
| *Cimetidine* | −1.35 |
| *Cloxyquin* | −0.37 |
| *Cycloserine** | 0.45 |
| *Desomorphine** | 0.20 |
| *Didanosine* | −0.22 |
| *Dioxaphetyl butyrate** | 0.43 |
| *Doxofylline* | −0.95 |
| *Ebrotidine* | −1.79 |
| *Efavirenz** | 0.54 |
| *Emtricitabine* | −0.12 |
| *Ephedrine** | 0.21 |
| *Ertapenem* | −2.09 |
| *Famciclovir* | −0.48 |

Compounds from the INACTIVE category are written in italic. An asterisk indicates misclassifications. Chemical nomenclature is used when generic name does not exist.

Swiss mice weighing between 18 and 23 g at the time of testing were used as experimental animals. Mice are housed in colony cages on a 12-h light/dark cycle, and provided with food and water ad libitum. The compounds were dissolved in 30% PEG 400.A maximal volume of 10 ml/kg of the freshly made solutions was injected intraperitoneally (i.p.).

**Table 4.** Results of the virtual screening for forty of the screened compounds from Merck Index 13th

| Compound | Therapeutic category/use | DF value |
|---|---|---|
| Abietic acid | Manufacture of ester gums | 0.37 |
| Buformin | Antidiabetic | 0.16 |
| Bufan | Insecticide | 0.68 |
| Bupivacaine | Anesthetic | 0.22 |
| Cicletanine | Antihypertensive | 0.26 |
| Diphenidol | Antiemetic | 0.39 |
| Demegestone | Progestogen | 0.43 |
| Dropropizine | Antitussive | 0.03 |
| Elenolide | — | 0.39 |
| Eltoprazine | Serenic | 0.26 |
| Guanadrel | Antihypertensive | 0.19 |
| Hexestrol | Estrogen; antineoplastic | 0.15 |
| Idazoxan | Antiparkinsonian | 0.38 |
| Idrocilamide | Muscle relaxant | 0.31 |
| Isosorbide | Diuretic | 0.68 |
| Mequitazine | Antihistaminic | 0.60 |
| Metapramine | Antidepressant | 0.64 |
| Stanolone | Androgen | 0.59 |
| Tetraethylphthalamide | Analeptic | 0.74 |
| Zotepine | Antipsychotic | 0.13 |
| Abecarnil | Anxiolytic | −1.14 |
| Abikoviromycin | Antiviral | −0.31 |
| Acediasulfone | Antibacterial | −0.26 |
| Acetamidoeugenol | Anesthetic | −0.43 |
| Buparvaquone | Antiprotozoal | −0.05 |
| Cilostazol | Antithrombotic | −1.40 |
| Droxidopa | Antiparkinsonian | −0.47 |
| Dulcin | Sweetener | −0.46 |
| Eicosapentaenoic acid | Antihyperlipoproteinimic | −1.40 |
| Histamine | Antineoplastic | −0.23 |
| Hydnocarpic acid | Antibacterial (leprostatic) | −0.10 |
| Hydralazine | Antihypertensive | −0.24 |
| Hydroxyamphetamine | Adrenergic, mydriatic | −0.07 |
| Levobunolol | Antiglaucoma | −0.30 |
| Mephenesin | Muscle relaxant | −0.11 |
| Nialamide | Antidepressant | −1.39 |
| Perazine | Antipsychotic | −0.08 |
| Succinylsulfathiazole | Antibacterial | −1.36 |
| Zalcitabine | Antiviral | −0.11 |
| Zomepirac | Analgesic | −0.65 |

Therapeutic categories or uses (according to Merck Index) and DF values are presented for each of the compounds. Twenty of them (on the left half) have been classified as potential anticonvulsants, with DF value above zero, and the remaining 20 (on the right half) as non-anticonvulsants.

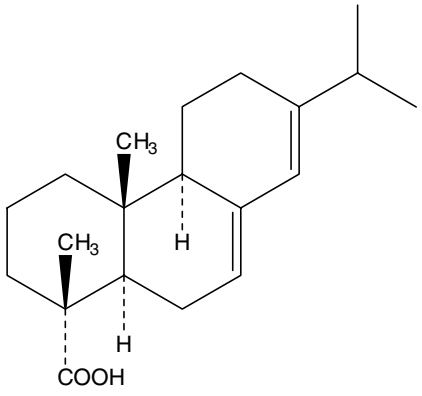


**Figure 5.** Structure of abietic acid.

**Table 5.** Biological data determined (mice) for abietic acid

| Dose (mg/kg) | Administration via | MES test | | PTZ test | | Rotorod test | |
|---|---|---|---|---|---|---|---|
| | | 0.5 h | 4 h | 0.5 h | 4 h | 0.5 h | 4 h |
| 30 | Ip | 0/3 | 3/8 | 0/3 | 0/3 | 0/6 | 0/11 |
| | Oral | 0/3 | 1/3 | 0/3 | 0/3 | 0/6 | 0/6 |
| 100 | Ip | 0/3 | 2/8 | 0/3 | 0/3 | 0/6 | 0/11 |
| | Oral | 0/3 | 1/3 | 0/3 | 0/3 | 0/6 | 0/6 |

Maximal electroshock seizures were elicited in mice by delivering a 60 Hz/50 mA electrical stimulus for 0.2 s via ear clip electrodes. A drop of saline solution was applied on each ear before placing the electrodes to ensure adequate electrical contact. In these conditions, maximal seizures are produced in virtually all normal mice. The maximal seizure typically consists of a short period of tonic flexion followed by a longer period of tonic extension of the hind limbs and a final clonic episode. Blockade of the hind limbs' tonic extensor component due to the drug treatment is taken as the end point. The tonic component is considered abolished if the hindleg tonic extension does not exceed a 90° angle with the trunk.

The PTZ tests identify substances that raise the seizure threshold. The freshly made solution of PTZ (1.7% in 0.9% saline solution) is administered subcutaneously (s.c.) into a loose fold of skin in the midline of the neck in a volume of 5 ml/kg body weight. Animals are observed for at least 30 min after s.c. injection of PTZ for the presence or absence of a convulsive episode persisting for at least 5 s. Absence of a clonic seizure indicates protection.

The RotoRod test was used to determine the possible neurotoxic effects of abietic acid.

Results are listed in Table 5. Abietic acid showed protection in the MES test, both ip and po, at 4 h and 30 and 100 mg/kg. Non-activity was observed at 0.5 or 4 h in PTZ test. The latter result is consequent to the fact that the training set from which the DF was derived includes drugs active in the MES test, but inactive in the PTZ test.

The ability of the generated DF to differentiate anticonvulsants with activity in the MES test from non-anticonvulsants was established through the validation steps and by the identification of a new anticonvulsant compound active at 30 and 100 mg/kg. Abietic acid may be a candidate for structural optimization and development of new anticonvulsant-compounds series. LDA based on 2D descriptors was confirmed as a powerful tool to be applied in the rational discovery of new therapeutic agents.

## Acknowledgments

Alan Talevi would like to thanks the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Eduardo A. Castro is a member of CONICET. Luis E. Bruno-Blanch thanks the Facultad de Ciencias Exactas de la Universidad Nacional de La Plata (Incentivos UNLP) and the Agencia Nacional de Promoción Científica y Tecnológica (PICT BIB 1728/06-11985).

## References and notes

1. World Health Organization Fact sheet n° 265: Mental and neurological disorders. December 2001.
2. Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Loiseau, P.; Perucca, E. *Epilepsy Res.* **2002**, *51*, 31.
3. Marrero-Ponce, Y.; Machado Tugores, Y.; Pereira, D. N.; Escario, J. A.; Barrio, A. G.; Nogal-Ruiz, J. J.; Ochoa, C.; Aran, V. J.; Martínez-Fernández, A. R.; García-Sánchez, R. N.; Montero-Torres, A.; Torrens, F.; Meneses-Marcel, A. *Curr. Drug. Discov. Technol.* **2005**, *2*, 245.
4. Montero-Torres, A.; García-Sánchez, R. M.; Machado-Tugores, Y.; Nogal-Ruiz, J. J.; Martínez-Fernández, A. R.; Aran, V. J.; Ochoa, C.; Meneses-Marcel, A.; Torrens, F. *Eur. J. Med. Chem.* **2006**, *41*, 483.
5. Marrero-Ponce, Y.; Cabrera-Pérez, M. A.; Romero-Zaldivar, V.; González-Díaz, H.; Torrens, F. *J. Pharm. Pharm. Sci.* **2004**, *7*, 186.
6. García-García, A.; Gálvez, J.; de Julián-Ortiz, J. V.; García-Domenech, R.; Muñoz, C.; Guna1, R.; Borrás, R. *J. Antimicrob. Chemother.* **2004**, *53*, 65.
7. Julián-Ortiz, J. V.; Gálvez, J.; Muñoz Collado, C.; García-Domenech, R.; Gimeno-Cardona, C. *J. Med. Chem.* **1999**, *42*, 3308.
8. Prieto, J. J.; Talevi, A.; Bruno-Blanch, L. E. *Mol. Div.* **2006**, *10*, 361.
9. Talevi, A.; Bellera, C. L.; Castro, E. A.; Bruno-Blanch, L. E. *Drugs Future*. **2006**, *31 (Suppl. A) Abstracts from the XIX International Symposium on Medicinal Chemistry*, 188.
10. Talete srl DRAGON for Windows (Software for Molecular Descriptors Calculation) Version 4.0, **2003**. www.talete.mi.it.
11. Raychaudhury, C.; Ray, S. K.; Ghosh, J. J.; Roy, A. B.; Basak, S. C. *J. Comput. Chem.* **1984**, *5*, 581.
12. Magnuson, D. R.; Harriss, V. K.; Basak, S. C. Studies in physical and theoretical chemistry. In King, R. B., Ed.; Elsevier: Amsterdam, 1983; p 178.
13. Geary, R. C. *Incorp. Statist.* **1954**, *5*, 115.
14. Gálvez, J.; García-Domenech, R.; Alapont de Gregorio, C.; De Julián-Ortiz, J. V.; Popa, L. *J. Mol. Graph* **1996**, *14*, 272.
15. Yasri, A.; Hartsough, D. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1218.
16. Obniska, J.; Kaminski, K.; Zagorska, A.; Dzierzawska-Majewska, A.; Karolak-Wojciechowska, J. *J. Fluorine Chem.* **2006**, *127*, 417.
17. Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Loiseau, P.; Perucca, E. *Epilep. Res.* **1999**, *34*, 1.
18. De Sarro, G.; Ferreri, G.; Gareri, P.; Russo, E.; De Sarro, A.; Gitto, R.; Chimirri, A. *Pharmacol. Biochem. Behav.* **2003**, *74*, 595.
19. Ferreri, G.; Chimirri, A.; Russo, E.; Gitto, R.; Gareri, P.; De Sarro, A.; De Sarro, G. *Pharmacol. Biochem. Behav.* **2004**, *77*, 85.
20. Obniska, J.; Jurczyk, S.; Zejc, A.; Kamiński, K.; Tatarczyñska, E.; Stachowicz, K. *Pharmacol. Rep.* **2005**, *57*, 170.
21. Schenck, H. A.; Lenkowski, P. W.; Choudhury-Mukherjee, I.; Ko, S.; Stables, J. P.; Patel, M. K.; Brown, M. L. *Bioorg. Med. Chem.* **2004**, *12*, 979.
22. Beguin, C.; LeTiran, A.; Stables, J. P.; Voyksnerc, R. D.; Kohnd, H. *Bioorg. Med. Chem.* **2004**, *12*, 3079.
23. Kaymakcıoglu, B. K.; Rollas, S.; Korcegez, E.; Arıcıoglu, F. *Eur. J. Pharm. Sci.* **2005**, *26*, 97.

24. The Merck Index, Encyclopedia of Chemicals, Drugs and Biologicals, 13th ed. Merck and Co. Withehouse Station, New Jersey, 2001.
25. Fernández, M. A.; Tornos, M. P.; García, M. D.; de las Heras, B.; Villar, A. M.; Sáenz, M. T. *J. Pharm. Pharmacol.* **2001**, *53*, 867.
26. Liu, T. P.; Gao, C. Z.; Feng, L. Z. J. *Tradit. Chin. Med.* **1985**, *5*, 115.
27. Stables, J. P.; Kupferberg, H. J.; Gladding, R. Molecular and cellular targets for antiepileptic drugs. In Avanzini, G., Regesta, G., Tanganelli, P., Avoli, M., Eds.; John Libbey: London, 1997; p 191.