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Development and Validation of a Pharmacophore-Based QSAR Model for the Prediction of CNS Activity

Rafael Gozalbes,*[a, b] Frédérique Barbosa,[a, c] Eric Nicolai,[a, d] Dragos Horvath,[a, e] and Nicolas Froloff[a]

A QSAR model aimed at predicting central nervous system (CNS) activity was developed based on the structure–activity relationships of compounds from an in-house database of "drug-like" molecules. These compounds were initially identified as "CNS-active" or "CNS-inactive", and pharmacophore 3D descriptors were calculated from multiple conformations for each structure. A linear discriminant analysis (LDA) was performed on this structure–activity matrix, and this QSAR model was able to correctly classify approximately 80% in both a learning set and a validation set. For validation purposes, the LDA model was applied to compounds for which the blood–brain barrier (BBB) penetration was known, and all of them were correctly predicted. The model was also applied to 960 other in-house compounds for which in vitro binding tests were performed on 20 receptors known to be present at the CNS level, and a high correlation was observed between compounds predicted as CNS-active and experimental hits. Finally, the model was also applied to a set of 700 structures with known CNS activity or inactivity randomly chosen from public sources, and more than 70% of the compounds were correctly classified, including novel CNS chemotypes. These results demonstrate the applicability of the model to novel chemical structures and its usefulness for designing original CNS-focused compound libraries.

Introduction

Many studies have been devoted to the prediction of blood–brain barrier (BBB) permeability,[1–5] often from limited datasets (20–100 compounds, usually with low structural diversity), thus limiting any reliable application of these models to new compounds. This drawback could be partially overcome if prediction of activity or inactivity toward the central nervous system (CNS) instead of toward BBB permeability was attempted from therapeutic indication data extracted from large databases such as Comprehensive Medicinal Chemistry (CMC), the World Drug Index (WDI), or the MDL Drug Data Report (MDDR).[6–8] The main drawback of this approach is the significant heterogeneity of the biological activities defined in these general chemical databases (the data are usually collected from different bibliographic sources and hence are not homogeneous), which implies a high risk of misclassification (for example, it can be assumed that some compounds in the CNS-inactive class may actually have CNS activity).[6]

Taking the above considerations into account, we based our strategy on BioPrint (http://www.cerep.fr/cerep/users/pages/ProductsServices/bioprintservices.asp),[9–14] an in-house database that includes more than 2500 commercially available drugs, clinical candidates, and reference compounds systematically tested in more than 180 assays (receptors, enzymes, ion channels, cellular function, and in vitro ADME). In addition, the database contains compound-specific therapeutic class and clinical information obtained from published sources.[9–14] A QSAR model for the prediction of CNS activity/inactivity was constructed by considering 978 compounds for which well-established CNS activity/inactivity information is available and proprietary 3D pharmacophore descriptors known as fuzzy bipolar pharmacophore autocorrelograms (FBPAs).[15] This QSAR model was developed by linear discriminant analysis (LDA), a pattern recognition method that provides a classification model based on the combination of variables which best predict the category to which a given object belongs.[16] The independent variables in this study were the calculated FBPA descriptors, and the discriminant property was the CNS activity/inactivity.

The main advantage of this approach is the decreased risk of misclassification of compounds as CNS active or inactive, as the database used for developing the model is constituted by drugs with well-characterized therapeutic activities and ad-

[a] *Dr. R. Gozalbes, Dr. F. Barbosa, Dr. E. Nicolaï, Dr. D. Horvath, Dr. N. Froloff*
Medicinal Chemistry and Molecular Modeling Department, CEREP
19 avenue du Québec, 91951 Courtaboeuf Cedex (France)

[b] *Dr. R. Gozalbes*
current address: Structural Biology Laboratory
Centro de Investigación Príncipe Felipe (CIPF)
Avda. Autopista del Saler 16, 46012 Valencia (Spain)
Fax: (+34) 96 328 97 01
E-mail: rgozalbes@cipf.es

[c] *Dr. F. Barbosa*
current address: Molecular Modeling Department, Novexel
102 avenue Gaston Roussel, 93230 Romainville (France)

[d] *Dr. E. Nicolaï*
current address: Medicinal Chemistry Department
Sanofi–Aventis R&D
10 rue des Carrières, 92500 Rueil-Malmaison (France)

[e] *Dr. D. Horvath*
current address: Laboratoire d'Infochimie, UMR 7177 CNRS
Université Louis Pasteur
4 rue Blaise Pascal, 67000 Strasbourg (France)

verse effects. In this context, we have included as CNS-active compounds those usually administered for their peripheral action that have well-known central effects. As a clear example, cocaine is described in BioPrint as a "local anesthetic", but we have included it in the group of CNS-active compounds.

The LDA model is intended to predict CNS-active compounds, that is, molecules that are able to pass the BBB and to act at some level on various CNS receptors. To verify that the model accounts for both claims, we performed two complementary analyses: 1) To verify that the model considers the ability of compounds to pass the BBB, we analyzed the distribution of the physicochemical parameters usually cited as relevant for passing the BBB in our database, and we also applied the model to compounds with chemically similar structures but with different BBB-permeating abilities; and 2) to ensure the reliability of the model for identifying potential CNS receptor binders, we applied it to 960 compounds not included in BioPrint and for which we recently performed experimental binding tests against a panel of 21 different G-protein-coupled receptors (GPCRs), 20 of which are known to be present at the CNS level.[12,13]

Finally, we applied our model to 700 structures corresponding to compounds from which information about their CNS activity/inactivity was extracted from external sources such as the Merck Index[17] and the Drug Data Report,[18] with none of these compounds being included in BioPrint. This test allowed us to verify two different aspects of the model: its ability to simultaneously detect compounds that are able to pass the BBB and to bind CNS receptors, as well as its ability to detect novel CNS features and original CNS chemotypes interacting with a large variety of CNS receptors.

Results and Discussion

The LDA was performed on a randomly chosen "learning set" (LS) of 665 compounds (68% of the total data set), and 313 compounds were consequentially reserved as a validation set (VS), thus representing 32% of the molecules. The distribution of active and inactive compounds in both LS and VS was similar, as in both cases approximately 25% of compounds were CNS active: 125 and 68 compounds in the LS and VS, respectively (Table 1).

Table 1. Data-point distribution of compounds in the learning set (LS), validation set (VS), and the entire BioPrint database.

	Number of Compounds	
	CNS Actives	CNS Inactives
LS (665 compds)	125	540
VS (313 compds)	68	245
Whole database (978 compds)	193	785

The best discriminant equation, according to which compounds were labeled by the statistical software used[19] as active if $LDA_{CNS} > 0$ and as inactive if $LDA_{CNS} < 0$, was composed of 21 FBPA descriptors; the most important of these are listed in Table 2. The statistical parameters of the equation were: $n = 978$, $F = 13.72$, *Wilk's* $U = 0.69$.

Table 2. Most important descriptors identified by the LDA model for their relation to CNS activity/inactivity.[a]

LDA > 0[b]	LDA < 0[c]
Hp-HD4	Hp-HA8
Ar-PC5	Ar-HA4
Ar-PC6	HA-NC4
HA-NC10	HD-HD4
PC-PC4	

[a] Hp = hydrophobic, Ar = aromatic, HA = hydrogen bond acceptor, HD = hydrogen bond donor, PC = positive charge, NC = negative charge; the numbers correspond to the interatomic distance bins of 1 Å width, ranging from 3 to 15 Å. [b] Descriptors with a positive contribution to the LDA value. [c] Descriptors with a negative contribution to the LDA value.

Table 3 lists the number and percentages of compounds correctly classified in the LS and VS, and Figure 1 shows a graphical representation of the percentages of classification in both sets. From the above results, the LDA equation can be considered as statistically significant and reliable, because approximately 80% of compounds in both the LS and VS were correctly predicted.

Table 3. Confusion matrix summarizing the number and percentages of compounds correctly classified after application of the LDA equation to the LS and VS.[a]

		LDA Calculated Classification		
		$Actives_{pred}$	$Inactives_{pred}$	
LS	$Actives_{exptl}$	100	25	Sn = 80.0%
	$Inactives_{exptl}$	97	443	Sp = 82.0%
		PPV = 81.6%	NPV = 80.4%	A = 81.6%
VS	$Actives_{exptl}$	52	16	Sn = 76.5%
	$Inactives_{exptl}$	47	198	Sp = 80.8%
		PPV = 79.9%	NPV = 77.5%	A = 79.9%

[a] Sn: "Sensitivity" (percentage of experimental positives predicted as positives); Sp: "Specificity" (percentage of correctly predicted as non-binders); PPV: "Positive probability value" (probability that a predicted binder will actually be a binder); NPV: "Negative probability value" (probability that a predicted non-binder will actually be a non-binder); A: "Accuracy" (overall percentage of correctly predicted compounds).

The highest probability of finding CNS activity is for compounds with LDA values greater than zero (76.5% of CNS-active compounds and only 19.2% of CNS-inactive compounds from the VS are in this region). The highest activity enrichment (the ratio between CNS-active and inactive compounds) is obtained for LDA values between 3 and 4: 16% of CNS-active compounds and 1.1% of inactive compounds in the VS, thus representing a 14.5-fold enrichment of CNS actives versus CNS inactives.

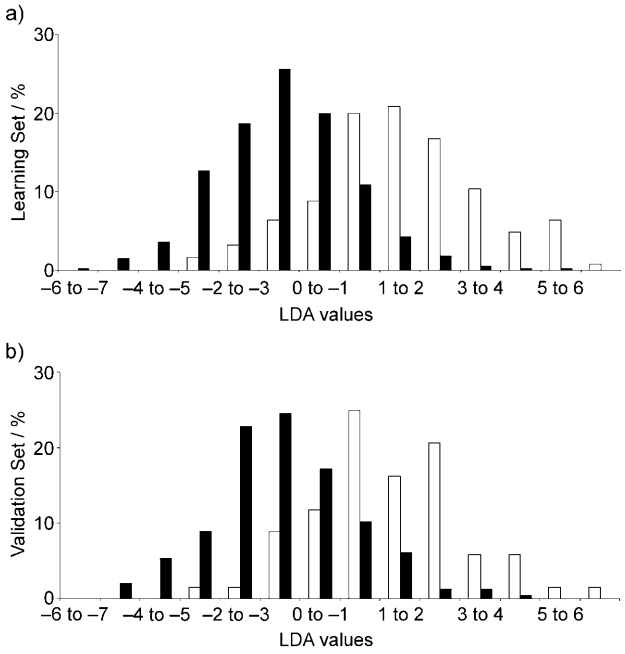

Figure 1. Graphical representation of the percentages of CNS-active (white bars) and inactive (black bars) compounds with respect to LDA values in the a) learning and b) validation sets.

Analysis of the model quality in application to external compounds

Ability of the model to predict BBB permeability

We analyzed the physicochemical properties of the dataset used to develop the model (978 compounds from BioPrint): Figure 2 shows the distribution of parameters that usually are cited as relevant for passing the BBB,[20–22] and it can be observed that in all cases the percentage of compounds with commonly considered "preferred" parameters ($M_r < 400$, $PSA_{calcd} < 80$ Å^2, $\log D > 0$, and $\log P_{ab} > 1$ in Caco-2 human intestinal cell lines at pH 7.4) is clearly higher for the CNS-active compounds which were used in the elaboration of the LDA model than for the CNS-inactive compounds.

We also challenged the model to predict BBB permeability for compounds with chemically similar structures but different BBB-passage behavior. As an example, Figure 3a shows the results for alvimopan, a peripherally selective μ-opioid receptor antagonist,[23,24] and four similar compounds that, along with alvimopan, share a common chemical skeleton (with a central nitrogen atom and two terminal aromatic ring systems) and which are known for their ability to pass the BBB. The LDA model correctly detects alvimopan as inactive at the CNS level, while all of the other four compounds are correctly predicted as active. Figure 3b shows the various LDA values obtained with four arylacetamides that have been described as κ-opioid receptor agonist ligands.[24,25] U50 488 and U69 593 are able to

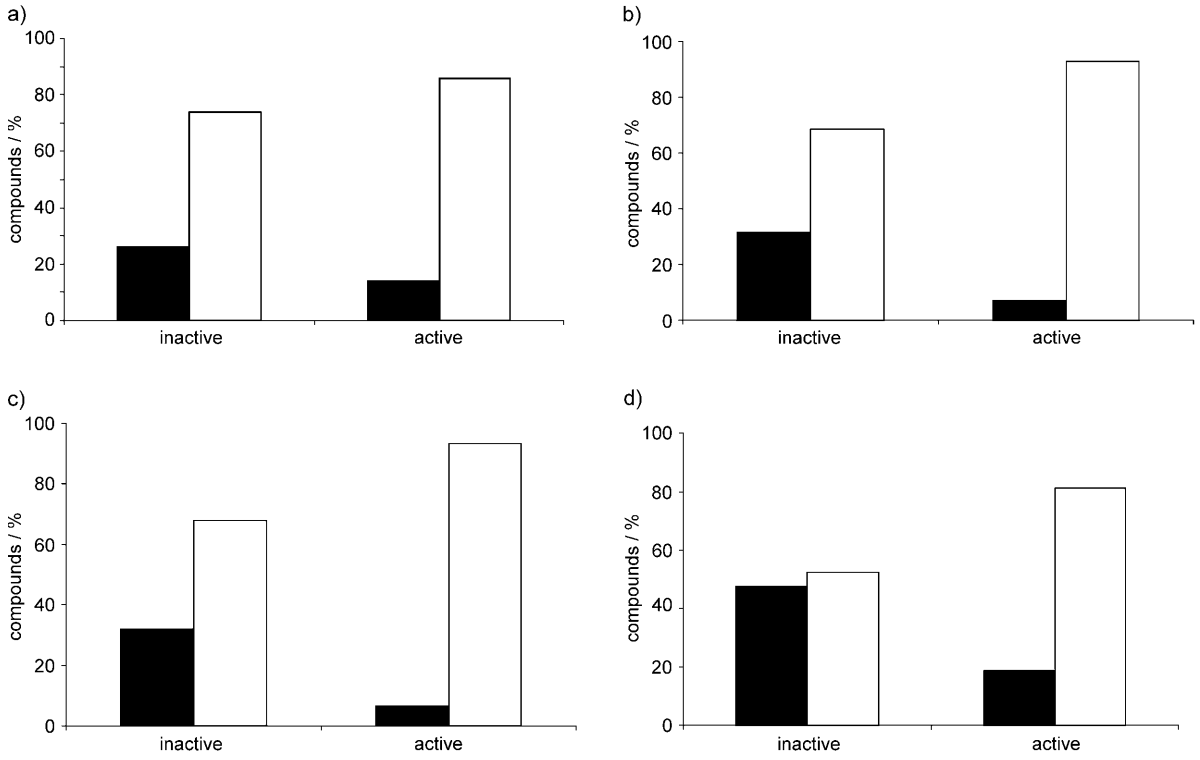

Figure 2. Statistics related to the 978 BioPrint compounds used for the development of the LDA model. a) Distribution of M_r values for CNS-active and inactive compounds; ■: $M_r > 400$, □: $M_r < 400$. b) Distribution of calculated PSA values for CNS-active and inactive compounds; ■: PSA > 80 Å^2, □: PSA < 80 Å^2. c) Distribution of experimental $\log D$ values for CNS-active and inactive compounds; ■: $\log D < 0$, □: $\log D > 0$. d) Distribution of experimental $\log P_{ab}$ values for CNS-active and inactive compounds; ■: $\log P_{ab} < 1$, □: $\log P_{ab} > 1$.

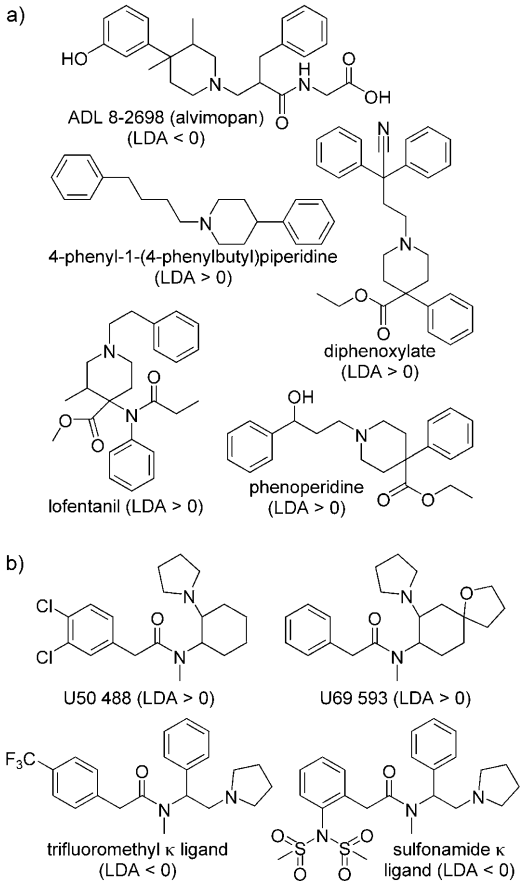

Figure 3. Compounds with chemically similar structures but with various BBB behaviors. a) The μ-opioid receptor antagonist alvimopan and related structures; b) κ-opioid receptor agonist ligands.

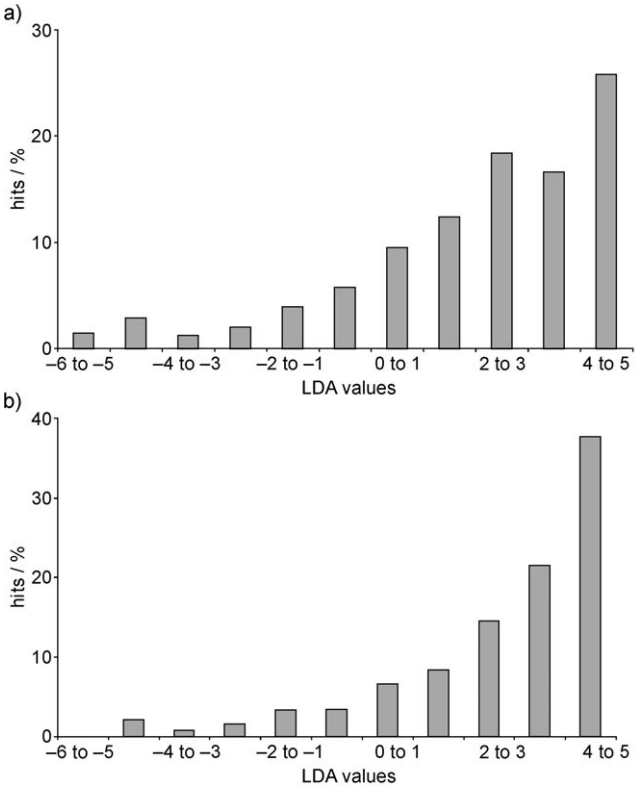

Figure 4. Results of the application of the LDA model to 960 compounds tested in 20 GPCRs known to be present at the CNS level. a) Percentages of "hits" at 90% inhibition; b) percentages of "hits" in at least three receptors at 90% inhibition.

pass the BBB, and as expected, LDA values are positive for them. In contrast, when the model is applied to structures designed to increase lipophilicity in order to avoid BBB passage (by inclusion of a trifluoromethyl (bottom left) or sulfonamide group (bottom right)[25]), the LDA values become negative, thus confirming the predictive accuracy of the model.

Ability of the model to predict the binding of compounds to receptors present in the CNS

We applied the LDA model to 960 in-house compounds not included in BioPrint for which we have performed experimental binding tests against a panel of 20 GPCRs known to be present in the CNS.[12,13] Results of this analysis (Figure 4a) show that 82.7% of the 381 experimental hits—compounds leading to at least 90% inhibition of reference radioligand binding at a concentration of 10 μM in a given test—correspond to compounds with LDA > 0 (that is, compounds predicted as CNS-active). Figure 4b shows the distribution of compounds that inhibit at least three CNS-related receptors: 88.7% of these have a calculated LDA value greater than zero, and the highest hit enrichment is obtained for compounds with LDA values between 4 and 5.

Ability of the model to predict compounds that are structurally different or with original chemotypes

The LDA model was also applied to 700 randomly chosen compounds from the Merck Index and the Drug Data Report, including 380 CNS-active and 320 CNS-inactive compounds (Table 4). More than 70% of these compounds were correctly identified as CNS-active or inactive compounds. Among 286 compounds that are predicted as CNS active, 232 have reported CNS activity (Table 4), thus confirming the predictive ability of the model for new compounds and its applicability to the

Table 4. Confusion matrix of correct predictions when applying the LDA-model to an external set of 700 compounds randomly selected from the Merck Index and the Drug Data Report.[a]

		LDA Predictions		
		$Actives_{pred}$	$Inactives_{pred}$	
Merck Index and DDR Set	$Actives_{exptl}$	232	148	Sn = 61.1%
	$Inactive_{exptl}$	54	266	Sp = 83.1%
		PPV = 78.3%	NPV = 68.1%	A = 71.1%

[a] Sn: "Sensitivity" (percentage of experimental positives predicted as positives); Sp: "Specificity" (percentage of correctly predicted as non-binders); PPV: "Positive probability value" (probability that a predicted binder will actually be a binder); NPV: "Negative probability value" (probability that a predicted non-binder will actually be a non-binder); A: "Accuracy" (overall percentage of correctly predicted compounds).

design of CNS-targeted libraries. Figure 5 shows that compounds which are difficult to predict as active at the CNS level owing to the presence of certain unfavorable groups (such as the sulfonamide group of UK-373911) or original features (as in SB-277211, WO-0034263, and EP 1010694) were correctly identified by the model. Particularly interesting is the case of IL-488, as this compound inhibits a class-B GPCR (CRF), and it presents a completely new and original chemotype.

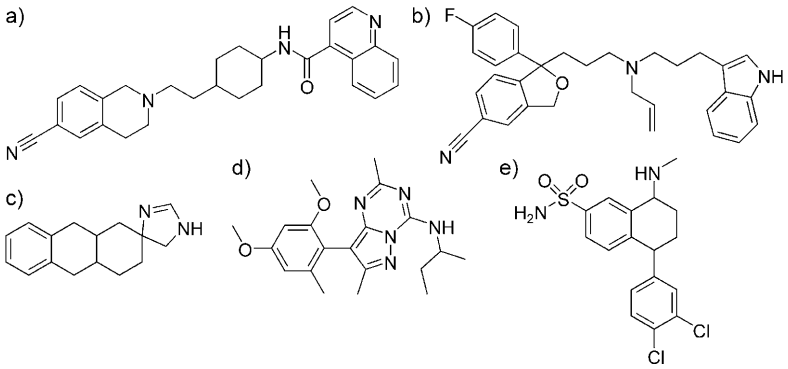

Figure 5. Some newly discovered CNS-compounds with original chemotypes that are correctly predicted as CNS-actives by the QSAR model (LDA values >0): a) SB-277211 (SmithKline Beecham); dopamine D3 receptor antagonist with high brain penetration, a potential antipsychotic agent. b) WO-0034263 (H. Lundbeck A/S); 5-HT_{1A} receptor ligand and 5-HT reuptake inhibitor, potentially useful for the treatment of depression. c) EP 1010694, FR 2787450, JP 2000178257 (ADIR); α2-adrenoceptor antagonist and monoamine reuptake inhibitor with potential for the treatment of depression, phobia, and Parkinson's disease. d) IL-488 (DuPont Pharmaceuticals); CRF receptor antagonist with potential use as an anxiolytic. e) UK-373911 (Pfizer); serotonin reuptake inhibitor with potential use as an antidepressant.

Conclusions

Herein we present a pharmacophore-based QSAR model for the prediction of CNS activity using BioPrint in vivo information. The main advantages of this model over other published QSAR studies are that: 1) it is based exclusively on a set of drugs with well-known clinical therapeutic and adverse effects, thus decreasing the risk of misclassification of compounds (for example, the group of CNS actives includes compounds known for their peripheral activity but with well-characterized central effects); and 2) the LDA model was developed using pharmacophore 3D descriptors from multiple conformations for each structure, thus allowing the retrieval of novel and original chemotypes. The aim was to minimize the risk of obtaining a model that describes chemical classes already known to be CNS active or inactive, and to favor the selection of 3D descriptors of CNS activity/inactivity of general use for the design of original CNS-focused compound libraries.

The model presented herein has been extensively validated by application to other compounds for which we performed experimental binding assays, or for which CNS activity or inactivity is known (data from the Merck Index and the Drug Data Report). In all cases a strong correlation was obtained between predictions and experimental findings, thus demonstrating the ability of the model to predict novel chemical structures. Interestingly, the LDA model recognizes not only compounds with a high probability of binding CNS receptors—which is demonstrated by the analysis of the 960 molecules tested on 21 CNS-related receptors—but also compounds that pass the BBB, as shown by the analysis of several compounds with chemically similar structures but different BBB-permeating behaviors. In fact, this can be considered an expected result, considering the physicochemical properties of the dataset used to develop the model (Figure 2).

Because the learning database includes highly diverse compounds that act in various therapeutic CNS areas, the QSAR model was also expected to identify potential hits for a large variety of CNS targets, including original CNS chemotypes, as has been confirmed by the analysis of 700 compounds from the Merck Index and the Drug Data Report. As most of the CNS-active compounds used to develop the model are known as aminergic GPCR ligands, one can consider that the model would recognize GPCR aminergic chemotypes in particular. Moreover, we have shown that the use of pharmacophore descriptors allows the retrieval of chemotypes other than those present in the learning database. In conclusion, this LDA model is a useful tool for the systematic application to new compounds (such as compounds for which CNS secondary effects are highly undesirable) or for the design and development of CNS-focused libraries.

Experimental Section

Distribution of compounds used to develop the QSAR model

The classification of compounds as "CNS active" or "CNS inactive" was performed by using the detailed clinical annotation of drugs (therapeutic indications, mechanisms of action, and adverse drug reactions), which is part of the BioPrint database.[9–14] A total of 978 compounds from BioPrint were identified as either "CNS-active" [193 molecules labeled with CNS-related activities or having well-described central side effects: analgesic (narcotic), anesthetic (general), antiparkinsonian, antipsychotic (neuroleptic), sedative–hypnotic, stimulant (central), anti-anxiety, anticonvulsant, antidepressant, anti-maniac, antipsychotic, antitussive, anorexic, antidyskinetic, anti-emetic, antimigraine, emetic, antivertigo, neuroprotectant] or "CNS-inactive" (785 compounds with non-CNS-related therapeutic activities and few or none reported secondary CNS effects).

Descriptors

The elaboration of a QSAR model requires encoding compound structures as molecular descriptors, which capture information on structural features responsible for the activity in a numerical form. The proprietary descriptors used in this work were fuzzy bipolar pharmacophore autocorrelograms (FBPAs).[15] These descriptors are 3D terms that carry information about the nature and spatial distribution of the various pharmacophore features in a molecule. For generating the FBPA of a compound, its constituent atoms are first classified according to their features (hydrophobicity, aromaticity, hydrogen bond donor or acceptor propensity, positive or negative

charge). Any atom may possess one or more such features, detected by a feature assignment routine according to empirical rules. There are 21 pairs from these six features (hydrophobic–hydrophobic, hydrophobic–aromatic, etc.) for two-point pharmacophores. All the atom pairs that occur in a molecule are first assigned to one of these 21 pharmacophore-pair categories and are further broken down into 12 interatomic distance bins of 1 Å width, in a range of 3–15 Å. This defines a total of 252 groups to which an atom pair would belong; the fingerprint is thus a 252-dimensional vector in which each component represents the number of atom pairs that belong to the given group, averaged over a diverse sample of 3D conformers.[15]

Statistical analysis: LDA

The FBPA descriptors were calculated for the set of 978 compounds, and an LDA analysis was performed to establish a general model for separating CNS from non-CNS molecules. The software used for the LDA analysis was the BMDP 7M package.[19] The variables to be used in the linear classification functions are selected in a stepwise manner: at each step the variable that makes the greatest contribution to the separation of the categories is entered into the discriminant equation (or the variable that makes the smallest contribution is removed from the pool of candidate variables). The method used to select the descriptors was based on the F-Snedecor parameter, which allows the assessment of relative importance among the candidate variables.[16,26] The classification criterion was the minimal Mahalanobis distance, which is the distance of each compound to the mean of all compounds in the descriptor space.[26] The quality of the discriminant equation was evaluated using the *Wilk's U* statistical parameter, a multivariate analysis of variance that tests the equality of group means for the variable(s) in the discriminant equation.[16]

In vitro assays

To validate the performance of the LDA equation to capture compounds able to bind CNS receptors, the model was applied to another set of 960 compounds, which were profiled at 10 μM against a set of 21 GPCRs,[12,13] 20 of which are known to be present in the CNS. The affinities of the compounds for each receptor were determined in radioligand binding assays performed homogeneously following standard operating procedures.

Protocols for binding assays included a minimum of eight control wells (for determining nonspecific and total binding) and a standard reference compound, which was tested in each experiment at several concentrations, from which its IC_{50} value was calculated.

Total binding was determined in the presence of the vehicle of test compounds. Nonspecific binding was determined in the presence of an excess of an appropriate compound (usually the endogenous ligand of the receptor). Membrane homogenates were incubated in the absence or presence of the test compound. Following the incubation, the samples were filtered rapidly under vacuum through glass fiber filters, dried, and then counted for radioactivity in a scintillation counter using a scintillation cocktail. The affinity of the test compound was expressed as the percent inhibition of the specific binding of the control radioligand.

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