# Comparative Molecular Field Analysis-Based Prediction of Drug Affinities at Recombinant D1A Dopamine Receptors[†]

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Determination of quantitative structure–activity relationships (QSAR) for affinity at particular dopamine (DA) receptors has become an even greater priority with the cloning of five DA receptor subtypes. The use of agonist affinity at recombinant receptors selectively expressed in clonal cells as the dependent variable in QSAR presents a unique opportunity for accuracy and precision in measurement of biological values. Bound conformations of 11 agonists (for which both affinity data at the recombinant D1A DA receptor and stereochemical configurations were available) were determined by alignment with a template compound, SKF38393, which shows high affinity and selectivity for D1A receptors and is fairly rigid in structure. These aligned structures suggested a 3-point pharmacophore map (one cationic nitrogen and two electronegative centers) of the D1A DA receptor. This map shows both similarities and differences when compared with a previously reported D2 DA receptor pharmacophore map based on biological data from rat brain and with a recently published map of the native D1 DA receptor using several semirigid compounds. Log($1/K_d$) values at recombinant D1A DA receptors were used as the target property for a CoMFA (comparative molecular field analysis) of the 11 aligned structures. The resulting CoMFA model yielded a cross-validated $r^2$ ($q^2$) value of 0.829 and a simple $r^2 = 0.96$. In contrast, when a CoMFA model was developed for 10 of these compounds using striatal D1 $K_d$ values, the $q^2$ value was reduced to 0.178. These results are consistent with the idea that drug affinity data obtained from clonal cells expressing recombinant receptors may be superior to that obtained using heterogeneous mixtures of native receptors prepared from brain membranes. The predictive utility of the CoMFA model was evaluated using several high-affinity dopamine agonists and *m*- and *p*-tyramine, two compounds with a single hydroxyl group on the aromatic ring. Predictions were fairly accurate for all compounds but the two tyramines.

## Introduction

The recent cloning and sequencing of the genes for several dopamine (DA) receptors have enhanced the already extensive interest in these extremely important proteins.[1–5] DA systems and their associated receptors in the brain are important in modulating motor, endocrine, and emotional functions.[6,7] Furthermore, both DA neurons and DA receptors are markedly reduced by normal aging and Parkinson's disease[8,9] and have been implicated in a variety of other disorders, including schizophrenia and drug abuse.[10] Therefore, the accurate determination of quantitative structure–activity relationship (QSAR) information concerning drug properties at DA receptors has great clinical significance.

The DA D1 receptor behaves similarly to the prototype G-protein-linked $\beta$2-adrenergic receptor system.[11–14] The D1 (D1A) and the more recently discovered D5 (D1B) DA receptors are both positively coupled to adenylate cyclase and constitute a D1/D5 subfamily.[5,13,15,16] In brain tissue and clonal cell lines, GTP is required for D1 and D5 receptor stimulation of adenylate cyclase activity. While the DA and $\beta$2-adrenergic receptors share a general topological and functional similarity and moderate homology, the relative importance of some conserved amino acid residues differs.[17] Therefore, QSAR information concerning drug properties at D1A DA receptors can also help to resolve the roles of the various conserved amino acids in this novel protein.

Associated with the recent increase in molecular pharmacological studies has been the publication of a number of stimulating papers attempting to model DA receptors using computational chemistry approaches.[18–23] While molecular modeling and molecular biological studies of receptor proteins provide exciting information concerning amino acid residues possibly involved in drug binding and activation of the receptor by drugs, alternative computational approaches allow a direct study of the aspects of drug structure which are most closely associated with particular biological target properties.[24,25] One of the most promising of these computational approaches is the comparative molecular field
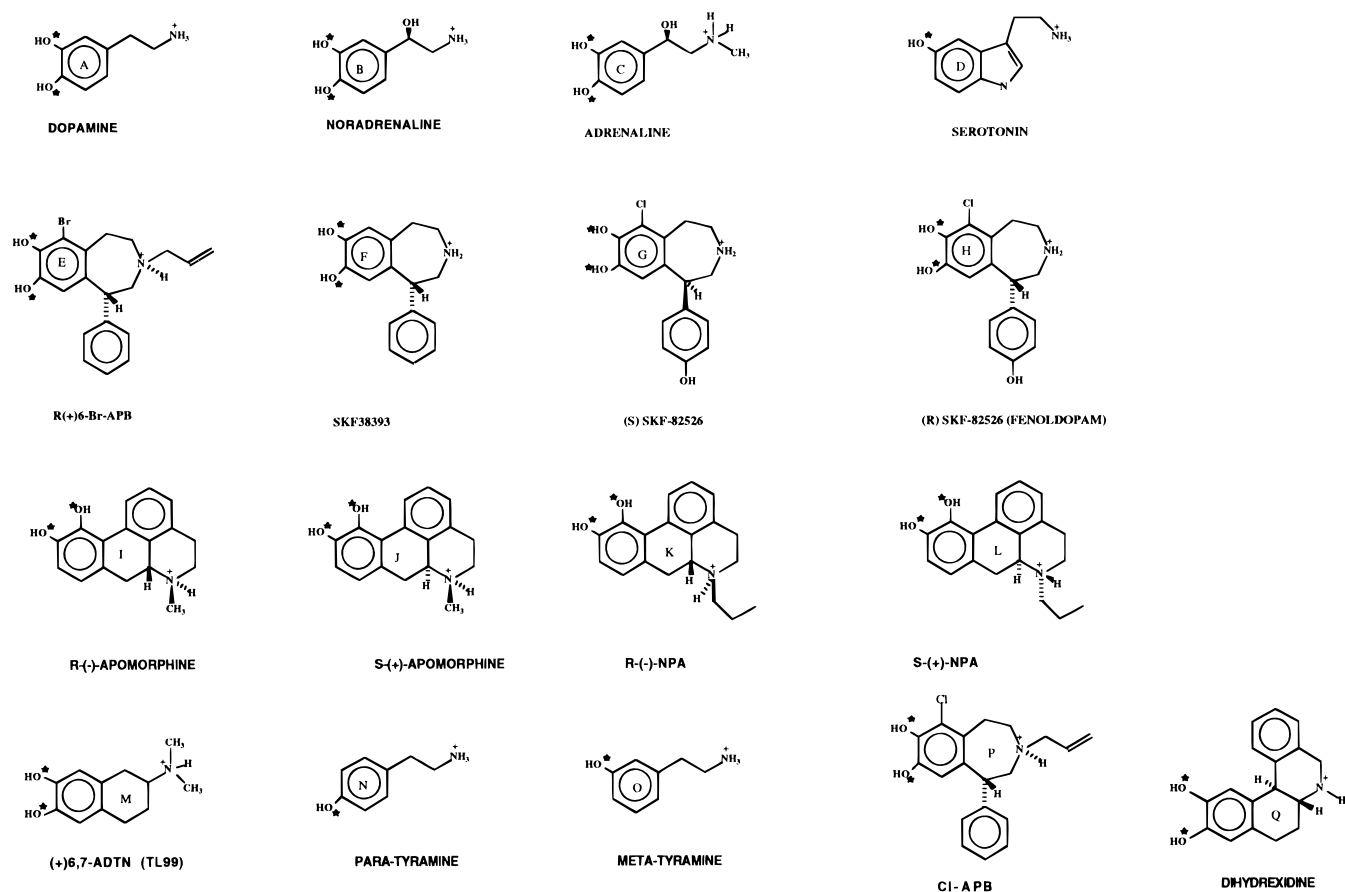
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**Figure 1.** Structures of drugs in the training and test sets. A star corresponds to hydroxyl groups used in establishing the pharmacophore map.

analysis (CoMFA) procedure,[26] incorporating partial least-squares (PLS) regression.[27]

Recent CoMFA studies have attempted to predict pharmacological properties of drugs acting at a variety of G-protein-coupled receptors[28,29] including those for DA.[30] However, these were not based on drug binding affinities obtained from studies of recombinant receptors expressed in clonal cells. This is of concern, since virtually all tissues express more than one receptor to which at least some of the drugs within the published data sets are known to bind with varying affinities (*e.g.*, D1A, D1B, and perhaps 5-HT2 receptors when labeled with [³H]SCH23390). This results in experimentally determined affinity values which deviate to some extent from true values at a single receptor and would, thus, be expected to distort predictions from CoMFA. In contrast, the target property reported here represents drug affinity at a recombinant receptor transiently expressed in a cell line, usually COS7. Control COS7 cells do not exhibit high-affinity [³H]SCH23390 binding[13] and do not appear to possess any dopamine receptors. Thus, the dependent variable of the present CoMFA study is uncontaminated by drug binding to receptors other than the target receptor. Data for one of the compounds in the training set (Br-APB) and several compounds for which affinities were predicted using the CoMFA-based model (Cl-APB, (*S*)-(+)-APO, (*S*)-(+)-NPA, DHX, *m*-tyramine, and *p*-tyramine) were obtained from recombinant D1A receptors stably expressed in C6 glioma cells;[31] *m*- and *p*-tyramine were evaluated in the present study because the single hydroxyl group on the aromatic ring may be relevant to which particular serine residue of the three conserved

serines in transmembrane region 5 of the D1A is utilized in binding.[17]

The active analog approach (AAA) and field fit methods were used to determine the bound conformations of 11 agonists. CoMFA was used to relate statistically measured agonist affinities at the recombinant D1A DA receptor to the steric and electrostatic fields of 11 aligned structures. These compounds included all agents for which detailed structural information (including chirality) was known and for which binding affinities at recombinant D1A receptors had been measured.

## Methods

**Literature Data on Drug Affinities at Recombinant D1A Dopamine Receptors.** The training set consisted of a structurally diverse set of drugs (Figure 1) which included all 11 known agonists for which both stereochemical information and $K_d$ values at recombinant D1A receptors are known. These agents included drugs with widely varying affinities at the recombinant D1A DA receptor, transiently expressed in COS7 cells or (for Br-APB) stably expressed in C6 glioma cells (Table 1). $K_d$ values for drugs included in the analysis ranged from 20 to 50 000 nM. Wherever possible, data were used from a single source[13] because those authors tested the largest number of agonist drugs. Together, these values represent the best current estimates to date of the affinities of these drugs at the D1A receptor, since COS7 cells appear not to express any endogenous DA receptor. Therefore, possible complications due to drug affinities at other DA receptors have been eliminated.

**Measurement of Drug Affinity at Recombinant D1A DA Receptors.** *m*- And *p*-tyramine are compounds containing a single hydroxyl oxygen attached to the aromatic ring. Thus, prediction of the affinity at recombinant D1A receptors would

**Table 1.** Literature Data—Drug Affinities at Recombinant and Striatal Dopamine Receptors

A. Training Set

| molecule | D1A $K_d$ (nM) recombin | log($1/K_d$) | pred log($1/K_d$) | D2A $K_d$ (nM) recombin | striatal D1 $K_L$ (nM) | striatal D2 $K_L$ (nM) |
|---|---|---|---|---|---|---|
| dopamine | 2500[b] | −3.39 | −3.47 | 17 000,[d]1705[e] | 580 | 4300 |
| noradrenaline | 50 000[a] | −4.69 | −4.65 | | 4141 | 126 000 |
| (R)-(+)-6-Br-APB | 384[c,i] | −2.58 | −2.16 | | | |
| (R)-apomorphine | 680[a] | −2.83 | −3.11 | 24[e,j] | 206 | 127 |
| (S)-SKF82526 | 1818[a] | −3.26 | −3.07 | | 1335 | |
| (R)-NPA | 1816[a] | −3.26 | −3.13 | 20[f] | 625 | 23 |
| (+)-6,7-ADTN | 4600[a] | −3.66 | −3.70 | | 734[g] | 463[g] |
| serotonin | 9690[a] | −3.99 | −4.13 | | 6543[k] | 183 000 |
| adrenaline | 55 000[b] | −4.74 | −4.65 | | 980 | 128 000 |
| SKF38393 | 150[a] | −2.18 | −2.29 | 9500[f] | 381 | |
| (R)-SKF82526[h] | 28[a] | −1.45 | −1.65 | | 21 | 1000 |

B. Test Set

| molecule | D1A $K_L$ obsd (nM) | log($1/K_L$) | D1A log($1/K_d$) predicted |
|---|---|---|---|
| Cl-APB | 83[36] (for racemate) | −1.92 | −1.69 |
| (S)-APO | 1200 ± 100 | −3.079 18 | −3.0064 |
| (S)-NPA | 5200 ± 1000 | −3.716 | −2.992 59 |
| DHX | 1200 | −3.079 18 | −3.127 79 |
| m-tyramine | 48 000 ± 6100 | −4.68 | −3.54 |
| p-tyramine | 390 000 ± 44 000 | −5.59 | −3.52 |
| (R)-NPA | 280 | −2.447 16 | −3.13 |

$^{a-g}$ $K_d$ values for the above compounds were obtained from the following sources: (a) Sunahara *et al.*, 1990[13]; (b) Dearry *et al.*,1990;[11] (c) Wilcox *et al.*, 1993[46] (note this value was later corrected to 0.43 ± 0.29 $\mu$M; Mak *et al.*, 1995[31]); (d) Bunzow *et al.*, 1988;[47] (e) Sokoloff *et al.*, 1990;[48] (f) Cox *et al.*, 1992;[17] (g) Seeman and Niznik, 1988.[42] Sources a and b utilized recombinant D1A receptors transiently expressed in COS7 cells. Source c utilized recombinant D1A receptors stably expressed in C6 glioma cells. $^h$ Fenoldopam. $^i$ In C6 cells transfected with the D1A receptor, this compound shows a $K_L$ = 3 nM, whereas the $K_L$ for the recombinant D2A receptor in C6 cells is 350 nM (Neve, unpublished observations). $^j$ Apparent high-affinity agonist binding constant. $^k$ Apparent one-site agonist binding constant.

be of interest using a CoMFA-based approach. Unfortunately, the affinity of these two compounds had not previously been measured at recombinant D1A receptors. $K_d$ values for (S)-APO, (S)-NPA, m-tyramine, p-tyramine, and DHX were determined using the methods described below. C6 glioma cells expressing the rhesus macaque D1A receptor were grown and prepared for radioligand binding experiments as described previously.[33,34] Confluent cells were lysed by replacing the medium with ice-cold hypotonic buffer (1 mM Na$^+$-HEPES and 2 mM EDTA). After 10−20 min, the cells were scraped off the plate into centrifuge tubes and centrifuged at 17 000 rpm for 20 min. The crude membrane fraction was resuspended with a Brinkman polytron homogenizer (setting 6 for 10 s) in assay buffer (50 mM Tris-HCl, pH 7.4, 0.9% NaCl, 1 mM EDTA, 0.025% ascorbic acid, and 0.001% bovine serum albumin). Determination of the affinity of the agonists was carried out in an assay volume of 250 $\mu$L, including cell membranes, radioligand ([$^3$H]SCH23390, 2 nM), GTP (100 $\mu$M), (+)-butaclamol, and test drugs. (+)-Butaclamol (2 $\mu$M) was used to define nonspecific binding. The assays were incubated at 30 °C for 1 h and stopped by dilution with ice-cold wash buffer (10 mM Tris-HCl, pH 7.4, and 0.9% NaCl) and filtration with a Tomtec 96-well cell harvester. Samples were counted in a Wallac 1205 Betaplate scintillation counter. Competition curves were analyzed by nonlinear regression using Prism (GraphPad Software, Inc.).

**Drugs and Reagents for the C6 Studies.** DHX was a generous gift from Dr. Richard Mailman (University of North Carolina). (+)-Butaclamol, m-tyramine, (S)-(+)-APO, and (S)-(+)-NPA were obtained from Research Biochemicals International (Natick, MA). [$^3$H]SCH23390 was obtained from Amersham, and other drugs and reagents were from Sigma Chemical Co.

**Pharmacophore Information.** Currently, the 3D structure of the D1A receptor is unknown, precluding rigorous calculations of intermolecular interactions. However, prior to the cloning and sequencing of D2A and D1A receptors, Seeman had described the structural requirements for classically defined DA "D2" agonists in brain using standard SAR information.[35] On the basis of the pharmacophore information from that work and recent site-directed mutagenic studies of the D1A (D1 in old terminology) and D2A (D2 in older

terminology) DA receptors,[17,36] we tentatively identified the following pharmacophoric features for agonist binding to the D1A DA receptor: (a) the distances from two oxygens marked with stars (Figure 1) to the cationic nitrogen and (b) the height of the cationic nitrogen above the plane of the ring to which the oxygens are bonded. A pharmacophore map was obtained from the set of aligned structures having $K_d$ values <5 $\mu$M at the D1A receptor (DA, (R)-(+)-6-Br-APB, SKF38393, (R)-apomorphine, (R)-SKF82526 (fenoldopam), (R)-NPA, and (+)-6,7-ADTN). Also, for serotonin, which does not possess all three pharmacophoric groups, we aligned the oxygen with the m-hydroxyl of DA, based on the relatively higher affinity of m-tyramine than p-tyramine for the recombinant D1A DA receptor (present results). The affinity values were expressed as log($1/K_d$), since CoMFA fields represent an enthalpic aspect of the overall free energy of drug−receptor interaction and the relationship between free energy and the equilibrium binding constant is logarithmic.

**Molecular Modeling.** SYBYL 6.1[37] was used for the drug modeling reported in the present study. Initial structures were generated using CONCORD (v 3.0.1)[38,39] and optimized using MNDO.[41] Since ligands probably do not bind to the receptor in their global minimum energy conformations, the AAA[40] was used to identify the set of candidate conformations of each molecule which are within the region of conformation space (*i.e.,* distance space) accessible to all of the compounds.

Alignment of the compounds in a CoMFA training set involves two tasks: (a) determination of a particular (bound) conformation and (b) alignment of all chosen conformations in a common orientation. We selected SKF38393 as the template for prescribing the bound conformation of the flexible ligands because it is a fairly rigid ligand which displays high affinity for the D1A receptor and is quite selective for binding to native D1 vs D2 receptors (Table 1).

The standard ("rigid") field fit method examines the previously generated candidate conformers and selects the conformation of a given molecule for which the external fields (steric and electrostatic, the latter being derived from the MNDO-calculated charges[41]) most closely resemble those of a template molecule. It does so by orienting the test compound conformer to the template conformer without altering the conformation and then measuring the field similarity between the two
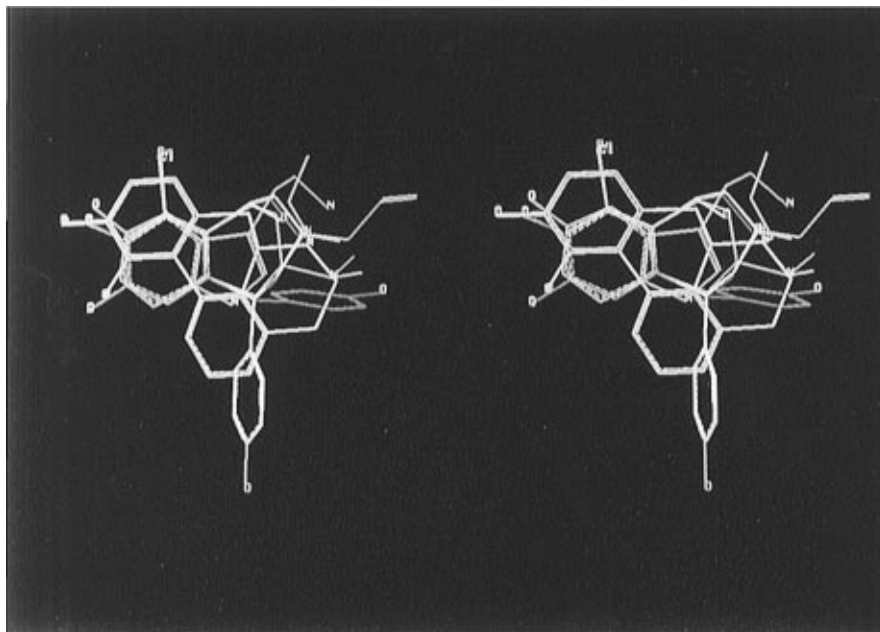
**Figure 2.** Structures of the aligned drugs of the training set. The alignment for the set of agonists resulting from the AAA and field fit is shown, using SKF38393 as the template. Shown in the figure are the high-affinity compounds (*R*)-apomorphine (pink), Br-APB (white), and fenoldopam (green), with the template SKF38393 (yellow). For comparison, the alignment of the low-affinity compounds serotonin (orange) and (*S*)-SKF82526 (blue) are also presented.

conformers. In the present work, a flexible field fit method was used to select the particular (bound) conformation. A flexible field fit procedure (fitting to the field of the SKF38393 template) was performed on all molecules and yielded template-aligned conformations with acceptable conformational energies. This was done by performing a MAXIMIN2 energy minimization with a field fit energy penalty, after the test conformer to template orientation and field similarity assessment had been performed. Because this procedure can alter the final conformation of the test compound, it is important to determine the final conformational energy in relation to the original local minimal energy. For the compounds of the training set, final conformations had an energy only 1.7 kcal/mol above the initial energy on average, with a range from 0 to 6.1 kcal/mol energy difference. This set of aligned structures was used for CoMFA (Figure 2).

CoMFA[26] uses PLS to regress a target property (*e.g.*, log(1/$K_d$)) against predictors calculated as steric and electrostatic components of the intermolecular interaction potential (field) evaluated at the grid points of a 3D lattice containing each member of a training set of aligned ligand structures. Cramer *et al.*[26a] have reviewed the importance of various parametric decisions which must be made during the course of a CoMFA to optimize the standard error of prediction.[26b] Accordingly, we have systematically investigated the effects of changing several such parameters, including grid step size (1 or 2 Å), probe atom type ($H^+$, $O^-$, $C_{sp^3}^+$), several different grid box origins to vary the probe atom position, and varying the column filtering values (1.0, 2.0, or 3.0 kcal/mol; Tables 3 and 4). We used the "leave-one-out" method for cross-validation analysis.

## Results

**Alignment.** The final alignment for the set of agonists resulting from the AAA and field fit is shown in Figure 2, using SKF38393 as the template. The difference between the nearest local minimum energy and the energy of the conformer selected for alignment averaged 1.7 kcal/mol. Shown in the figure are the high-affinity compounds (*R*)-apomorphine (pink), Br-APB (white), and fenoldopam (green), with the template SKF38393 (yellow). For comparison, the alignment of the lower affinity compounds serotonin (orange) and (*S*)-SKF82526 (blue) are also presented.
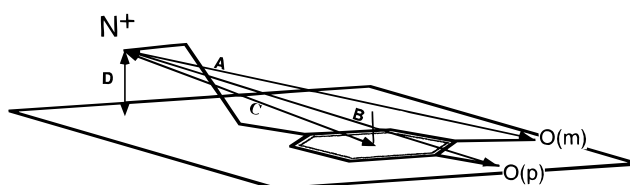


**Figure 3.** Pharmacophore map for drugs of the training set having $K_d$ values for the D1A receptor <5 $\mu$M.

**Pharmacophore Maps for Affinity at Dopamine Receptors.** As shown in Figure 3, for the higher affinity compounds, the distance between the cationic nitrogen and the *m*-hydroxyl oxygen ranged from 6.8 to 7.9 Å (distance A), with the highest affinity compound (fenoldopam) having a distance of 6.8 Å and the lowest affinity compound (ADTN) having a distance of 7.9 Å (Table 2). The distance between the cationic nitrogen and the *p*-hydroxyl oxygen ranged from 6.4 to 7.4 Å (distance B), with fenoldopam having a distance of 6.9 Å and ADTN having a distance of 7.4 Å. Thus, the distances between the cationic nitrogen and each hydroxyl group were more similar for fenoldopam than for ADTN. The height of the cationic nitrogen above the plane of the catechol ring ranged from 1.0 to 1.4 Å (distance D) for the compounds of the training set. This distance was found to be 1.3 Å for fenoldopam and only 1.04 Å for ADTN. The N$^+$−centroid distance (distance B) shown in Figure 3 was *not* a distance criterion used in the initial conformational search. Instead, this distance was computed from the aligned structures to facilitate the comparison of our pharmacophore map with that proposed by Alkorta and Viller.[45a] This distance ranged from 4.3−5.2 Å (average 4.65 Å), with fenoldopam having a distance of 4.34 Å vs a distance of 5.18 Å for ADTN. Distances shown in Figure 3 are given for each compound in the training set in Table 2.

The D1A pharmacophore map, obtained by Alkorta and Viller[45a] using a set of 10 semirigid drugs with affinities based on measurements on native receptors

**Table 2.** Pharmacophoric Features of Compounds in the Training Set

| molecule | N−O(*m*) (Å) | N−O(*p*) (Å) | height (Å) | N−centroid (Å) |
|---|---|---|---|---|
| dopamine | 6.82 | 6.87 | 1.30 | 4.34 |
| Br-APB | 6.90 | 6.94 | 1.20 | 4.40 |
| (*R*)-APO | 7.82 | 6.42 | 1.21 | 5.16 |
| SKF38393 | 6.82 | 6.87 | 1.26 | 4.33 |
| (*S*)-SKF82526 | 6.81 | 6.81 | 1.41 | 4.32 |
| (*R*)-SKF82526[a] | 6.82 | 6.88 | 1.30 | 4.34 |
| ADTN | 7.89 | 7.43 | 1.04 | 5.18 |
| (*R*)-NPA | 7.85 | 6.41 | 1.38 | 5.15 |
| serotonin | 7.91 | | 1.16 | 5.88 |
| norepinephrine | 6.82 | 6.87 | 1.26 | 4.33 |
| epinephrine | 6.82 | 6.87 | 1.26 | 4.33 |

[a] Fenoldopam.

prepared from mammalian brain tissue, was evaluated in relation to that obtained in the present study. For the compounds of their training set, these authors reported a height of the cationic nitrogen above the plane of the catechol ring of 1.2 Å (distance D in Figure 3) vs the 1.0−1.4 Å reported here and a distance between the nitrogen and the centroid of the ring of 4.85 Å (distance C) vs the 4.65 Å average reported here. Also for comparison, a pharmacophore map derived by Seeman[35a] using classical SAR methods for agonist binding to the native striatal D2 DA receptor is represented by Figure 3. In that report the distance between the cationic nitrogen and the *m*-hydroxyl oxygen ranged from 6.5 to 7.3 Å (distance A in Figure 3, vs 6.8−7.9 Å for the corresponding distance we found for compounds binding to the D1A receptor). This suggests that a distance between these two pharmacophoric features slightly greater than that found for high-affinity D2 binding might facilitate agonist affinity at the D1A receptor. Interestingly, Seeman reported a distance between the cationic nitrogen and the plane of the catechol ring of 0.6 Å for agonists acting at D2 receptors (distance D) in contrast to the 1.0−1.4 Å range for the corresponding distance we found for agonist binding to the D1A DA receptor.

**Comparative Molecular Field Analysis.** The cross-validated $r^2$ ($q^2$) values which resulted from the various CoMFA options for $K_d$ at recombinant D1A receptors as the target property are shown in Table 3. Using the default CoMFA settings, which included both steric and electrostatic fields, we observed a standard error of prediction of 0.691 with two principal components and a $q^2$ of 0.621. The choice of CoMFA options described below was based upon minimizing the standard error of prediction rather than simply maximizing the $q^2$ value.[45c] This resulted in using a smaller number of PLS principal components (as appropriate for our relatively small data set). Interestingly, when evaluating the contributions of steric and electrostatic fields to the CoMFA model, the largest $q^2$ was obtained using only the electrostatic field. Using $1/r$ as the value for the dielectric function produced a higher $q^2$ value than setting the value equal to a constant of 1.0. The $q^2$ value was also improved by using 50 kcal/mol as the energy cutoff option for each interaction field. Decreasing the grid step size from 3 to 2 to 1 Å had negligible effects on the $q^2$ but increased CPU time substantially. Changing the column filtering (minimum $\sigma$) to exclude those interaction field points which contribute little to the CoMFA model had relatively little effect. However, when the column filtering was reduced to 0.0 kcal/mol

(eliminating no field points from consideration in the regression equation), the CPU time was increased dramatically, for relatively little improvement in the $q^2$ value. Therefore, we elected to use a value of 2.0 Å for the grid step size and 1.0 kcal/mol for the column filtering. Changing the type of probe atom also was found to have had no effect on the $q^2$ value because the steric field (dependent on the probe radius) was not used in the CoMFA model (and change of probe merely affects the sign of the electrostatic field). In contrast to the minimal effect of varying several of the other CoMFA options, varying the position of the probe atom by translating the CoMFA grid box origin (by 0.5 Å at a time in *x*-, *y*-, and *z*-axes) had a significant influence on the $q^2$ value (from 0.277 to 0.829). The maximum $q^2$ model also had the minimum value for the standard error of prediction (0.464).

The final model, without cross-validation and with two principal components, was obtained using the following options: only the electrostatic field, a 50 kcal/mol field energy cutoff, $1/r$ for the dielectric function, 2.0 Å step size, 1.0 kcal/mol column filtering, a $C_{sp^3}^{+}$ probe atom, and a grid box translated from SYBYL's default position 1.0 Å in the *X* direction relative to the orientation of our aligned structures. This model had an $r^2$ value 0.96. Figure 4 shows the relationship between actual log(1/$K_d$) values for the compounds of the training set and the values predicted by the CoMFA model.

**Contour Map.** Figure 5 shows the contour map of our final CoMFA model. From a statistical perspective, the electrostatic field contributed more to predicting the binding affinities than did the steric field or both fields. For the final CoMFA model, only the electrostatic field was utilized, since this improved the standard error of prediction and the $q^2$ value. This may reflect the fact that we did not have sterically diverse compounds in the training set. For example, the training set included only a single pair of *R*- and *S*-enantiomers. However, this model also highlighted the region of the pendant aromatic ring as well as the protonated nitrogen in contributing to high affinity.

**Predictive Ability of the Model and Comparisons of Enantiomers.** Using the procedures described in the Methods section, the affinities of test compounds at recombinant D1A receptors stably expressed in C6 glioma cells were obtained in the presence of sodium and GTP (Table 1B). The $K_L$ of DHX at recombinant D1A receptors stably expressed in C6 cells was found to be 1200 nM. The $K_d$ of Cl-APB was obtained from the literature as 83 nM[36] or 20 nM for the racemate.[34]

We observed a small, but fairly systematic, difference between the predicted $K_d$ and the measured $K_L$ values for the compounds of the test set. One possible basis for this variation may be that the test compound affinities were essentially all measured using transiently expressed receptors in COS7 cells and without the inclusion of saturating GTP concentrations to shift all the agonist binding to the low-affinity form. Furthermore, the density of the receptors in the COS7 cells was in the pmol/mg of protein range, leading to an inappropriate stoichiometry of receptor coupling to G-protein. In contrast, the affinities for the test compounds were obtained from a more physiological system in which stable expression of a moderate density of

**Table 3.** Effects of CoMFA Options on Cross-Validated $r^2$ ($q^2$) Values for Affinities at Recombinant D1A Dopamine Receptors

| fields | energy cutoffs (kcal/mol) | dielectric function | CoMFA region | minimum $\sigma$ | step size (Å) | probe atom type | $q^2$ | no. of components (std error pred) | no. of columns used |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Field | | | | | |
| both[b] | 30/30 | $1/r$ | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | 0.621 | 2(0.691) | 278 |
| elec[a] | 30 | $1/r$ | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | 0.810 | 2(0.489) | 117 |
| steric | 30 | $1/r$ | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | 0.606 | 2(0.704) | 97 |
| | | | | Energy Cutoff and Dielectric Function | | | | | |
| elec | 30 | 1.0 | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | -0.449 | 2(1.35) | 4 |
| elec | 30 | $1/r$ | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | 0.810 | 2(0.489) | 117 |
| elec | 50 | 1.0 | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | 0.512 | 2(0.783) | 172 |
| elec | 50 | $1/r$ | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | 0.814 | 2(0.484) | 124 |
| | | | | Minimum $\sigma$ | | | | | |
| elec | 50 | $1/r$ | default | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.817 | 2(0.480) | 217 |
| elec | 50 | $1/r$ | default | 2.0 | 2.0 | $C_{sp^3}{}^+$ | 0.814 | 2(0.484) | 124 |
| elec | 50 | $1/r$ | default | 3.0 | 2.0 | $C_{sp^3}{}^+$ | 0.813 | 2(0.485) | 92 |
| elec | 50 | $1/r$ | default | 4.0 | 2.0 | $C_{sp^3}{}^+$ | 0.811 | 2(0.488) | 78 |
| | | | | Varying Probe Atom Position by Translating CoMFA Region | | | | | |
| elec[a] | 50 | $1/r$ | $-0.5$ in $X$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.782 | 2(0.523) | 221 |
| elec | 50 | $1/r$ | 0.5 in $X$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.800 | 2(0.502) | 224 |
| elec | 50 | $1/r$ | 1.0 in $X$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.829 | 2(0.464) | 221 |
| elec | 50 | $1/r$ | $-0.5$A in $Y$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.740 | 3(0.612) | 220 |
| elec | 50 | $1/r$ | 0.5A in $Y$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.746 | 3(0.604) | 220 |
| elec | 50 | $1/r$ | 1.0A in $Y$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.734 | 5(0.732) | 221 |
| elec | 50 | $1/r$ | $-0.5$A in $Z$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.490 | 2(0.801) | 217 |
| elec | 50 | $1/r$ | 0.5A in $Z$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.729 | 2(0.584) | 220 |
| elec | 50 | $1/r$ | 1.0A in $Z$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.277 | 2(0.954) | 210 |
| elec | 50 | $1/r$ | $-0.5$A in $XYZ$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.752 | 3(0.597) | 217 |
| elec | 50 | $1/r$ | 0.5A in $XYZ$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.764 | 2(0.545) | 223 |
| elec | 50 | $1/r$ | 1.0A in $XYZ$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.726 | 5(0.743) | 214 |
| | | | | Grid Step Size | | | | | |
| elec | 50 | $1/r$ | 1.0A in $X$ | 1.0 | 1.0 | $C_{sp^3}{}^+$ | 0.721 | 2(0.592) | 1752 |
| elec | 50 | $1/r$ | 1.0A in $X$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.829 | 2(0.464) | 224 |
| elec | 50 | $1/r$ | 1.0A in $X$ | 1.0 | 3.0 | $C_{sp^3}{}^+$ | 0.740 | 3(0.612) | 63 |
| | | | | Probe Atom Type | | | | | |
| elec | 50 | $1/r$ | 1.0A in $X$ | 1.0 | 2.0 | $C_{sp^3}{}^+$ | 0.829 | 2(0.464) | 224 |
| elec | 50 | $1/r$ | 1.0A in $X$ | 1.0 | 2.0 | $O^-$ | 0.829 | 2(0.464) | 224 |
| elec | 50 | $1/r$ | 1.0A in $X$ | 1.0 | 2.0 | $H^+$ | 0.829 | 2(0.464) | 224 |

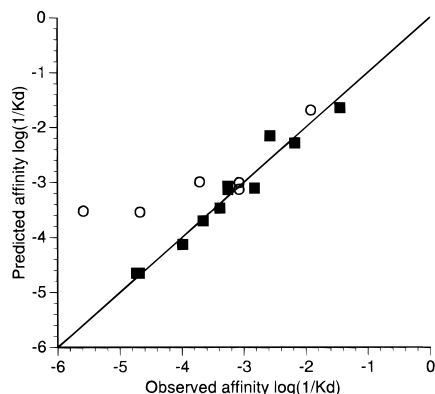[a] Elec = electrostatic. [b] Values in this row represent the default CoMFA options in SYBYL.



**Figure 4.** Relationship between actual and predicted affinity values at recombinant D1A DA receptors for the compounds of the training set. Members of the 11-member training set are denoted by filled squares. Also shown, as unfilled circles, are the compounds for which $K_d$ was predicted (Cl-APB, (S)-APO, (S)-NPA, DHX, m-tyramine, and p-tyramine).

receptors in C6 cells and assays done in the presence of excess GTP were used. The predicted $K_d$ value for Cl-APB was 49 nM, in fairly good agreement with the reported values. Predictions for (S)-APO and (S)-NPA were 1015 and 983 nM, respectively, while that for DHX was 1342 nM, also in fairly good agreement with the measured affinity (Figure 4). The predicted values for m- and p-tyramine (3466 and 3343 nM, respectively) were in relatively poor agreement with the measured values of 48 000 and 390 000 nM, respectively. Graphi-cal indications of these relationships are provided in Figure 4. We have suggested one possible reason for the observation that, whereas the predictions of the model were generally reasonable, except for the two tyramines, most of the predicted $K_d$ values were slightly lower than the observed $K_L$ values. However, when we measured the affinity of one of the training set compounds, (R)-NPA, we found that its affinity at the D1A receptor stably expressed in C6 cells was 280 nM (log(1/$K_L$) = $-2.45$) rather than the value of 1820 nM reported for transiently expressed D1A receptors in COS7 cells. This highlights the fact that future studies will need to incorporate $K_L$ measurements for both compounds of the training set and test set.

As evident from Figure 1, only one pair of enantiomers was included in the primary training set of 11 compounds ((R)- and (S)-SKF82526). As indicated above, we measured the $K_L$ values of (S)-APO and (S)-NPA to allow comparison of two additional pairs of enantiomers ((R)- and (S)-APO, (R)- and (S)-NPA). For all three pairs of enantiomers, higher affinity is *observed* with the R-isomer. The observed log(1/$K_d$) values were $-1.45$ and $-3.26$, whereas the predicted values were $-1.65$ and $-3.07$ for (R)- and (S)-SKF82526, respectively. However, the model *predicted* higher affinity for (S)-APO than for (R)-APO and for (S)-NPA than for (R)-NPA. Observed log(1/$K_L$) values were $-2.83$ and $-3.08$, whereas predicted values from the CoMFA model were $-3.11$ and $-3.01$ for (R)- and (S)-APO, respectively. The observed log(1/$K_L$) values were $-3.26$ and $-3.72$, while
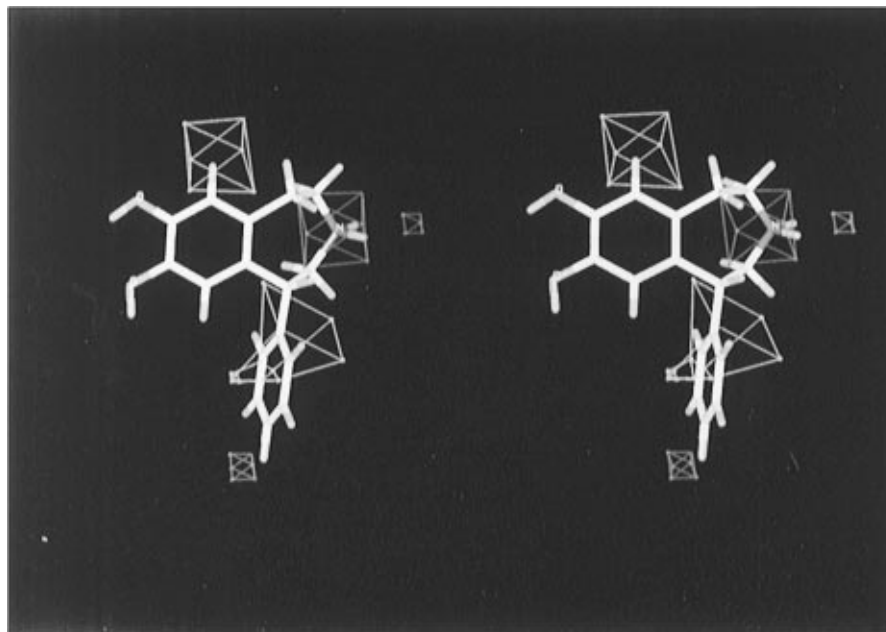
**Figure 5.** Contour map of electrostatic field (standard deviation times coefficient) from CoMFA model with the template compound SKF38393. Positive electrostatic charge is favored (blue) or unfavored (red) for high affinity.

the predicted values were $-3.13$ and $-2.99$ for ($R$)- and ($S$)-NPA, respectively.

An effect associated with the pairs of isomers was the significantly smaller plane heights in the $R$- vs $S$-enantiomer. The height of the nitrogen above the plane of the catechol ring is 1.21 Å for ($R$)-APO vs 2.26 Å for ($S$)-APO. For the $N$-propyl compounds, the corresponding $N^+$−plane distances were 1.38 Å for ($R$)-NPA and 2.46 Å for ($S$)-NPA. Only for ($R$)-SKF82526 (fenoldopam) and ($S$)-SKF82526 were the differences in plane heights between isomers more modest—1.30 Å for ($R$)-SKF82526 vs 1.41 Å for ($S$)-SKF82526.

**Predictive Ability of the Model for Affinities at Native Receptors.** A major hypothesis of the present work was that, whatever the absolute utility of the model, the relative utility would be greater when $K_d$ values at recombinant D1A receptors were used as the dependent variable rather than $K_d$ values at native striatal D1 receptors. This was tested directly for a subset of 10 compounds of the original training set for which striatal $K_d$ values have been reported in the literature[42] (Table 1). Keeping the alignment of these compounds and the CoMFA options unchanged, the $q^2$ value, based on these "native tissue" data, was only 0.066. When alternative CoMFA options were explored to minimize the standard error of prediction as above, as shown in Table 4, the $q^2$ value was increased only to 0.178.

## Discussion

Our results suggest that optimal agonist binding to the D1A receptor requires a significantly greater distance between the plane of the ring and the position of the cationic nitrogen than that previously suggested by standard SAR for optimal binding to the "D2" DA receptor (defined using pharmacological criteria prior to the cloning and sequencing of the receptor gene). Specifically, Seeman's[35a] description of the structural requirements for a "D2" DA agonist suggested that the height of the cationic nitrogen above the plane of the aromatic ring is *ca.* 0.6 vs $1.0-1.4$ Å for the D1A

receptor in the present study. If this difference could be confirmed by a conformational analysis of ligands acting at the D2A receptor, similar to that reported here for the D1A receptor, such results could be exploited in the development of agents binding preferentially to the D2A vs D1A receptor. Note that the present results also provide an explanation for the historical difficulty of developing D1-selective agonists. Compounds with sufficient flexibility to achieve the plane height optimal for D1A binding could also assume a flatter configuration consistent with high D2A affinity, unless conformational constraints are present by design. Conversely, compounds for which the energetically more favorable conformations were essentially planar would tend to bind well to D2 receptors but be much less likely to be able to assume a conformation favorable for interactions with the D1A active site. Preliminary pharmacophoric data using a set of agonists with varying affinities at the recombinant D2A receptor seem to confirm this impression.[35b]

In contrast to the differences in plane height, our results suggested that the distance between the primary H-bond site and cationic nitrogen required for optimal binding ($6.8-7.9$ Å; Figure 3, distance A) is essentially the same as that suggested by Seeman for the "D2" receptor ($6.5-7.3$ Å). While the distance between the cationic nitrogen and the *p*-hydroxyl oxygen of the aromatic ring was not reported for the D2 receptor, we found this distance to be similar to that between the nitrogen and *m*-hydroxyl oxygen at the D1A receptor ($6.4-7.4$ and $6.8-7.9$ Å, respectively). It is of interest that the highest affinity compound in the training set (fenoldopam) had very similar distances between nitrogen and the *m*- and *p*-hydroxyls (6.82 and 6.88 Å, respectively). This suggests that those compounds with greater symmetry for these pharmacophoric features might be expected to show higher affinity than those compounds which could not adopt such symmetric conformations without considerable increase in conformational energy.

**Table 4.** Effects of Various CoMFA Options on Cross-Validated $r^2$ ($q^2$) Values at Striatal D1 Receptors

| fields | energy cutoffs (kcal/mol) | dielectric function | minimum $\sigma$ | CoMFA region | step size (Å) | probe atom type | $q^2$ | no. of components (std error pred) | no. of columns used |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Field | | | | | |
| steric | 30 | $1/r$ | 2.0 | default | 2.0 | $C_{sp^3}^+$ | −0.072 | 1(0.760) | 89 |
| elec[b] | 30 | $1/r$ | 2.0 | default | 2.0 | $C_{sp^3}^+$ | 0.139 | 1(0.681) | 119 |
| both[a] | 30/30 | $1/r$ | 2.0 | default | 2.0 | $C_{sp^3}^+$ | −0.047 | 1(0.751) | 160 |
| | | | | Energy Cutoff and Dielectric Function | | | | | |
| elec | 30 | 1.0 | 2.0 | default | 2.0 | $C_{sp^3}^+$ | −0.270 | 1(0.827) | 4 |
| elec | 30 | $1/r$ | 2.0 | default | 2.0 | $C_{sp^3}^+$ | 0.139 | 1(0.681) | 119 |
| elec | 50 | 1.0 | 2.0 | default | 2.0 | $C_{sp^3}^+$ | 0.168 | 1(0.670) | 187 |
| elec | 50 | $1/r$ | 2.0 | default | 2.0 | $C_{sp^3}^+$ | 0.055 | 1(0.714) | 127 |
| | | | | Minimum $\sigma$ | | | | | |
| elec | 50 | 1.0 | 1.0 | default | 2.0 | $C_{sp^3}^+$ | 0.178 | 1(0.666) | 701 |
| elec | 50 | 1.0 | 2.0 | default | 2.0 | $C_{sp^3}^+$ | 0.168 | 1(0.670) | 187 |
| elec | 50 | 1.0 | 3.0 | default | 2.0 | $C_{sp^3}^+$ | 0.168 | 2(0.716) | 26 |
| elec | 50 | 1.0 | 4.0 | default | 2.0 | $C_{sp^3}^+$ | 0.132 | 2(0.731) | 11 |
| | | | | Varying Probe Atom Position by Translating CoMFA Region | | | | | |
| elec | 50 | 1.0 | 1.0 | −0.5a in $X$ | 2.0 | $C_{sp^3}^+$ | 0.020 | 1(0.727) | 703 |
| elec | 50 | 1.0 | 1.0 | 0.5a in $X$ | 2.0 | $C_{sp^3}^+$ | 0.093 | 1(0.699) | 706 |
| elec | 50 | 1.0 | 1.0 | 1.0a in $X$ | 2.0 | $C_{sp^3}^+$ | −0.086 | 1(0.765) | 709 |
| elec | 50 | 1.0 | 1.0 | −0.5a in $Y$ | 2.0 | $C_{sp^3}^+$ | −0.064 | 1(0.757) | 686 |
| elec | 50 | 1.0 | 1.0 | 0.5a in $Y$ | 2.0 | $C_{sp^3}^+$ | 0.156 | 1(0.675) | 715 |
| elec | 50 | 1.0 | 1.0 | 1.0a in $Y$ | 2.0 | $C_{sp^3}^+$ | −0.086 | 1(0.765) | 717 |
| elec | 50 | 1.0 | 1.0 | −0.5a in $Z$ | 2.0 | $C_{sp^3}^+$ | 0.084 | 1(0.703) | 703 |
| elec | 50 | 1.0 | 1.0 | 0.5a in $Z$ | 2.0 | $C_{sp^3}^+$ | 0.017 | 1(0.728) | 694 |
| elec | 50 | 1.0 | 1.0 | 1.0a in $Z$ | 2.0 | $C_{sp^3}^+$ | 0.007 | 1(0.731) | 678 |
| elec | 50 | 1.0 | 1.0 | −0.5a in $XYZ$ | 2.0 | $C_{sp^3}^+$ | −0.138 | 1(0.783) | 708 |
| elec | 50 | 1.0 | 1.0 | 0.5a in $XYZ$ | 2.0 | $C_{sp^3}^+$ | 0.056 | 1(0.714) | 704 |
| elec | 50 | 1.0 | 1.0 | 1.0 in $XYZ$ | 2.0 | $C_{sp^3}^+$ | 0.162 | 1(0.672) | 701 |
| | | | | Grid Step Size | | | | | |
| elec | 50 | 1.0 | 1.0 | default | 1.0 | $C_{sp^3}^+$ | 0.086 | 1(0.702) | 5331 |
| elec | 50 | 1.0 | 1.0 | default | 2.0 | $C_{sp^3}^+$ | 0.178 | 1(0.666) | 701 |
| elec | 50 | 1.0 | 1.0 | default | 3.0 | $C_{sp^3}^+$ | 0.150 | 1(0.677) | 225 |
| | | | | Probe Atom Type | | | | | |
| elec | 50 | 1.0 | 1.0 | default | 2.0 | $C_{sp^3}^+$ | 0.178 | 1(0.666) | 701 |
| elec | 50 | 1.0 | 1.0 | default | 2.0 | $O^-$ | 0.178 | 1(0.666) | 701 |
| elec | 50 | 1.0 | 1.0 | default | 2.0 | $H^+$ | 0.178 | 1(0.666) | 701 |

[a] CoMFA default option. [b] Elec: electrostatic.

The predictive utility of the CoMFA model for several dopaminergic agonists not in the training set (Cl-APB, (*S*)-APO, (*S*)-NPA, and DHX) was fairly good (Figure 4). For Cl-APB, this was not surprising considering the similarity between the structure of that agent and Br-APB in the training set. Similarly, DHX, although quite rigid, has identical pharmacophores with DA, (*R*)-APO, and (*R*)-NPA and, thus, has many aspects of its structure in common with training set compounds. Perhaps somewhat more surprising was the small absolute deviation of observed from predicted values for (*S*)-APO and (*S*)-NPA affinities, given that only one pair of enantiomers ((*R*)- and (*S*)-SKF82526) was present in the training set. Whereas we suggested three pharmacophores as a basis for the initial alignment of the drugs of the training set, the final CoMFA model (Figure 5) highlights the region of the pendant aromatic ring and cationic nitrogen. Similarly, the importance of a pendant aromatic ring as a pharmacophore for native D1 receptors was suggested earlier by Bowen, Mailman, and colleagues.[45b] In contrast, the utility for predicting log($1/K_d$) for both *m*-tyramine and *p*-tyramine was poor. One possible reason for this is that serotonin (with higher observed affinity) was the only compound in the training set which provided information about the contribution of a single hydroxyl group in the relevant position in 3D space to binding affinity. In contrast, both epinephrine and norepinephrine have two hydroxyl groups on the aromatic ring and very low D1A affinity. As is evident from an examination of Figure 5, neither

the *m*- nor *p*-hydroxyl group on the catechol ring played a prominent role in differentiating among the compounds of the training set in our final CoMFA model. This is due to the fact that the compounds of the training set were quite well aligned in the model and, so, generated similar fields at the lattice points of the grid box. Therefore, the PLS procedure would not have utilized information on these hydroxyls. Our final CoMFA model is unable to account for a possible differential contribution of catechol hydroxyl groups to D1A binding affinity.

The prediction of the $K_d$ values for the set of test compounds as a whole was close to the observed $K_L$ values. However, the *steric* differences between the *R*- and *S*-enantiomers clearly were not incorporated into our final CoMFA model. This may have been due, in part, to the fact that we had only a single pair of *R*- and *S*-enantiomers included in our training set. Even though our CoMFA results show relatively small standard errors of prediction and high $q^2$ values, there is still residual (unexplained) variance in the target properties. One reason for the unexplained variance is that the CoMFA describes only one of several factors contributing to the free energy of the overall binding process. The observed binding constant (just like the overall free energy of interaction) depends not only on the intermolecular interaction energy (which *is* addressed in CoMFA) but also on the conformational energy, interaction entropy, and free energy of requisite desolvation. These later contributions are not considered in CoMFA

and may contribute to the unexplained variance of our CoMFA models.

The minimum value for the standard error of prediction (0.464 with two principal components) and the maximum values for the cross-validated $r^2$ ($q^2$) and simple $r^2$ of 0.829 and 0.96, respectively, were obtained for a CoMFA model of 11 agonists with affinity at D1A dopamine receptors when the dependent variable was $\log(1/K_d)$ measured at the recombinant receptor. This small training set included all agents for which both chiral information and $K_d$ values at recombinant D1A DA receptors were known. In contrast, the $q^2$ value for a CoMFA model derived for a subset of 10 of these compounds for which striatal $K_d$ values at the native D1 DA receptor were available reached a maximum $q^2$ of only 0.178. Together, these results suggest that the use of $K_d$ values derived from cell lines expressing a target recombinant receptor may represent a significant improvement over similar values derived from measurements made using membranes containing the native receptor. We suggest that a major reason for the improved utility of the $K_d$ data derived from recombinant receptors lies in the fact that the compounds of the training set bind only to the targeted receptor in the clonal expression system. In contrast, striatum contains multiple receptor subtypes (*e.g.,* D1A, D1B, and 5HT2A) to which the commonly used radioligand ([$^3$H]SCH23390) and many of the agents bind with substantial affinity.[42−44] We suggest that for large training sets, the precision of $K_d$ measurement would be further improved by the use of clonal cell systems which *stably* express the recombinant receptor in question, since all cell batches would contain not only the same receptor but also at similar density.

## Summary

Overall, the present results suggest that use of drug affinity data obtained at recombinant receptors may represent a significant improvement over more traditional target data for CoMFA obtained at native receptors in brain. In most tissues, the drugs of the training set may bind to confounding receptor sites and/or interact with interconvertible affinity states of the target receptor. The results indicate that low standard errors of prediction plus high $q^2$ values may be obtained using a very small training set when care is taken in the initial alignment and evaluation of the CoMFA options and when accurate target property data are available. Comparison of pharmacophore maps obtained in the present study of recombinant D1A DA receptors with published maps for native D1 and D2 receptors suggests that compounds with similar distances between the cationic nitrogen and *p*- and *m*-hydroxyl oxygens may be favored for higher D1A affinity. In addition, significantly greater height of the cationic nitrogen above the plane of the catechol ring is consistent with higher D1A, but not D2, binding affinity and suggests that development of D2A, perhaps to a greater extent than D1A, agonists with *in vivo* selectivity in intact cells may be a rational goal for drug development.

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