ABSTRACT

COMPARATIVE MOLECULAR FIELD ANALYSIS (CoMFA) OF PROTONATED METHYLPHENIDATE PHENYL-SUBSTITUTED ANALOGS

by
Kathleen Mary Gilbert

Protonated methylphenidate (pMP) and several phenyl-substituted pMP analogs were analyzed using Comparative Molecular Field Analysis (CoMFA) to develop a pharmacophore for dopamine transporter (DAT) binding. This research is a part of an interdisciplinary study on using methylphenidate (MP) analogs to block the binding of cocaine to the DAT as a treatment for addiction.

A random search conformational analysis using key pMP torsional angles was performed to create conformer families representing possible bioactive conformations. The lowest energy pMP conformer of each family was used as a template to create phenyl-substituted pMP analogs.

Partial least squares analysis was used to determine the combination of electrostatic and steric cutoffs that yielded the highest predictability (q^2). q^2 values above 0.5 were achieved for all conformer families. The best model was used to propose a pharmacophore to predict DAT binding affinity. The results were compared to a previous CoMFA study on neutral MP.

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by
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COMPARATIVE MOLECULAR FIELD ANALYSIS (CoMFA) OF PROTONATED METHYLPHENIDATE PHENYL-SUBSTITUTED ANALOGS

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To Kelly and the boys

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TABLE OF CONTENTS

Chapter	Page
1 INTRODUCTION	1
2 BACKGROUND	3
2.1 Overview	3
2.2 Cocaine and MP Act on the DAT	3
2.3 Pharmacophore Development	6
2.4 Review of Molecular Modeling	10
2.5 Review of QSAR Techniques	11
2.6 Review of CoMFA	12
2.7 Review of PLS Statistical Method	16
3 METHODS	20
3.1 Assumptions	20
3.2 Random Search Conformational Analysis	21
3.3 Analog Creation and Alignment	23
3.4 Molecular Spreadsheet™ Creation and Manipulation	25
3.5 CoMFA and PLS Calculations	26
4 RESULTS	28
4.1 Random Search Conformational Analysis	28
4.2 Analog Creation and Alignment	30
4.3 Part I CoMFA Results	35
4.4 Part II CoMFA Results	38

TABLE OF CONTENTS
(Continued)

Chapter	Page
4.5 Best PLS Model	39
5 CONCLUSIONS	43
6 DISCUSSION AND PERSPECTIVES	45
6.1 Comparison of Conformer Families	45
6.2 Comparison of Relative Energies	47
6.3 Comparison of q^2 and r^2	47
6.4 Potential for Future Work	47
APPENDIX A - SOFTWARE SPECIFICATIONS	49
APPENDIX B - SAMPLE LINES FROM SLN MACRO	50
APPENDIX C - PART I CoMFA RESULTS: FAMILIES 2 THROUGH 11	51
REFERENCES	61

LIST OF TABLES

Table **Page**

2.1 IC_{50} and pIC_{50} Values for MP Analogs 13

4.1 pMP Conformers Found Using Sybyl®'s Random Search Utility 31

4.2 Energies for MP Analog Conformations Studied 33

4.3 Best q^2 (CV PLS) and r^2 (Full PLS) Values – Part I CoMFA 38

6.1 Comparison of pMP and nMP Conformer Families 46

LIST OF FIGURES

Figure	Page
2.1 Two-dimensional Structure of DAT	5
2.2 Schematic of DAT Action in Response to Cocaine	6
2.3 pMP Molecule with Torsional Angles Used in Proposed Pharmacophore	7
2.4 Examples of Torsional Angles	8
2.5 Methylphenidate Analogs	9
2.6 Schematic of CoMFA Method	15
3.1 Starting Conformation for Random Search of pMP	21
4.1 Potential Energy Surface for pMP Conformers	29
4.2 pMP Conformer Dendrogram	29
4.3 Structure of the Eleven Template pMP Conformers	32
4.4 Conformations of Family 1+ and Family 1- MP Analogs	35
4.5 Part I CoMFA Results – Family 1	36
4.6 Part II CoMFA Results – Family 1	40
4.7 Part II CoMFA Results – Family 4	41
4.8 Actual pIC_{50} versus Predicted pIC_{50} for Family 1-	42
5.1 pMP Molecule with Torsional Angles Used in the Proposed Pharmacophore	44

CHAPTER 1

INTRODUCTION

Cocaine abuse has proven to be an ongoing problem in the United States. Since one of cocaine's modes of action is on the dopamine transporter (DAT) system, researchers have looked for other compounds that bind to the DAT, but do not inhibit dopamine (DA) reuptake as much as cocaine. Such compounds have the potential for use in cocaine addiction treatment, by blocking cocaine from the DAT binding site. DA reuptake inhibition causes an excess of DA in the synaptic cleft, which is related to cocaine's addictive effects.

Methylphenidate (MP) has been researched both as a direct substitute for cocaine in addicted patients, and as a target for research on the molecular and cellular level. It is also known for binding to the DAT, as well as being a DA reuptake inhibitor. Whether the neutral or protonated form of MP binds to the DAT has not been confirmed; this could be important in developing a model of receptor-ligand interaction for MP binding to the DAT.

A pharmacophore model forms the basis of the molecular modeling studies performed previously by the Venanzi group on neutral methylphenidate (nMP) and performed here on protonated methylphenidate (pMP) [Misra, 1999]. The pharmacophore describes the 3-dimensional orientation of the important functional groups of the MP molecule as related to its binding to the DAT and is based on the bioactive conformation of MP. It has been postulated that the torsional angles associated with the piperidine and the phenyl groups on MP are related to its binding affinity.

Molecular modeling of ligands has been used to develop receptor models for other receptors and transporters, including the nicotinic receptor and the serotonin transporter [Waters, 1988; Barker, 1998]. Molecular modeling techniques have been paired with statistical treatments in order to develop quantitative structure-activity relationships (QSAR) for sets of chemical compounds. The QSAR technique used here is Comparative Molecular Field Analysis (CoMFA) [Cramer, 1988]. This technique uses the values for steric and electrostatic potentials at various points around a set of analogs to find an acceptable model for one or more molecular properties. CoMFA was used on a set of pMP phenyl-substituted analogs to develop a model for the relative binding of a compound to the DAT as a function of its steric and electrostatic interactions with a probe atom. Potential bioactive conformations were determined using a random search conformational analysis of four torsional angles performed by Milind Misra of the Venanzi group, and clustering on two torsional angles was used to divide conformers into logical families. Each family was further split into two subfamilies, one (-) for a negative torsional angle on phenyl group substituents, and one (+) for a positive angle on phenyl group substituents. The low energy conformer in each subfamily was studied using CoMFA techniques with Tripos' Sybyl® software.

The results of the studies were compiled and compared. The predictability and goodness-of-fit of the developed models were reviewed, and a pharmacophore was proposed. Conclusions were drawn based on the results found, and suggestions for further study are given. The pMP CoMFA results were compared to the results of the nMP CoMFA's performed previously by the Venanzi group. The use of this study in terms of the interdisciplinary MP study is discussed.

CHAPTER 2

BACKGROUND

2.1 Overview

Cocaine abuse is a problem in the U.S. and around the world. Initial research into the abuse of cocaine revealed its addictive effects on the human body. Further research has been complicated by the fact that cocaine works on several neurotransmitter systems in the body, and more recently has been shown to affect signal transduction cascades and the brain on a more basic level [Volkow, 1999; Steinberg, 2001]. Cocaine's addictive effects are thought to be related to its binding action to several neurotransmitter receptors, namely the DA and serotonin transporters [Gatley, 1996; Lieske, 1998].

This section will establish MP as a starting point to find analogs that may block cocaine from binding the DAT, and allow DA uptake, without causing any deleterious side effects. In addition, background information on molecular modeling and CoMFA is reviewed. The partial least squares (PLS), also known as projections to latent structures, statistical method used for model creation is described.

2.2 Cocaine and MP Act on the DAT

Cocaine is a DA reuptake inhibitor, binding to the DAT in a way that blocks DA from being transported into the pre-synaptic nerve terminal, thus leaving an excess of DA in the synaptic junction. The use of cocaine results in addiction and other negative effects; this has led researchers to look into other compounds that bind to the DAT in order to help people break free of their cocaine addiction [Grabowski, 1997]. MP (α-phenyl-2-

piperidineacetic acid methyl ester) is also a DA reuptake inhibitor, and can displace cocaine from its binding site on the DAT [Deutsch, 1996]. MP appears not to be addictive. It has been studied as a potential solution for cocaine addiction because it has little effect on other neurotransmitter systems that appear to play a role in cocaine's addictive properties. MP exhibits minimal binding to the serotonin transporter, but cocaine binds as well to the serotonin transporter as it binds to the DAT [Gatley, 1996].

MP was first synthesized over 55 years ago [Glaser, 1998]. It is most widely known as the active ingredient in Ritalin and generic equivalents, used for Attention Deficit Hyperactivity Disorder (ADHD), narcolepsy, and depression [Thai, 1998; Volkow, 1999]. Research has shown that the most active form of MP is the *threo (2R,3R)* stereoisomer. It is not known whether the protonated or neutral form of MP binds to the DAT.

The definite locations and configurations of the binding sites on the DAT have not been determined. A two-dimensional map (Figure 2.1) showing 12 transmembrane regions was developed by researchers [Giros, 1993]. Although the third transmembrane region has been postulated as having a role in cocaine binding to the DAT [Lee, 1998] and a 3-dimensional model of the DAT has been proposed [Edvardsen, 1994], the lack of an X-ray crystal structure of the DAT makes research on the mechanisms of the DAT and its interaction with ligands more difficult.

Therefore, the binding of ligands to the DAT has been studied using indirect techniques. The exact mechanism of its interaction with the DAT has not been confirmed, but MP has been the subject of laboratory, preclinical, and clinical trials [Grabowski, 1997; Roache, 2000]. There have been several studies on the physiological

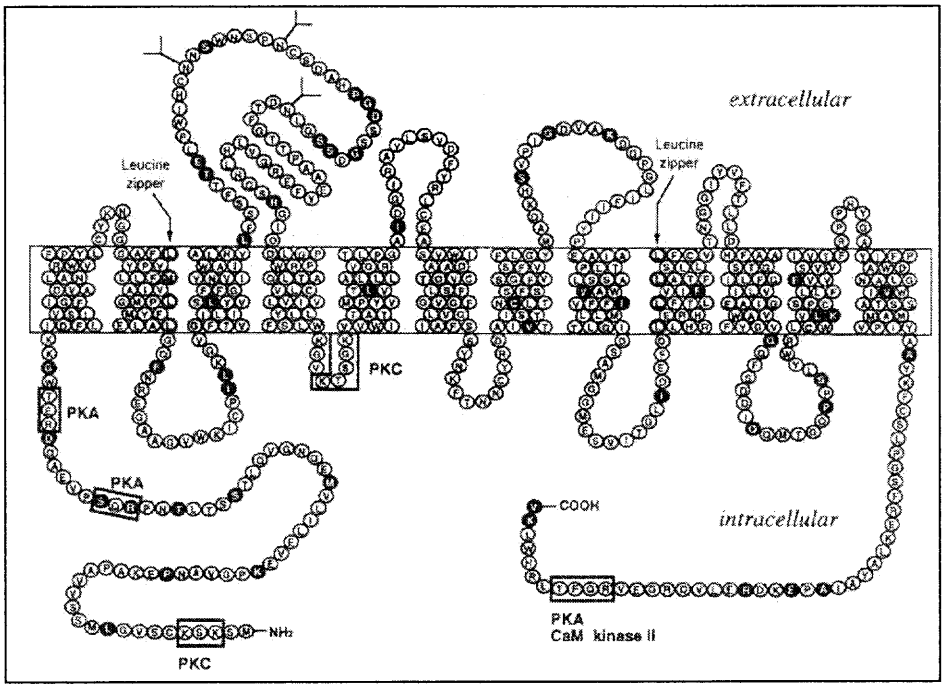


Figure 2.1 Two-dimensional Structure of DAT

effects of MP administration on cocaine-addicted patients [Segal, 1999; Roache, 2000]. It has been found that MP blocks DA reuptake as well as cocaine does [Deutsch, 1996]. However, various studies of MP's mode of interaction with the DAT have not defined what the binding site and exact mode of action are.

It has been proposed that MP could be used to displace or block cocaine (that is, act as a cocaine antagonist). After DA is secreted at the synaptic junction, the DAT takes up DA into the presynaptic terminals from which is was released in order to shut off the signal [Lee, 1998]. Reuptake inhibitors like cocaine and MP decrease the reuptake of DA. This leads to an increased effect by the same amount of DA released at the synaptic junction because the DA has more time to bind to the postsynaptic receptors. Figure 2.2 shows a representation of the effects on DA release and reuptake of cocaine as a typical DA reuptake inhibitor.

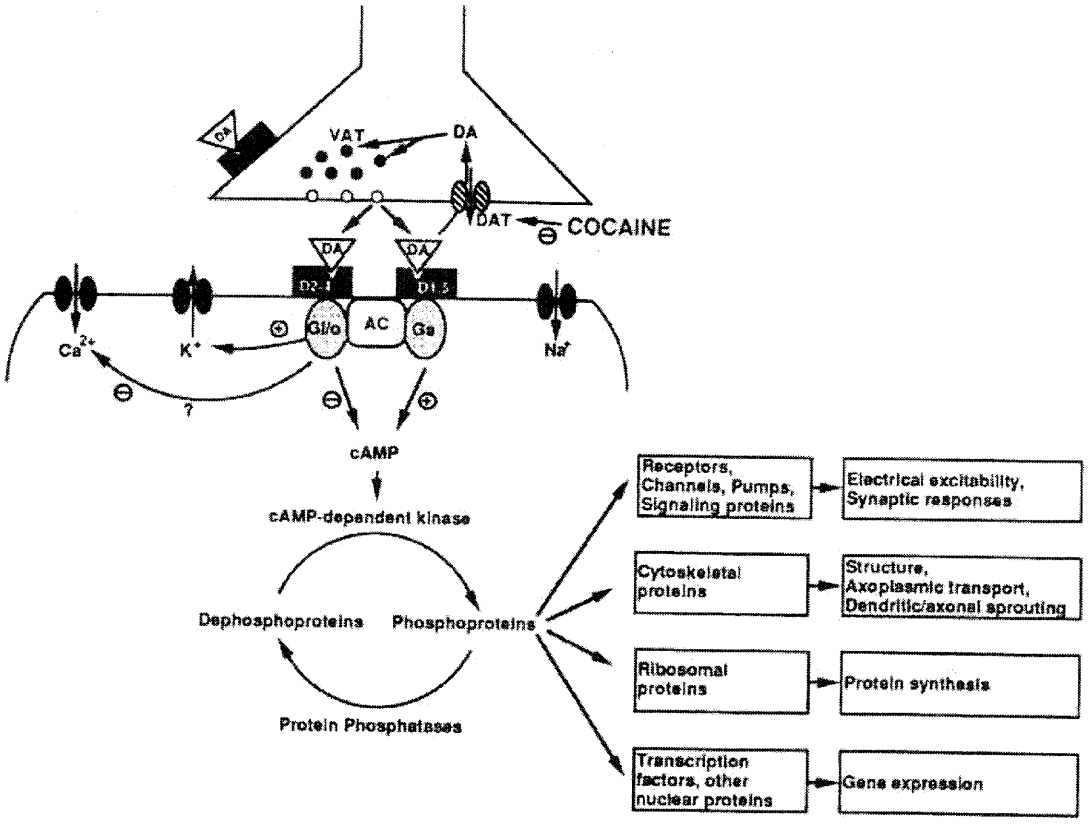


Figure 2.2 Schematic of DAT Action in Response to Cocaine

This project addresses the hypothesis that the protonated form of *(2R,3R)-threo*-methylphenidate is the active form of the molecule, and thus can be used to develop an improved pharmacophore for MP analogs' DAT binding affinities. A pharmacophore model will be developed based on the results of a molecular modeling study of pMP.

2.3 Pharmacophore Development

Molecular modeling techniques will be used to understand the DAT binding affinity of MP analogs. A pharmacophore will be proposed for binding of MP analogs to the cocaine binding site on the DAT. Molecular recognition, i.e., complementarity in molecular shape and molecular electrostatics between the ligand and the binding site, is

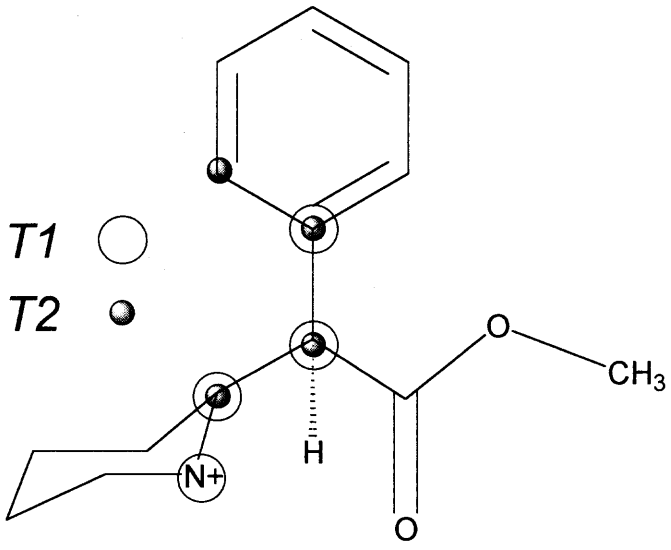
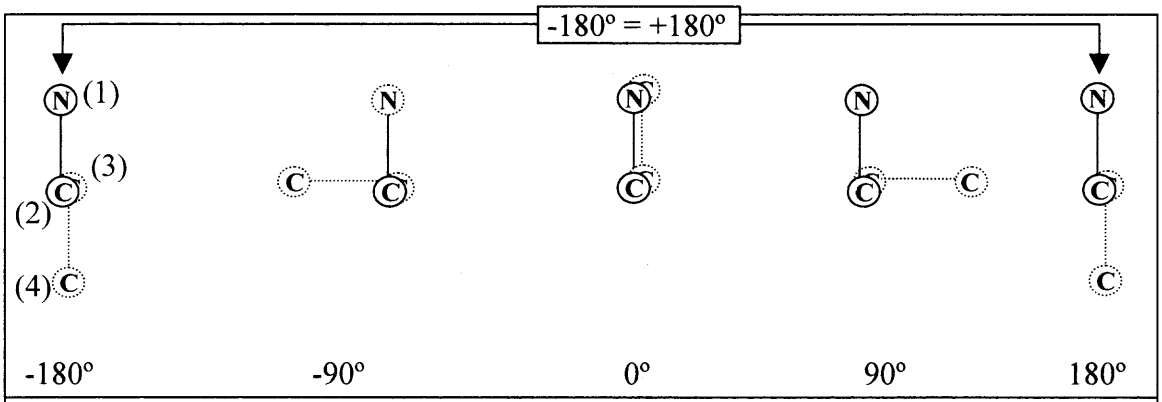


Figure 2.3 pMP Molecule with Torsional Angles Used in Proposed Pharmacophore

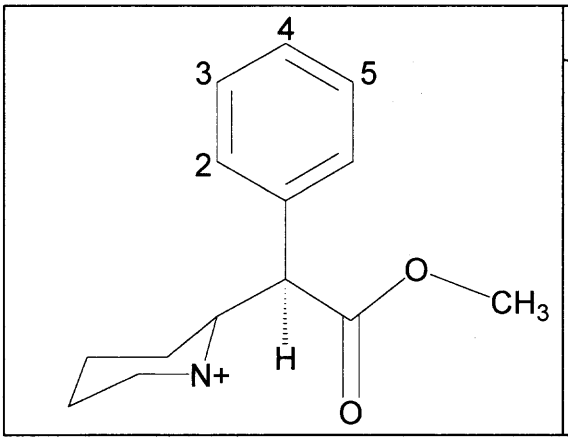
the basis of the pharmacophore. Typical pharmacophores might include the distance between key atoms, bond angles relating three adjacent atoms, or torsional angles relating four adjacent atoms. Figure 2.3 shows the pMP molecule and the framework for the proposed pharmacophore. The torsional angles T1 and T2 were selected as the key attributes of the molecule because they control the relative orientation of MP's piperidine nitrogen and phenyl ring. Research suggests that the relative orientations of cocaine's nitrogen and phenyl ring are important for its binding to the DAT [Lieske, 1998]. The role of the ester side chain in binding affinity for the DAT is in question, and it is not addressed.



-180° = piperidine nitrogen *anti* to carbon attaching phenyl ring to central carbon -90° = piperidine nitrogen 90° ahead of carbon attaching phenyl ring to central carbon 0° = piperidine nitrogen eclipsing carbon attaching phenyl ring to central carbon 90° = piperidine nitrogen 90° behind carbon attaching phenyl ring to central carbon 180° = piperidine nitrogen *anti* to carbon attaching phenyl ring to central carbon	
T1 : angle between piperidine nitrogen (1), carbon attaching piperidine ring to central carbon (2), central carbon (3), and carbon attaching phenyl ring to central carbon (4).	Front atoms have solid lines, back atoms have dashed lines.

Figure 2.4 Examples of Torsional Angles

Torsional angles are defined by four atoms, looking down the line between the second and third atom in the four. When the first and fourth atoms are eclipsed, the torsional angle t is 0°. Positive angles are achieved when the fourth atom (the last atom) has rotated clockwise. Negative angles are when the fourth atom has rotated counterclockwise. For *(2R,3R)-threo*-methylphenidate, T1 rotates the large ester and phenyl side chains away from the piperidine nitrogen. A positive T2 brings a 2- or 3-position phenyl ring substituent towards the ester side chain, and a negative T2 moves them away from the ester side chain. Figure 2.4 shows a representation of positive and negative T1 torsional angles as an example.



0-MP {None}			
2-Br 2-Cl 2-F 2-OCH_3 2-OH 2-naphthyl	3-Br 3-Cl 3-F 3-OCH_3 3-OH 3-CH_3 3-NH_2	4-Br 4-Cl 4-F 4-I 4-OCH_3 4-OH 4-CH3 4-NH_2 4-CF_3 4-C_2H_5 4-NO_2 4-t-butyl	3,4-Cl 3,4-OCH_3 3,5-Cl 3,5-CH_3

Figure 2.5 Methylphenidate Analogs

These torsional angles can be directly related to compounds in MP's structural family or indirectly related to other compounds that might bind to the DAT. An example of an indirect relationship might be the distances between key atoms that are produced in certain T1 and T2 combinations. This indirect relationship would form the basis of expanding the pharmacophore to also address molecules of significantly different structure than MP.

The development of the pharmacophore will be accomplished by study of 30 analogs in the methylphenidate family. Figure 2.5 shows the structure of methylphenidate with the substituents of each analog noted. The 30 analogs were selected based on a range of binding affinities to the DAT. The 2-naphthyl analog is listed separately from the 2- analogs because it is not attached to the 2- carbon, it is attached to the 3 and 4 carbons.

2.4 Review of Molecular Modeling

Molecular modeling of ligands in conjunction with a pharmacophore has been used to develop receptor models for other receptors, including the nicotinic acetylcholine receptor and the serotonin transporter [Waters, 1988; Barker, 1998]. One popular technique is Comparative Molecular Field Analysis (CoMFA). This technique can be used to develop quantitative structure-activity relationships (QSAR) for a set of chemical compounds.

Molecular modeling has been used since the 1980's to explore and research chemical structures. One type of molecular modeling is molecular mechanics, which uses empirical force field parameters to define the shape, potentials, and interactions of a given molecule. The computer program Sybyl® uses the Tripos force field to calculate the structure and attributes of molecules [Clark, 1989]. Molecular mechanics uses classical mechanical forms, such as springs with differing elasticity constants, to represent different atoms. Parameters are derived by fitting equations to experimental results.

Molecular mechanics methods use a series of equations to derive the locations of atoms in a molecular structure. There are various molecular mechanics methods, but all use a total energy equation calculated as a result of different intra- and inter- atomic forces in the molecule. The equation used to calculate the total energy of a molecule using the Tripos force field is as follows:

$$E = \Sigma E_{str} + \Sigma E_{bend} + \Sigma E_{oop} + \Sigma E_{tors} + \Sigma E_{vdw} + \Sigma E_{ele} \qquad (2.1)$$

where E_{str} is the energy of a bond stretched or compressed from its natural bond length, E_{bend} is the energy of bending bond angles from their natural values, E_{oop} is the energy of

bending planar atoms out of the plane, E_{tors} is the energy due to twisting (torsion) about bonds, and E_{vdw} is the energy due to van der Waals forces between non-bonded atoms. E_{ele} is the energy due to electrostatic interactions. One of the important points of the use of empirical molecular modeling energy calculations is that the absolute values of the energies are meaningless, but the relative energies for the same molecule, and conformational energy differences between different molecules do have meaning [Stanley, 2000]. Thus these techniques lend themselves to mathematical comparison of molecules in order to predict relative activities of the compounds.

2.5 Review of QSAR Techniques

Quantitative structure-activity relationship (QSAR, also known as quantitative structure-property relationship, QSPR) techniques have been developed over the past several decades. Probably the earliest structure-activity relationship work used mathematical methods before the use of computers to postulate the effect of changing substituents on the activity of chemical analogs [Free, 1964; Martin, 1998]. QSAR equations can be derived empirically from molecular descriptors either arbitrarily or from a predetermined set. QSAR techniques provide insight into how the molecular and submolecular attributes of a molecule relate to physical, chemical, or biological properties [Hawkins, 2001]. QSAR has been used for such varying purposes as predicting the toxicity to aquatic life of a substance to predicting the flavor of whiskeys. However, the most common usage has been in the chemical industry to predict the chemical properties of compounds related by structure, use, or property.

2.6 Review of CoMFA

The CoMFA technique was first used to predict the binding of steroid molecules to carrier proteins [Cramer, 1988]. Since then, CoMFA has been used more generally by researchers to determine the structure-activity relationship between a set of compounds and one or more properties of the compounds. The steric bulk and electrostatic potential fields at various grid points, as seen by a probe atom, are used to describe the structure of each compound. Cutoffs are used to ensure that large values for steric and electrostatic potentials are not overweighted. The cutoff tells the program that any value above the cutoff is set to the cutoff value. There is one steric potential cutoff, one electrostatic potential cutoff, and a column filtering cutoff (σ) to even out variations within a CoMFA column. Each Sybyl CoMFA column shown in the molecular spreadsheet contains a volume estimate of each molecule based on the number of lattice points in the molecule. The column shown represents the thousands of steric or electrostatic potential values that make up the CoMFA field.

PLS is used to determine the best model for the property data. In this case, the property data used is the IC_{50}'s of the MP analogs, transformed by taking the negative logarithm of the IC_{50} in nanomoles. The IC_{50} shown is the amount of compound necessary to displace half of the [^{3}H]WIN 35,428 from the binding site on the DAT. This data approximates the ability of the MP analog to bind to the DAT. The IC_{50} is the inverse of the binding affinity.

The MP analog binding data was experimentally determined by the Schweri group at Mercer University School of Medicine using rat brain synaptosomes [Schweri, personal communication]. Table 2.1 shows the IC_{50} data for the 30 analogs. The 3-

Table 2.1 IC_{50} and pIC_{50} Values for MP Analogs

Analog	WIN 35,428 IC_{50}* (nM)	pIC_{50} (-log IC_{50}(M))
0-MP	83.0	7.081
2-Br	1865.0	5.729
2-Cl	1946.7	5.711
2-F	1415.0	5.849
2-naphthyl	11.0	7.959
2-OCH_3	100666.7	3.997
2-OH	23050.0	4.637
3-Br	4.2	8.377
3-CH_3	21.4	7.670
3-Cl	5.1	8.292
3-F	40.5	7.392
3-NH_2	265.0	6.577
3-OCH_3	287.5	6.541
3-OH	321.0	6.493
3,4-Cl	5.3	8.276
3,4-OCH_3	910.0	6.092
3,5-CH_3	4685	5.329
3,5-Cl	65.6	7.183
4-Br	6.9	8.161
4-C_2H_5	736.7	6.133
4-CF_3	615.0	6.211
4-CH_3	33.0	7.481
4-Cl	20.6	7.686
4-F	35.0	7.456
4-I	14.0	7.854
4-NH_2	34.5	7.462
4-NO_2	493.8	6.306
4-OCH_3	83.0	7.081
4-OH	98.0	7.009
4-*t*-butyl	13450.0	4.871
* M. M. Schweri, personal communication.		

substituted analogs show better (lower) IC_{50}s than 2- substituted analogs for all substituents, which might indicate an unstable steric interaction with the DAT for the 2-substituted analogs. There are quite a few analogs that have reasonable binding affinities that may be potential research targets. It appears from the 2-naphthyl analog IC_{50} that there is a significant binding pocket available for large substituents in certain locations.

The CoMFA techniques used in this study are explained in detail in the Methods section, and the details of specific software parameters is contained in Appendix A. The computer software Sybyl® was used to perform the CoMFA studies which resulted in PLS models for the binding affinity of the MP analogs to the DAT.

The procedure attempts to correlate a set of chemical structures and their properties with known biological activities, in this case the structures and properties of conformers of pMP and its analogs and their binding affinities at the DAT. The software program is used calculate the steric and electrostatic potential fields in response to a probe atom interacting with each pMP analog conformer. A statistical method is used to determine if a highly predictive ($q^2 > 0.5$) model of the structure-activity relationship can be found. A model is deemed satisfactory if the property of interest can be estimated using the CoMFA data and the model.

Figure 2.6 shows a schematic of the CoMFA procedure. In the QSAR table shown, *Cpd1*, *Cpd2*, etc. are the pMP analogs to be studied. *Bio* is the –log IC_{50} for each analog, a logarithmic transformation of the IC_{50}. *S001* through *S998* and *E001* through *E998* represent the steric and electrostatic interaction energies between the probe atom and each pMP analog conformer at each of the grid points within the structure. The resulting equation shows the model for the biological activity (pIC_{50}) as a weighted sum of the steric and electrostatic interaction energies. Because there can be thousands of data points to be analyzed for inclusion in the model, a statistical method such as PLS must be used to make sense of the data.

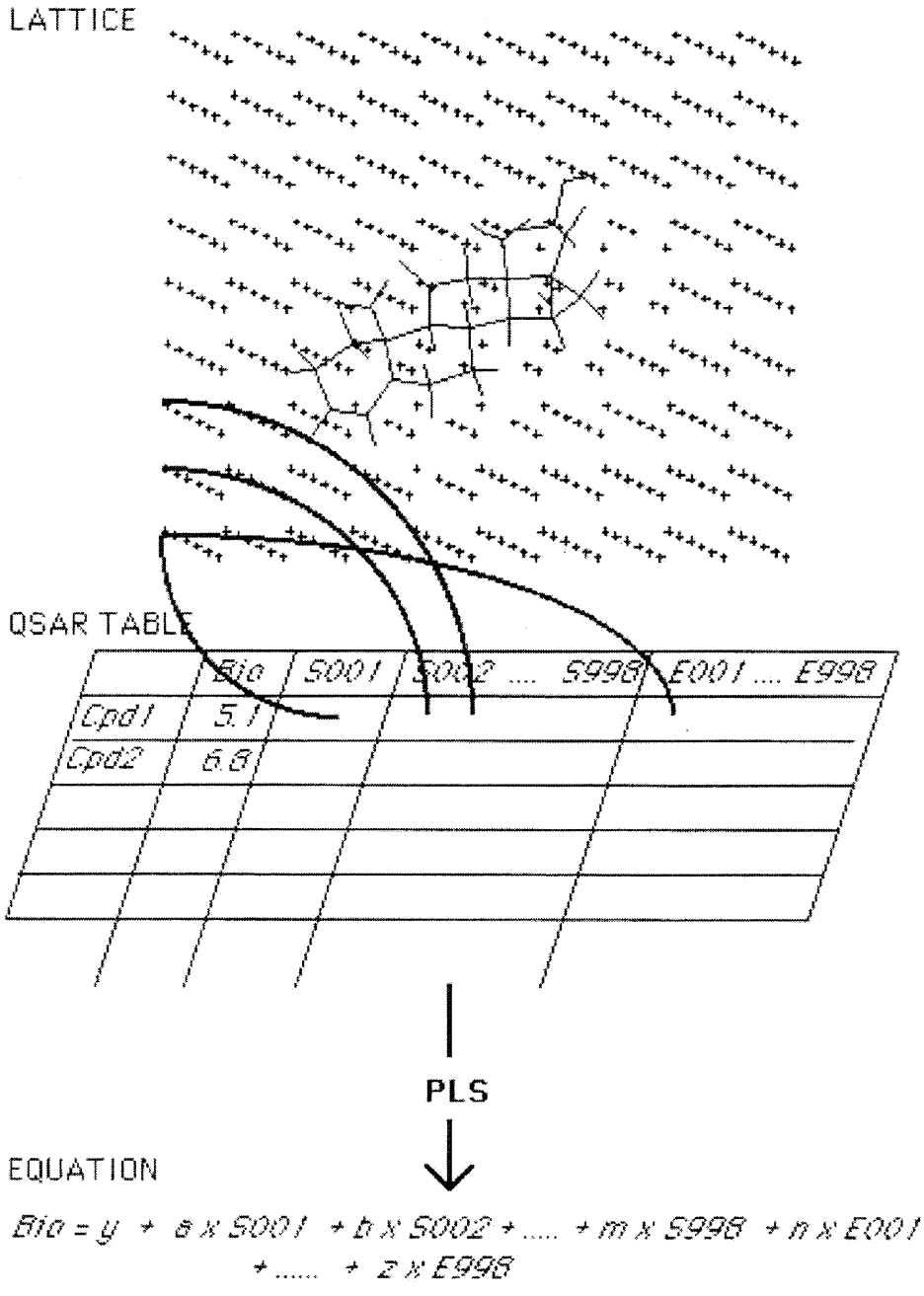


Figure 2.6 Schematic of CoMFA Method

2.7 Review of PLS Statistical Method

One of the chief reasons that CoMFA is a method of choice for researchers is the use of the PLS multivariate statistical method, developed by Wold and others in the early 1980's [Wold, 1993]. This method, based on perturbation theory, allows the user to manipulate and interpret the thousands of pieces of steric and electrostatic potential data produced by the CoMFA procedure. PLS is also known as "projections to latent structures," a term which is based on the methods used to fit the data to a modified least squares algorithm. The PLS analysis can be performed on a set of x variables ("predictors" – may be referred to as "independent variables," but independence of each value is not necessary to the PLS model and probably not the case in the CoMFA data) to relate them to a given set of y variables ("response" or "dependent variables").

There are a series of steps to follow when performing a PLS analysis:

(1) Form new variables t_a from the x variables.

(2) Use the set of new t_a variables to predict the y variables; if there are more than one set of y variables, the y variables need to be transformed as well.

Repeat (1) and (2) until t_a variables are created which are not predictively significant.

Each time these steps are carried out, a set of t_a variables are created which represent a component of the PLS model. These variables t_a are known as "scores" or "latent variables," and they are the key to the PLS method. A PLS component therefore represents a level of complexity of the model – as the number of components increases, so does the complexity of the model. In the extreme case, a model could have one less component than the number of objects (molecules) in the study, which would essentially model the variability due to each molecule. For that reason, many researchers limit the

number of components that can be produced by the PLS algorithm, based on the total number of molecules in the CoMFA. At the other extreme, a one component model essentially reduces to a linear least squares model.

In order to test whether a new set of t_a variables significantly contributes to the PLS model, cross-validation is used. Cross-validation (CV) is the method of dividing data into groups and developing models for each reduced set of data, minus one of the groups for each iteration. For example, if there were five groups, five iterations would be performed, one with Group 1 left out, the second with Group 2 left out, etc. The leave-one-out (LOO) approach is a form of CV where the number of groups equals the number of objects. In this study, the number of objects equals the number of analogs, 30. The LOO technique is used; therefore, 30 PLS runs were made, each with one analog left out, for each cross-validated PLS (CV PLS) analysis. These CV PLS analyses produce a value for the predictability of the model, q^2.

Two correlation coefficients were of interest in the PLS studies. Predictability, q^2, is calculated from the CV PLS results:

$$q^2 = 1 - [PRESS / SS(Y)] \qquad (2.2)$$

where: q^2 = predictability, a dimensionless quantity, generally between 0 and 1

PRESS = predictive residual sums of squares formed from all of the sums of squares of the residuals for each cross-validation run

(residual = actual value – predicted value)

SS(Y) = sum of squares of the y variables

This value q^2 is only calculated for the cross-validated PLS runs. The CV PLS runs were also used to determine the optimal number of components to use in the non-validated PLS runs.

The non-validated PLS run is performed on all of the data as a group, with no row being left out. This produces the second correlation coefficient, r^2, which represents the "goodness of fit" of the model. It is calculated using the following formula:

$$r^2 = 1 - [SS(F) / SS(Y)] \quad (2.3)$$

where: r^2 = goodness of fit, dimensionless, generally between 0 and 1

SS(F) = sum of squares of the y residuals

SS(Y) = sum of squares of the y variables

According to Wold, the pair of q^2 and r^2 values should be within 0.15 of each other to indicate model stability, although this limit is not observed when a survey of CoMFA studies was reviewed. Rather, most results showed r^2's in the range of 0.9 or above, with many above 0.95 [Kim, 1998]. A model can be predictive, but not fit the line well, or it could fit a line well and not be predictive. Some sources assume that a q^2 above 0.3 is significant [Martin, 1996], but a q^2 of 0.5 is more likely to be significant and not due to chance or error.

Finally, other statistical parameters can be calculated from the non-validated PLS runs. The standard error (SE) is an estimate of the error in prediction. An F test is also performed by the Sybyl® program. The F value is generically defined as follows:

$$F\ value = (Results) / (1 - Results) \quad (2.4)$$

where: F value = statistical F test result

Results = normalized results of model

In this application, the F value is used to compare the results of similar studies for relative significance of the models. Use of the PLS method avoids the problem of variable selection or variable reduction for the x data set. This statistical method has made CoMFA and other complicated modeling techniques possible.

CHAPTER 3

METHODS

The project is a series of CoMFA studies on phenyl-substituted analogs of pMP, in support of other MP work done by the Venanzi group. The MP analogs studied (Figure 2.5) were synthesized by Howard Deutsch's group at the Georgia Institute of Technology and their binding affinities experimentally determined by the Schweri group at Mercer University School of Medicine. The series of steps followed were similar to those performed by Milind Misra in his Master's thesis work on nMP MP analogs at NJIT to ensure direct comparison of the nMP and pMP results [Misra, 1999].

3.1 Assumptions

There are several assumptions that are integral to this work. The assumptions allow for the limited nature of this study, and may be reassessed for future work. First, it is assumed that the piperidine nitrogen and phenyl ring are important molecular recognition features for the binding of MP analogs to the DAT. Torsional angles T1 and T2 control the spatial orientation of these important features. Second, the ester side chain feature of the MP molecule, as described by T3 and T4, is used to find the low energy conformers of pMP, but is not used to model the binding affinity of the MP analogs. It is also assumed that the binding conformation, also known as the bioactive conformation, is a local minimum within 20 kcal/mol of the global energy minimum (GEM). A further assumption is that the conformational potential energy surface of each analog is similar to

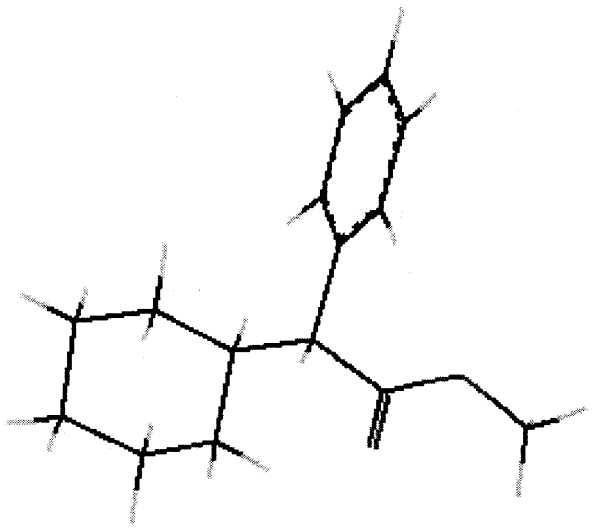


Figure 3.1 Starting Conformation for Random Search of pMP

that of pMP, allowing the analog structures to be built from representative pMP structures. Complicated studies where each MP analog has a random search conformational analysis performed are being done by the Venanzi group, but the time and computational expense of the undertaking is prohibitive for this project.

3.2 Random Search Conformational Analysis

The Random Search conformational analysis function of Sybyl® was used to probe the potential energy surface of pMP. The starting point for the random search was created by Milind Misra. The nMP conformer used was the GEM derived from his previous study of the neutral species [Misra, 1999]. The conformational analysis starting point is shown in Figure 3.1. The nMP structure was modified to add a proton to the piperidine nitrogen, thus creating the pMP molecule that has a charge of +1. Gasteiger-Hückel charges were calculated for the molecule before the conformational analysis was begun.

Using this as a starting point, a random search of four torsional angles, T1, T2, T3, and T4, was performed using Sybyl®. T1 and T2 are identified in Figure 2.3, and T3 and T4 are associated with the ester side chain. T3 is formed by the carbon that attaches the piperidine ring to the central carbon, the central carbon, the carbonyl carbon in the ester side chain, and the ether oxygen. T4 is formed by the central carbon, the carbonyl carbon in the ester side chain, the ether oxygen, and the carbon in the methyl group of the ester side chain. It is assumed that the orientation of the ester side chain is of minor importance to the pharmacophore.

The random search conformational analysis function discarded all conformers that were more than 20 kilocalories (kcal) from the GEM structure found, using an iterative process that redefines the GEM if a conformer with a lower energy structure than the current GEM is identified through the random search. This 20 kcal cutoff was selected in order to include a range of low energy conformations because the bioactive conformer is not necessarily the lowest energy structure [Misra, 1999] .

These results were compiled using hierarchical clustering to create a dendrogram, which is a branched diagram showing the level of relationship between conformers. Sybyl® has a hierarchical clustering function that creates dendrograms based on the columns of data selected. In this case, T1 and T2 were used to cluster the data and divide the conformers into logical families. T2 was limited to a range of 180° because the phenyl ring is symmetrical, and the carbons on either side of the unsubstituted pMP molecule are equivalent. The possibility of a phenyl-substituted analog having a substituent on either side of the phenyl ring is addressed in the next part of this section.

These families appear as groups of points on the 2-dimensional slice of the pMP molecule potential energy surface represented by a plot of T1 versus T2. Each family represents a potential bioactive conformation for MP at the DAT binding site. The data was analyzed visually through the dendrogram and analytically by importing torsional angle data into Microsoft Excel. In the dendrogram, the height difference between the conformers and groups of conformers is proportional to the degree of difference in T1 and T2 between the conformations. The number of families was selected using both methods, and the conformers were divided up into these families. The lowest energy conformer in each family was selected as a template for each family. This was done because the assumption is that the conformers in a family are "equivalent," so the lowest energy conformer would be the "local energy well" for the family of conformers.

3.3 Analog Creation and Alignment

The template conformer for each family was used as the starting structure for each of the analogs. There are 30 analogs in total, shown in Figure 2.5, with one being the pMP molecule (identified by {None} in the figure - all groups on the phenyl ring are hydrogens). The other 29 analogs are single or double phenyl-substituted analogs. The analogs with 2-, 3-, or 3,4- substituents correspond to unsymmetrical phenyl rings. This was addressed by further dividing up each family into a plus ("+") and minus ("-") subfamily. Each "+" subfamily includes the MP template, all symmetrical phenyl-substituted analogs, and the unsymmetrical phenyl-substituted analogs with a positive T2. Each "-" subfamily includes the MP template, all symmetrical phenyl-substituted analogs, and the unsymmetrical phenyl-substituted analogs with a negative T2.

Therefore the "+" and "-" subfamilies have more than half of their members in common. It is expected therefore that the difference in q^2 between subfamilies is due only to the effects of unsymmetrical analogs.

Each analog was created using Sybyl®'s molecule editing function. Since the pMP conformers were produced from the same structure, all of the atoms were numbered as in the starting structure. This allowed the creation of a Sybyl® line notation (SLN) macro to automate the analog generation process. A sample of SLN from this macro is included in Appendix B.

The generation of analogs was further complicated by multi-atom substituents. Some groups, such as $-NO_2$, are planar so their conformation is pre-defined. Other multi-atom substituents, such as $-OCH_3$, required conformational searching about rotatable bonds. This was done using Sybyl®'s Systematic Search conformational analysis function. The only atoms allowed to move in the search were those in the multi-atom substituent. The remaining atoms were kept in their original conformation. The lowest energy conformation was then selected from the systematic search results and used in the studies. Gasteiger-Hückel charges were then calculated for all molecules before analog alignment proceeded, with the net charge on all molecules being +1.

The analogs produced using this procedure were separated into directories, equivalent to Sybyl® molecular databases, and aligned with regards to the CoMFA grid. The Inertial Alignment option in Sybyl®'s Align Database command was used, with the template pMP conformer in each family used as a basis. This option aligns the molecules in a database along the dipole of the template, as opposed to the As Is option which keeps the original location of the template. The Inertial Alignment option allows results to be

replicated without original coordinate data. The central atom and the three surrounding carbon atoms were used to realign the molecules after aligning the pMP template molecule based on its inertial moment. The relative alignment is meant to cancel the effects of the main structure of the pMP and is exact in this case because each analog was created from the pMP template without modification to the main structure. Thus the results would only be based on the differences in phenyl ring substituents.

3.4 Molecular Spreadsheet™ Creation and Manipulation

A Molecular Spreadsheet™ was created for each database of molecules. A database was used for each set of molecules studied, two databases per family. A SLN macro was used to add energy and IC_{50} data to each spreadsheet. The macro also calculated the pIC_{50} from the IC_{50} data. The pIC_{50} is the negative logarithm of the IC_{50} in moles, using a similar transformation as pH (which is the negative logarithm of the concentration of hydrogen ions in a solution). Table 2.1 in the Background section shows the IC_{50}'s and pIC_{50}'s for the 30 analogs. CoMFA columns were added using the Insert Column menu command. Each CoMFA column was named to identify the steric and electrostatic cutoffs used in the CoMFA. For example, a CoMFA column with 40 kcal/mol steric potential cutoff and 50 kcal/mol electrostatic potential cutoff was named S040E050. This allowed great ease in creation of CoMFA columns using a macro, as well as performing PLS studies without having to return to the table to select a new CoMFA column.

3.5 CoMFA and PLS Calculations

Two sets of CoMFA were performed using different ranges of steric and electrostatic cutoffs. The cutoffs for the Part I CoMFA ranged from 10 to 50 kcal/mol by 10's for both the steric and electrostatic potential, giving 25 CoMFA columns. Part II CoMFA studies were performed on the lowest energy conformer family and the family with the highest q^2 identified in Part I. These studies were performed in the same manner as in Part I, with the larger range of cutoffs. The studies were separated because of the large amount of data required for the more comprehensive studies.

The Part II CoMFA ranged from 10 to 250 kcal/mol for the steric potential cutoffs and from 10 to 60 kcal/mol by 10's for the electrostatic potential cutoffs. The electrostatic cutoffs in Part II ran from 10 to 60 by 10's, 80 to 200 by 20's, then 225 and 250. These values were selected to encompass the range used in the nMP studies. The CoMFA columns were calculated using Sybyl®'s default settings, as noted in Appendix A.

PLS runs were performed on the CoMFA columns. Cross-validated PLS runs (CV PLS) were performed using 2 kcal/mol for the column filtering value (σ). If the energy variance for a particular grid point is more than σ, it will be ignored in the PLS run. Thus setting σ=0 will include the most grid points for consideration, and higher settings for σ will ignore potentially more grid points. The CoMFA column with the best q^2 produced by the CV PLS was selected to perform full PLS runs. These were done at three values of σ, 0, 1, and 2 kcal/mol. The number of PLS components were limited to 10 in both cases in order to limit the complexity of the models generated.

The CoMFA results were reviewed for the predictability and goodness-of-fit of the best model found. This information was used to propose a pharmacophore for MP binding to the DAT system. The results were also compared to the results of similar nMP studies.

CHAPTER 4

RESULTS

4.1 Random Search Conformational Analysis

The results of the random search on the four torsional angles of interest in the pMP molecule indicated 28 conformers (including the starting conformation) within a 20 kcal/mol range of energies of the pMP GEM. Note that the starting conformation that was based on nMP did not turn out to be the GEM for pMP. These 28 conformers were then broken up into families based on torsional angles T1 and T2 as shown in Figure 2.3. Note that T2 has only values from – 180° to 0° (or 0° to +180°) since the phenyl ring is symmetrical. Figure 4.1 shows the T1 versus T2 "slice" through the potential energy surface identifying 28 low energy conformers. The potential energy surface indicates T1 and T2 combinations that are favorable and unfavorable. Areas where no points appear are energetically unfavorable and conformations with those T1 and T2 values are improbable. T2 values appear in groups, indicating that there are few favorable orientations of the phenyl ring, which is expected because of its relative bulkiness.

Sybyl®'s Hierarchical Clustering tool produced the dendrogram shown in Figure 4.2. The line indicates where the dendrogram was "cut" to produce 11 families. Eleven families were selected based on the range of T1 and T2 within the families being "reasonable" (11.73° for T1 and 9.30° for T2, less than 10% difference (±5%) within each family). Families 7 and 8 appear close in Figure 4.1, but going from 10 to 11 families changes from a T1 range over 21° to a T1 range of around 12°. The lowest energy conformation in each family was identified and selected as the family template.

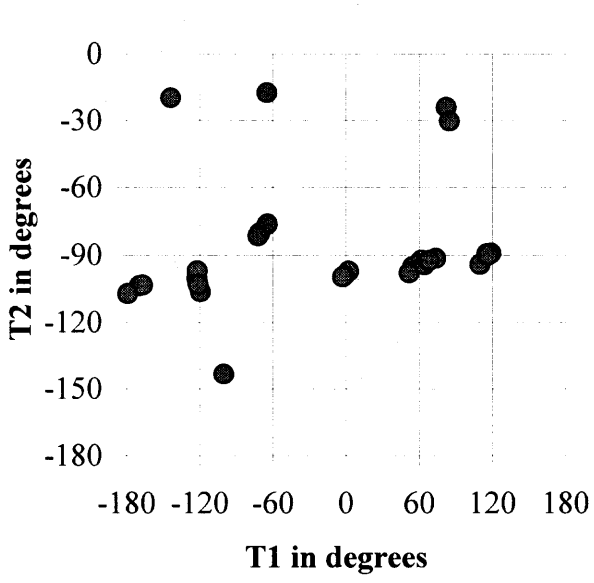


Figure 4.1 Potential Energy Surface for pMP Conformers

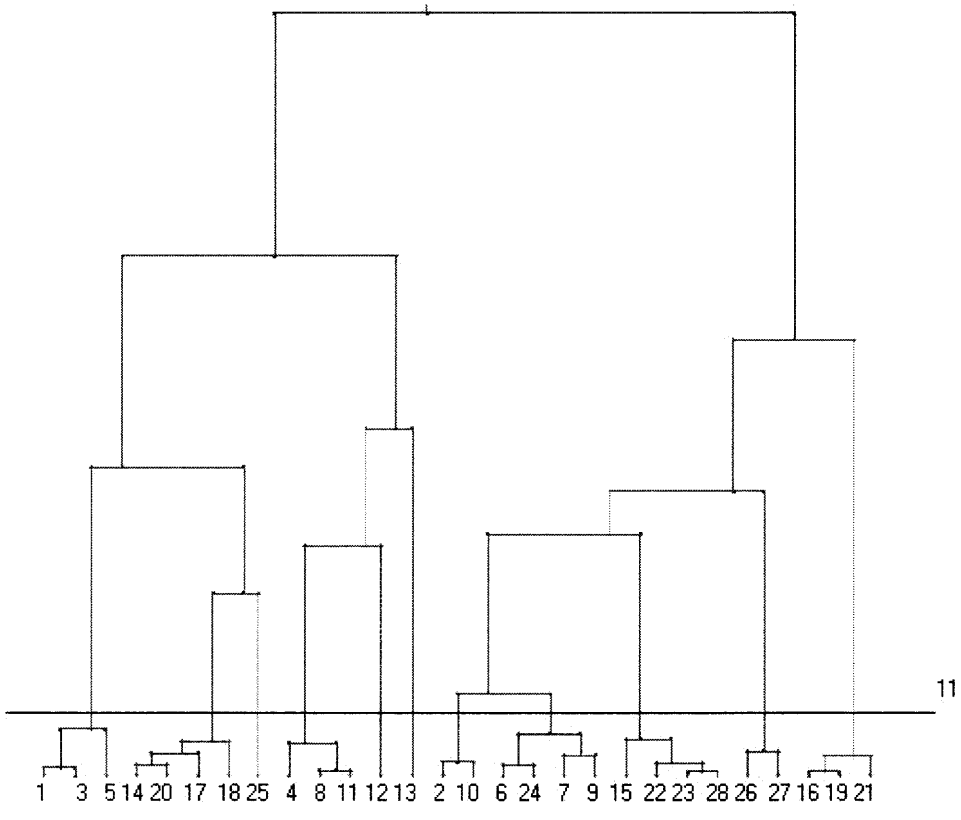


Figure 4.2 pMP Conformer Dendrogram

Table 4.1 shows the conformers divided up into families, with the template conformers identified. The table includes the values for the key torsional angles T1 and T2, as well as the energies relative to the pMP GEM (Family 4, Conformer 8). Conformer numbers were assigned arbitrarily by Sybyl®, in order of generation in the random search conformational analysis. Relative energies within each family were usually no more than 5 kcal/mol.

Figure 4.3 shows the conformations of the 11 template pMP molecules. They were aligned before the images were separated in order to compare the conformations. The center box shows all of the template conformers aligned by the central carbon atom and the three surrounding carbon atoms. This figure is for visual comparison only, as the families were not combined in any study. Only one pMP conformation was studied in each CoMFA run. The pMP conformations that were produced in this study will be compared to the nMP conformations identified in earlier studies.

4.2 Analog Creation and Alignment

Analogs were built based on each template structure. In this study, the MP analog conformations are created from pMP templates. The potential energy surface for each analog is assumed to be similar to the pMP potential energy surface shown in Figure 4.1. It is noted that that the results of a thorough conformational analysis of each of the 30 analogs might yield somewhat different results for individual conformations found, but it is assumed that the families used here encompass all possible energetically favorable conformations (energies within approximately 20 kcal/mol of the GEM conformations).

Table 4.1 pMP Conformers Found Using Sybyl®'s Random Search Utility

Family	Conformer #	T1 (°)	T2 (°)	Relative Energy (kcal/mol)
1	**1**	**-169.17**	**-103.6**	**0.662**
	3	-166.72	-103.3	5.475
	5	-179.04	-107.2	4.908
2	**14**	**-122.09**	**-100.3**	**3.282**
	17	-121.83	-97	8.081
	18	-119.1	-106.3	8.095
	20	-120.92	-103.1	8.542
3	**25**	**-100.12**	**-143.3**	**4.209**
4	**8**	**-70.85**	**-80.3**	**0.000**
	4	-64.44	-76.4	3.129
	11	-72.13	-81.5	4.593
5	**12**	**-64.74**	**-17.4**	**3.216**
6	**13**	**-143.64**	**-19.7**	**7.876**
7	**2**	**54.63**	**-95.2**	**9.328**
	10	51.79	-97.9	10.889
8	**6**	**61.92**	**-92.4**	**8.134**
	24	64.4	-94.4	10.723
	7	73.65	-91.5	10.530
	9	68.06	-92.5	14.409
9	**23**	**115.44**	**-89.5**	**13.071**
	22	118.97	-89.2	13.926
	15	110.08	-94.2	14.391
	28	116.64	-90.1	19.485
10	**26**	**82.37**	**-24.0**	**16.819**
	27	84.98	-30.2	17.653
11	**21**	**2.35**	**-97.2**	**13.616**
	19	-1.25	-99.2	13.617
	16	-2.52	-99.8	13.653

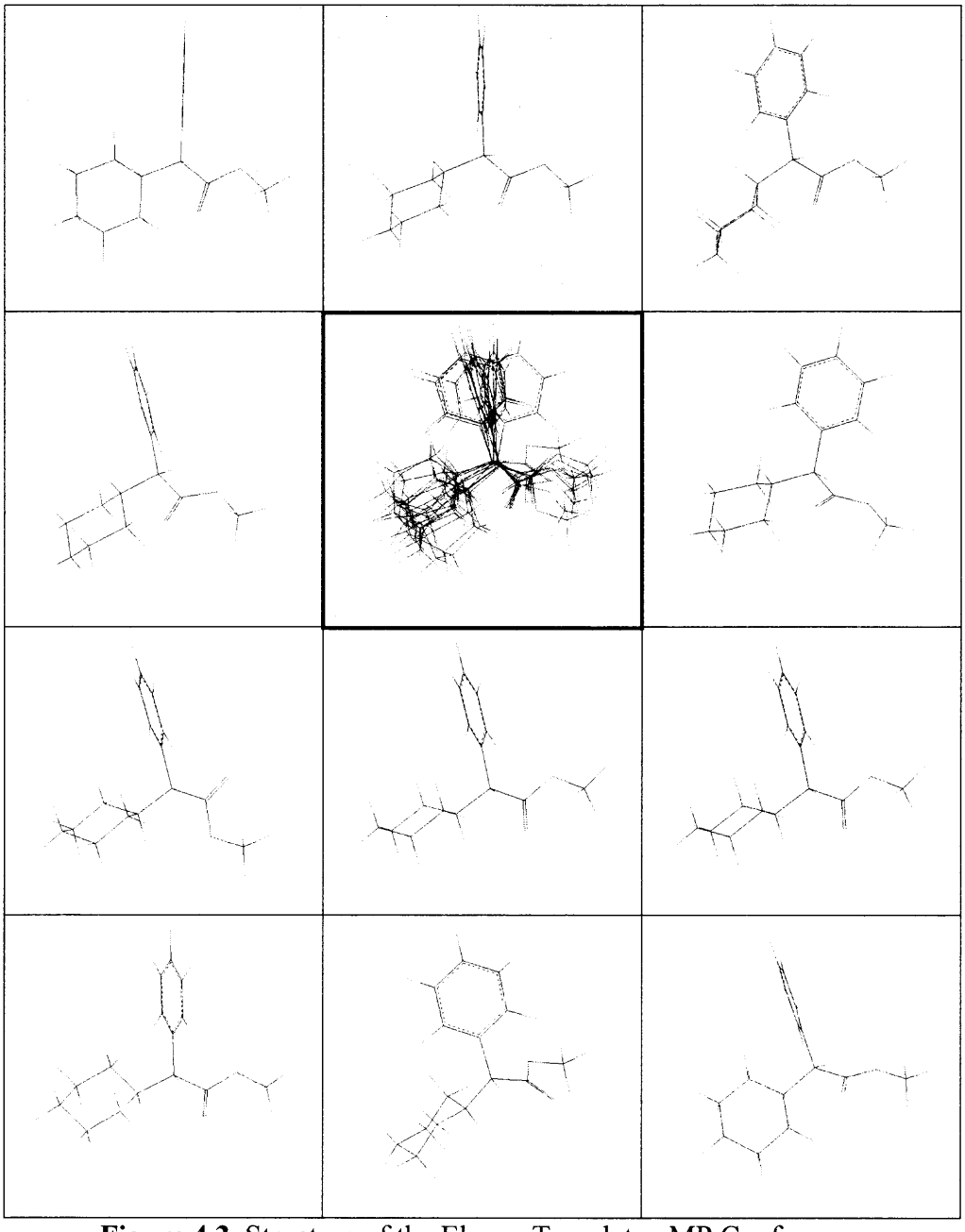


Figure 4.3 Structure of the Eleven Template pMP Conformers

The energies of all of the molecules used in these studies are shown in Table 4.2. The table is set up to show the analogs with a negative T2 value, then the analogs where the substituents are symmetrical. Finally, the analogs where T2 are positive are listed. For each study performed, the negative or positive T2 analogs were combined with the

Table 4.2 Energies for MP Analog Conformations Studied

	Family										
Analog	1	2	3	4	5	6	7	8	9	10	11
-2-Br	0.91	3.76	5.86	**0.00**	42.36	66.65	10.10	8.51	12.66	***262.25***	14.88
-2-Cl	0.62	3.48	5.26	**0.00**	23.37	37.25	9.46	7.87	12.19	***122.20***	14.63
-2-F	0.11	3.07	4.57	**0.00**	2.18	8.10	8.27	6.72	11.32	11.92	***14.34***
-2-OCH_3	4.76	3.43	***26.12***	**0.00**	20.23	11.25	7.08	5.38	11.23	15.14	13.48
-2-OH	0.58	3.47	4.97	**0.00**	3.09	10.48	7.82	6.16	11.47	13.18	***14.04***
-2-naphthyl	0.54	3.24	4.27	**0.00**	3.17	7.84	9.15	7.97	12.87	***16.70***	13.61
-3-Br	0.42	3.13	4.09	**0.00**	3.05	7.56	9.25	8.06	12.82	***16.47***	13.76
-3-Cl	0.41	3.09	4.01	**0.00**	3.10	7.57	9.35	8.15	12.85	***16.50***	13.83
-3-F	0.27	2.95	3.77	**0.00**	3.17	7.45	9.54	8.31	12.81	***16.46***	14.06
-3-OCH_3	0.71	3.29	4.02	**0.00**	3.19	7.97	9.29	8.06	13.10	***16.77***	13.64
-3-OH	0.64	3.24	4.03	**0.00**	3.16	7.86	9.39	8.13	13.03	***16.68***	13.78
-3-CH_3	0.82	3.38	4.19	**0.00**	3.41	8.16	9.48	8.23	13.25	***17.01***	13.59
-3-NH_2	0.85	3.36	4.07	**0.00**	3.66	8.37	9.69	8.40	13.39	***17.32***	13.70
-3,4-Cl	0.18	2.92	3.85	**0.00**	3.06	7.34	9.35	8.16	12.67	***16.33***	14.01
-3,4-OCH_3	0.48	3.09	3.74	**0.00**	3.08	7.74	9.47	8.13	12.92	***16.48***	13.97
0-MP	0.66	3.28	4.21	**0.00**	3.22	7.87	9.33	8.14	13.07	***16.82***	13.62
4-Br	0.46	3.13	4.09	**0.00**	3.18	7.67	9.31	8.13	12.90	***16.66***	13.75
4-Cl	0.43	3.11	4.06	**0.00**	3.16	7.65	9.33	8.14	12.89	***16.64***	13.80
4-F	0.29	2.99	3.94	**0.00**	3.12	7.50	9.36	8.17	12.79	***16.53***	13.97
4-I	0.45	3.13	4.08	**0.00**	3.18	7.67	9.30	8.12	12.89	***16.65***	13.75
4-OCH_3	0.67	3.29	4.27	**0.00**	3.27	7.97	9.27	8.11	13.07	***16.88***	13.65
4-OH	0.61	3.23	4.11	**0.00**	3.16	7.80	9.29	8.12	13.00	***16.69***	13.76
4-CH_3	0.80	3.39	4.26	**0.00**	3.21	7.98	9.34	8.12	13.17	***16.85***	13.55
4-NH_2	0.79	3.37	4.24	**0.00**	3.20	7.96	9.37	8.14	13.17	***16.85***	13.61
4-CF_3	**0.00**	2.90	4.01	0.39	3.41	7.13	9.55	8.51	12.59	***16.37***	14.55
4-C_2H_5	0.78	3.39	4.26	**0.00**	3.22	7.99	9.32	8.10	13.13	***16.87***	13.54
4-NO_2	0.37	3.04	3.92	**0.00**	3.06	7.50	9.39	8.18	12.85	***16.47***	13.99
4-*t*-butyl	0.07	2.92	3.79	**0.00**	2.83	6.72	9.03	8.04	12.52	***15.74***	13.78
3,5-Cl	0.15	2.89	3.82	**0.00**	3.02	7.30	9.28	8.08	12.62	***16.26***	14.04
3,5-CH_3	0.91	3.47	4.37	**0.00**	3.28	8.26	9.30	8.00	13.19	***16.90***	13.45
+2-Br	**0.00**	4.62	***82.87***	10.58	31.97	74.74	16.71	11.55	13.99	22.90	36.07
+2-Cl	**0.00**	3.24	***38.62***	5.53	17.53	40.59	13.74	9.93	13.54	20.36	25.56
+2-F	**0.00**	2.05	0.04	0.60	4.99	10.28	10.93	8.88	13.37	***17.61***	16.23
+2-OCH_3	0.89	2.09	**0.00**	2.42	3.97	10.44	11.76	11.36	***16.79***	16.68	14.87
+2-OH	**0.00**	1.84	0.26	0.52	5.93	12.42	8.71	8.53	13.39	***18.30***	15.07
+2-naphthyl	0.56	3.19	4.16	**0.00**	3.39	7.95	9.38	8.16	13.00	***16.95***	13.62
+3-Br	0.45	3.11	4.04	**0.00**	3.24	7.70	9.32	8.12	12.90	***16.70***	13.78
+3-Cl	0.40	3.08	4.02	**0.00**	3.13	7.61	9.26	8.07	12.83	***16.58***	13.82
+3-F	0.19	2.92	3.90	**0.00**	2.91	7.32	9.12	7.95	12.62	***16.26***	14.00
+3-OCH3	0.69	3.32	4.40	**0.00**	3.25	8.05	8.96	7.89	12.95	***16.58***	13.56
+3-OH	0.61	3.22	4.12	**0.00**	3.10	7.84	9.17	7.98	12.97	***16.63***	13.74
+3-CH_3	0.75	3.37	4.38	**0.00**	3.08	7.97	9.14	7.90	13.01	***16.70***	13.46
+3-NH_2	0.70	3.36	4.49	**0.00**	2.90	7.91	8.98	7.74	12.88	***16.52***	13.45
+3,4-Cl	0.17	2.90	3.87	**0.00**	3.07	7.37	9.25	8.07	12.65	***16.39***	14.00
+3,4-OCH_3	0.44	3.11	4.13	**0.00**	2.93	7.66	9.07	7.91	12.73	***16.22***	13.88

Note: **Bold** numbers are minimum for row (analog). ***Bold italic*** numbers are maximum for row (analog).

symmetrical substituent analogs (that is, the upper 30 MP analogs would be in the negative subfamily, and the lower 30 analogs would be in the positive subfamily, with the middle 15 being represented in each subfamily).

Across each row, the energies shown are relative to the lowest energy conformation for each analog. Thus, many of the energy values for pMP analogs based on the pMP GEM, Conformer 8 in Family 4, are zero, indicating that of the conformations found, the conformation based on the Family 4 template is the lowest relative energy. There were a few relative lowest energy conformations in Family 1, and one in Family 3. There is no assumption made in terms of absolute GEM for each MP analog, these results are only relative to the conformations found. Zero value analog conformations are shown in bold type.

The conformations with the largest energies relative to the set of analog conformations created are shown in bold italics. Families 3 and 11 have some analogs with the highest relative energy compared to the rest of the conformations. Family 9 has one analog with the highest relative energy among the families. Family 10 had many analogs that were in the highest energy conformation of all families.

Two examples of the overlap of the pMP analogs within a subfamily are shown in Figure 4.4. Aligning the molecules on the central carbon and its surrounding carbons produced a good visual alignment, canceling out the effects of the ester side chain by exactly aligning them for each analog. The relative alignment is exact because the pMP analogs were built from the same template.

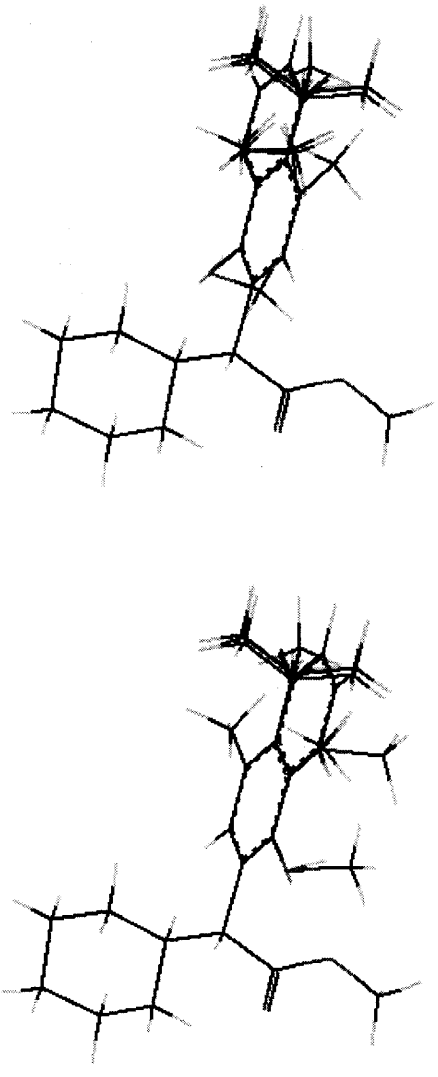
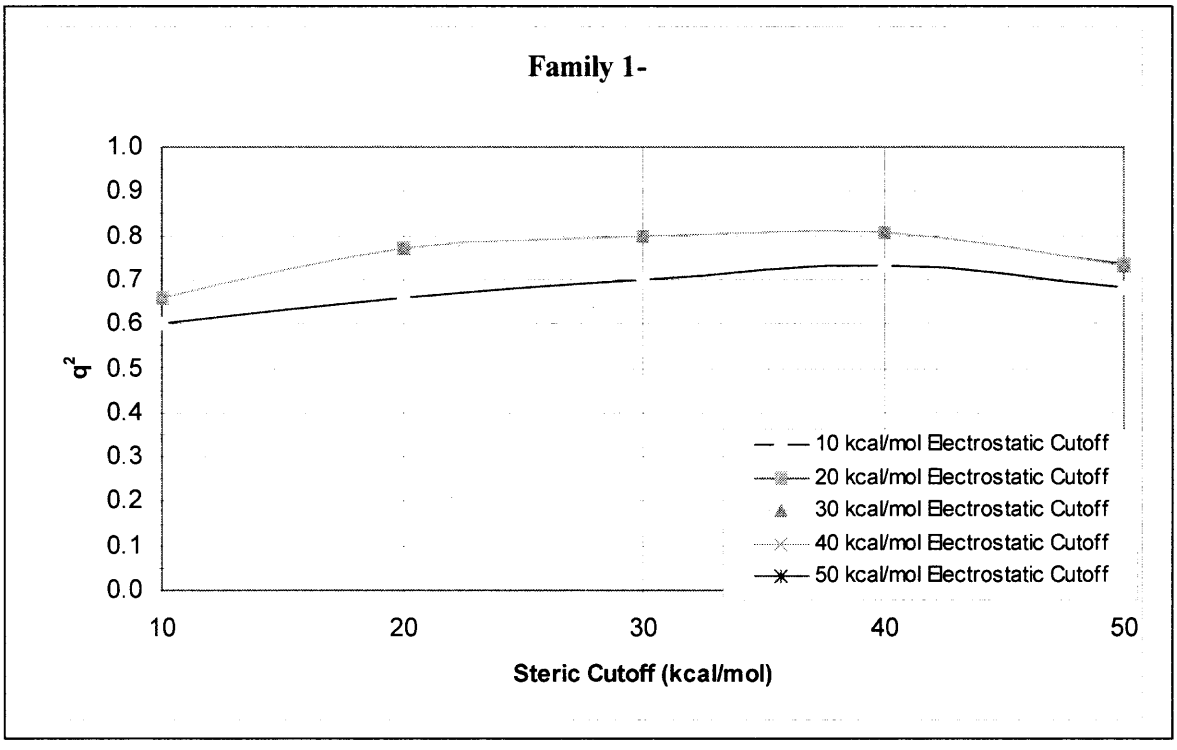


Figure 4.4 Conformations of Family 1+ and Family 1- MP Analogs

4.3 Part I CoMFA Results

The Part I CoMFA were performed using 25 CoMFA columns as identified in the Methods section. Figure 4.5 contains graphs of the results of the Part I CoMFA for Family 1- and Family 1+. The overlap of the graphs for each electrostatic cutoff indicates that there is little effect of raising the cutoff at this range. The results for the remaining families are contained in Appendix C.



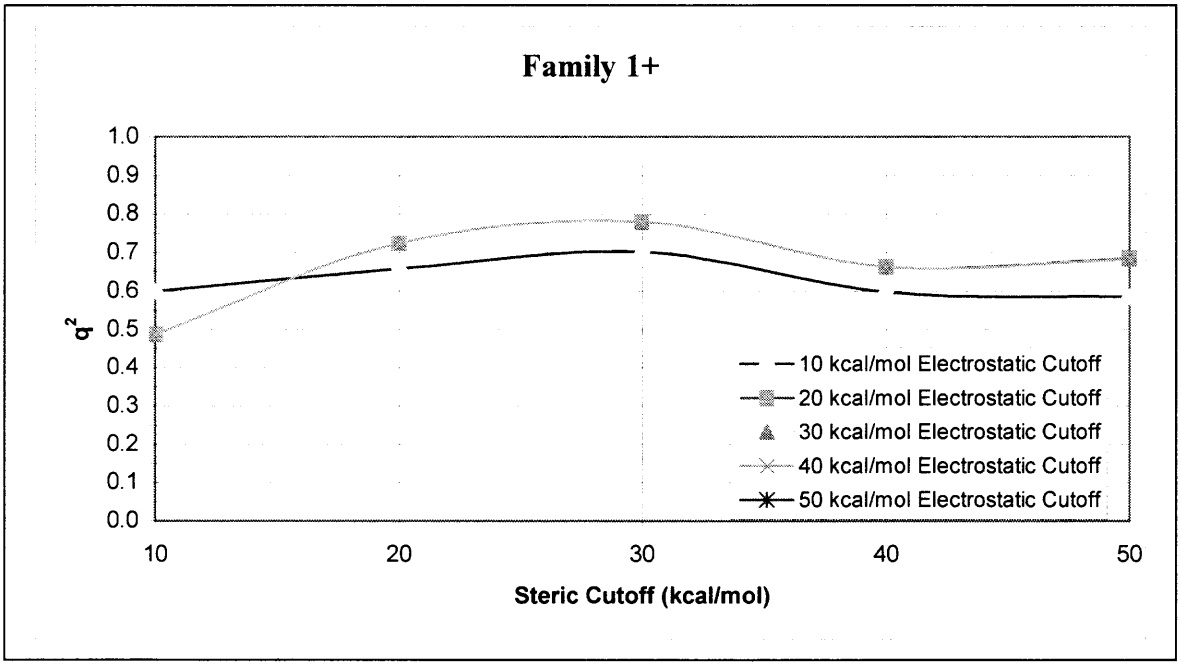


Figure 4.5 Part I CoMFA Results – Family 1

In some cases, the data for different electrostatic cutoffs, as represented by the different curves on a graph, were the same and overlapped. In Family 1-, the results for 10 kcal/mol and 20 kcal/mol electrostatic cutoffs were different, but the results for the remaining electrostatic cutoffs were almost identical and thus overlapped. This can be interpreted as there being no electrostatic potential values above a certain point, or a large jump in the electrostatic potential values, such that increments between potentials are larger than 10 kcal/mol.

Table 4.3 contains the data for the best q^2 and r^2 obtained for the 22 subfamilies, as well as the number of components and the steric (denoted "S" in the table) and electrostatic (denoted "ES" in the table) cutoffs in kcal/mol. The range of q^2 was from a minimum of 0.532 for Family 10- to a maximum of 0.808 for Family 1-, indicating a high predictability for all families. The values for r^2 ranged from 0.900 to 0.995, indicating a high goodness-of-fit for all of the families.

In Table 4.3, the % steric value is the percentage of the best PLS model that is based on steric potential values of grid points. The % electrostatic value is the percentage of the best PLS model that is based on electrostatic potential values of grid points. It could be expected that the results of the pMP CoMFA should show more electrostatic effects than the nMP results, but the proportions of % electrostatic to % steric are similar.

The ranges of q^2 showed that models with a high degree of predictability (q^2 more than 0.5) were achieved with all template molecules within 20 kcal/mol of the pMP GEM. Three of the four best q^2 were achieved with subfamilies from Family 4 (based on the GEM) and Family 1 (based a pMP template with an energy 0.662 kcal/mol more than the GEM).

Table 4.3 Best q^2 (CV PLS) and r^2 (Full PLS) Values – Part I CoMFA

Fam.	T1	T2	Best q^2	Comp.	Cutoffs	r^2	% Steric	% Electrostatic
1-	-169.17	-103.6	**0.808**	10	S040E20	0.992	56.2%	43.8%
1+	-169.17	+103.6	0.779	6	S030E20	0.977	59.3%	40.7%
2-	-122.09	-100.3	0.613	4	S040E20	0.900	64.7%	35.3%
2+	-122.09	+100.3	0.676	5	S020E20	0.949	65.8%	34.2%
3-	-100.12	-143.3	0.568	5	S050E30	0.953	68.6%	31.4%
3+	-100.12	+143.3	0.703	10	S020E40	0.992	69.3%	30.7%
4-	-70.85	-80.3	0.710	7	S050E30	0.977	63.4%	36.6%
4+	-70.85	+80.3	0.573	7	S010E10	0.957	65.2%	34.8%
5-	-64.74	-17.4	0.757	10	S050E30	0.995	68.3%	31.7%
5+	-64.74	+17.4	0.574	9	S030E10	0.992	69.0%	31.0%
6-	-143.64	-19.7	0.732	10	S030E20	0.991	70.4%	29.6%
6+	-143.64	+19.7	0.694	9	S040E20	0.991	51.7%	48.3%
7-	54.63	-95.2	0.632	10	S050E20	0.991	61.0%	39.0%
7+	54.63	+95.2	0.650	4	S010E20	0.915	66.8%	33.2%
8-	61.92	-92.4	0.607	5	S050E30	0.937	73.2%	26.8%
8+	61.92	+92.4	0.608	7	S040E10	0.962	59.5%	40.5%
9-	115.44	-89.5	0.661	5	S010E20	0.923	72.2%	27.8%
9+	115.44	+89.5	0.689	9	S020E10	0.990	64.8%	35.2%
10-	82.37	-24.0	0.532	10	S040E30	0.985	69.0%	31.0%
10+	82.37	+24.0	0.660	7	S040E10	0.977	49.8%	50.2%
11-	2.35	-97.2	0.785	10	S040E20	0.994	63.5%	36.5%
11+	2.35	+97.2	0.586	5	S020E20	0.952	62.4%	37.6%

4.4 Part II CoMFA Results

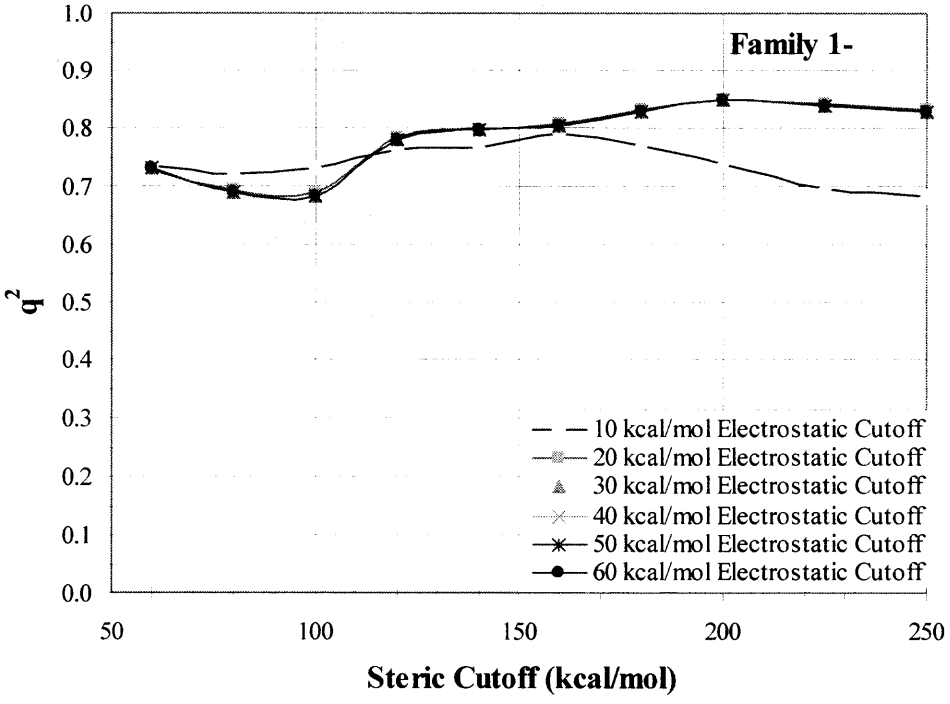
Two families were selected for the Part II CoMFA. Family 4 was selected because it is based on the pMP GEM conformer. Family 1 was selected because one of its subfamilies yielded the highest q^2 from the CV PLS runs, which indicates a high level of predictability in the model. It is also a good candidate for the bioactive conformation because the energy of its template is only 0.662 kcal/mol above that of the GEM.

Figure 4.6 and Figure 4.7 show the results of the Part II CoMFA. For Family 1+, the model with the highest predictability (q^2 = 0.779) was reached again with another set of steric and electrostatic cutoffs. The lower set of cutoffs was used for the full PLS runs to use less data points and therefore yield a simpler model. For Family 1-, the combination of 200 kcal/mol steric potential cutoff and 20 kcal/mol electrostatic potential cutoff yielded an excellent predictability (q^2 = 0.850), improved from a maximum q^2 of 0.800 in the Part I CoMFA. The best PLS had 10 components, indicating a somewhat complex model, but still within the limit set in this study. The PLS model was 56.2 % due to steric interactions and 43.8 % due to electrostatic interactions. The σ value yielding the best model was set to filter grid points with steric and electrostatic potentials within 1 kcal/mol of each other. The F value was 244.908, among the highest in the study but not the highest value. r^2 was 0.992, showing a high goodness-of-fit, which might indicate overfitting if the q^2 value was lower, but should be acceptable with a high q^2.

The results for the Part II CoMFA of Family 4 yielded a best q^2 less than 0.8. The Family 4 PLS models had approximately the same amount of steric and electrostatic contribution as the Family 1 PLS models.

4.5 Best PLS Model

Family 1- yielded the best model of MP analogs' binding affinities for the DAT. Figure 4.8 shows a plot of the actual (experimental value) pIC50's and the pIC50's predicted by the S200E020 (that is steric cutoff = 200 kcal/mol and electrostatic cutoff = 20 kcal/mol) σ=2 model for Family 1-. Each point represents a pMP analog. The points fit the



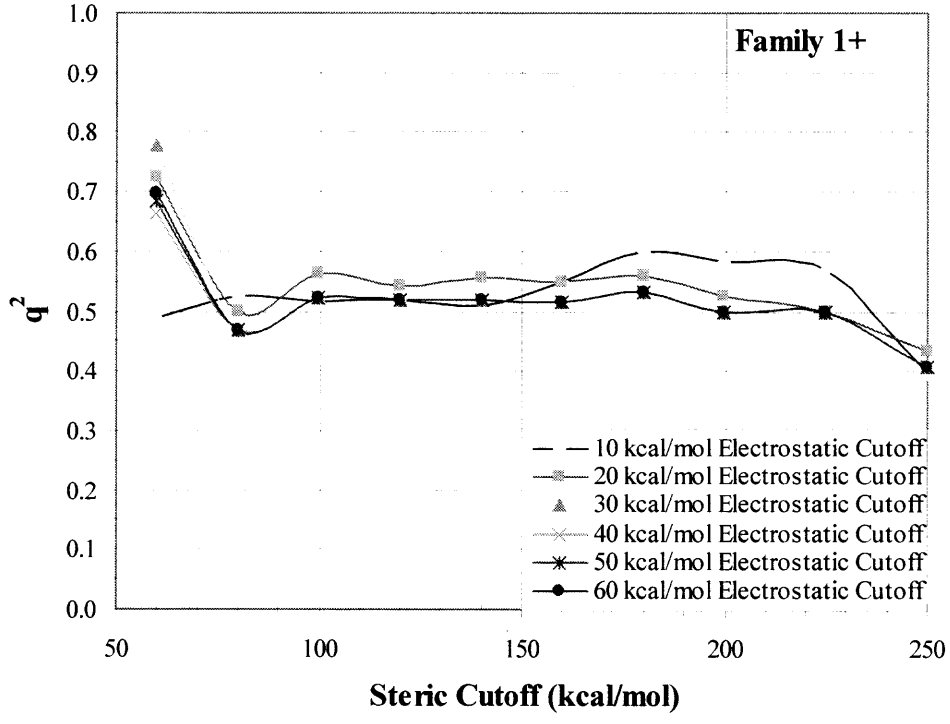


Figure 4.6 Part II CoMFA Results – Family 1

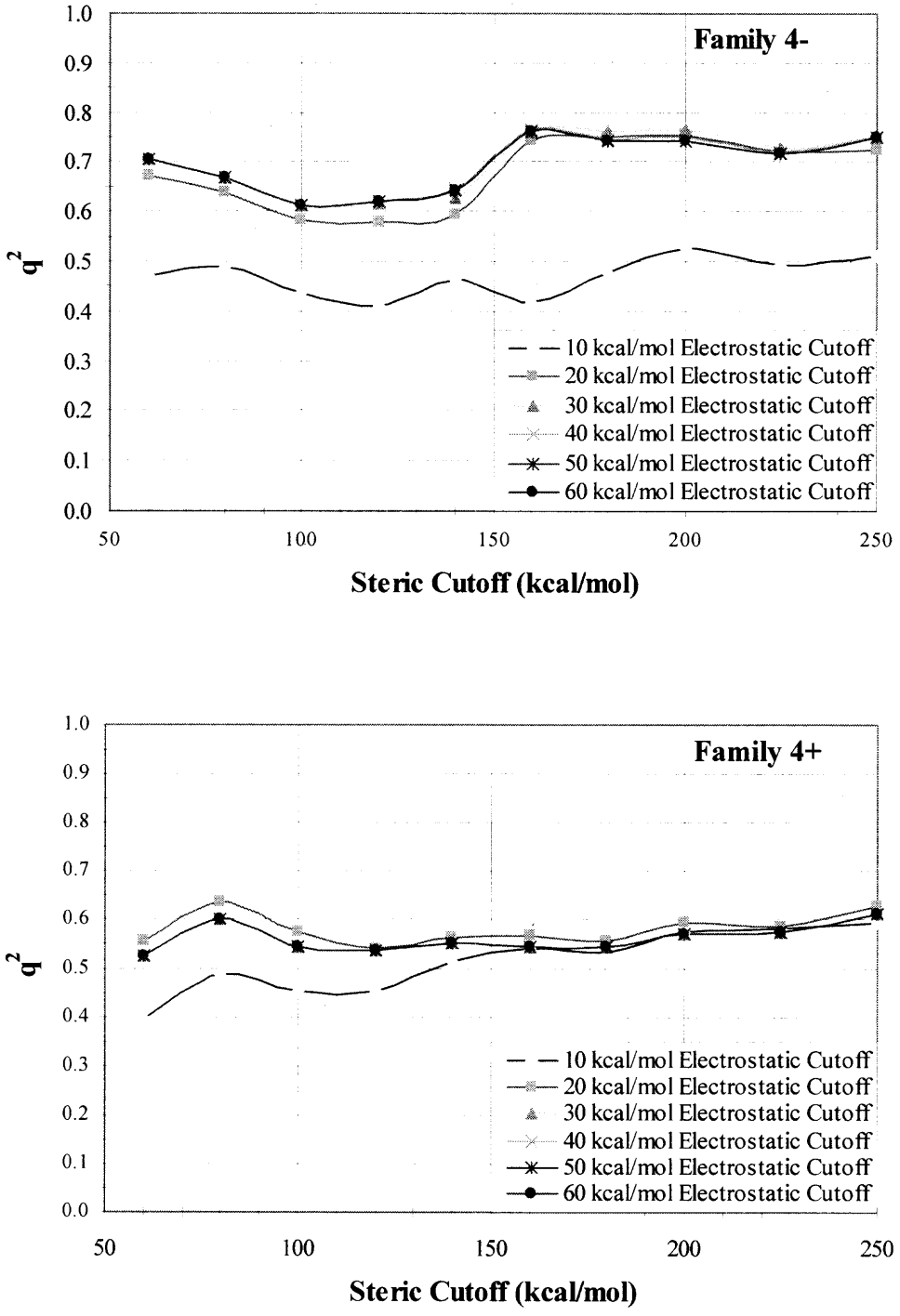


Figure 4.7 Part II CoMFA Results – Family 4

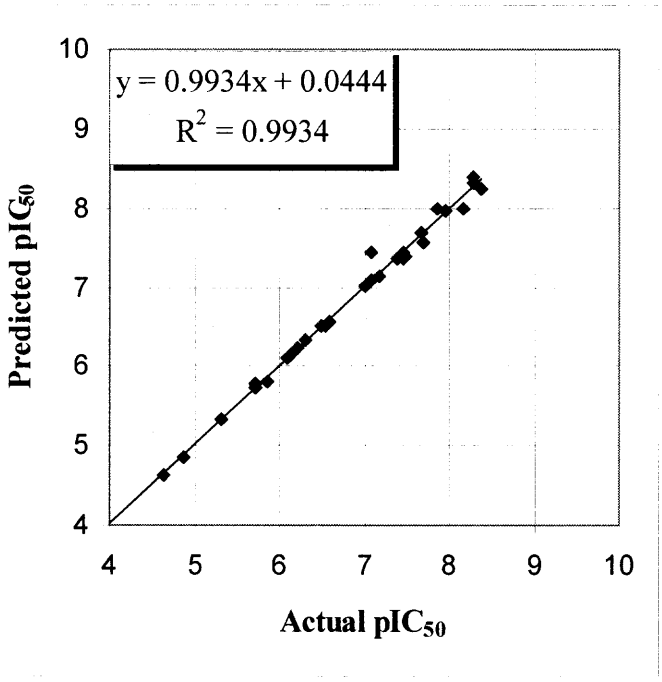


Figure 4.8 Actual pIC_{50} versus Predicted pIC_{50} for Family 1-S200E020 σ=2 Model

diagonal well (correlation $R^2 > 0.99$) and are evenly dispersed. One point near an actual $pIC_{50} = 7$ is somewhat of an outlier; this point is the template molecule with no substituents. Since this study focuses on phenyl ring substituents, it may not be as useful to predict the pIC_{50} for the unsubstituted phenyl ring. This model will be used to propose a pharmacophore for MP analogs' binding affinities with the DAT.

CHAPTER 5

CONCLUSIONS

CoMFA of the phenyl-substituted analogs of pMP provided statistically predictable and well-fit models. The proposed bioactive conformation was selected using the best q^2 (predictability) value from two conformations that were studied in detail. The proposed MP analog DAT binding pharmacophore based on the Family 1- PLS model has the torsional angles T1~170° and T2 ~ -100°. This would be qualitatively described as the phenyl group almost *anti* to the piperidine ring, relative to the central carbon, and the phenyl group rotated to be perpendicular to the piperidine ring, with any substituents oriented away from the ester side chain.

The pharmacophore can be described more generally by the distance between the piperidine nitrogen and the third carbon on the phenyl ring. The third carbon was selected because the best IC_{50} was seen with 3-Br. This value is 7.55 Å in the selected model. The IC_{50} data further indicates that an electronegative substituent in the third position on the phenyl ring improves binding. Figure 5.1 shows the two different descriptions of the pharmacophore.

The pMP CoMFA studies produced models that were highly predictive of the DAT binding affinity of the 30 analogs studied, as experimentally measured as the ability of an MP analog to displace [^{3}H] WIN 35,428 IC_{50} from the DAT. Although the meaning in terms of general trends is difficult to interpret, the PLS model could be used to estimate the pIC_{50} of new compounds. The results indicate that the Inertial alignment method used in this study was satisfactory, since highly predictive models were

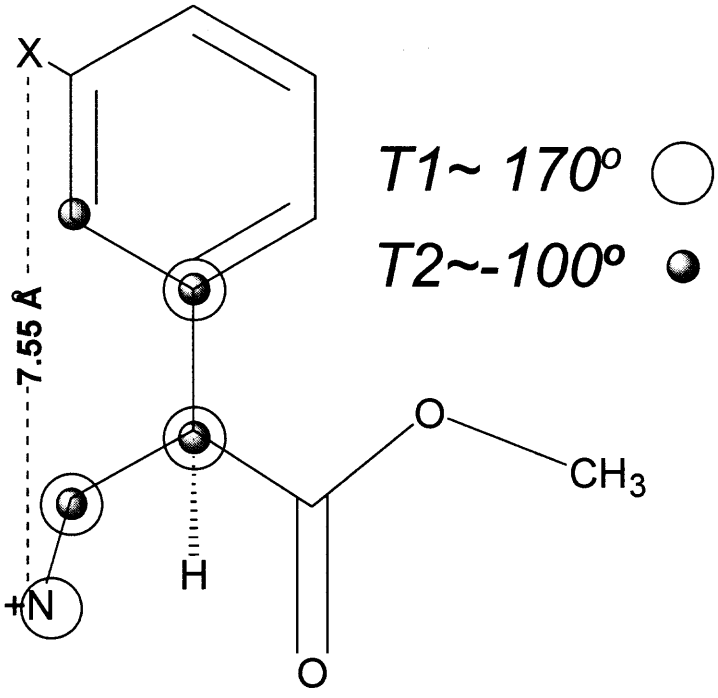


Figure 5.1 pMP Molecule with Torsional Angles Used in the Proposed Pharmacophore

produced. The alignment was simplified because of the 30 analogs are closely related; studies with more varied sets of more flexible compounds would require more detailed relative and absolute alignment considerations.

CHAPTER 6

DISCUSSION AND PERSPECTIVES

These results help determine key factors in the binding of MP and related structures. These results are compared here to the nMP CoMFA results in three ways, comparing conformer families, relative energies of analogs, and q^2 and r^2 results. It was found that the nMP and pMP CoMFA studies produced similar results, with no new conformations identified by protonating the molecule.

6.1 Comparison of Conformer Families

Table 6.1 shows the data for the templates for the nMP and pMP CoMFA. The Study Relative Energy shown is relative to the other MP conformations found in each study. The energies for nMP families are compared to other nMP families and the energies for the pMP families are compared to other pMP families; they are not compared between the nMP and pMP studies. Although there is one additional family used in the pMP studies, the nMP and pMP conformer families are relatively similar and pMP Family 7 and pMP Family 8 could have been combined for this study (see Figure 4.1).

nMP Family 3 (T1=19.6° and T2=-132.9°) is the most different from the other nMP and pMP families. pMP Family 2 (T1=-70.9° and T2=-80.5°) was the most different of the pMP families. The pMP families studied in Part II CoMFA were similar to nMP families. GEM pMP Family 4 (T1=-70.9° and T2=-80.5°) was very similar to nMP Family 5 (T1=-66.8°, T2=-76.5°). pMP Family 1 (T1=-169.17°, T2=-103.6°) was similar

Table 6.1 Comparison of pMP and nMP Conformer Families

Family	#	T1 (°)	T2 (°)	Study Relative Energy (kcal/mol) and Ranking
pMP 1	1	-169.17	-103.6	0.662 (pMP Rank 2)
nMP 9	*14*	*175.6*	*-105.7*	*0.00 (nMP Rank 1)*
nMP 10	*8*	*173.4*	*-73.32*	*14.6 (nMP Rank 10)*
pMP 2	14	-122.09	-100.3	3.282 (pMP Rank 4)
pMP 3	25	-100.12	-143.3	4.209 (pMP Rank 5)
nMP 6	*50*	*-103.4*	*-139.94*	*5.08 (nMP Rank 6)*
pMP 4	8	-70.85	-80.3	0.000 (pMP Rank 1)
nMP 5	*12*	*-66.8*	*-76.5*	*2.30 (nMP Rank 3)*
pMP 5	12	-64.74	-17.4	3.216 (pMP Rank 3)
nMP 7	*21*	*-70.4*	*-8.79*	*3.69 (nMP Rank 4)*
pMP 6	13	-143.64	-19.7	7.876 (pMP Rank 6)
nMP 8	*63*	*-139.5*	*-27.7*	*7.05 (nMP Rank 9)*
pMP 7	2	54.63	-95.2	9.328 (pMP Rank 8)
pMP 8	6	61.92	-92.4	8.134 (pMP Rank 7)
nMP 1	*9*	*63.9*	*-85.3*	*1.22 (nMP Rank 2)*
pMP 9	23	115.44	-89.5	13.071 (pMP Rank 9)
pMP 10	26	82.37	-24	16.819 (pMP Rank 11)
nMP 2	*18*	*58.9*	*-13.6*	*3.71 (nMP Rank 5)*
pMP 11	21	2.35	-97.2	13.616 (pMP Rank 10)
nMP 3	**35**	*19.6*	*-132.9*	*5.21 (nMP Rank 7)*
nMP 4	*55*	*-13.8*	*-116.57*	*6.91 (nMP Rank 8)*

to nMP Family 9 (T1=175.6°, T2=-105.7°). The GEM conformations were different for the nMP and pMP studies. The proposed bioactive conformation found in the pMP studies was within 6.5° for T1 and 2.1° for T2 as compared to the GEM for the nMP study. nMP Conformer 50 was selected as the most probable bioactive conformation for the nMP study, but its nearest counterpart pMP Conformer 25 does not appear to be the bioactive conformation from the results of this study. The nMP GEM and pMP Conformer 1 both produced relatively high q^2 values in their respective studies, which supports the pharmacophore proposed here.

6.2 Comparison of Relative Energies

The creation of conformers of analogs allows comparison across a set of analog conformers. For the nMP analogs, the lowest relative energies were seen with Family 9, although for unsymmetrical analogs there was a split between Family 9's positive T2 and negative T1 subfamilies for the lowest energies. The highest energies were found with nMP Family 10 for the most part. nMP Family 3+ showed the highest energies for 2-Br and 2-Cl analogs, and Family 2- showed the highest energy for the 3,4-OCH_3 analog.

6.3 Comparison of q^2 and r^2

The highest q^2 achieved in this study was 0.850 for Family 1-. The highest q^2 achieved in the nMP CoMFA was 0.765. It is not clear in the literature whether a difference of 0.085 is significant, but it can be said qualitatively that the CoMFA results for pMP correlate better with the WIN 35,428 IC_{50} DAT binding data than the nMP CoMFA results. It is obvious from the close relationships between the nMP and pMP conformer families that there is not a major difference in the neutral and protonated conformations. The differences in the predictiveness and goodness-of-fit of the models could be due to different electrostatic potential fields, but further studies are needed to confirm this.

6.4 Potential for Future Work

Further analysis and development of the pharmacophore with other DA reuptake inhibitors would be useful. Another interesting study would be to compare the pharmacophore to those for other neurotransmitter systems, or develop pharmacophores

in a similar way for those systems. Synthesis and testing of a rigid analog that has several key features of the proposed pharmacophore would be useful to experimentally check these results.

Another area of research could be using molecular modeling to look into the role of zinc atoms in DA reuptake inhibitor binding. Radiolabelled DA and WIN 35,428 experiments have been performed using zinc atoms, but it is not clear what level of molecular modeling research has been done in this area (Norregard, 1998). Molecular modeling studies are best carried out in tandem with experimental work to take full advantage of latest research techniques and the multi-disciplinary approach to scientific problems.

APPENDIX A

SOFTWARE SPECIFICATIONS

This appendix contains the software specifications and parameters used for the Sybyl molecular modeling program used to complete the CoMFA studies of the pMP analogs.

Software: Sybyl®/Base Version 6.4

Manufacturer: Tripos, Inc., St. Louis, Missouri, USA

Platform: SGI Irix 6.5

Sybyl® CoMFA and PLS Parameters:

All settings left as default, except for changes in electrostatic and steric cutoffs.

CoMFA Field Class: Tripos Standard

Field Values: Type(s): Both
Dielectric: Distance
Smoothing: None

Drop Electrostatics: Within Steric Cutoff for Each Row

Transition: Smooth

Region: Create Automatically

PLS: "Use SAMPLS" box unchecked

For cross-validated (leave-one-out) runs:
(Maximum Number of) Components: 10
"Leave-One-Out" box checked

For full PLS runs:
No Validation checked
Components: (as found in best cross-validated run)

APPENDIX B

SAMPLE LINES FROM SLN MACRO

In this appendix, a portion of an SLN macro is included as an example of the macros used to create the pMP analogs.

```
# Macro Name: makeFamilies.spl
# Author: K. Gilbert (based on Milind Misra's modifyType.spl) (New Jersey Institute of Technology)
# Date: March 6, 2001
# Function: To create 2,3,4,34,35 analogs of a database of methylphenidate conformers.
# Note: have source database (all.mdb) open when running the program.

# UIMS DEFINE MACRO makeFamilies SYBYLBASIC YES

for i in %range(1 %count(%database(*)))
# get rid of last molecule
  close database
  zap m1
# first set up family database
  database create %mol_info($i name)
# do first set of 2- analogues
# 2-BrA
  database open all.mdb update
  database get "%arg($i %database(*))" m1
  database close
  modify atom type 36 "Br"
  modify molecule name m1 "2-Br_A" & %mol_info($i name)
  database add m1
  zap m1
# 2-ClA
  database open all.mdb update
  database get "%arg($i %database(*))" m1
  database close
  modify atom type 36 "Cl"
  modify molecule name m1 "2-Cl_A" & %mol_info($i name)
  database add m1
  zap m1
```

(program continues in a similar manner for each analog)

APPENDIX C

PART I CoMFA RESULTS: FAMILIES 2 THROUGH 11

Part I CoMFA results not contained in the main text are included in this appendix.

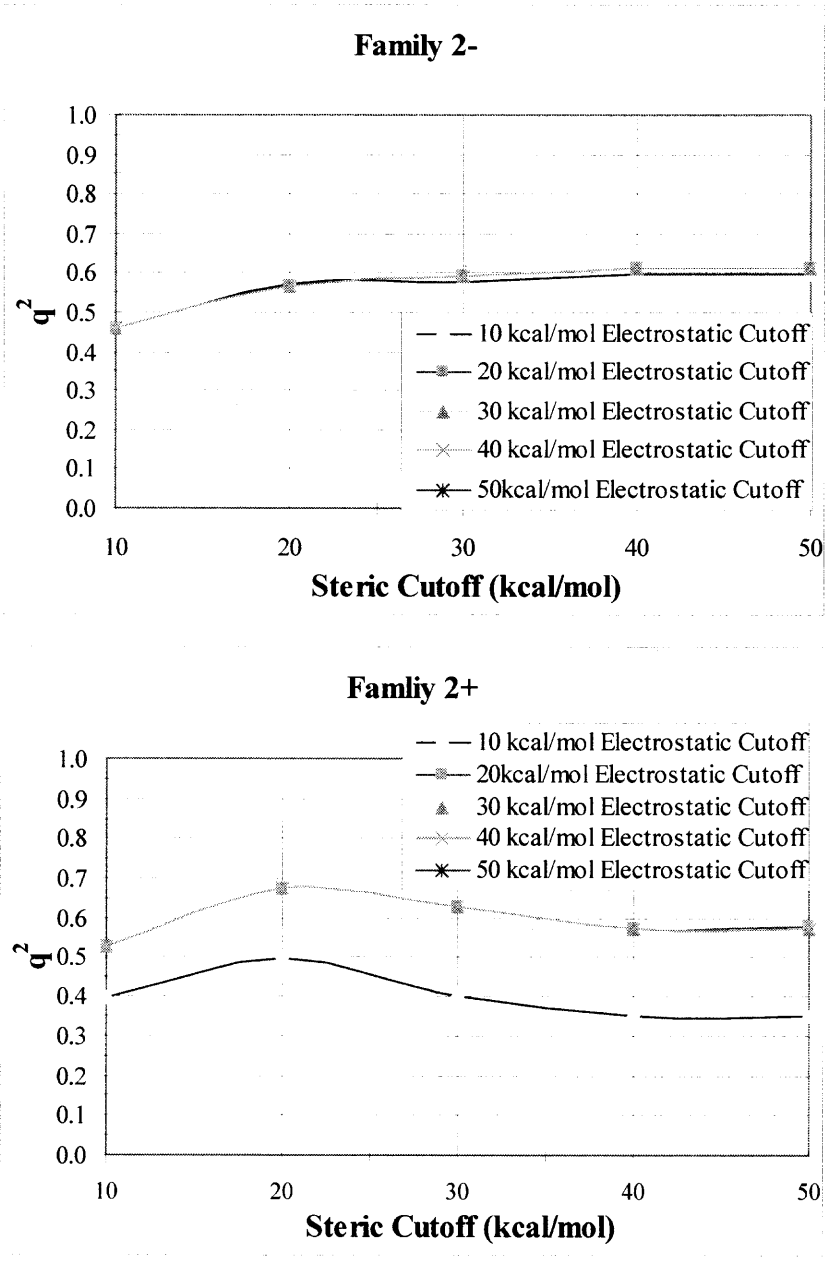


Figure C.1 Part I CoMFA Results – Family 2

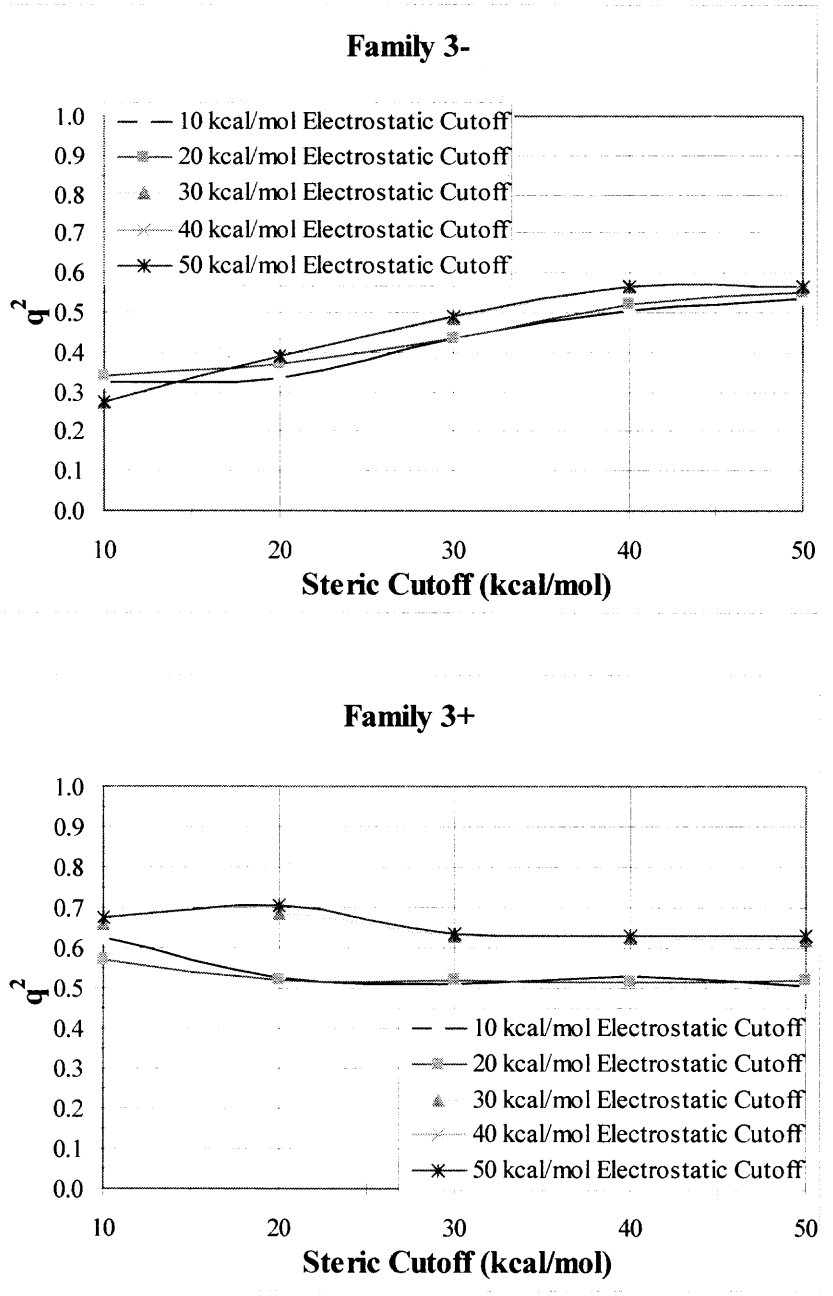
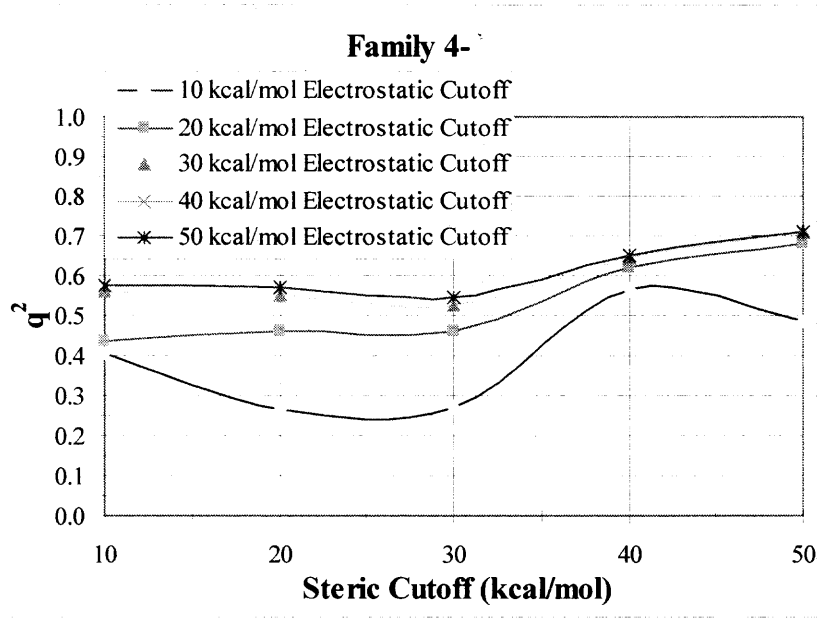


Figure C.2 Part I CoMFA Results – Family 3



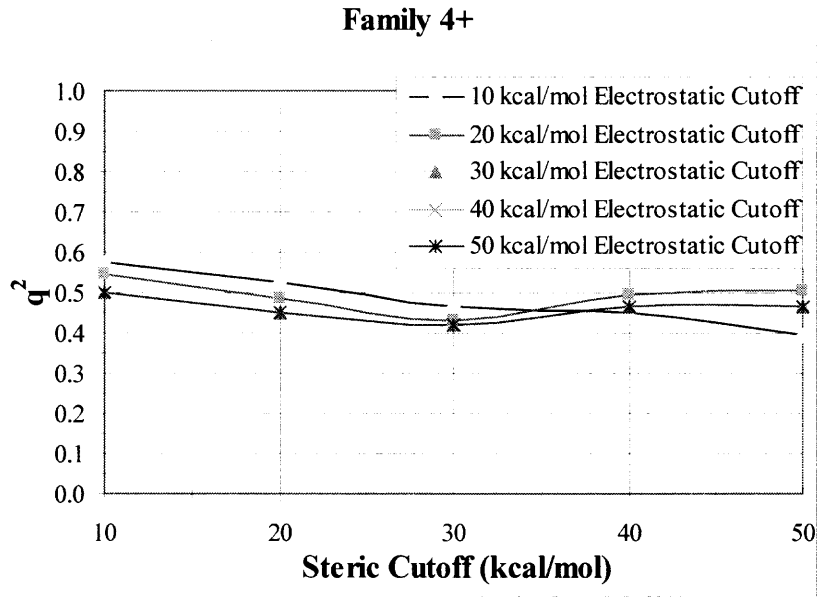


Figure C.3 Part I CoMFA Results – Family 4

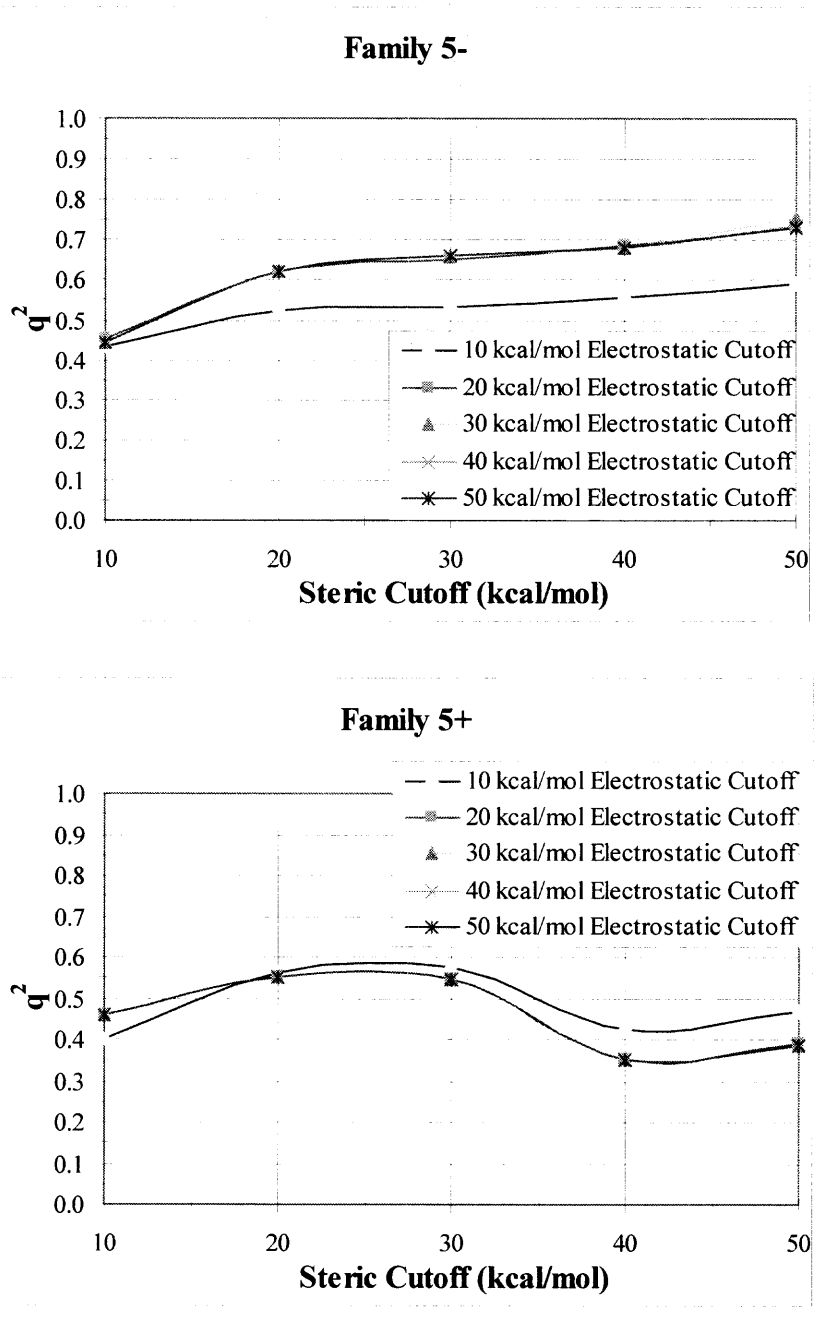


Figure C.4 Part I CoMFA Results – Family 5

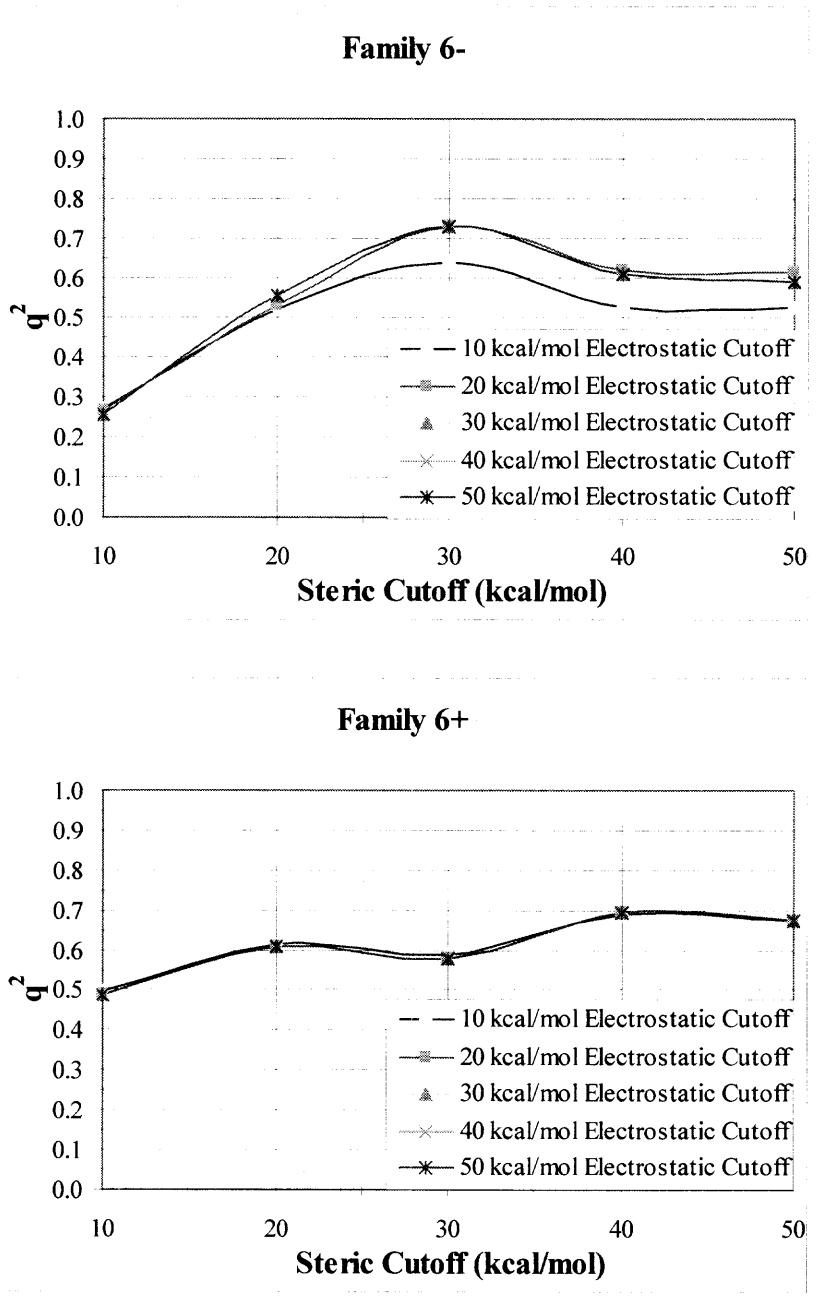


Figure C.5 Part I CoMFA Results – Family 6

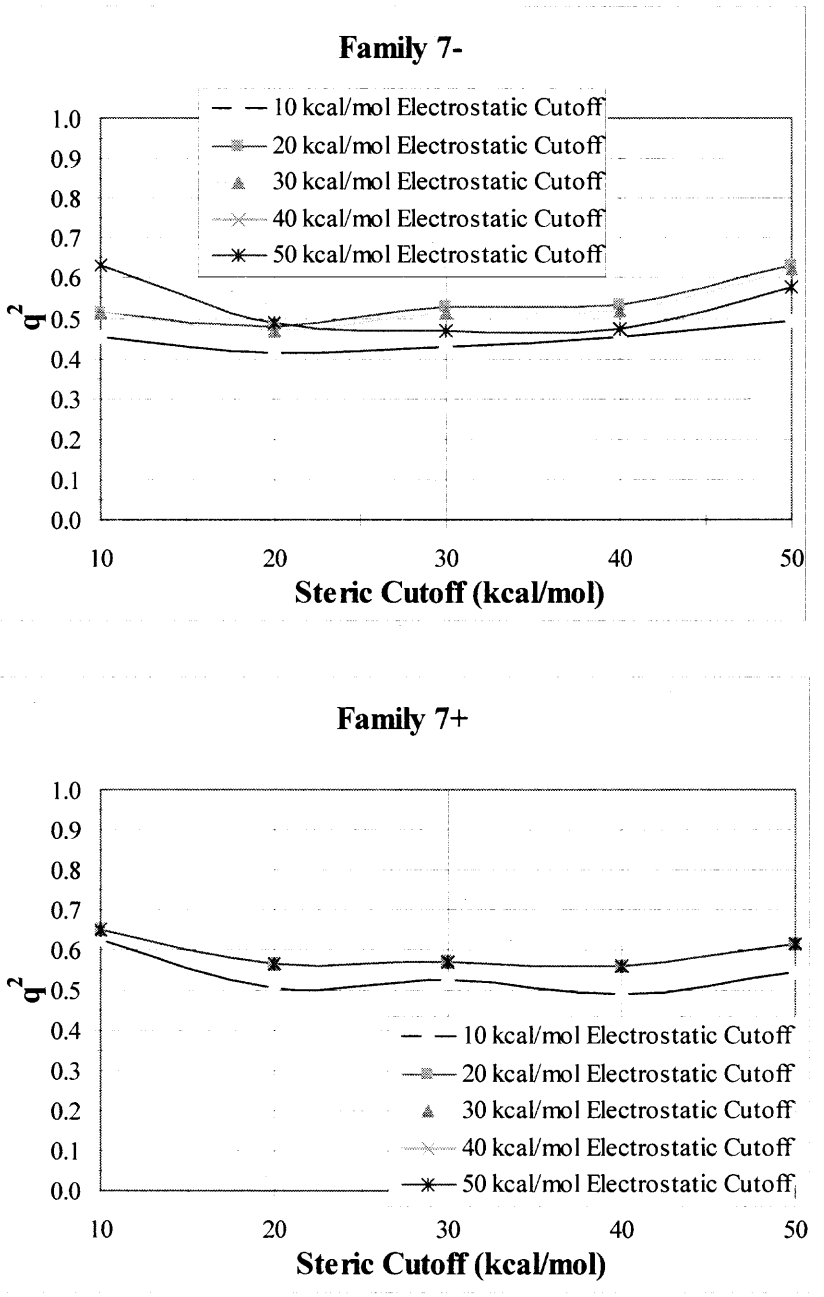


Figure C.6 Part I CoMFA Results – Family 7

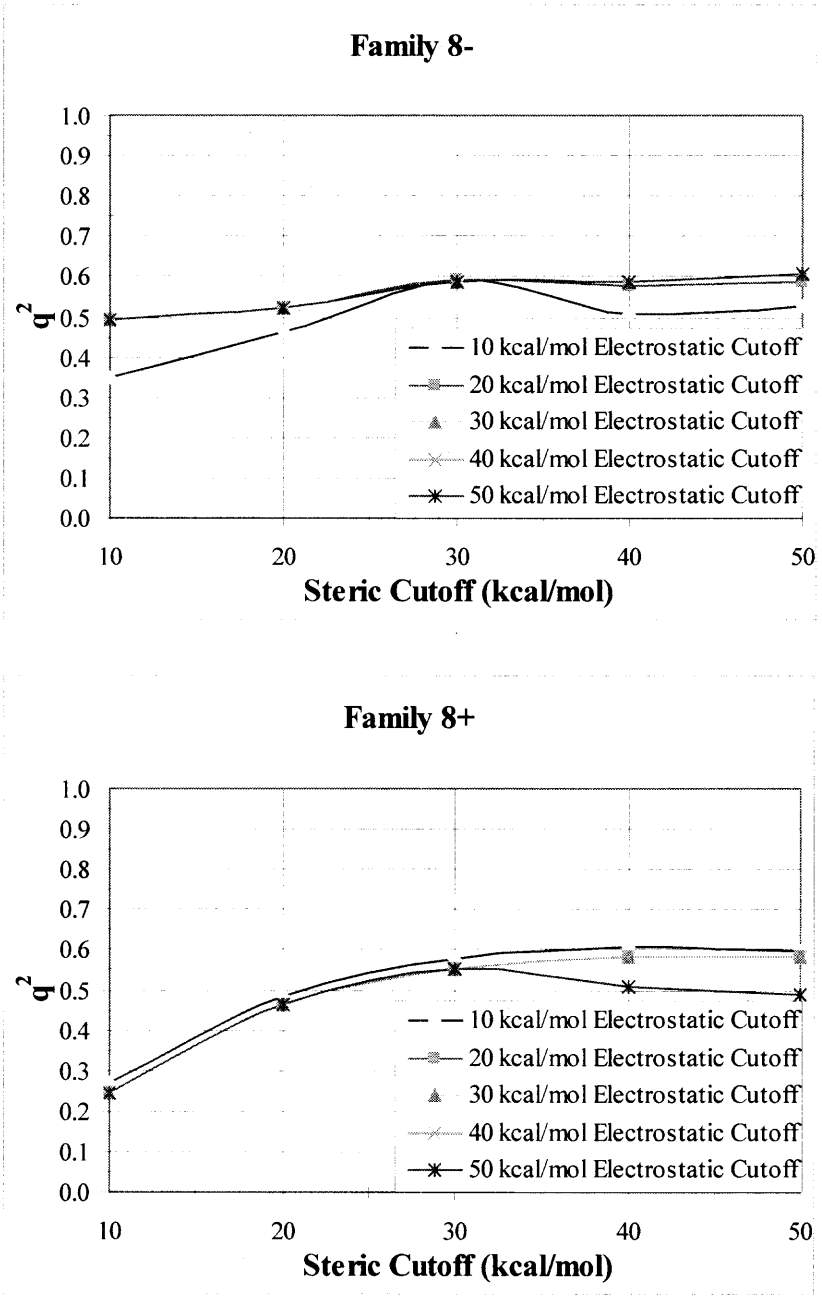


Figure C.7 Part I CoMFA Results – Family 8

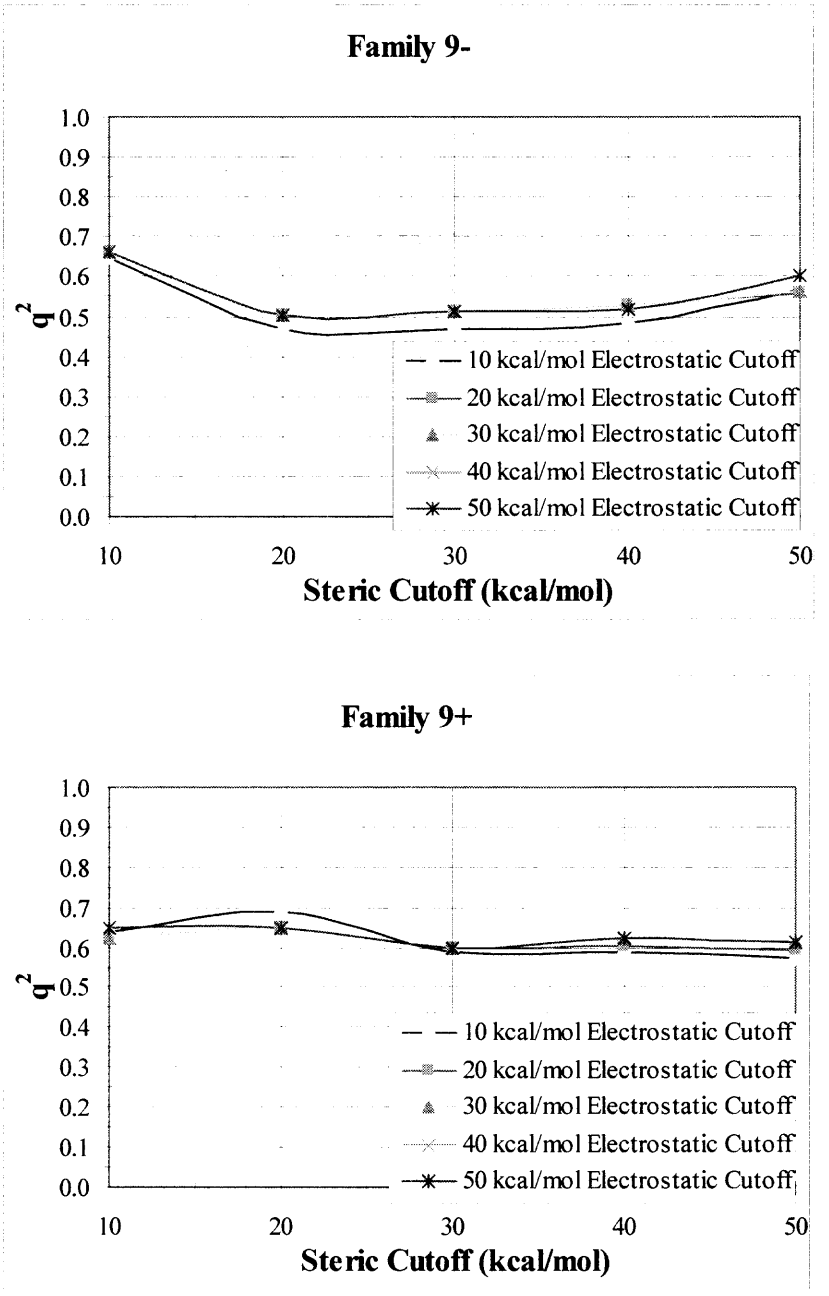


Figure C.8 Part I CoMFA Results – Family 9

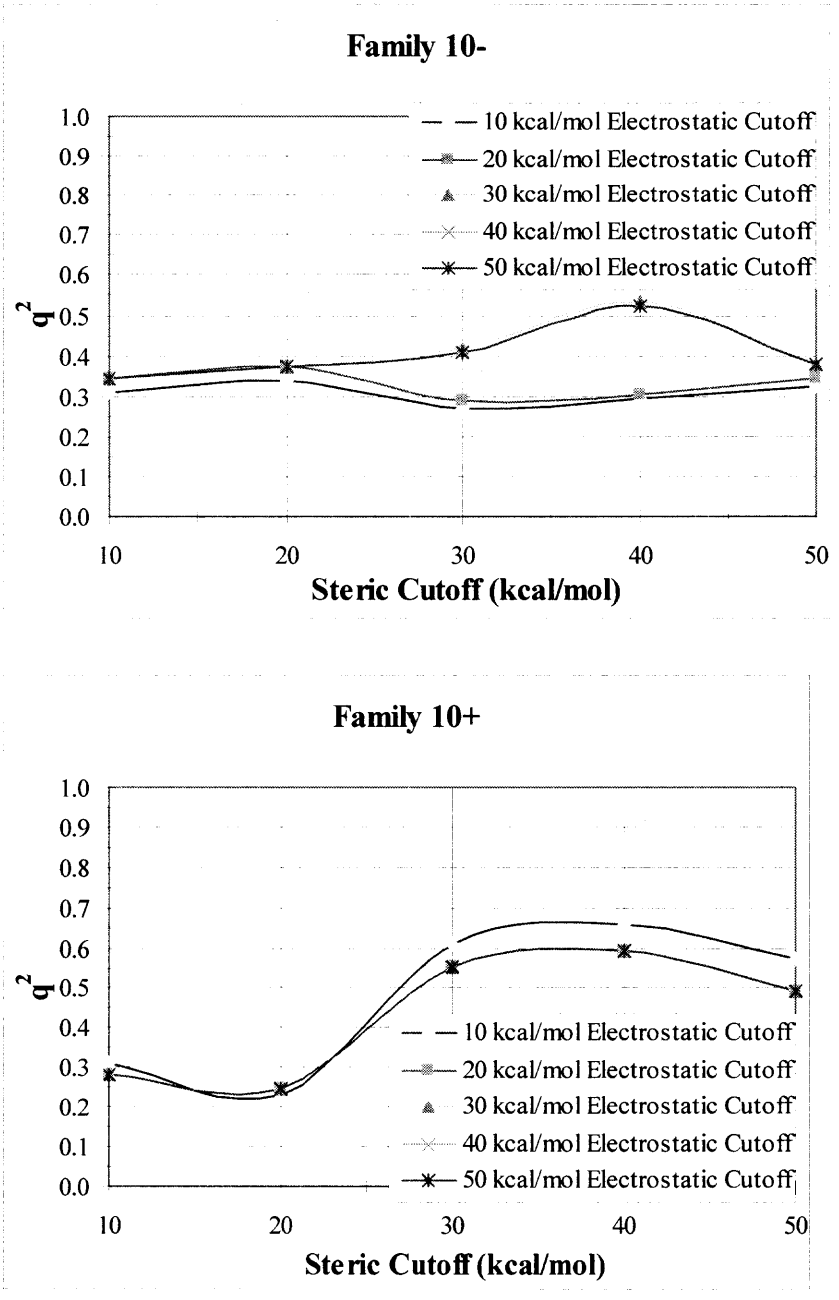


Figure C.9 Part I CoMFA Results – Family 10

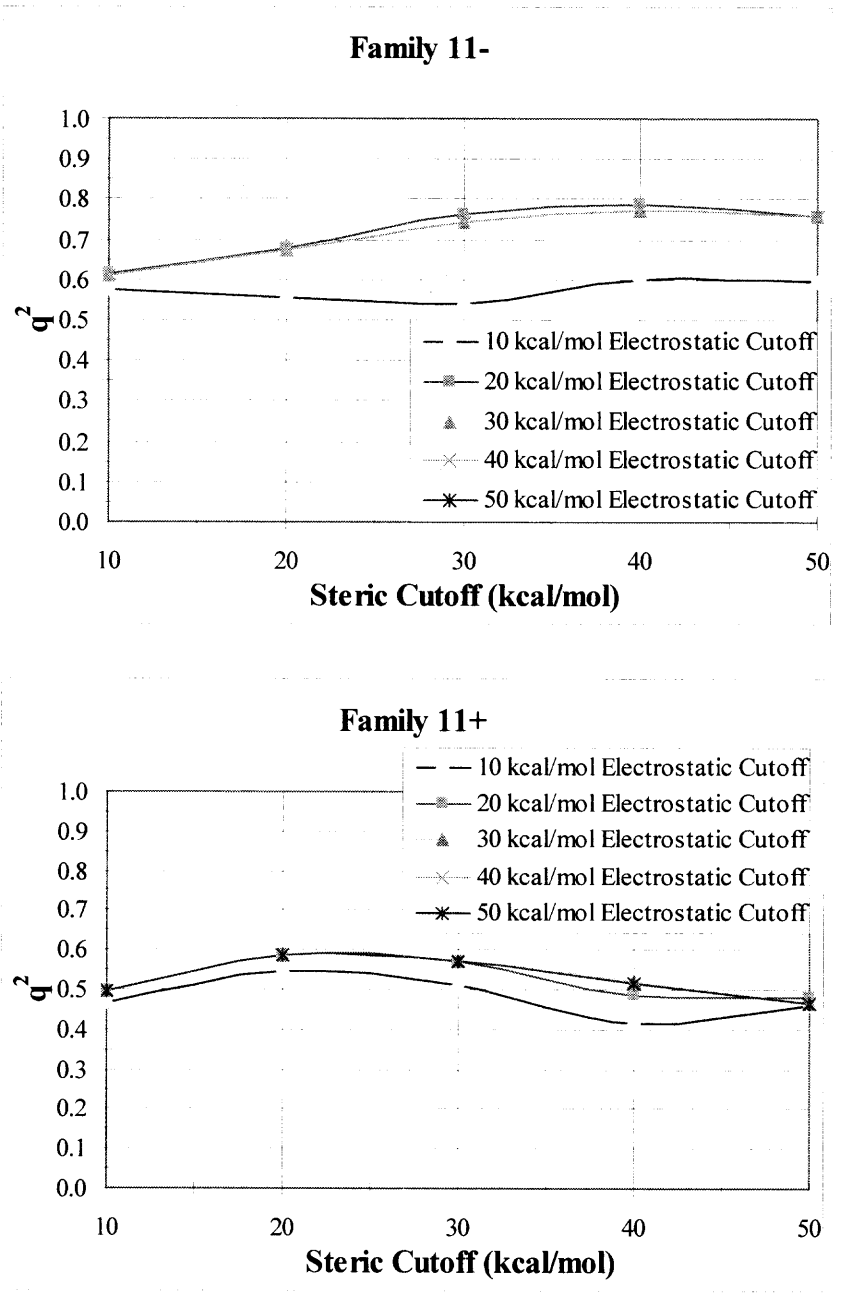


Figure C.10 Part I CoMFA Results – Family 11

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