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Development of pharmacophore and CoMFA studies for sigma2 receptor ligands

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ABSTRACT

DEVELOPMENT OF PHARMACOPHORE AND CoMFA STUDIES FOR σ_2 RECEPTOR LIGANDS

by
Laura Ann Wirpsza

This study describes the development of a pharmacophore and CoMFA model for sigma 2 (σ_2) receptor ligands. CoMFA studies were performed for 32 bioactive σ_2 receptor ligands using the radioligand [H^3] (+) DTG in the presence of pentazocine. The pharmacophore was derived using Distance Comparisons (DISCOtech) from eight partially to highly active σ_2 receptor ligands. All 32 compounds were calculated in three methods: AM1, HF/3-21G*, and B3LYP/3-21G* methods. These methods run in Gaussian 98 determined the geometry optimization and electrostatic charges for each molecule. CoMFA maps were developed using SYBYL ver. 7.2 to compare the electrostatic and steric properties of each calculation and molecule. With "leave-one-out" cross validation, the numbers of optimal components was determined. No cross validation was performed in a training set using the optimal components for each analysis. After the completion of a test set, it was verified that CoMFA models derived from HF/3-21G* optimized geometries and atomic charges are more reliable in predicting the bioactivities of σ_2 receptor ligands. Using the HF/3-21G* analysis, new active σ_2 receptor ligands were designed and pK_i values were predicted. It was determined that active σ_2 receptor ligands require localization on the benzene ring contributed through an electron withdrawing group.

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by
Laura Ann Wirpsza

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APPROVAL PAGE

DEVELOPMENT OF PHARMACOPHORE AND CoMFA STUDIES FOR σ_2 RECEPTOR LIGANDS

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Riddle: *"What has roots as nobody sees,*
Is taller than trees,
Up, up it goes,
And yet never grows?"
J.R. Tolkien, *The Hobbit*

"The future belongs to those who believe in the beauty of their dreams."
Eleanor Roosevelt

Mom and Dad, thank you for your support of my education throughout the years. I could have never come this far without both of you. Lilly, my beloved sister, your logical outlook on life always helps to keep me grounded. My young at heart grandparents, you taught me to work as hard as you play.

"Good friends, good books and a sleepy conscience: this is ideal life."
Mark Twain

My family of friends from Susquehanna University, I am forever treasuring your words of 'wisdom.' To my new friends at NJIT, thank you for the laughter and joyful memories.

"A scientist in his laboratory is not a mere technician: he is also a child confronting natural phenomena that impress him as though they were fairy tales."
Marie Curie

My professors, old and new, have guided me through a magical world of biology and chemistry. You have inspired me and shared my appreciation for the world at the atomic level. I am surer than ever of what my path will be. Words cannot express my joy and appreciation. Thank you again.

"First say to yourself what you would be; and then do what you have to do."
Epictetus

Answer: Mountain

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CHAPTER 1

OUTLINE

Molecular Modeling has been an essential tool for the development of drug design using various methods. Two of these methods: pharmacophore derivation and CoMFA (Comparative Molecular Field Analysis), determine the molecular structure and function for biological receptors. There has been a significant amount of study on σ_1 receptor ligands, and the σ_1 receptor has been isolated and cloned. However, the second subtype, σ_2 receptor, has fewer active ligands and only one is commercially available. This research was conducted to determine a pharmacophore model for σ_2 receptor ligands and to perform a CoMFA study using various partially to highly active σ_2 compounds.

The objectives of the research are:

1) To derive a pharmacophore model for σ_2 receptor ligands, using highly active compounds from different classes.

2) To perform an alignment of 32 compounds to perform a validated CoMFA study for σ_2 receptor ligands.

3) To compare semi-empirical, density functional, and *ab initio* calculations to the CoMFA studies on σ_2 ligands.

4) To design new σ_2 ligands from CoMFA results.

Chapter 1 presents an outline and objectives of this research.

Chapter 2 contains QSAR methodology. Section 2.1 describes biological activity. Section 2.2 gives approaches to developing a QSAR methodology with sections focusing

on Alignment, Partial Least Squares, and Electrostatic and Steric Properties.

Chapter 3 contains CoMFA studies using semi-empirical, density functional, *ab initio* calculation methods and a pharmacophore derivation using DISCOtech on σ_2 receptor ligands. Section 3.1 shows a selection of σ_2 ligands, choice of conformation, a pharmacophore derivation using DISCOtech, geometry optimization and atomic charges calculations, alignments of optimized structures, and CoMFA models. Section 3.2 contains a discussion of pharmacophore results, comparative molecular field analyses, validation of CoMFA models, and design of new ligands. Section 3.3 presents a summary of σ_2 receptor ligands.

Chapter 4 presents a general conclusion from this study. Suggestions for future work can be found in Section 4.1.

CHAPTER 2

BACKGROUND

2.1 Bioactivity of Receptor Ligands

Inhibition of enzymes is found through interactions with their respective ligands. The type of inhibition is determined by the substrate interaction with the enzyme. Equation 1 presents the rate between an enzyme (E) and inhibitor (I) forming an enzyme inhibitor (EI) complex. This three species model, (E, I, EI) represent the case with σ_2 receptors and ligand interactions.

$$E + I \underset{Ki}{\rightleftharpoons} EI \qquad (2.1)$$

Competitive inhibition is the focus in this study using the true equilibrium constant (K_i= [E][I]/[EI]), termed the inhibition constant [1, 2]. The pK_i (pK_i =-log [K_i]) used was collected from various *in vitro* and *in vivo* studies with a range of activity and selectivity.

2.2 QSAR Methodology

To determine the molecular shape in a biological system, QSAR methods are used. First, a structure is energy minimized using a program such as AM1 and the electron distribution in the compound is calculated. The program, SYBYL, places the molecules in a box of points, producing a lattice to represent hypothetical receptor space and surface. In the data matrix, steric, electrostatic, and hydrophobic interaction energies are calculated [2]. These calculations are validated using Partial Least Squares and cross

validation methods to determine the accuracy of prediction for biological activity viewed through a CoMFA contour map [3, 4].

2.2.1 Alignment

Two questions need to be asked during a CoMFA study: 1) what is the conformation of the molecule when bound to its biological target? 2) How can a CoMFA map describe the electrostatic charges and steric properties of the molecules? The first step in the CoMFA is to energy-minimize the molecule using different techniques going from simpler approximations (molecular mechanics) to more sophisticated methods (semi empirical calculations, *ab initio* optimizations). Since they do not have many conformations, rigid compounds act as a template when overlapping non rigid molecules. Flexible molecules have local energy minimum conformations. For establishing a realistic representation, molecules used in the CoMFA study have the following requirements; one or more rotatable bonds, belonging to different chemical series that bind to the macromolecular target. To find similarities between ligands a consistent alignment is essential. Differences in field values at each lattice point should reflect differences in structure only, not chance variations in model geometry [3, 4].

The main techniques commonly used are classical fit of selected atom pairs, or fitting steric and (or) electrostatic fields by minimizing the differences at each lattice point between a candidate and a template molecule. The Field Fit method has been found to compute better cross-validated correlations than alignments based on crystal evidence. This function minimizes the entropy contribution of free energy of binding by forcing the aligned molecules to share a common global shape and location in the 3D lattice [3, 4].

Some problems with alignment include the use of different structural classes of ligands that bind to the receptor [3].

2.2.2 Partial Least Squares

A QSAR study cannot be successful if the structural limits do not relate to the differences in biological activity. For each compound in the CoMFA study, a structural column records the intensity of a particular type of interaction, at a distinct point in space, with a probe atom of specified charge and steric properties. The electrostatic effects are calculated and values are recorded into a table [4].

The resulting linear equation presents a relation between the biological activities and the intensity of the exerted fields. Partial Least Squares (PLS) generates a better set of coefficients (extract a new component), criterion maximized to the degree of commonality between all structural limit columns and experimental data. During the evaluation phase of a PLS iteration, the criterion for acceptance of the principal component generated is based on the improvement of prediction of the biological data. PLS is assessed through cross validation, where one or more of the active ligands are omitted. The resulting equation is used to predict the biological activity value for the omitted compounds. PLS is used to calculate PRESS (Predictive Residual Sum of Squares) expressed as q^2 or r^2 values. Values of q^2 can range from 1.0 to less than zero. A value of 1.0 indicates a perfect prediction while negative values of q^2 arise from accumulated prediction error which is greater than 'no model at all.' The q^2 value and its associated residual graph represents the merit and robustness of a QSAR for predicting

the activity of the molecules. The r^2 is a measurement of how well a particular model reproduces or fits the input data. In this case, PRESS is replaced by the sum of squares of the differences between the least-squares fit and the experimental observations [4].

One disadvantage of PLS is that the magnitude of one variable relative to another can strongly affect the resulting factors, so that the question of 'scaling' is an important one [4].

2.2.3 Distribution of Steric and Electrostatic Properties

Understanding natural molecules through the influence of their binding, leads to the design of new molecules that have similar pharmacological effects. Any potential drug molecule must be engineered to bind tightly to the desired target receptor with assets such as; non-toxicity, chemical stability, and bioavailability. As a result of studying the electrostatic charges and steric properties, a cohesive interaction between ligand and receptor can be found [1, 5].

The contribution of electrostatics to binding is non-intuitive. Placing a polar or charged chemical group in the prospective drug molecule may create favorable interactions with the target molecule and solvent. This trade-off between favorable interaction and unfavorable desolvation free energies is the concern of charge optimization. Optimizing the ligand charge distribution ensures that electrostatics contributes favorably to the binding process. Optimization can affect computed binding affinities through different levels of calculations and alignment of functional groups[4].

Potentially repulsive interaction energy also known as steric energy is another essential portion of receptor ligand interaction. Studying the intramolecular and

intermolecular interactions of a molecule one can understand the steric contributions of ligands [2].

CHAPTER 3

DEVELOPMENT OF PHARMACOPHORE AND CoMFA STUDY FOR SIGMA 2 RECEPTOR LIGANDS

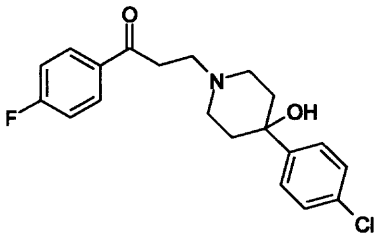
3.1 Introduction

Since the 1970s σ receptors have been the focus of physiological studies [6]. Originally, these compounds were mistaken for opioid and phencyclidine (PCP) receptors [6-10]. Once classified and initially studied, σ receptors were separated into three subgroups: σ_1, σ_2, and σ_3 . σ_1 Receptors have been cloned with the molecular weight of ~25 kDa. Presently the σ_2 receptor has a molecular weight of 18~21.5 kDa [7, 12] and has not been cloned. The σ_3 receptor has been shown to modulate tyrosine hydroxylase (TH) and dopamine synthesis in striatum, represented by a separate class of ligands [12]. These receptors have a widespread distribution in the central nervous system (CNS) and are found in a range of organs and tissues [7].

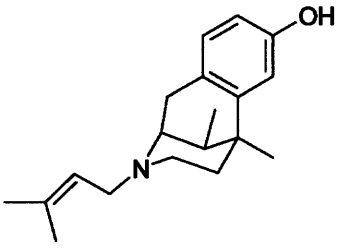
σ_1 Receptors are associated with CNS disorders such as depression, schizophrenia, and dementia. Agonists are valued as neuroprotective agents, while antagonists may help alleviate cocaine addiction [8]. The pharmacophore for σ_1 has been derived and many active ligands have been synthesized through various sources [13]. Manallack used various classes of molecules to determine the first σ_1 pharmacophore [6]. This model was further developed by Gund and Shukla in 1991 using electrostatic and steric calculations [14]. Glennon and coworkers [15,16] made further revisions by presenting a general structural feature with two hydrophobic regions and an amine site (distance from the primary hydrophobic region to the amine is 6-10 Å, distance from the

secondary hydrophobic region to the amine center is 2.5-3.9 Å). Gund and coworkers [17] developed a σ_1 pharmacophore using more recent ligands. They determined four necessary regions and a nitrogen atom: R1 (0.85, 7.26, 0.30); R2 (5.47, 2.40, -1.51) R3 (-2.57, 4.82, -7.10); N (-0.71,3.29,-6.40); carbon centroid (3.16,4.83, -0.60), where R1, R2 were constructed onto the aromatic ring of each compound to present hydrophobic interactions with the receptor; and R3 represented a hydrogen bond between the nitrogen atom and the receptor. These models present two hydrophobic centers with nitrogen donating lone pair electrons to the σ_1 receptor. A few examples of highly active σ_1 compounds include: PD144418, Spipethiane, Haloperidol, (+)-Pentazocine, and PRE084 (Table 3.1) [13-17].

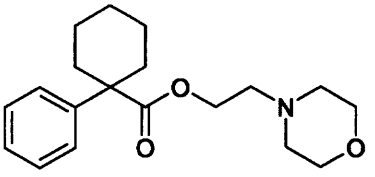
Table 3.1 Binding and Functional Data of Known Active σ_1 Receptor Ligands [17]



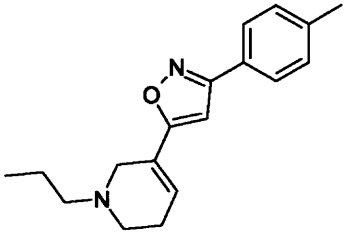
Haloperidol



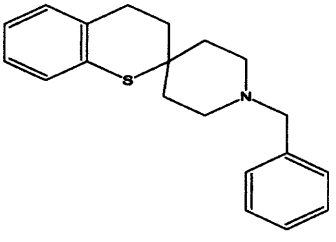
(+) Penatzocine



PRE084



PD144418

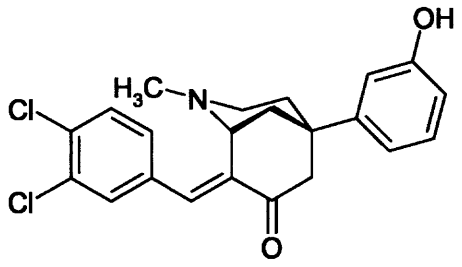


Spipethiane

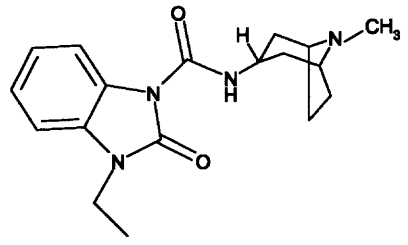
Compounds	σ_1 K_i (nM)	σ_2 K_i (nM)	σ_1/σ_2
Haloperidol	1.2±0.20	26 ± 5.4	22
(+)Pentazocine	5.8±1.0	1253±519	0.0046
PRE084	44	NA	NA
PD144418	0.08	1377	$5.8x10^{-5}$
Spipethiane	0.5±0.02	416±43	0.0012

New research has presented several active σ_2 receptor ligands as well as suggesting a potential role of the σ_2 receptor in regulation of cellular proliferation and apoptosis [2, 6]. The regulation of cell proliferation consists of intracellular membrane bound σ_2 receptors, localized on organelles known to store calcium. These receptors also can cause the release of calcium, used in cell signaling and (or) for the induction of apoptosis. σ_2 Agonists may also be useful as anti-neoplastic agents [11]. Compounds with moderate σ_2 activity include phenyl morphan CD-184 **28** [7, 10] (Table 3.2), trishomocubane derivatives **29** [18] (Table 3.2), BIMU-1 **5** [7, 19] (Table 3.1). 1,3-di (2-tolyl) guanidine (DTG) **27** is the standard for nonspecific binding of σ receptors (Table 3.2) [2-12]. Recently, there has been a pharmacophore developed for the σ_2 receptor using GRIND (GRid INdependent Descriptors), a program that does not use alignment, for a series of α-tropanyl ligands. The model was able to prove two hydrophobic areas and an H-bond donor receptor region with non-covalent bonds present. PLS models for the σ_2 affinity had r^2=0.83, q^2=0.63 [20]. There has been little further development for a pharmacophore of σ_2.

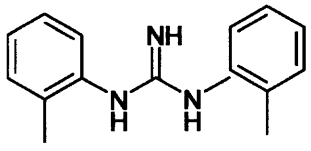
Table 3.2 Binding and Functional Data of Rigid σ_2 Receptor Ligands



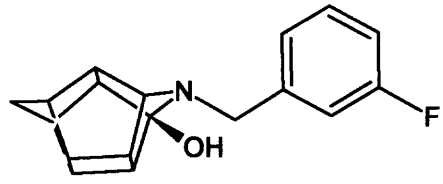
CB-184 (28)



BIMU-1 (5)



1,3-di (2-tolyl) guanidine (DTG) (27)



ANSTO-19 (Trishomocubane Derivative) (29)

Compounds	Configuration	σ_1 K_i(nM)	σ_2 K_i (nM)	σ_1/σ_2
28*	(+)-1R,5R	7436±308	13.4±2.0	554.93 [7]
5*†	-	6300	32 ± 15.2	- [19]
27	-	-	28.2 ± 1.4	- [10]
29*	-	152±1	20±4	7.60 [18]

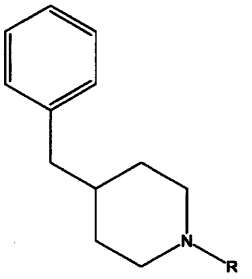
* Indicates included in Pharmacophore Derivation. † Indicates included in CoMFA test set.

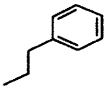
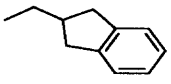
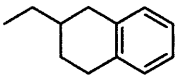
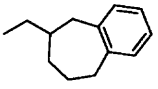
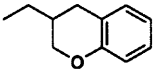
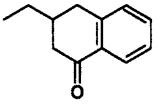
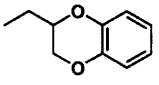
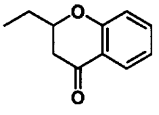
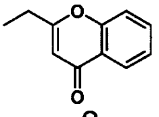
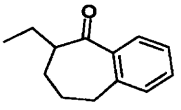
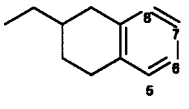
Pharmacophore identification methods apply to a series of molecules which are known to bind to a receptor, even when the three dimensional structure of the receptor is unknown. Common features from the active compounds are aligned to determine possible conformations and the minimum requirement for binding. Superimposing moderately to highly active compounds with rigid compounds helps to better define the geometry of the pharmacophore for the ligands, since in flexible ligands there are numerous conformational possibilities [20]. Comparative Molecular Field Analysis (CoMFA) is able to calculate bioactivity properties of various σ_2 ligands obtained from different

functional groups of the compounds. Using the training sets of five different molecules, the models are verified based on q^2 and r^2 values using the alignment from the pharmacophore model [14].

Current development in σ_2 receptor ligands have presented three series of compounds that are moderate to highly bioactive: 1-aralkyl-4-benzylpiperidine [9], 1-aralkyl-4-benzylpiperazine [9], 1-cyclohexylpiperazines [10] and N-substituted 9-azabicylo [3.3.1] nonan-3α-yl carbamate [7] analogues. These new compounds are flexible and have many conformations (Tables 3.3-3.6). Recently, PB28 **2**, a member of the 1-cyclohexylpiperazine class (Table 3.5), has been synthesized and represents one of the first highly potent σ_2 receptor ligand agonists [21]. Location of different substituents groups and hydrophobic groups affect the binding to the σ_2 receptor.

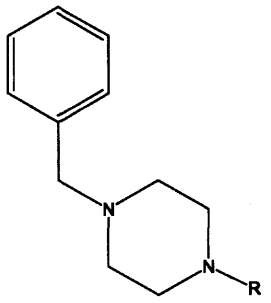
Table 3.3 Binding and Functional Data of 1-aralkyl-4-benzylpiperidine Derivatives [9]

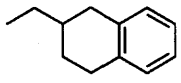
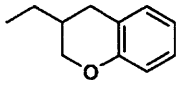
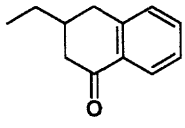
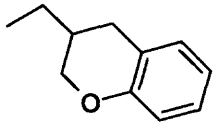
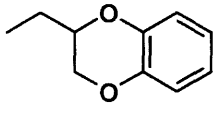
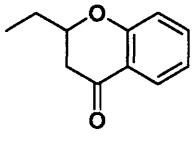
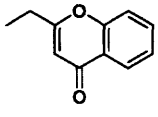
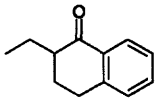
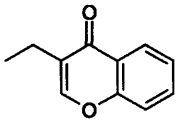


Compounds	R group	σ_1 K_i (nM)	σ_2 K_i (nM)	σ_1/σ_2	σ_2/σ_1
17*		1.40	0.49	2.857	0.4
18		1.40	12.2	0.11	9
19*		1.50	2.52	0.60	2
20		28.7	47.3	0.61	2
22†		2.50	5.98	0.42	2
23		1.40	4.63	0.30	3
24		16.2	28.4	0.57	2
25		1.40	7.90	0.178	6
26		115	285	0.40	2
14		36.4	89.3	0.41	2
15		9.4	11.0	0.85	1
16	7-OCH_3 8-OCH_3	36.3	75.0	0.48	2

* Indicates included in Pharmacophore Derivation. † Indicates included in CoMFA test set.

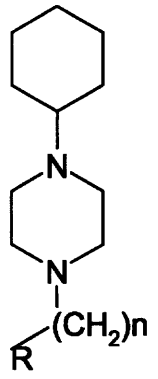
Table 3.4 Binding and Functional Data of 1-aralkyl-4-benzylpiperazine Derivatives [9]



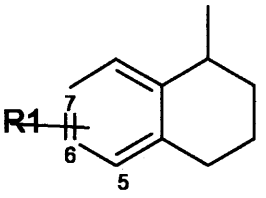
Compounds	R	σ_1 K_i (nM)	σ_2 K_i (nM)	σ_1/σ_2	σ_2/σ_1
21*		0.80	1.70	0.47	2
8		0.30	1.48	0.20	5
9		0.30	1.59	0.19	5
10		0.30	3.02	0.10	10
11		15.4	25.6	0.60	2
12†		1.20	4.75	0.25	4
13		2.66	5.35	0.50	2
6		0.40	1.40	0.29	3
7		3.80	14.1	0.27	4

* Indicates included in Pharmacophore Derivation. † Indicates included in CoMFA test set.

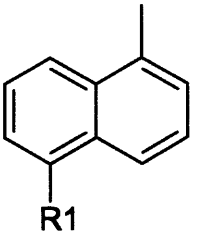
Table 3.5 Binding and Functional Data of 1-cyclohexylpiperazine Derivatives [10]



R=



A

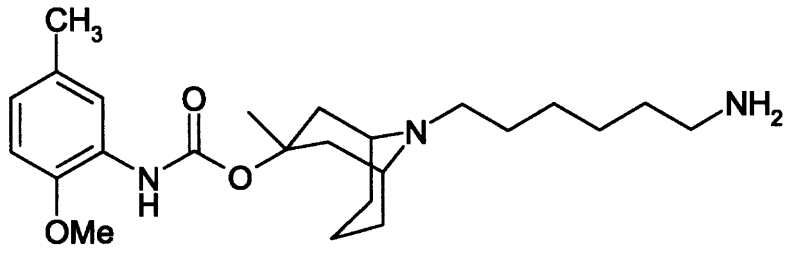


B

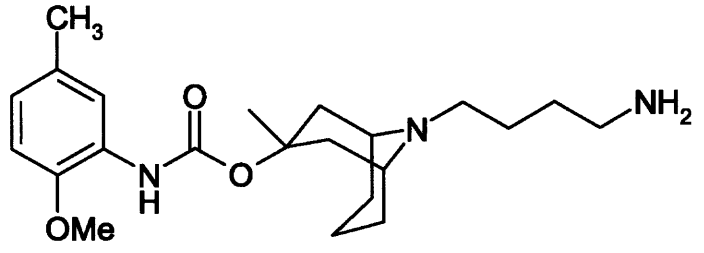
Compounds	R	R^1	n	σ_1 K_i (nM)	σ_2 K_i (nM)	σ_1/σ_2
1	A	H	3	0.61 ±0.08	0.68±0.03	0.89
2*	A	5-OCH_3	3	13.6±1.9	0.34±0.02	40
3†	B	H	3	2.16±0.63	0.69±0.08	3.1
4	B	H	5	2.40±0.47	0.57±0.08	4.2

* Indicates included in Pharmacophore Derivation. † Indicates included in CoMFA test set.

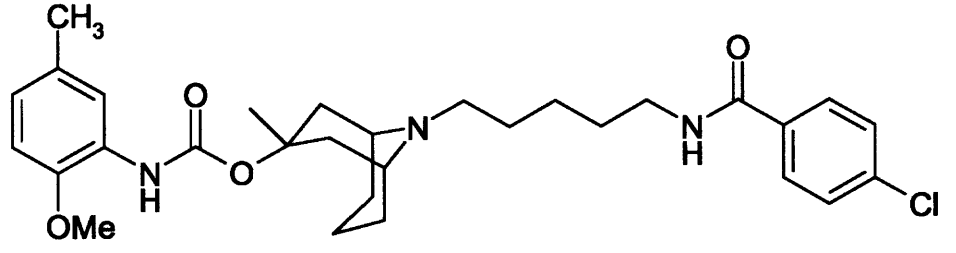
Table 3.6 Binding and Functional Data of N-(9-(6-aminohexyl)-9-azabicyclo [3.3.1] nonan-3α-yl)-N'-(methoxy-5-methylphenyl) carbamate [7]



31



30



32

Compounds	σ_1 K_i (nM)	σ_2 K_i (nM)	σ_1/σ_2
30	2490±271	12.94±0.46	193
31*†	1418±439	5.19±0.80	273
32	1397±64	3.56±0.08	392

* Indicates included in Pharmacophore Derivation. † Indicates included in CoMFA test set.

3.2 Materials and Methods

The initial conformer searches and pharmacophore derivation was performed by DISCOtech using SYBYL 7.2 [23]. The program GALAHAD [24] found within SYBYL 7.2 was unsuccessful in developing a pharmacophore. All CoMFA models were derived using SYBYL 7.2 [23]. Activity of compounds is based on the rate of dissociation between the inhibitor and σ_2 receptor (K_i). Active compounds from each group were selected for a CoMFA study using Gaussian 98 [26] for geometry optimization. Atomic charges were calculated using AM1, HF/3-21G*, and B3LYP/3-21G* calculations. These limits assist in the future development for other σ_2 ligands.

3.2.1 Pharmacophore Derivation

3.2.1.1 Selection of Ligands. For the derivation of the pharmacophore, eight moderately to highly active compounds were used: three rigid and five flexible. The three rigid ligands were selected for setting limitations on the pharmacophore and are listed in Tables 3.2: Trishomocubane analogue **29** [18, 27], CB-184 **28** [26], and BIMU-I **5** [7, 19]. Since σ_2 sites have much more restrictive structural requirements for high affinity binding, [28] consideration of the stereochemistry, rigidity, and alkyl chain length are essential when selecting compounds for the development of a pharmacophore model. Furthermore, consideration of structural diversity and understanding of biological activities are necessary [29 - 34].

Non rigid ligands listed in Tables 3.2 - 3.5 were selected based on their σ_2 affinity values: N-(9-(6-aminohexyl)-9-azabicyclo [3.3.1] nonan-3α-yl)-N'-(methoxy-5-

methylphenyl) carbamate **31** [7], 1-aralkyl-4-benzylpiperidine **17** and **19** [9], 1-aralkyl-4-benzylpiperazine **21** [9], and 1-cyclohexylpiperazines **2** [10]. All values were obtained using [^{3}H] DTG in the presence of 1 μM (+)-pentazocine. [^{3}H] (+) pentazocine was used as the radiolabel for σ_1 sites [7, 9, 10, 25]. Histogram pictures of training and test sets are shown in Figure 3.1. The range of binding affinities for the training set was -2.64 to 0.469 log units, and -1.292 to 0.16 log units for the test set.

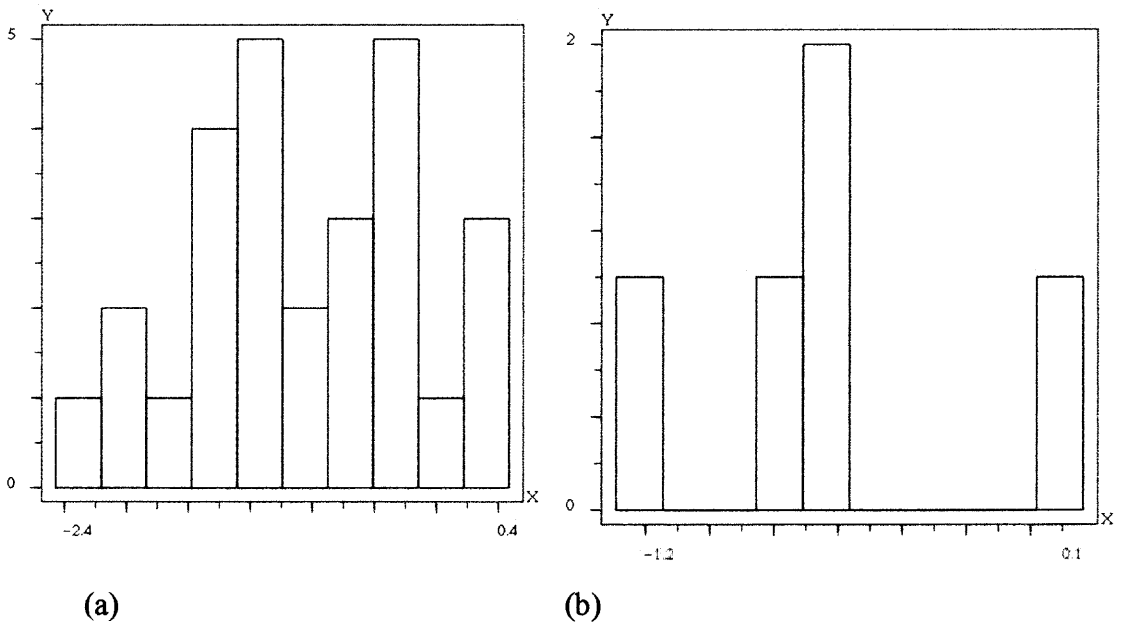


Figure 3.1 Histogram of pK_i (abscissa) vs. number of molecules (ordinate).
(a) Training set, (b) Test set.

3.2.1.2 Choice of Initial Conformations. Conformations of the ligands were generated using DISCOtech which aligned them by the selection of similar functional groups within each compound. Two hydrophobic centers were selected, one nitrogen and one acceptor site. Initial structures were built in SYBYL 7.2 using default bond distances

and angles. These structures were then minimized using MAXIMIN2 within Tripos which uses a distance-dependent dielectric function.

DISCOtech recognizes each molecule by ligand points and site points. Ligand point consisted of a nitrogen center, lone pair of electrons, and two hydrophobic centers for each compound. Conformational flexibility is handled by computing a series of low energy conformations for each molecule with each conformer being treated as a rigid body during the alignment step. The molecule with the fewest conformations is used as a reference. DISCOtech takes each conformation of the reference molecule in turn and compares it to all conformations of the other molecules [17, 22]. The resulting pharmacophore is a four point system with two hydrophobic regions, a nitrogen center that is approximately 2.90+/- 2.50 Å (Figure 3.2).

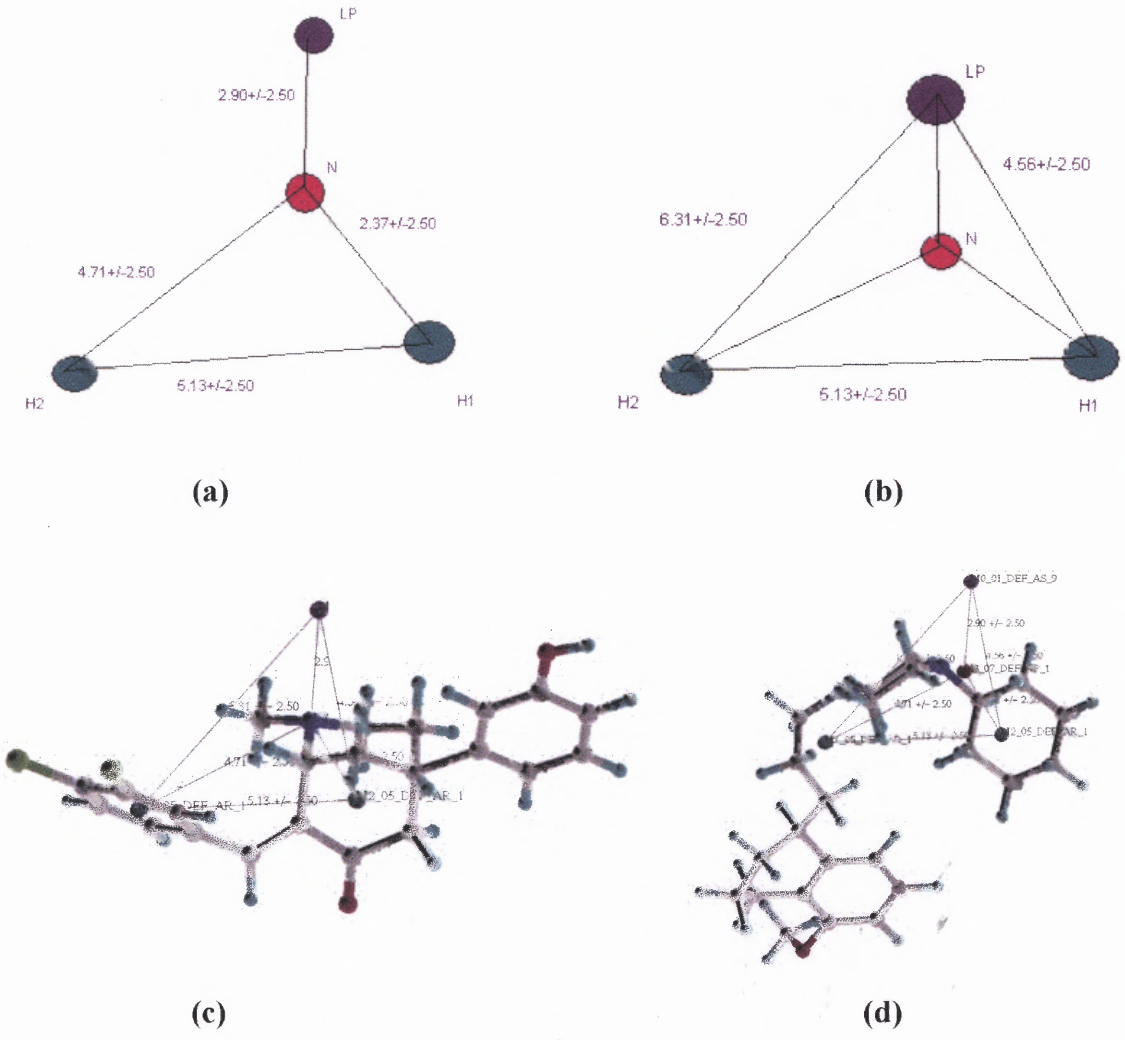
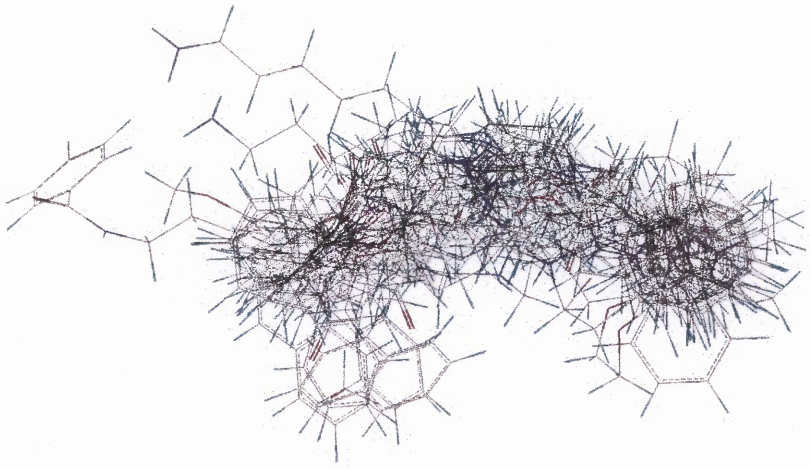


Figure 3.2 DISCOtech Pharmacophore Dimensions with hydrophobic (H1 and H2) regions, nitrogen (N), and Lone Pair (LP) (a and b). Contains σ_2 receptor ligands **2** (c) and **28** (d).

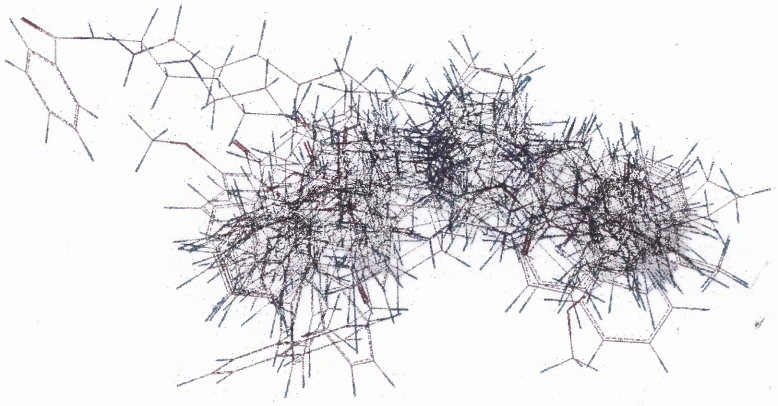
3.2.2 CoMFA Studies

3.2.2.1 Geometry Optimization and Electrostatic Studies. Conformers derived by DISCOtech were optimized using *ab initio* HF/3-21G*, B3LYP/3-21G [17] or with semi-empirical AM1 calculations for 32 compounds (Tables 3.2- 3.7). Electrostatic charges for these geometries were derived by semi-empirical AM1, *ab initio* HF/3-21G* and density functional B3LYP/3-21G according to Mulliken population using Gaussian 98.

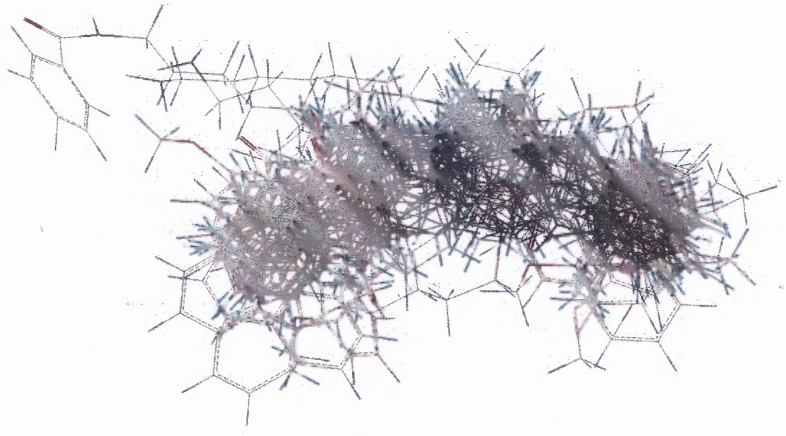
3.2.2.2 Alignment. Alignment of presumed bound conformations of the training set compounds was an essential prelude to the Comparative Molecular Field Analysis (CoMFA) study [30-35]. The three optimization calculations were aligned by a fit function in SYBYL 7.2 using a template compound **28** in Table 3.2 and the generated pharmacophore. Each of the 32 molecules were aligned according to class and overall geometry using AM1, HF/3-21G*, and B3LYP/3-21G* methods using the ALIGN DATABASE and "Field Fit" functions [27]. Geometry optimization performed by Gaussian 98 ensured a reliable CoMFA using Partial Square Least (PLS) validation q^2 and r^2. K_i values were converted to pK_i values (pK_i = -log [K_i]) [1, 30]. Using a K_i value, a ligand can be classified based on its selectivity between the two subtypes: σ_1 and σ_2 [16, 30-35]. Alignments used in the CoMFA model are found in Figure 3.3.



(a)



(b)



(c)

Figure 3.3 Alignments of all 32 molecules optimized using: AM1 (a), HF/3-21G* (b) and B3LYP/3-21G* (c) methods.

3.2.2.3 CoMFA Model. CoMFA columns were generated using the Tripos Standard CoMFA field class. CoMFA dielectric function of 1/r; a dielectric constant ε of 1 extends every molecule 4.0 Å in all directions. A default of 30 kcal/mol energy cutoff for steric and electrostatic fields was used. The CoMFA standard scaling was used. The CoMFA column with literature pK_i values generated PLS cross validation and validated results used to predict the bioactivity of ligands (Figure 3.5) [32, 33]. A test set of five molecules were used to predict pK_i values for each of the three calculations (Table 3.9). The training set values for the three calculations are found in Table 3.10.

PLS analysis diminishes target property against predictors, calculating steric and electrostatic components of the intermolecular interaction field. The SAMPLS (SAMple-distance PLS) algorithm developed by Bruce Bush [30] was used to determine "leave-one-out" cross-validation q^2 value. The method for cross-validation was used to 1) to find if the CoMFA model was productively useful and 2) to decide how many components to use for the best model. The number of optimal components was considered by the 5% rule; if the q^2 increases by at least 5% upon increasing the number of components by one, it is justified to add an additional component. A high q^2 calculated with the training set only shows a good internal validation, not an automatic high predictive ability for an external test set [31]. Therefore, the PLS analysis was repeated without cross-validation using the optimum number of three components. This final analysis yielded a predictive model, and a CoMFA coefficient contour plot for the steric and electrostatic potential contributions. The CoMFA model displayed the spatial distribution of important steric and electrostatic properties affecting the ligands (Figure 3.4).

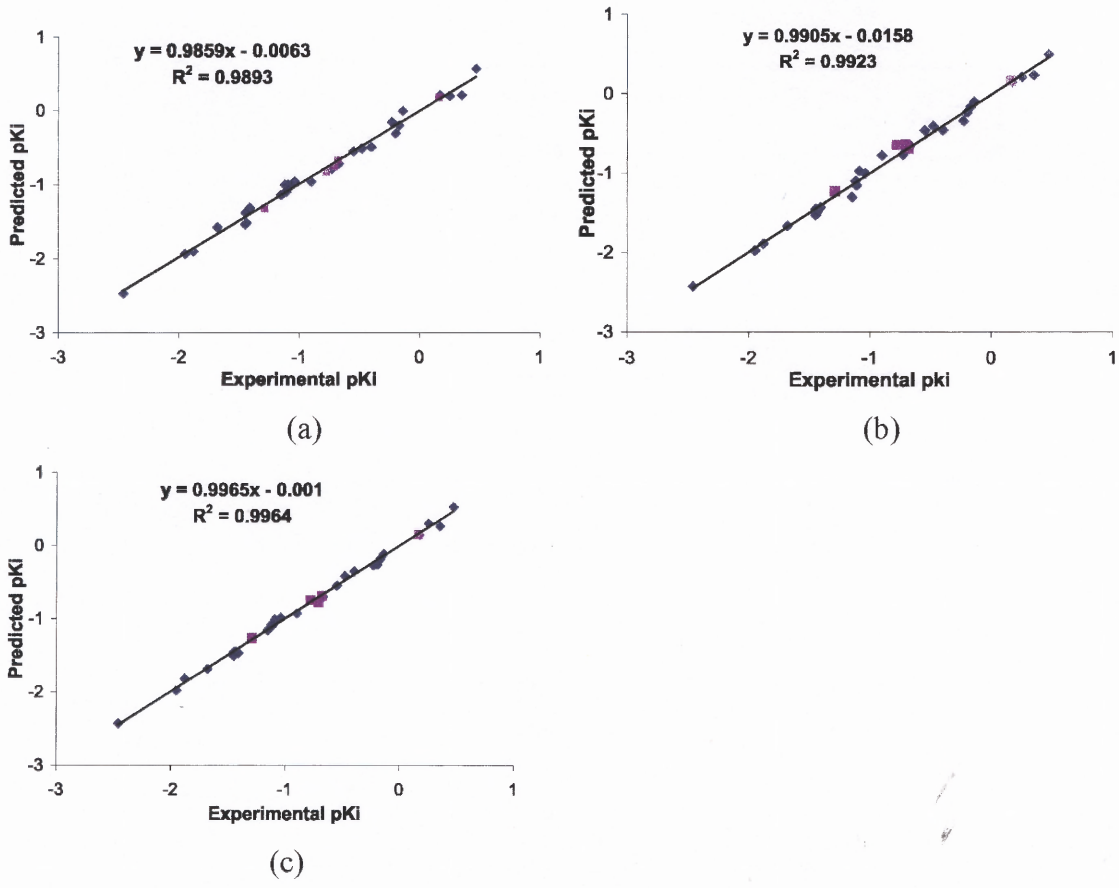
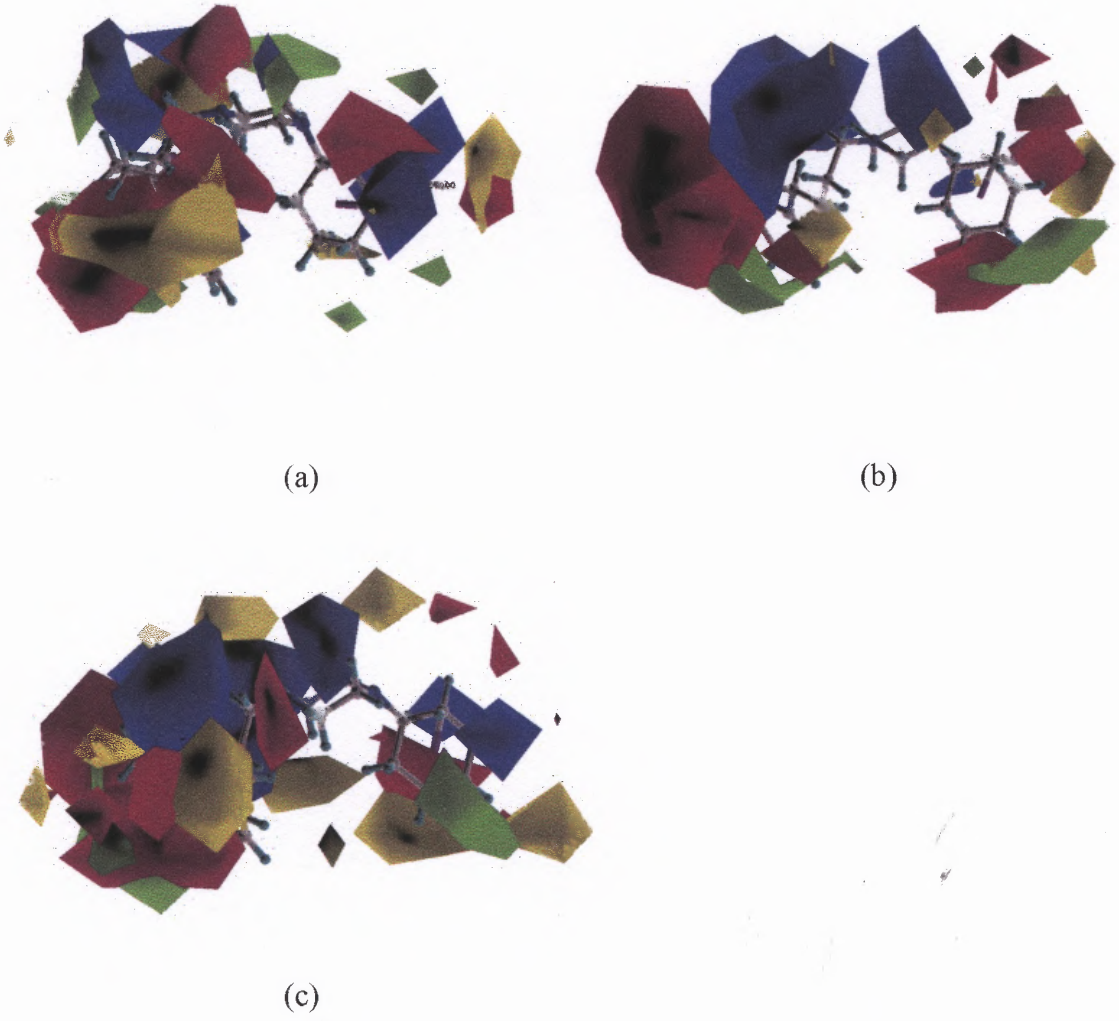


Figure 3.4 Graph of experimental (pK_i =-log [K_i]) vs. predicted bioactivity by the CoMFA model using different calculation methods AM1 (a) HF/3-21G* (b) and B3LYP/ 3-21G* (c).

*Blue indicates training set while pink indicates test set.



(a) (b)

(c)

Figure 3.5 CoMFA contour maps for Compound **2**, derived by σ_2 receptor ligands using various charge and geometry optimization methods.

Geometry optimizations and atomic charges were calculated in AM1 (a), HF/3-21G (b) and B3LYP/ 3-21G* (c) methods.

3.3 Results and Discussions

3.3.1 Pharmacophore

If a ligand has a very low K_i for the target enzyme, then the enzyme ligand complex formation with the target enzyme will be favored and the selective affinity will be enhanced. Nahas [36] was able to determine, when looking at N-(3-phenylpropyl)-N'-benzylpiperazines, that hydrophobic groups are necessary for the σ_2 ligands. The pharmacophore designed by DISCOtech is a four point arrangement and includes the: nitrogen, lone pair of electrons approximately 2.90 Å from the nitrogen, and two hydrophobic regions approximately 6.31 Å apart (Figure 3.2 a). Figure 3.2b and 3.2c present Compounds **2** (b) and **28** (c) with the pharmacophore dimensions.

3.3.2 Comparative Molecular Field Analysis

A structural diversity and homogenous range of affinities is necessary to obtain a significant 3D-QSAR study using the CoMFA method. The CoMFA model required 1 or 3 optimal components in different calculations to explain the variance in binding affinity to σ_2 receptor in Table 3.7. The highest q^2 (0.567) was obtained for HF/3-21G* optimized geometries and atomic charge calculations. The CoMFA models of AM1 optimized geometries produced lower q^2 (0.321) at one component. This suggests that HF/3-21G* presents a more accurate variance in activity among similar σ_2 receptor ligands than AM1 optimized geometries and charges. CoMFA model with B3LYP/3-21G* calculated geometries and electrostatic charges produced a moderate q^2 (0.382) with three

components values in comparison to HF/3-21G* with a range of (0.510-0.567) in three components (Table 3.7).

The cross-validation "leave-one-out" confirmed the predictive ability of the CoMFA model. PLS analysis performed without any validation produced the best R^2 and standard errors of predicted pK_i values. R^2 measures for HF/3-21G* and B3LYP/3-21G* methods were 0.991 and 0.996 respectively, and standard errors of estimates were 0.073 and 0.048. AM1 gave the lowest r^2 value (0.989) and standard error of estimate of 0.081 (Table 3.8). The relationship between calculated and measured pK_i values (predicted) for non-cross validated analysis is located in Table 3.9 and 3.10.

Table 3.7 Optimal Component Number and q^2 by "One-Leave-Out" using SAMPLS [30] by the Training Set of 27 Molecules

Theory	Terminology	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5
AM1	standard error	**0.636**	0.702	0.765	0.810	0.847
	q2 (PRESS)	**0.321**	0.206	0.096	0.030	-0.012
HF	standard error	0.661	0.547	**0.530**	0.555	0.590
	q2 (PRESS)	0.374	0.518	**0.567**	0.545	0.510
B3	standard error	0.663	0.629	**0.633**	0.651	0.666
	q2 (PRESS)	0.261	0.363	**0.382**	0.374	0.375

Table 3.8 QSAR Reports by Non-Crossvalidation using SAMPLS [30] by the Training Set of 27 Molecules

Theory	S.E.	R^2	F Values	Steric.	Electro.
AM1	0.081	0.989	(n1=5, n2=26) 486.325	0.368	0.632
HF/3-21G*	0.073	0.991	(n1=5, n2=26) 592.092	0.383	0.617
B3LYP/3-21G*	0.048	0.996	(n1=5, n2=26) 1371.654	0.316	0.684

Standard error of estimation. R^2 of non-crossvalidation using training set of 27 molecules in Table 3.2-3.5. Steric and Electrostatic contributions to this CoMFA field.

Table 3.9 Experimental and Predicted Bioactivities (pK_i) by the Training Set of 27 Molecules using Various Calculation Methods

Compounds	Lit. pKi	AM1	HF/3-21G*	B3LYP/3-21G*
		Predicted pKi		
1	0.17	0.208	0.147	0.147
2	0.47	0.568	0.493	0.529
4	0.25	0.198	0.211	0.305
5	-1.44	-1.517	-1.49	-1.442
6	-0.14	-0.003	-0.103	-0.113
7	-1.15	-1.139	-1.301	-1.155
8	-0.17	-0.2	-0.164	-0.181
9	-0.2	-0.309	-0.233	-0.262
10	-0.48	-0.51	-0.409	-0.417
11	-1.41	-1.31	-1.427	-1.47
13	-0.73	-0.788	-0.768	-0.771
14	-1.95	-1.934	-1.976	-1.982
15	-1.04	-0.953	-1.002	-0.986
16	-1.88	-1.902	-1.89	-1.814
17	0.35	0.211	0.233	0.265
18	-1.09	-0.999	-0.974	-1.011
19	-0.4	-0.492	-0.459	-0.354
20	-1.68	-1.572	-1.664	-1.685
21	-0.23	-0.154	-0.345	-0.271
23	-0.67	-0.718	-0.652	-0.698
24	-1.45	-1.539	-1.53	-1.507
25	-0.9	-0.958	-0.779	-0.927
26	-2.46	-2.473	-2.426	-2.431
27	-1.45	-1.379	-1.45	-1.455
28	-1.12	-1	-1.099	-1.08
30	-1.11	-1.098	-1.157	-1.092
32	-0.55	-0.55	-0.461	-0.549

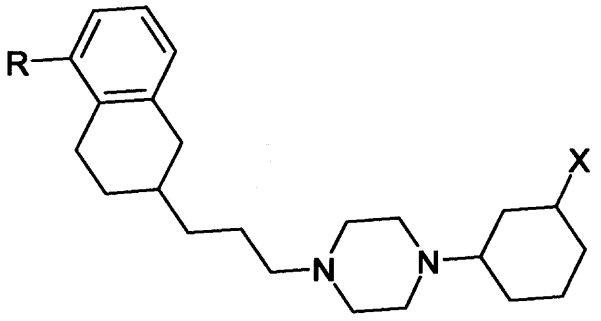
Table 3.10 Experimental and Predicted Binding Affinities (pK_i) by Test Set of Five Molecules using Various Calculation Methods

Compound	Lit. pKi	AM1	HF/3-21G*	B3LYP/3-21G*
3	0.16	0.181	0.161	0.157
12	-0.68	-0.665	-0.688	-0.688
22	-0.78	-0.826	-0.646	-0.743
29	-1.29	-1.318	-1.229	-1.264
31	-0.71	-0.757	-0.644	-0.777

3.3.4 Design of New Ligands

Using the spatial distribution of steric and electrostatic properties, the design of new ligands and prediction of activities is possible from CoMFA calculations (Figure 3.5). The contour maps of steric fields are shown in yellow and green. Green areas (80% contribution) are regions where more bulky substitutions are desired, and yellow (20% contribution) are regions where less bulk is favorable for higher σ_2 activity. The contour maps of electrostatic fields are presented in red and blue; red areas (80% contribution) are regions that favor more negative charge, and blue areas (20% contribution) are regions that favor more positive charge for higher σ_2 activity. AM1 failed to predict PB28 in proper ranges (Table 3.11).

Table 3.11 Prediction of Bioactivity of New Ligands



Compounds	Lit. pK_i	R	X	AM1	HF/3-21G*	B3lYP/3-21G*
2 (PB28)	0.469	OCH_3	H	0.468	0.493	0.529
33	-	OCH_3	No cyclohexane	0.072	0.422	0.241
34	-	OCH_3	F	0.441	0.668	0.490
37	-	OCH_3	Cl	0.730	0.544	0.466
38	-	OCH_2CH_3	H	0.258	0.441	0.165
48	-	$OCCl_3$	H	0.369	0.217	0.132
49	-	CO(t-butyl)	H	0.505	0.513	0.573

Recently, PB28 **2** was found to be one of the few highly selective σ_2 receptor ligands [5, 14]. Six structures were suggested (Table 3.11), and predicted pK_i values were calculated using AM1, HF/3-21G*, and B3LYP/3-21G. Since the HF/3-21G* model had the highest q^2 value, the predicted bioactivity of the new ligands is more probable. Two locations on the CoMFA maps were investigated to determine the activity of potentially new compounds (Table 3.11). The first region was on the cyclohexane nearest the piperazine ring, while the second portion was on the benzocyclohexane. Many σ_2 receptor ligands have focused on alterations for the second region and have had little success [2-5, 14]. Using the derived validated CoMFA model, the study of various other substituent groups can be determined efficiently in less amount of time.

Among these new structures, **33** and **34** show the highest predicted values according to the HF/3-21G* model (Table 3.11). When comparing compound **33** to compound **2**, the original ligand had a higher predicted pK_i value in the three different calculations. Therefore σ_2 receptors are more active with the additional substituents on the cyclohexane ring. In compound **34** (predicted $pK_i = 0.668$), which has a substitution of a fluorine on the cyclohexane showed the best predicted pK_i value using the HF/ 3-21G* PLS analysis. **37** increased the size of the halogen with chlorine showing a drop in the predicted activity (0.544). However, these two compounds are more active than the original structure through the ideal location for the substituents in the CoMFA map. Therefore, there is a possibility of future development of σ_2 receptor ligands by using small substituents on the cyclohexane to increase the selectivity and affinity.

To determine the pK_i of the benzocyclohexane substituents, many of the ligands mentioned above had been predicted using the CoMFA [2-5, 14]. The model showed a large area of positive and negative charge and bulky substituents surrounding the second region. Various substituents at the methoxy (CH_3O) location on the aromatic ring were tested focusing on these characteristics. Substituents with a bulkier groups such as methoxy (OCH_3), ethyloxy (OCH_2CH_3), methoxy trichloride ($OCCl_3$), and acyl tert-butyl (C=O [t-butyl]) provide a higher affinity for the receptor. The addition of the larger group generates a localization of electrons on the oxygen. This increases the negativity in the desired area of the molecules. These compounds had an active range from 0.217 to 0.668 (Table 3.11). Therefore, substituents within this size range which have this electronegative effect, may be active or have a higher partial affinity with the σ_2 receptor.

Other compounds that were suggested and tested in the CoMFA model showed a significant drop in activity (Appendix). These substituents were electron withdrawing or had smaller substituents. For instance, compound **35** (predicted pK_i = -0.140) with the hydroxyl (OH) and compound **41** (-0.145) with the bromine (Br) (Appendix).

CHAPTER 4

CONCLUSIONS

This study demonstrated the modified pharmacophore of σ_2 receptor ligands using DISCOtech with four points (nitrogen, lone pair of electrons, and two centers of hydrophobic rings). An alignment of 32 compounds using AM1, HF/3-21G*, and B3LYP/3-21G* optimized geometries and atomic charges were calculated from methods on Mulliken populations and the predicting ability of the CoMFA model. Within this study, HF/3-21G* showed the best reliable ability to predict affinities for the CoMFA. Two possible sights of modification to a highly active compound **2** were located on the cyclohexane and cyclohexanebenzene. Both locations showed the addition of electron donating groups may produce ligands to be more active for the σ_2 receptor.

With the recent development of PB28, this flexible molecule should be considered in future studies of σ_2 receptor ligands. Design and development should focus on a rigid highly active ligand. Another avenue of development should focus on the development of naphthalene in place of the benzocyclohexane and substituents with electron donating groups. Finally, a direct comparison between the σ_1 and σ_2 pharmacophore may be an effective method to determine more σ_2 selective receptor ligands.

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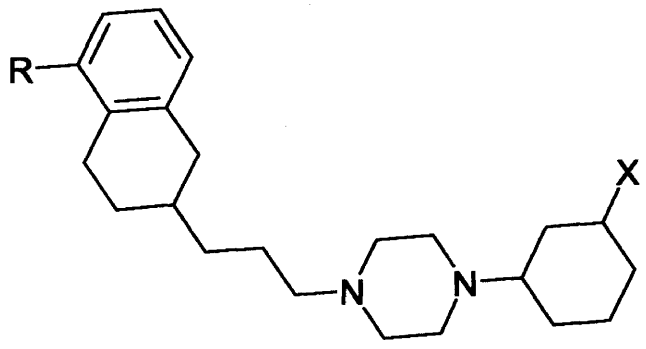
APPENDIX

NEW POTENTIAL σ_2 RECEPTOR LIGANDS

This table presents new potential σ_2 receptor ligands that were calculated to be less active than PB28.

Table A.1 Prediction of Bioactivity of New Ligands That Are Not Active



Compounds	Lit. pK_i	R	X	AM1	HF/3-21G*	B3lYP/3-21G*
2 (PB28)	0.469	OCH_3	H	0.468	0.493	0.529
35	-	OH	H	0.406	-0.139	-0.469
36	-	NO_2	H	0.457	-0.212	-0.009
39	-	NH_2	H	0.336	-0.235	-0.221
40	-	Cl	H	0.368	-0.346	-0.276
41	-	Br	H	0.338	-0.376	-0.406
42	-	COOH	H	0.504	-0.399	-0.604
43	-	OCH_3	COOH	0.059	-0.514	0.376
44	-	SH	H	0.339	-0.513	-0.38
45	-	OCH_3	SH	0.319	-0.422	0.338
46	-	COH	H	0.128	-0.209	0.475
47	-	OCH_3	COH	0.405	-0.341	0.399
50	-	T-butyl	H	0.309	-0.960	-0.372

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